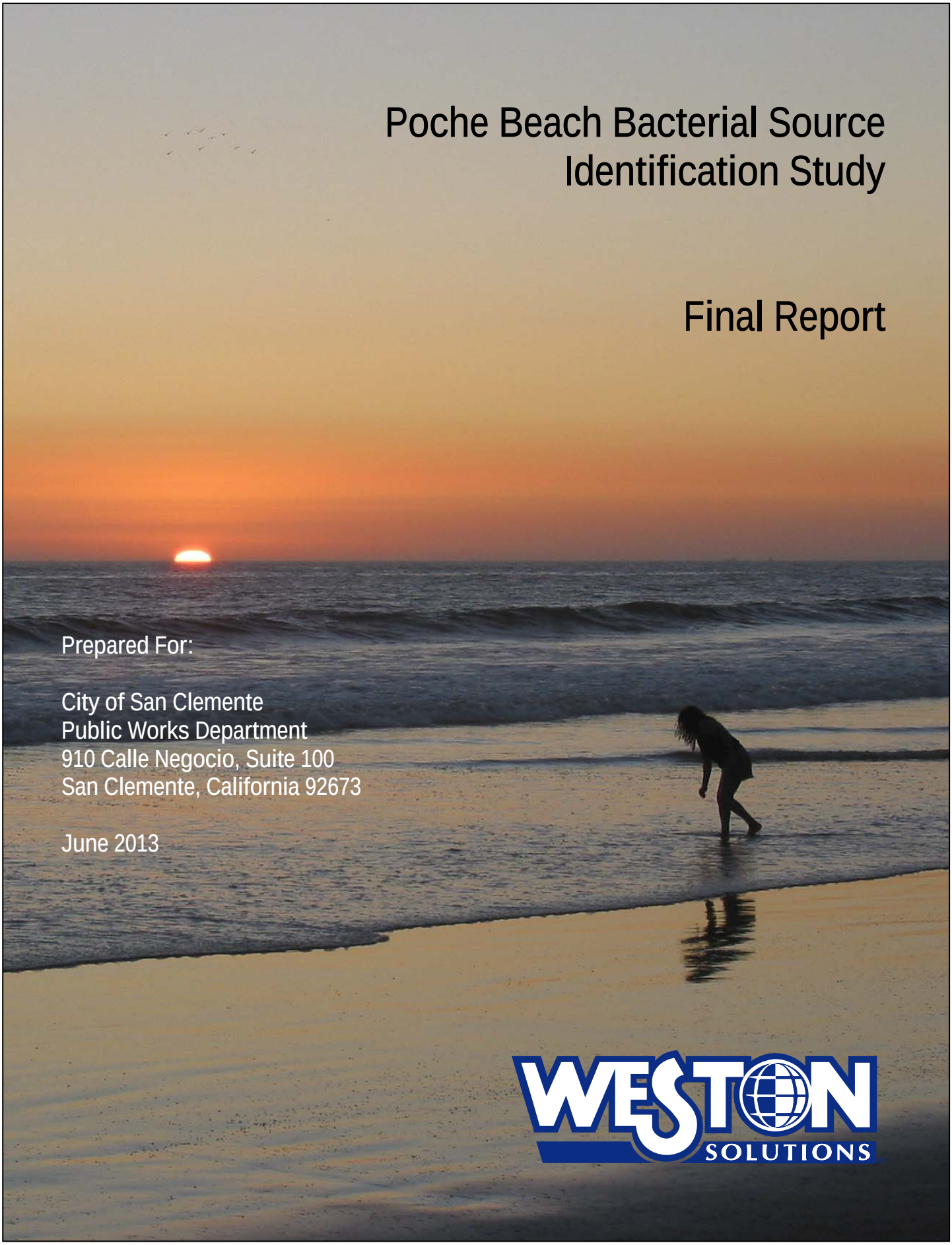

Poche Beach Bacterial Source Identification Study

Final Report

Prepared For:

City of San Clemente
Public Works Department
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June 2013

WESTON SOLUTIONS

Poche Beach Bacterial Source Identification Study
Final Report

Prepared For:

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Public Works Department
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Table of Contents

TABLE OF CONTENTS

Table of Contents

Table of Contents

Table of Contents

LIST OF FIGURES

LIST OF TABLES

Table of Contents

LIST OF ACRONYMS

%	percent
µM	micrometer
AB411	Assembly Bill 411
BMP	Best Management Practice
CCTV	closed circuit television
cfs	cubic feet per second
COC	chain-of-custody
CRM	Certified Reference Material
Ct	cycle threshold
DNA	deoxyribonucleic acid
DNQ	detectable but not quantifiable
DO	dissolved oxygen
ELAP	Environmental Laboratory Accreditation Program
EMA	EnviroMatrix Laboratory
EPA	U.S. Environmental Protection Agency
EQV	equivocal
FRAM	ferroelectric random access memory
ft	feet
g	gram
HDPE	high density polyethylene
LCM	Laboratory Control Material
LLOQ	lower limit of quantification
LOD	limit of detection
m	meter
mg/L	milligrams per liter
mL	milliliter
MLLW	mean lower low water
MPN	most probably number
MS4	Municipal Separate Storm Sewer System
MST	Microbial Source Tracking
ND	not detected
NTU	Nephelometric Turbidity Units
PBS	Phosphate Buffered Saline
PCR	polymerase chain reaction
ppt	parts per thousand

LIST OF ACRONYMS (CONTINUED)

PVC	polyvinyl chloride
PVD	physical vapor deposition
QA/QC	quality assurance/quality control
QAPP	Quality Assurance Project Plan
ROQ	range of quantification
SIPP	Source Identification Pilot Program
SOP	Standard Operating Procedure
SWAMP	Surface Water Ambient Monitoring Program
TDS	total dissolved solids
TKN	total Kjeldahl nitrogen
TP	total phosphorous
TSS	Total suspended solids
USGS	U.S. Geological Survey
UV	ultraviolet
WESTON®	Weston Solutions, Inc.
µS/cm	microsiemens per centimeter

EXECUTIVE SUMMARY

Poche Beach, located in San Clemente, California, is one of the only beaches in Orange County, California, that regularly exceeds Assembly Bill 411 (AB411) bacterial water quality standards and has been listed as one of the top ten "Beach Bummer" sites in Heal the Bay's Annual Beach Report Card. In order to improve water quality at the beach, the City of San Clemente (the City) felt that it was important to understand the bacterial sources and transport mechanisms at play within the Prima Deshecha Cañada Watershed that drains to the beach. The City contracted with Weston Solutions, Inc. (WESTON®) to design and conduct a bacterial source tracking study. The current source tracking study further explores the findings of a previous bacteria study performed in 2005 and 2006 for Orange County by examining likely bacterial sources at Poche Beach and sources in the overall Prima Deshecha Cañada Watershed.

The Poche Beach Bacterial Source Identification Study consists of several independent but interlinked studies. The studies were designed to determine the location and magnitude of bacterial sources and transport mechanisms both within Prima Deshecha Cañada Watershed and along Poche Beach and to assess the effectiveness of existing Best Management Practice (BMP). The overall study is comprised of the following major elements:

- Sanitary Survey Investigation.
- Biofilm Study.
- Groundwater Study.
- Bioswale BMP Effectiveness Study.
- Scour Pond/Beach Environment Study.
- Human Bacterial Source Identification Survey.

The Poche Beach Bacterial Source Identification Study report is organized into 11 sections, including this executive summary. The executive summary provides a synopsis of the organization of the study and report, the major findings of the study, the study conclusions, and the recommendations to the City for reducing bacterial loads in the watershed and at Poche Beach.

Section 1 discusses previous studies, the site description, project scope, AB411 criteria, general methods, and the report organization. Sections 2 through 6 provide study-specific summaries of each of the individual elements that comprise the Poche Beach Bacterial Source Identification Study, as listed above. Each section includes an overview of the reason for conducting the particular study, materials and methods used to collect data and carry out the study, results of data analysis, discussion of results, and conclusions.

Section 8 provides conclusions from the study based on the findings from the individual study elements. Section 9 includes recommendations to the City of San Clemente for reducing bacterial levels. Section 10 contains a list of references to literature cited in the report.

The appendices include the original and adapted scopes of work, analytical laboratory reports, chain-of-custody (COC) forms used in field and laboratory sampling, field data sheets, and a report for the Poche Beach Human Bacterial Source ID Study, which was an additional study carried out to assess the presence of bacteria from human sources in one drainage of the Prima Deshecha Cañada Watershed.

Sanitary Survey

A sanitary survey of the Prima Deshecha Cañada Watershed was designed to measure bacterial concentrations at various points along the Mainstem Channel to determine where the largest bacterial inputs occurred and to compare bacterial concentrations and loads to those measured in the 2006 study (WESTON, 2006). For this study, sampling points were kept identical to those used in the 2006 study and flow meters were installed throughout the Mainstem Channel and at the base of Cascadita Channel to assess sub-watershed load and flow contributions.

Water chemistry, bacterial concentrations, flow, and bacterial loads were examined in the watershed over the course of a year from November 2010 through December 2011. The sanitary study consisted of two 24-hour monitoring events, conducted on December 14, 2010 and July 21, 2011, and continuous flow monitoring throughout the year at the six sample locations.
The following are the major findings of the sanitary survey investigation:

- A strong diurnal pattern was observed in flow; flows at night and early morning were two to three times greater than flows during the day. This pattern is consistent with residential and commercial irrigation, which typically peaks at night in urbanized watersheds.
- Flow was found to be greatest at Sites 5 and 7 near the base of the watershed across all months, and was particularly elevated in February and March relative to the other monitored sites.
- The relative contribution of flow from the upper watershed appears to have decreased since 2006, but the results were highly seasonal.
- Flow in the Cascadita Channel appears to have decreased since 2006.
- Fecal coliform and enterococci concentrations were greatest in the upper and middle watershed and were highest in the early morning hours. These results are similar to those in the 2006 study.
- Fecal coliform and enterococci concentrations were lowest at Sites 6 and 7 at the base of the watershed.

Biofilm Study

A biofilm study was designed to determine whether bacterial regrowth within the storm drain itself is a significant source of bacteria in the watershed. The configuration of the Mainstem Channel appears conducive for bacterial growth because the mainly underground configuration affords water exposure to solar ultraviolet (UV) radiation in only a few short sections and the channel remains perpetually wet and littered with organic material. A small-scale biofilm study conducted during the 2005 to 2006 survey, however, did not find the channel to be a significant source of bacteria. Confirmation of that finding was needed to rule out biofilm as a bacterial source. In this study, several small concrete discs (coupons) were attached to the channel bottom in two storm drains within the Mainstem Channel to measure biofilm in the Municipal Separate Storm Sewer System (MS4). The coupons were removed at intervals of 8 days, 7 weeks, 10

weeks, and 6 months. The discs were enumerated for total coliforms, fecal coliforms, and enterococci. The major findings of the study are presented below:

- Regrowth of total coliform, fecal coliform, and enterococci occurred at all sites within the Mainstem and Cascadita Channels.
- Colonization of the concrete substrate of the coupons occurred rapidly (within 8 or 9 days of deployment).
- The microbial communities that contained the three types of indicator bacteria were maintained over time (the6-month time frame of the study) under conditions found in the storm drain system.
- Biofilm concentrations of all three indicators were highest in the upper Mainstem Channel sites.
- Biofilm concentrations of all three indicators were lowest at the Cascadita Channel (Site 6) during nearly all sampling events.
- The results indicated that the biofilm within the Cascadita and Mainstem Channels could serve as a continual reservoir of indicator bacteria and a source of indicator bacteria to the ocean receiving waters at Poche Beach.

Groundwater Study

The results of the 2005 to 2006 bacterial source identification study suggested that the majority of bacterial loads and concentrations in the Cascadita and Mainstem Channels originated at the top and mid points of the watershed from side inlets downstream of the Prima Deshecha Landfill, possibly as a result of groundwater intrusion and over-irrigation. A groundwater study was designed to determine whether groundwater intrusion either contributes bacteria or provides a transport mechanism for bacteria to (MS4) within the watershed. To make this determination, temporary groundwater monitoring wells were installed at four locations and monitored over a one-year period for indicator bacteria, nutrients, and general chemistry. The major findings of the study are presented below.

- Groundwater at the sites monitored did not contain elevated levels of indicator bacteria and did not appear to be a direct source of bacteria to the watershed. Concentrations of all three indicator bacteria were largely at or below detection limits in the majority of samples collected.
- Although bacterial levels in groundwater were low, groundwater infiltration into the storm drain network likely helps maintain an atmosphere conducive to bacterial regrowth inside the channel.
- Total phosphorous (TP) concentrations were greater than the water quality benchmark in all groundwater samples collected, suggesting that groundwater influx contributes to elevated TP levels in the channel, which may enhance regrowth of indicator bacteria.

Bioswale BMP Effectiveness Study

Shorecliffs Golf Course is located within the middle and lower portion of the Prima Deshecha Cañada Watershed and covers approximately 20% of the drainage. In the late 1990s, a bioswale was installed at the golf course as a BMP in order to assess whether the diversion of the Mainstem Channel runoff through the bioswale would reduce nutrient and bacterial loads to the lower portion of the watershed and ultimately to Poche Beach. For the current study, the

bioswale BMP effectiveness study was designed to assess the effectiveness of the bioswale BMP. Water samples were collected at the inlet and the outlet of the bioswale and were analyzed for enterococci, total and fecal coliforms, nutrients, and general water quality. The major findings of the study are presented below:

- Indicator bacteria concentrations and loads were lower at the outlet of the bioswale than the inlet, suggesting that the bioswale may have a limited positive effect in reducing bacterial levels in the watershed.
- Concentrations of ammonia, nitrite, cadmium, and nickel (total and dissolved) decreased from upstream to downstream in the first section of the bioswale, suggesting that the bioswale had been effective in reducing concentrations of these constituents.
- Flow was greater at the outlet of the bioswale than from the inlet, suggesting an increase in surface flow from irrigation practices or surfacing groundwater in the lower portion of the bioswale.

Scour Pond and Beach Environment Study

The scour pond located at the terminus of the Prima Deshecha Cañada Mainstem Channel and the fresh water discharge to the ocean is thought to attract large numbers of birds that periodically congregate at Poche Beach. Because the birds defecate on the beach sands, they are thought to be a source of indicator bacteria in the ocean receiving waters at Poche Beach. Although the scour pond is predominantly fresh water, it is influenced during high spring tide events, which allow for some exchange with seawater. The Scour Pond and Beach Environment Study was designed to measure indicator bacteria concentrations in various areas of the scour pond, along adjacent areas of the sandy beach, and in the ocean water immediately in front of the scour pond over the course of a tidal cycle to assess proportional bacterial contributions of the scour pond itself and of the sand where the birds congregate. In this portion of the study both traditional culture techniques for fecal indicator bacteria (total coliform, fecal coliform, and enterococci) and microbial source tracking (MST) were used. MST methods have been developed to discriminate between human and non-human sources of fecal contamination. Real-time polymerase chain reaction (PCR) MST assays designed to detect human-associated (HF183) and gull-associated (Gull2Taqman) fecal contamination were used. In addition, samples were analyzed for the general *Bacteroidales* marker to detect *Bacteroides* spp., anaerobic bacteria that are predominant in warm-blooded animals (including humans). Three scour pond surveys were conducted. The major findings of the scour pond and beach environment study are presented below:

- Both beach and scour pond sand had low concentrations of indicator bacteria during Survey 1 (at or below detection limit in most samples), suggesting that they did not serve as a major reservoir of bacteria during the time of the survey (January 2011). Indicator bacteria concentrations were elevated in the scour pond compared to ocean samples during this event.
- During Survey 2, the concentrations of indicator bacteria at Site 7 were lower than those in the scour pond, tidal creek draining the scour pond, and ocean receiving waters, suggesting the presence of regrowth or bacterial contributions from sources other than the watershed.

- During Survey 3, indicator bacteria concentrations in the ocean receiving waters were greater than those at the other sites, suggesting a source of bacteria in the ocean receiving waters other than the watershed.
- During Survey 2, gull MST results showed that bacteria originating from birds were found in all samples collected during all sampling periods, except Site 7 where the marker was found only in the 7 a.m. sample. Quantifiable levels of the gull marker were measured at all five sites, with ocean samples having the most frequent occurrence of detection and the highest concentration (the 7 a.m. sample). Concentrations of the gull marker were fairly consistent across all sites with a tendency to be higher at the tidal creek and adjacent ocean site.
- During Survey 3, the gull marker frequency of detection and concentration was greatest for ocean samples compared to the other sites. The gull marker was detected at all beach sites during every monitoring period. The gull marker concentrations in the 1.p.m. samples tended to decrease from north to south. The gull marker was detected in scour pond and tidal creek samples, but concentrations were low, with all but one tidal creek sample returning detectable but not quantifiable (DNQ) results. No samples were positive for the gull marker in samples collected from Site 7, located at the base of the watershed.
- The results of Surveys 1 and 2 suggest that birds on the beach were a source of fecal bacteria in the receiving waters at Poche Beach. Furthermore, enterococci and fecal coliform concentrations were correlated to the gull marker concentrations for ocean samples collected adjacent to the scour pond. This relationship was weak when all ocean samples were used, lending support to the theory that fresh water in the scour pond may act to congregate birds.
- A canine-associated *Bacteroides* marker was detected from all sites during Survey 3, suggesting that bacteria originating from canines (coyotes cannot be ruled out) are present in the watershed. A total of 12 samples were positive for the canine marker: four at Site 7, three each in the scour pond and tidal creek, and one each in the ocean and at the UV treatment discharge. Of these, only Site 7 and the Ocean sample during the 1 p.m. monitoring period had concentrations high enough for quantification.
- A human-associated *Bacteroides* marker was detected at Site 7 (located at the base of the watershed), suggesting that bacteria originating from human sources were present in the watershed during Surveys 2 and 3. However, the low frequency of occurrence (3 positive samples among 48) and low concentrations (2 out of 3 sample concentrations were below the statistical detection limit) coupled with a lack of positive results for the human marker in the scour pond, tidal creek, or ocean samples suggests that bacteria originating from humans had, at most, a minimal impact on indicator bacteria levels in the ocean receiving waters at Poche Beach.

Human Bacterial Source Identification Survey

The Poche Beach Human Bacterial Source Identification Survey was a limited survey of a small drainage within the Prima Deshecha Cañada Watershed that was designed to complement the larger scale effort of the Poche Beach Bacterial Source Identification Study. One of the major goals of the survey was to determine the extent to which indicator bacteria (total coliforms, fecal coliforms, and enterococci) originating from human origin (e.g., sewage, homeless population,) might be impacting water quality at Poche Beach. This study used real-time PCR to analyze samples for a human-associated *Bacteroides* MST marker.

The major findings of the Human Bacterial Source Identification Survey presented below are based on the data collected as part of the investigation with the knowledge that additional data may need to be collected to verify the study conclusions:

- The results of the Poche Beach Human Bacterial Source Identification Survey indicated that flow from the PB-5UP-SDS storm drain was positive for the human-associated *Bacteroides* marker, suggesting that bacteria originating from anthropogenic sources were present at the time the sample was collected.
- The negative results at Mainstem Channel sites upstream and downstream of the storm drain, the scour pond, and ocean receiving waters at Poche Beach suggest that any source originating from the storm drain outfall did not appear to have a measurable impact downstream on the day the samples were collected.
- The lack of positive results for human marker at any of the sites in a follow-up survey suggests that bacteria from human origin that may have been present in the drainage of PB-5UP-SDS during the initial survey was ephemeral in nature. Overall, the data did not provide evidence of a chronic source, such as leaking sewage infrastructure.
- The results of the closed circuit television (CCTV) investigation conducted by the City were consistent with the lack of positive results for the human-associated *Bacteroides* analyses conducted by WESTON and suggested that leaking sewage infrastructure within the drainage was not a likely source of indicator bacteria in the receiving waters of the Mainstem Channel and Poche Beach.
- The presence of a positive result for the human-associated *Bacteroides* marker in bird feces needs further investigation to identify potential sources or causes.
- Additional surveys would be needed to rule out any human sources of indicator bacteria in the PB-5UP-SDS drainage or other areas within the Prima Deshecha Cañada Watershed.

Summary of Recommendations

Although these results indicate the presence of human sources of bacteria in this drainage, the overall low frequency of occurrence of human-associated MST marker suggests that human sources were not the primary contributor to the high levels of indicator bacteria measured in the watershed. Based on the findings of the various studies conducted over the course of this project, some basic recommendations can be made. There are three major areas that should be considered in order to reduce bacterial concentrations in the receiving waters of Poche Beach:

- **Reduce flows from excess irrigation**—The results of the sanitary surveys and biofilm studies indicate that excess irrigation is likely a major source of flow in the Prima Deshecha Cañada Watershed. A constant flow of water helps maintain a well-developed biofilm in the Mainstem Channel, which is likely a source of indicator bacteria to the ocean receiving waters at Poche Beach. Therefore, reducing over-irrigation in the watershed may help reduce bacterial levels at Poche Beach.

- **Address the scour pond configuration**—The scour pond surveys revealed that the scour pond at Poche Beach is at least 15 feet deep. This large depression at the base of the watershed provides an environment that may be conducive to growth of indicator bacteria and a fresh water source by which to attract birds. Additional studies should be

considered to address the configuration of the scour pond, address the limited public access to Poche Beach, and reduce flows to the ocean that appear to attract gulls.

- **Reduce the impact of birds at the beach**—The scour pond studies reveal that fecal material from gulls are a likely source of indicator bacteria in the receiving waters. Management plans to reduce the impact of gulls on indicator bacteria in the receiving waters should be considered.

1.0 INTRODUCTION

Poche Beach, located in San Clemente, California, is one of the only beaches in Orange County, California that regularly exceeds Assembly Bill 411 (AB411) bacterial water quality standards and has been listed as one of the top ten "Beach Bummer" sites in Heal the Bay's Annual Beach Report Card. In order to improve the water quality at the beach, the City of San Clemente felt that it was important to understand the bacterial sources and transport mechanisms at play within the Prima Deshecha Cañada Watershed that drains to the beach, and to this end, contracted Weston Solutions, Inc. (WESTON®) to design a bacterial source tracking study. The current source tracking study further explores the findings of a previous bacteria study performed from 2005 to 2006 (WESTON, 2006) for the County of Orange by examining likely bacterial sources at Poche Beach as well as sources in the overall Prima Deshecha Cañada Watershed (Figure 1-1).

Based on the findings of the 2005-2006 study, both human activities and natural features of the watershed and beach were further investigated for their potential to be major sources, sinks, and/or transport mechanisms for bacteria. Specifically, these were defined to be the following:

- Sources - Bacterial transport via over-irrigation in the sub-watersheds that drain to the Mainstem and Cascadita Channel (a tributary drainage to the south of the Mainstem Channel)
- Bacterial regrowth in the scour pond, bacterial production via bird activity at the base of the watershed, and bacterial regrowth within the storm drain channels.
- Sinks - Bacterial reduction in the bioswale Best Management Practice (BMP) along the golf course.
- Transport - Bacteria transport via the influx of groundwater and/or spring water in the sub-watersheds.

1.1 Background and Problem Statement

Historically, Poche Beach has had more beach postings as a result of exceedances of AB411 bacterial standards (Table 1-1) for indicator bacteria (total coliforms, fecal coliforms, and enterococci) than any other beach in Orange County. The consistently poor water quality ratings at Poche Beach prompted a directive to be issued by the San Diego Regional Water Quality Control Board (Regional Board) in 2002 requiring that actions be taken by the City of San Clemente and the County of Orange to improve water quality and minimize bacterial exceedances.

Table 1-1. Assembly Bill 411 (AB411) Bacteriological Criteria for Recreational Beaches in California

Indicator Bacteria	30-Day Limit[1]	Single Sample Limit
Total Coliform	1,000 MPN[2]/100 mL	1,000 MPN/ 100 mL if Fecal > 10% of Total, or 10,000 MPN/100 mL[3]
Fecal Coliform	200 MPN/100 mL	400 MPN/100 mL
Enterococci	35 MPN/100 mL	104 MPN/100 mL

1 = 30-day limit is based on the geometric mean of at least five weekly samples.

2 = MPN is Most Probable Number
3 = Total coliform single sample limit of 10,000 MPN drops to 1,000 when the fecal coliform value is greater than 10% of total coliform value.

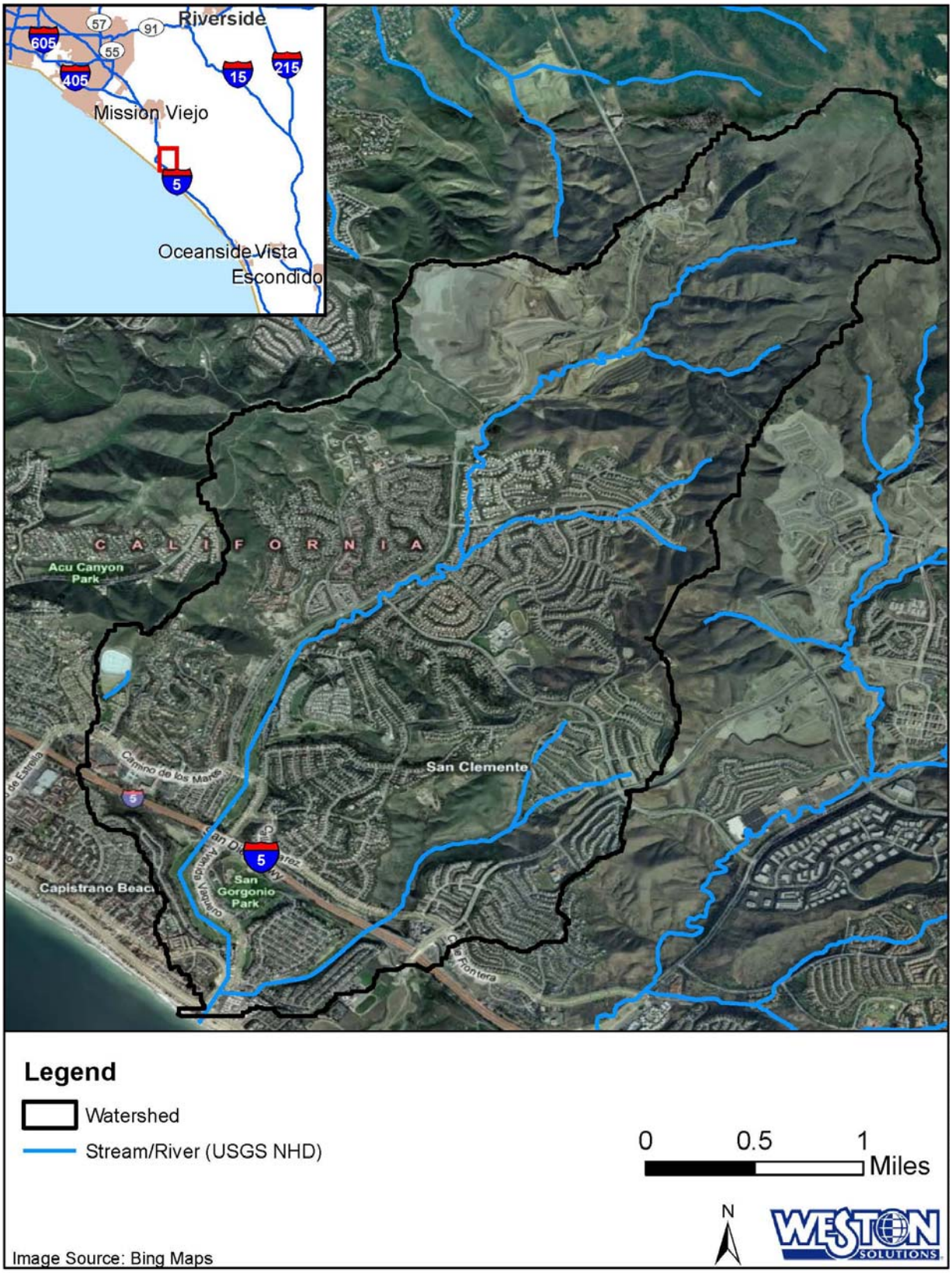


Figure 1-1. Prima Deshecha Cañada Watershed, Orange County, CA

In 2006, WESTON completed a bacterial source tracking investigation for the County of Orange of the Prima Deshecha Cañada Watershed that drains to Poche Beach (WESTON, 2006). The findings from the 2006 bacterial source tracking study are summarized below.

- The source of indicator bacteria in the Mainstem Channel of the watershed was primarily the inflow from tributary storm drain pipes that drain the surrounding watershed.
- The most significant contribution of flow and bacterial loading in the Mainstem Channel emanated from tributary pipes in the mid- to upper region of the watershed.
- The greatest flow throughout the channel occurred during the early to mid-morning hours and appeared to be the result of residential over-watering.
- The Cascadita Channel (which flows into the Mainstem Channel near the base of the watershed) contributed less than 1% of the bacterial load to the overall runoff.
- Indicator bacteria in the Prima Deshecha Cañada Watershed and the ocean receiving waters at Poche Beach did not appear to originate from human fecal sources.

The results from the 2006 study suggested that the majority of the indicator bacteria in the Mainstem Channel originated from over-irrigation in the upper watershed and that the initial load generated from these sources became amplified by processes such as re-growth within the storm drain system. Following this investigation, the City of San Clemente installed an ultraviolet (UV) treatment facility in 2007 at the base of the watershed to treat up to 1.1 million gallons of water per day flowing from the Mainstem Channel to Poche Beach (Figure 1-2).

Despite these actions by the City of San Clemente and the County of Orange, Poche Beach continued to be regularly posted for bacterial exceedances following the installation and operation of the UV treatment system. In 2010, WESTON was asked to conduct an additional study to assess the likely bacterial transport mechanisms and bacterial sources within the Prima Deshecha Cañada Watershed and along Poche Beach to prioritize future BMPs aimed at reducing bacterial loads and improving water quality.

Figure 1-2. Aerial View of Poche Beach Showing the Location of the UV Treatment System and Scour Pond at the Mouth of the Mainstem Drainage Channel

1.2 Project Description

The following five specific studies were designed to determine the location and magnitude of bacterial sources and transport mechanisms both within Prima Deshecha Cañada Watershed and along Poche Beach and to assess the effectiveness of an existing BMP. The findings of these studies will help the City of San Clemente to effectively implement BMPs that can be used to reduce bacterial loads in the near future and to improve water quality.

Sanitary Survey
A sanitary survey of the Prima Deshecha Cañada Watershed was designed to measure bacterial concentrations at various points along the Mainstem Channel to determine where the largest bacterial inputs occurred and to compare bacterial concentrations and loads to those measured in the 2006 study (WESTON, 2006). For this study, sampling points were kept identical to those used in the 2006 study and flow meters were installed throughout the Mainstem Channel and at the base of Cascadita Channel to assess sub-watershed load and flow contributions.

Biofilm Study
A biofilm study was designed to determine whether bacterial re-growth within the storm drain itself is a significant source of bacteria in the watershed. The configuration of the Mainstem Channel appears conducive for bacterial growth because the mainly underground configuration affords water exposure to solar UV radiation in only a few short sections and the channel remains perpetually wet and littered with organic material. A small-scale biofilm study conducted during the 2005 to 2006 survey, however, did not find the channel to be a significant source of bacteria. Confirmation of that finding was needed to rule out biofilm as a bacterial source. In this study, small in situ concrete tiles were used in two storm drains within the Mainstem Channel study to measure biofilm in the Municipal Separate Storm Sewer System (MS4).

Groundwater Study
A groundwater study was designed to determine whether groundwater intrusion may either contribute bacteria or provide a transport mechanism for bacteria within the Prima Deshecha Cañada Watershed. To make this determination, temporary groundwater monitoring wells were drilled at four locations within the watershed and were analyzed for bacteria, nutrients, and general chemistry.

Bioswale Study
A BMP effectiveness study was designed for the Shorecliffs Golf Course bioswale to assess the effectiveness of the bioswale for reducing bacteria and nutrient loads. To assess effectiveness, water samples were collected at the inlet and the outlet of the bioswale and were analyzed for enterococci, total and fecal coliforms, nutrients, and general water quality.

Scour Pond and Beach Environment Study
A Scour Pond and Beach Environment Study was designed to measure indicator bacteria concentrations within various areas of the scour pond as well as along adjacent areas of the sandy beach and in the ocean water immediately in front of the scour pond over the course of a tidal cycle. Sampling points for this study were located at multiple locations within the scour pond, along transects on the beach radiating out from the scour pond, and in the ocean receiving waters to assess proportional bacterial contributions of the scour pond itself and of the sand where the birds congregate. In this portion of the study both traditional culture techniques for fecal indicator bacteria (total coliform, fecal coliform, and enterococci) and microbial source tracking (MST) were used. MST methods have been developed to discriminate between human and non-human sources of fecal contamination (Boehm et al., 2013). Real-time polymerase chain reaction (PCR) MST assays designed to detect human-associated (HF183) and gull-associated (Gull2Taqman) fecal contamination were used. In addition, samples also were analyzed for the general *Bacteroidales* marker to detect *Bacteroides* spp., anaerobic bacteria that are a predominant resident in the feces of warm-blooded animals (including humans). Because *Bacteroides* spp. are obligate anaerobes, they are thought to provide a good indication of recent fecal pollution (Dick and Field, 2004).

Human Bacterial Source Identification Survey
The Poche Beach Human Bacterial Source Identification Survey was initiated in response to positive results in the human-associated *Bacteroides* samples collected as part of the *Poche Beach Bacterial Source Identification Study* (WESTON, 2012). These positive results were infrequent throughout the study and did not show a regular pattern suggestive of a consistent source, such as leaking sewage infrastructure. However, the City of San Clemente felt it was important to pursue an investigation of potential sources to assure the protection of human health. In addition, the County of Orange (County), an active stakeholder in the project, had identified a potential positive human-associated *Bacteroides* result from a sample collected from a storm drain that discharges to the Mainstem Channel in the Prima Deshecha Cañada Watershed (also known as the MO1 Channel).

A number of MST methods have been developed for discriminating between human and non-human sources of fecal contamination. Many MST methods take advantage of host-specific

genetic differences in the 16S rRNA gene of *Bacteroides* spp., anaerobic bacteria that are predominant in the feces of warm-blooded animals. Analysis for *Bacteroides* is thought to have advantages over standard enumeration of fecal indicator bacteria. *Bacteroides* are obligate anaerobes and, thus, should be unable to survive long outside of the intestinal tract and are thought to provide a good indicator of recent fecal pollution (Dick and Field, 2004). *Bacteroides* comprise approximately one-third of human fecal microflora (Noble et al., 2005). In this study, a real-time PCR assay was used to detect *Bacteroides* associated with the human gut.

Study Questions:

The following specific questions were posed for each of the areas described above:

Sanitary Survey Investigation

- What are the dry weather concentrations and loads in the Prima Deshecha Cañada Watershed and have they changed since the original 2006 study?

Biofilm Study

- Does the Municipal Separate Storm Sewer System (MS4) act as a biofilm reservoir for fecal indicator bacteria?
- If so, what are the concentrations per square inch?

Groundwater Study

- Is groundwater a source of bacteria to the MS4?

Bioswale BMP Effectiveness Study

- Is the bioswale effective in reducing bacterial concentrations and loads?
- How does operation of the bioswale impact bacterial loads and concentrations?

Scour Pond and Beach Environment Study

- What is the impact of the scour pond on bacterial concentrations at the beach?
- What is the impact of the sand in conjunction with the scour pond and the bird population on bacterial concentrations at the beach?
- What is the impact of sand only on bacterial concentrations at the beach?

Human Bacterial Source Identification Survey

- To what extent do bacteria from human origin impact the water quality at Poche Beach?

1.3 Watershed Description

The Prima Deshecha Cañada Watershed drains approximately 4,400 acres of land located in the City of San Clemente as well as a parcel of land located in the City of San Juan Capistrano. The Mainstem Channel storm drain is a highly channelized stream, most of which is lined with concrete. A significant portion of this conveyance is covered by a concrete ceiling, with the channel tunneling underground for distances of up to 1,000 feet (ft). Approximately 95 percent (%) of the Mainstem Channel is a covered reinforced concrete box, ranging in size from 8ft. by 8ft. to 20ft. by 11ft. The remaining 5% is an open trapezoidal concrete channel. Figure 1-3 shows

an open area of the channel just as it exits a covered portion. The Mainstem Channel receives year-round continual flow from surface runoff and/or groundwater infiltration. Dry weather flows in the downstream reaches of the Mainstem Channel are approximately 0.62 million gallons per day. The largest confluence into the Mainstem Channel is the Cascadita Channel that drains the Cascadita sub-watershed. The Cascadita Channel consists of a natural, soft-bottom channel that is exposed to sunlight and UV radiation.

Figure 1-3. Uncovered Portion of the Mainstem Channel

The runoff from the Prima Deshecha Cañada Mainstem Channel terminates at a scour pond that drains directly to Poche Beach (Figure 1-4). The scour pond is thought to be the main reason that large numbers of birds congregate at Poche Beach on a daily basis. The birds, primarily Herman's gulls (*Laurus heermanni*) and western gulls (*L. occidentalis*), drink and bathe in the mainly fresh water of the scour pond and forage along the adjoining beach sands. The birds defecate on the beach and, thus, are considered a potentially significant source for fecal indicator bacteria at Poche Beach.

Although the scour pond is predominantly fresh water, it is influenced by seawater through wave and tidal actions. The westernmost portion of the scour is tidally influenced and is typically washed out by incoming tides on a daily basis. In general, the scour pond remains roughly the same size throughout the year unless the County reshapes it during semiannual maintenance practices.

Figure 1-4. Scour Pond and Foraging Birds at Poche Beach

Land use within the Prima Deshecha Cañada Watershed is primarily residential with some small commercial businesses (Figure 1-5). There is one major industrial land use in the watershed, the Prima Deshecha Landfill, which is located near the top of the drainage and is owned and operated by the County of Orange Integrated Waste Management Department. Shorecliffs Golf Course, which has been operating since the 1960s, is located within the middle and lower portion of the watershed and covers approximately 20% of the drainage watershed. The landfill is fully permitted and in compliance with all environmental regulations and routinely monitors surface and groundwater downstream of the landfill. The landfill may also act as a bird attractant, as local residents have mentioned that birds regularly visit the landfill before flying downstream to congregate at the scour pond on Poche Beach. The landfill and undeveloped native land comprise almost 30% of the watershed area. There are no dry weather flows from the landfill or from the undeveloped areas at the top of the watershed. The remaining 50% of the Prima Deshecha Cañada Watershed consists of large, single-family homes.

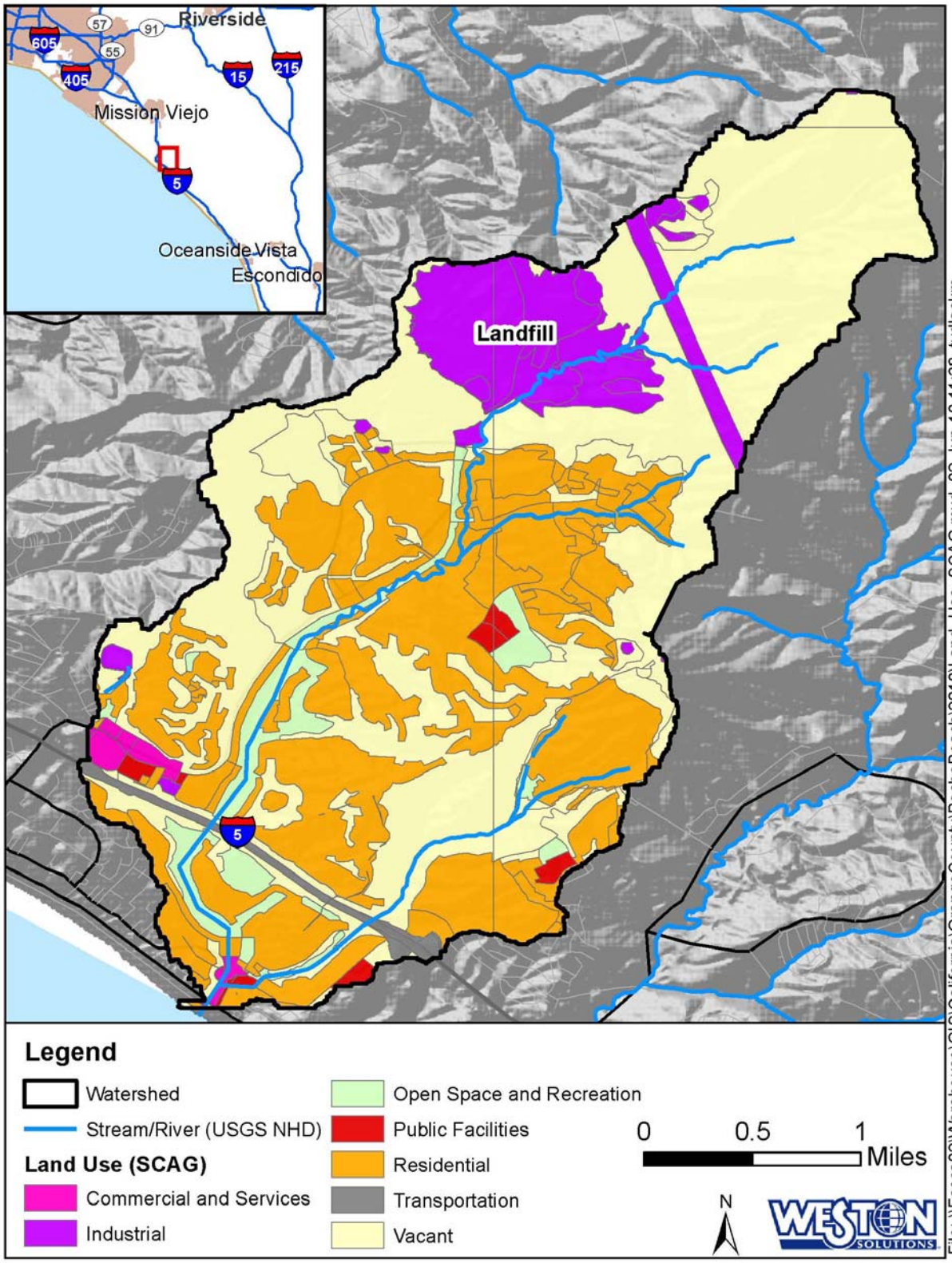


Figure 1-5. Land Use within the Prima Deshecha Cañada Watershed

1.4 Methods Overview

1.4.1 Sample Collection for Bacteria and MST

1.4.1.1 Sample Collection for Analysis of Bacteria by Culture

Water samples were collected in sterile, U.S. Environmental Protection Agency (EPA)-approved bottles containing sodium thiosulfate (to counteract any chlorine that might be present in the water). Sample containers were kept in clear resealable plastic bags until use. Just prior to sampling, the bag and sample container were opened. Both container and lid were held face-down to prevent airborne contamination. The bottle was filled and capped. No sediment or debris was allowed to enter the sample bottle. Sand and sediment samples were sometimes collected; method collection details are provided in those individual sections in which such samples are discussed.

Each field sample was labeled and identified with the project title, appropriate identification number, the date and time of sample collection, and preservation method. The sample container was then sealed in the resealable plastic bag. The samples were stored on ice in the dark from the time of sample collection until delivery to the analytical laboratory. All samples were delivered to the laboratory in time to meet the required 6-hour holding time.

1.4.1.2 Sample Collection for MST Analysis by PCR

Grab samples of water for MST analysis by real-time PCR were collected in the same area as those collected for analysis of bacteria by culture. Samples were collected in 250 milliliter (mL) sterile (irradiated), nuclease-free, plastic bottles. The bottle was labeled with a unique sample name, location, date, time, and name of collector using black, waterproof ink.

To verify proper sampling technique, field blanks were collected at a rate of 5% of the overall samples per field event. Field blanks were collected using the sampling technique described above except that reagent-grade, nuclease-free water was substituted for the water sample. Samples were delivered to the laboratory at the same time as the samples for bacteria analysis (described above).

Extreme care was taken to avoid sample contamination. Samples were collected exclusively by technicians specifically trained in the "clean hands" aseptic technique similar to that required by the Regional Board Surface Water Ambient Monitoring Program (SWAMP) protocols. Resealable plastic bags were used to double-bag the sample bottles before and after sampling. Sample handlers wore double gloves to allow for easy replacement of a contaminated glove. Gloves and outside plastic surfaces were sprayed with DNA *AWAY*™, a deoxyribonucleic acid (DNA) destabilizing reagent, and wiped dry with Kimwipes® prior to opening sample bottles to remove any potential contamination from human contact. While the sample container was open, the cap was held face-down to prevent aerial contamination. After sampling, excessive water was removed from the outside of the sample container, and using clean gloves, the outside of the sample bottle was sprayed with DNA *AWAY*™ and wiped dry prior to placing it in the inner resealable plastic bag. The sealed resealable plastic bags were placed in a clean, dedicated cooler with blue-ice and transported to WESTON's Molecular Biology Laboratory in Carlsbad, CA.

1.4.2 Analytical Methods for Bacteria and MST

1.4.2.1 Analytical Methods for Analysis of Bacteria by Culture

Analytical procedures performed by WESTON are summarized in this section. Standard Operating Procedures (SOPs) pertaining to these methods are found in the Weston Solutions, Inc. Microbial Sciences SOP manual as described in the Quality Assurance Project Plan (QAPP) for the study (WESTON, 2010). Samples were analyzed for total and fecal coliforms and enterococci by WESTON's in-house Microbiology Laboratory (Environmental Laboratory Accreditation Program (ELAP) - Certificate No. 2613). The laboratory methods used in this study are listed in Table 1-2.

For beach sand and sediment, samples were processed in accordance with Boehm et al. (2009). Briefly, a 250 mL bottle was tared on a weighing scale and a sand sample between 9.5 and 10.5 grams (g) was transferred into the bottle. Phosphate Buffered Saline (PBS) solution (1X, 60 mL) was added to the sample and the bottle was shaken vigorously by hand for two minutes. After the sand had settled 30 seconds, the eluant was transferred into a sterile bottle. The original bottle was rinsed with 40 mL of sterile PBS by swirling gently for 10 seconds, and the solution was decanted into the bottle containing the initial eluant. This water sample was analyzed for fecal indicator bacteria and values were normalized to the dry weight of the sediment sample. Dry weight was determined by drying sand (5-10 g, n=3) overnight in an oven set between 103-105 °C.

Table 1-2. Bacterial Parameters and Corresponding Analytical Methods

Analytical Parameter	Analytical Method	Units	Method Detection Limit	Laboratory Reporting Limit	Sample Volume	Container (#, size, type)	Preservation	Holding Time
Total Coliform	SM 9221 B	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Fecal Coliform	SM 9221 E	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Enterococci	Enterolert	MPN/ 100 mL	1 MPN	<10 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours

1.4.2.2 Analytical Methods for Analysis of MST Markers by PCR

Upon arrival at WESTON's Molecular Biology Laboratory, water samples for MST were stored at 4º C until filtration processing (6 to 24 hours after collection). The laboratory analysis procedures for MST samples included sample filtration, DNA extraction, and DNA amplification by real-time PCR.

Water samples were filtered (100 mL each) using a Pall vacuum manifold fitted with sterile, disposable Pall Microfunnels with Supor, 0.2 micrometer (µM)-pore size filters. In addition to field blanks (described in Section 1.4.3), a laboratory blank was processed for every set of MST samples. Laboratory blanks were filtered similarly to samples except that molecular-grade water was substituted for the water sample. For beach sand and sediment, samples were processed as described above for culture analysis and then filtration proceeded as described here for water samples.

After filtration, each filter was rolled and placed into a labeled 2 mL tube containing silica beads and lysis buffer provided in the GeneRite DNA-EZ Kit. Filters were rolled using forceps that had been dipped in bleach to denature residual DNA prior to ethanol/flame-sterilization. Filters were stored at -80°C until extracted (typically within 2 weeks). DNA was extracted and purified using the GeneRite DNA-EZ Kit according to the manufacturer's protocol. Purified DNA was stored at -80ºC until PCR analysis.

Extracted DNA was analyzed for MST by real-time PCR. Analysis included markers for general *Bacteroides* and human-, gull-, and canine-associated markers, as detailed in Table 1-3. Earliest MST investigations for human fecal contamination used a combination of *Bacteroides*-General and HF183 with melt assays. Later MST studies for human fecal contamination used the HF183 Taqman assay, as detailed in publications from the Source Identification Pilot Program (SIPP) (Boehm et al., 2013; Layton et al., 2013). The gull- and canine-associated markers are described in SIPP studies (Sinigalliano et al., 2013 and Schriewer et al., 2013, respectively) and Table 1-3. Samples collected during or before January 2011 were analyzed on a Cepheid Smart Cycler. Samples collected after that date were run on a BioRad CFX96 Real-time PCR Detection System with default quality control data analysis settings, baseline subtracted curve fit with fluorescence drift correction, and baseline threshold set to 100 (Layton et al., 2013).

DNA was quantified on a Nanodrop 2000 UV-Vis spectrophotometer (Thermo-Scientific, Wilmington, DE). Positive controls for human assays used *Bacteroides dorei* genomic DNA (DSM 17855; obtained through the DSMZ-German collection of microorganisms and cell cultures; www.dsmz.de). Positive controls for gull- and canine-assays consisted of plasmid DNA as described in the references for each assay (Table 1-3). Laboratory controls included the following: laboratory blanks, no-template controls, positive controls, and inhibition controls. See Section 1.4.3 for more information.

A full calibration curve was used for the gull and canine assays, yielding quantitative results. These assays were run with a 5-point calibration curve set up with more replicates at the low end to improve curve accuracy (e.g., 6 wells for the two lowest standards, triplicate for the middle, and duplicate for the two highest standards). Each run included its own standard curve. Standard curve outliers were removed based on default quality control settings for replicate precision, amplification efficiency, and linear fit. The detection limit used for samples collected on September 20, 2011 was 0.5 copies/µL rx (200 copies/100 mL sample) and for samples collected October 19, 2011 was 1.25 copies/ µL rx (500 copies/100 mL sample). The detection limit for the canine assay was 0.5 copies/ µL rx (200 copies/100 mL sample).

The limit of detection (LOD) was considered as the lowest standard dilution in which ≥50% of the replicates were positive. The C_t for the lower limit of quantification (LLOQ) was calculated as $C_{t\,LLOQ} = C_{t\,LOD}$ -1.645*SD, where SD = the standard deviation of the LOD standard replicates (AOAC, 2006; Armbruster and Pry, 2008; Burd, 2010; CODEX, 2010; Stewart, 2013). The LLOQ was calculated based on the standard curve metrics of that qPCR run (starting quantity = $10^{(Ct-b)/slope}$).

Samples with results that fell within the linear dynamic range of the standard curve (i.e., within the range of quantification [ROQ]) were considered detected. Samples in which the signal did not pass the cycle threshold (C_t) were considered not detected (ND). A result was considered detected but not quantifiable (DNQ) if it fell between the LLOQ and ROQ. A result was considered equivocal (EQV) if the value fell between the LOD and not detected (i.e., the Ct was greater than zero but below the limit of detection, as determined by a 50% amplification criteria). Determinations of positive or negative for replicate reactions showing mixed results were based on the criteria of Sinigalliano et al. (2013) and Schriewer et al. (2013) in which DNQ was called in cases in which ≥2/3 of the reactions were DNQ, and ND was called in cases in which ≥2/3 of the samples did not return a Ct value. If needed for calculation purposes, a Ct value of 40 was substituted for reactions that did not generate an amplicon (no Ct value) and calculations proceeded using the standard curve for that run (Boehm et al., 2013).

A full calibration curve generally was not used for the human MST assay, HF183 Taqman. In those cases, real-time PCR with end point analysis with default settings was used (end cycles to analyze: 5; cut off range: 10% unless lowest standard was not called as positive, in which case the cut off was adjusted down until the lowest standard curve was called). The results are presented as positive or negative for those assays.

Table 1-3. Microbial Source Tracking (MST) by Real-Time Polymerase Chain Reaction (PCR) Analyses

Target	Assay	Sequence 5'-3' (Final Conc, µM)	References	Conditions[a]
General *Bacteroides*	*Bacteroides* -General	Bac32F: AACGCTAGCTACAGGCTT (0.4) Bac708R: CAATCGGAGTTCTTCGTG (0.4) GenProbe: 6-FAM-CAATATTCCTCACT GCTGCCTCCCGTA-BHQ1 (0.2)	Bernhard and Field, 2000; Dick and Field, 2004	95°C, 2min; 40 cycles: 95°C, 15s; 60°C, 30s
Human *Bacteroides*	HF183 with melt	HF183F: ATCATGAGTTCACATGTCCG (0.4) Bac708R: CAATCGGAGTTCTTCGTG (0.4)	Bernhard and Field 2000; Layton et al., 2013	95°C, 15min; 50 cycles: 94°C, 30s; 54°C, 30s, 72°C, 45s; Melt: 60°C to 95°C at 0.2°/s
Human *Bacteroides*	HF183 Taqman	HF183F: ATCATGAGTTCACATGTCCG (1.2) BthetR1: CGTAGGAGTTTGGACCGTGT (1.2) BthetP1: 6FAM-CTGAGAGGAAGGTCC CCCACATTGGA-TAMRA (0.09)	Haugland et al., 2010; Layton et al., 2013	95°C, 20s; 40 cycles: 95°C, 1s; 60°C, 20s
Gull[b] *Catellicoccus marimammalium*	Gull2 TaqMan	Gull2forward: TGCATCGACCTAAAGTTTTGAG (0.9) Gull2reverse: GTCAAAGAGCGAGCAGTTACTA (0.9) Gull2probe: 6FAM-CTGAGAGGGTGATCGGCC ACATTGGGACT-BHQ1 (0.3)	Sinigalliano et al., 2013	95°C, 15min; 40 cycles: 95°C, 15s; 62°C, 1min
Canine *Bacteroides*	CanineBac	DF475F: CGCTTGTATGTACCGGTACG Bac708R: CAATCGGAGTTCTTCGTG CanineBact: 6FAM-ATTCGTGGTGTAGCG GTGAAATGCTTAG-BHQ1 (0.3)	Schriewer et al., 2013	95°C, 15min; 40 cycles: 95°C, 15s; 60°C, 30s

[a] Master Mix and thermocycler conditions typically consisted of Quanta-Perfecta QPCR Fastmix w/UNG (#84077) used on a BioRad CFX 96 thermocycler except for paired *Bacteroides*-General/ HF183 with melt assays, which were run on a Cepheid Smart Cycler. The master mix for the *Bacteroides*-General assay was Qiagen Quantitect Sybr Green (Cepheid #1017340). Reaction volumes were 25 micrograms per liter (µL).
[b] Also detected pigeon feces for samples collected from S. CA (Sinigalliano et al., 2013).

1.4.3 Quality Assurance/Quality Control Procedures for Bacteria and MST

For microbiological analysis, field blanks were collected at a rate of one sample per sampling event. Field blanks were used to ensure that no contamination originating from the collection, transport, or storage of environmental samples occurred. More detailed information regarding quality assurance/quality control (QA/QC) procedures are presented in the QAPP (WESTON, 2010).

For MST analyses, at least one sterile field blank was collected by each sampling field scientist during each sampling event, as described in Section 1.4.1.2. Once in the laboratory, care was taken to avoid contamination during sample processing. Surfaces and instruments were first cleaned with ethanol and DNA *AWAY*™. The outsides of the sample bottles were wiped down with DNA *AWAY*™ and dried with Kimwipes® prior to being brought to the filtration area.

Laboratory controls included the following: (1) laboratory blanks; (2) no-template controls; (3) positive controls; and (4) inhibition controls. In addition to field blanks, a laboratory blank was processed for every set of MST samples. Laboratory blanks were filtered similarly to samples, except that molecular-grade water was substituted for the water sample. No template controls (2 to 3 per plate) consisted of PCR reactions set up with molecular-grade water replacing sample DNA. Positive controls consisted of plasmid or genomic DNA (see Section 1.4.1.2).

Samples were tested for inhibition using a matrix spike consisting of *B. dorei* DNA added to HF183 Taqman PCR reactions that contained extracted sample DNA (not crude lysate) at full strength (1:1) and extract diluted 1:10 by molecular-grade water. Sample DNA was considered inhibited if the C_t between the undiluted and diluted extracts differed by more than 1.5 cycles. For the combination of *Bacteroides*-General/HF183 with melt assays, 10% of the samples used a matrix spike and, in addition, if any samples failed to amplify by the *Bacteroides*-General assay, the sample was specifically tested by the matrix spike method to ensure that negative results were not due to inhibition. For samples analyzed by only the HF183 Taqman assay, each sample was accompanied by a matrix spike. If results had indicated inhibition, the sample DNA would have been diluted 1:5 and re-analyzed. No inhibition was observed for the samples analyzed during this study.

A field or laboratory blank or no-template control found positive by PCR analysis would have invalidated the samples for that PCR set. No field or laboratory blanks tested positive by PCR during the entire course of this study. Lack of amplification of a positive control would have invalidated the PCR run, and the samples would have been analyzed again. No positive controls failed to amplify for the entire study (all sections of the Poche Beach Bacterial Source Identification Study report).

For quantitative MST, the default quality control settings on the CFX96 were used. Runs failing to meet these parameters (efficiency 90-110%, standard curve $r^2 \geq 0.980$) were re-analyzed.

1.4.4 Chain-of-Custody Procedures

Chain-of-custody (COC) procedures were used for all samples throughout the collection, transport, and analytical process. Samples were considered to be in custody if they were: (1) in the custodian's possession or view; (2) retained in a secured place (under lock) with restricted access; or (3) placed in a container and secured with an official seal such that the sample could not be reached without breaking the seal. The principal documents used to identify samples and to document possession were COC records (Appendix B) and field logs.

COC procedures were initiated during sample collection. A COC record was provided with each sample or group of samples. Each person who had custody of the samples signed the form and ensured the samples were not left unattended unless properly secured. Documentation of sample handling and custody includes the following:

- Sample identifier
- Sample collection date and time
- Any special notations on sample characteristics or analysis
- Initials of the person collecting the sample
- Date the sample was sent to the analytical laboratory

Completed COC forms were placed in a plastic envelope and kept inside the container containing the samples. Once delivered to the analytical laboratory, the COC form was signed by the laboratory personnel receiving the samples. The condition of the samples was noted and recorded by the receiver.

1.5 Report Organization

The Poche Beach Bacterial Source Identification Study report is organized into 11 sections, including this introduction. An executive summary at the beginning of the document summarizes the major findings of the study, the study conclusions, and recommendations to the City for reducing bacterial loads within the watershed and at Poche Beach.

Section 1 discusses previous studies, site description, project scope, AB411 criteria, and the report organization.

Sections 2 through 6 provide the study-specific summaries of each of the individual investigations that comprise the Poche Beach Bacterial Source Identification Study. These consist of the following:

- Section 2: Sanitary Survey Investigation.
- Section 3: Biofilm Study.
- Section 4: Groundwater Study.
- Section 5: Bioswale BMP Effectiveness Study.
- Section 6: Scour Pond/Beach Environment Study.
- Section 7: Human Bacterial Source Identification Survey.

Each section includes an overview of the reason for conducting the particular study, materials and methods used to collect data and carry out the study, results of data analysis, discussion of results, and conclusions.

Section 8 provides conclusions from the study based on the findings from the individual study elements.

Section 9 includes the recommendations to the City of San Clemente for reducing future bacterial levels at both Poche Beach and within the upper Prima Deshecha Cañada Watershed.

Section 10 contains the references to literature cited in this report.

The appendices include the original and adapted scopes of work, analytical laboratory reports, COC forms used in field and laboratory sampling, field data sheets, and a report for the Poche Beach Human Bacterial Source ID Study, which was an additional study carried out to assess the presence of bacteria from human sources in one drainage of the Prima Deshecha Cañada Watershed.

2.0 SANITARY SURVEY INVESTIGATION

2.1 Overview, Sanitary Survey

A sanitary survey designed for source identification was undertaken within the Prima Deshecha Cañada Watershed to determine dry weather bacterial concentrations and loads at different points along the Mainstem Channel. Study design allowed comparison of these concentrations and loads to those measured during the 2005 to 2006 survey to determine whether significant changes have occurred. Data collected from this portion of the overall study can help fill seasonal and temporal data gaps and provide a more robust dataset for assessing sources of bacteria in the watershed.

The results from the 2005 to 2006 survey indicated the following:

- Exceedances of bacterial water quality objectives at Poche Beach were greatest during the month of February and from July through October.
- Flows, concentrations, and loads were highest in the early morning.
- Flows, concentrations, and loads were greatest at the top of the watershed.
- The Cascadita Channel contributed a small percentage of the overall bacterial load.
- Human contamination was not a significant bacterial source based on MST using a human-associated *Bacteroides* assay.
- Over-irrigation was thought to be the major source of bacteria in the watershed.

In the 2010 to 2011 sanitary surveys, a subset of areas investigated in the 2005 to 2006 study were sampled, sites were added above and below the UV Treatment System (which was installed after the previous study), and flow monitoring was conducted in order to determine current bacterial concentrations and loads and to assess whether the findings of the previous study had changed.

2.2 Methods, Sanitary Survey

2.2.1 Field Methods

2.2.1.1 Site Locations

The sampling sites for the 2010 to 2011 Sanitary Survey were the same as those used during the 2005 to 2006 Bacterial Investigation. Sites 2, 3, 4, and 5 were located at regular intervals along the Mainstem Channel, whereas Site 7 was located at the base of the watershed just above the scour pond and below the point at which the Cascadita Channel merges with the Mainstem Channel (Table 2-1 and Figure 2-1). Site 6 was located at the bottom of the Cascadita Channel, just above the confluence with the Mainstem Channel.

Automated leveloggers (Solinst Barrologger Edge) were installed in November 2010 at each site to continuously monitor flow throughout the year. The datalogger at Site 6 was installed at the base of the Cascadita Channel, just upstream of the confluence with the Mainstem Channel. Site 7 was located in the Mainstem Channel downstream of the confluence with the Cascadita Channel. At this location, the channel is bifurcated into two channels separated by a concrete

wall (7 North and 7 South), both of which discharge to the scour pond. Two leveloggers were installed at this location: one in the southern half of the split channel, which receives approximately 75% of the flow from the Mainstem Channel mixed with 100% of the flow from the Cascadita Channel (Site 7 South) and one in the northern side of the split channel, which receives approximately 25% of the flow from Mainstem Channel (Site 7 North).

Table 2-1. Location and Description of Sanitary Survey Sites

Site	Site Name	Sampling	Flowmeter	Longitude	Latitude
2	Estancia	✓		-117.62972	33.47113
3	Calle Nuevo	✓	✓	-117.63952	33.46474
4	Ave. Vaquero	✓	✓	-117.64220	33.45667
5	Calle Grande Vista	✓	✓	-117.64380	33.44360
6	Villa Cascadita	✓	✓	-117.64342	33.44277
7	Base	✓	✓	-117.64489	33.44103

Figure 2-1. 2010-2011 Sanitary Survey Sites

2.2.1.2 Flow Monitoring

The Sanitary Survey Investigation used Solinst leveloggers, which are self-contained, automatic water level and temperature recording devices that contain a barometric pressure sensor, temperature thermistor, 10-year lithium battery, and a datalogger with ferroelectric random-access memory (FRAM) for saving up to 40,000 sets of individual data points. All components within the Solinst leveloggers are sealed within a 7/8-inch by 6.25 inch stainless steel housing with corrosion resistant Titanium based physical vapor deposition (PVD) coating. The dataloggers were programmed to record water depth and temperature continuously at 15-second intervals for the length of the study. A large storm on December 20, 2010 is believed to have dislodged the leveloggers at two of the sampling locations (Sites 4, 5, and 7). Leveloggers were re-installed at these sites on January 17, 2011.

Solinst Level logger for measuring stream stage

At each site, the levelogger was secured to the bottom of the channel as close as possible to the stream thalweg. Weirs or stilling wells were incorporated into the installation at some sites, depending on site conditions. Each levelogger was maintained in the field for a period of approximately 14 months from November 2010 through February 2012. Data collected by the levelogger was manually downloaded during regular site visits. In addition to downloading data, the site visits were used to assess the need for additional stream ratings (see below) and trouble shoot any flow or sampling-related issues. At the conclusion of the monitoring period, installed equipment was removed.

To convert stream stage data to continuous flow, a stream rating was conducted at each site during the initial installation and periodically throughout the study period, depending on changes in site conditions. The stream rating was conducted using standardized stream rating protocols developed by the U.S. Geological Survey (USGS; Rantz, 1982). To accurately measure flow in streams, the following three critical elements are needed to develop rating curves:

- An accurate survey of the stream channel cross section and longitudinal slope.
- Accurate level measurements based on a fixed point.
- Measurements of velocity and flows at base flow conditions.

The stream rating procedure is described below.

Channel Cross Section

Channel cross-section surveys were conducted at each monitoring site. The cross-section survey involves placing endpoints at the highest point of the channel on each bank. A tape is then stretched between the endpoints such that the zero end of the tape is attached to the endpoint on the left bank of the channel (looking downstream). Channel depth is measured by holding a stadia rod vertical and level from the channel bottom to the stretched tape. The channel depth

measurements are recorded incrementally at equal horizontal distances across the channel for a minimum of 20 measurements.

Channel Slope
Using a DeWalt™ Model DW092 transit level, a minimum of three elevations at increasing horizontal distances from the transit level were recorded in the channel bed. A minimum of five elevations were measured at sites with irregularly sloped or curved channel surfaces. The average channel slope was calculated from the survey data.

Stream Rating
To measure instantaneous flow during base flow conditions, a Marsh-McBirney Model 2000 Portable Flow meter connected by a cable to an electromagnetic open-channel velocity sensor was used. The velocity sensor was attached to a stainless-steel, top-setting wading rod. To make an instantaneous flow measurement, a tape measure was stretched across the stream perpendicular to flow and secured on both banks of the stream. The tape was suspended approximately 1 ft above the surface of the water. The distance on the tape directly above the waterline (where the water meets the bank) was recorded as the initial point. The first measurement was then made at the first point where there was adequate depth and measurable velocity. At this point, three measurements were made: water depth, velocity, and distance from the bank (i.e., the initial point). Subsequent depth, velocity, and distance measurements were made incrementally across the entire width of the channel so that a minimum of 20 points were measured at the site. Water depths were determined from calibrations on the wading rod in tenths of feet. Velocity measurements were made at each point along the transect by positioning the velocity sensor perpendicular to flow at 60% of the water depth (from the surface) to attain an average velocity. The top-setting wading rod is designed so the sensor can be conveniently positioned at the appropriate depth. Water velocity was measured in feet per second.

Data from the field measurements was entered into a computer spreadsheet that calculates the stream's cross-sectional profile from the depth and distance-from-bank measurements. Total flow across the channel was determined by integrating the velocity measurements over the cross-sectional surface area of the stream channel. The result is an instantaneous flow measurement in cubic feet per second (cfs).

Rating Curve
A rating table or curve is the relationship between stage and flow at a cross section of a river and reflects the particular geometry of the given cross section. The stream rating data may be used with a Manning's Equation to produce a rating curve for each sampling site. Each rating curve is calibrated using instantaneous flow measurements by adjusting the formula roughness coefficient. Alternatively, flow measurements may be collected using a stadia rod and velocity reading, as explained below.

Rating curves were modeled using site-specific survey information with Manning's Equation as defined by the USGS (Rantz, 1982). Using the direct measures of stream discharge collected during the base flow conditions, indirect stream discharge measurements were calculated using Manning's Equation. Manning's Equation is an empirical formula for open channel flow or for flow driven by gravity:

$$Q = VA = \left(\frac{1.49}{n}\right) AR^{\frac{2}{3}}\sqrt{S}$$

where
Q = Flow
n = Manning Roughness coefficient
A= Cross-sectional area
R = Hydraulic radius
S = Hydraulic slope

The hydraulic radius is derived as:

$$R = A/P$$

where
A = Cross-sectional area of flow (ft^2)
P = Wetted perimeter (ft)

The Manning's Equation was developed for conditions of uniform flow in which the water surface profile and energy gradient are parallel to the streambed and the area, hydraulic radius, and depth remain constant throughout the reach. Field surveys of the channel cross section and channel geometry of each site were conducted to compute the channel characteristics for each monitoring site.

Ratings curves may require periodic validation or re-calibration based on channel dimensions that may shift due to channel bed erosion or deposition throughout the year. During regular site maintenance visits, a visual assessment of the creek channel was conducted at each sampling location to determine whether additional stream ratings were needed. Once the rating curves were generated at each site, total flow across the channel was determined by measuring stream stage (i.e., water level) with the levelogger.

2.2.1.3 Sampling Events

All six sites were assessed during two dry weather sampling events, which occurred during the winter of 2010 (December 14) and the summer of 2011 (July 21). During the first event, sampling was performed over an 18-hour time period that covered early morning (approximately 6 a.m.), early afternoon (approximately noon), and evening (approximately 6 p.m.) flows. During the second event, sampling occurred over a 24-hour period and covered early morning, early afternoon, evening, and late night (approximately midnight) flows.

2.2.1.4 Sampling Frequency

For each sampling event, water samples were collected from all six of the sampling locations during multiple rounds of sampling that occurred every 6 hours. Sampling for each of the rounds typically took approximately 3 hours to complete, beginning at Site 2 at the top of the watershed

and proceeding downstream in the sequential order of the sites. Sample collection for each event began in the early morning (6 a.m. to 9 a.m.) and was followed by sample collection in the early afternoon (12 p.m. to 3 p.m.), and evening (6 p.m. to 9 p.m.). A late night round (midnight to 3 a.m.) also was collected during the July sampling event.

2.2.1.5 Sample Collection

Sample Collection for Analysis of Bacteria by Culture
Grab samples of water were collected at each of the six sampling locations (Table 2-1) from the center of the channel as described in Section 1.4.1.1.

Sample Collection for MST Analysis
Grab samples of water for MST analysis by real-time PCR were collected in the same area as described above for bacteria (Table 2-1). At each site, samples were collected from the center of the channel by dipping the sample bottle in the flowing water as described in Section 1.4.1.2.

Sample Collection for Analysis of Water Chemistry
Grab samples were collected during each of the sampling events from the six sampling locations (Table 2-1) for water chemistry analysis. Samples were collected in analyte-specific bottles from the center of the channel at each site every 3 hours for a total of eight samples per site. Just prior to sampling, the sample container was opened holding the container and lid face-down to prevent airborne contamination. The bottle was filled and capped. No sediment or debris was allowed to enter the sample bottle.

Each field sample was labeled and identified with the project title, sample identification number, date and time of sample collection, and initials of the sample collector. All samples were stored on ice in the dark from the time of sample collection until delivery to the analytical laboratory.

2.2.1.6 Field Measurements

At each sampling station, field water quality measurements were recorded with a YSI 6920 water quality data sonde and recorded on field data sheets (Appendix A). These measurements included temperature, conductivity, dissolved oxygen (DO), pH, salinity, and turbidity. In addition, water quality appearance (odor, color, floating materials, and turbidity), meteorological characteristics (wind, temperature, cloud cover), and physical conditions at the time of collection also were recorded on field data sheets.

2.2.2 Analytical Methods, Sanitary Survey

2.2.2.1 Total and Fecal Coliforms/Enterococci

Samples were analyzed for total and fecal coliforms and enterococci by WESTON's in-house Microbiology Laboratory (ELAP - Certificate No. 2613) as described in Section 1.4.2.1 and as listed in Table 2-2.

Table 2-2. Bacterial Parameters and Corresponding Analytical Methods

Analytical Parameter	Analytical Method	Units	Method Detection Limit	Laboratory Reporting Limit	Sample Volume	Container (#, size, type)	Preservation	Holding Time
Total Coliform	SM 9221 B	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Fecal Coliform	SM 9221 E	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Enterococci	Enterolert	MPN/ 100 mL	1 MPN	<10 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours

2.2.2.2 MST Markers

Samples were processed and DNA was extracted as described in Section 1.4.2.2. Extracted DNA was analyzed for MST by real-time PCR for general *Bacteroides* and human-associated markers using a combination of *Bacteroides*-General and HF183 with melt assays. Putative positive samples for human fecal contamination were re-analyzed using the HF183 Taqman assay (Boehm et al 2013, Layton et al., 2013) (Table 2-3). Laboratory controls were as described in Section 1.4.3.

Table 2-3. Laboratory Analytical Methods for Microbial Source Tracking (MST) by Real-Time Polymerase Chain Reaction (PCR) for Sanitary Survey Samples

Target	Assay	Sequence 5'-3' (Final Conc, µM)	References	Conditions[a]
General *Bacteroides*	*Bacteroides* -General	Bac32F: AACGCTAGCTACAGGCTT (0.4) Bac708R: CAATCGGAGTTCTTCGTG (0.4) GenProbe: 6-FAM-CAATATTCCTCACT GCTGCCTCCCGTA-BHQ1 (0.2)	Bernhard and Field, 2000; Dick and Field, 2004	95°C, 2 min; 40 cycles: 95°C, 15s; 60°C, 30s
Human *Bacteroides*	HF183 with melt	HF183F: ATCATGAGTTCACATGTCCG (0.4) Bac708R: CAATCGGAGTTCTTCGTG (0.4)	Bernhard and Field, 2000; Layton et. al., 2013	95°C, 15 min; 50 cycles: 94°C, 30s; 54°C, 30s, 72°C, 45s; Melt: 60°C to 95°C at 0.2°/s
Human *Bacteroides*	HF183 Taqman	HF183F: ATCATGAGTTCACATGTCCG (1.2) BthetR1: CGTAGGAGTTTGGACCGTGT (1.2) BthetP1: 6FAM-CTGAGAGGAAGGTCC CCCACATTGGA-TAMRA (0.09)	Haugland et al., 2010; Layton et al., 2013	95°C, 20s; 40 cycles: 95°C, 1s; 60°C, 20s

[a] Master Mix and thermocycler conditions typically consisted of Quanta-Perfecta QPCR Fastmix w/UNG (#84077) used on a BioRad CFX 96 thermocycler except for paired *Bacteroides*-General/ HF183 with melt assays, which were run on a Cepheid Smart Cycler. The master mix for the *Bacteroides*-General assay was Qiagen Quantitect Sybr Green (Cepheid #1017340). Reaction volumes were 25 µL.

2.2.2.3 Water Chemistry

Samples were analyzed for the following constituents: total and dissolved cadmium and nickel, ammonia-N, nitrate-N, nitrite-N, total Kjeldahl nitrogen (TKN), total orthophosphate, and total phosphorus by EnviroMatrix Laboratory (EMA) in San Diego, CA. The samples were analyzed according to prescribed methods as outlined by the EPA. The laboratory methods used in the process are listed in Table 2-4.

Table 2-4. Chemistry Parameters and Corresponding Analytical Methods

Analytical Parameter	Analytical Method	Units	Method Detection Limit	Laboratory Reporting Limit	Sample Volume	Container (#, size, type)	Preservation	Holding Time
Total and Dissolved Cadmium	EPA 200.8	µg/L	0.4	0.8	500 mL	1, 250mL plastic	Cool to 4ºC	6 Months
Total and Dissolved Nickel	EPA 200.8	µg/L	0.2	0.4	500 mL	1, 250mL plastic	Cool to 4ºC	6 Months
Ammonia - N	SM 4500-NH3 F	mg/L	0.01	0.05	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
Nitrate – N	SM 4500 NO3 E	mg/L	0.01	0.05	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
Nitrite - N	SM 4500 NO2 B	mg/L	0.01	0.05	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
TKN	SM 4500 N C	mg/L	0.456	0.0	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	28 days
Total Orthophosphate	SM 4500-P C	mg/L	0.01	0.01	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
Total Phosphorus	SM 4500 P E	mg/L	0.016	0.05	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours

mg/L = milligrams per liter
µg/L = micrograms per liter
HDPE = high density polyethylene

2.2.3 Quality Assurance/Quality Control Procedures

For chemical analyte (e.g., metals, nutrients) field sampling, a blank and duplicate sample were collected for every 20 field samples (i.e., 5%) following SWAMP QA/QC protocols. Field duplicates were collected simultaneously with the field sample. Field blanks were collected using the same methods as the field sample, but the sample bottle was filled with reagent-grade blank water. Duplicate and blank samples were analyzed in the laboratory for the same constituents as the field samples.

For chemical analysis, EMA employed replicate spikes to determine the precision and accuracy of an analysis when some or all of the parameters being determined were below the detection limit. One set of duplicate samples or spike duplicates, a Laboratory Control Material (LCM) or Certified Reference Material (CRM) sample, and a method blank also were analyzed with each batch of samples.

For microbiological and MST analyses, field blanks and controls were collected as described in Section 1.4.3.

2.2.4 Chain-of-Custody Procedures

COC procedures were used for all samples throughout the collection as described in Section 1.4.4.

2.3 Results, Sanitary Survey

The Poche Beach Sanitary Survey Investigation consisted of two dry weather sampling events at six locations along the Mainstem Channel in the Prima Deshecha Cañada Watershed. Both water quality and bacterial densities were monitored at each location. Flow measurements were collected from three locations during the December 14, 2010 monitoring event and at all locations during the July 22, 2011 monitoring event. From these investigations, bacterial loads were calculated and water quality and water chemistry were analyzed. This portion of the overall investigation helped to determine not only where elevated bacterial concentrations were located but also where groundwater intrusion may be occurring and the magnitude of the bacterial load at various points along the Mainstem Channel before it enters Poche Beach. A comparison of the volume of flow from the current study with the volume of flow from the 2006 study helped to determine whether irrigation runoff has undergone a reduction in recent years within the watershed. The results from these analyses are presented below.

2.3.1 Survey 1

Water chemistry and bacteria results from the December 14, 2010 monitoring event (Survey 1) are shown in Table 2-5. In general, all physical water quality parameters during the December 14, 2010 survey were within San Diego Basin Plan benchmarks. Conductivity and salinity were generally lower during the early morning hours (Round 1) than in the early afternoon and evening hours (Rounds 2 and 3) across all sites, especially in the upper watershed (Sites 2, 3, and 4). Conductivity ranged from 4,712 microsiemens per centimeter (µS/cm) at Site 2 during Round 1 to 13,145 µS/cm at Site 4 during Round 3, whereas salinity ranged from 2.54 parts per thousand (ppt) at Site 2 (Round 1) to 7.62 ppt at Site 4 (Round 3). DO peaked at a majority of sites during the early afternoon hours (Round 2), ranging from 7.8 milligrams per liter (mg/L) at Site 4 (Round 3) to 14.4 mg/L at Site 3 (Round 2), whereas water temperature, which was highest in the upper watershed at Sites 2 and 3, generally peaked during the early afternoon hours across all sites. With the exception of Site 4, turbidity was below 5 Nephelometric Turbidity Units (NTU) across nearly all sites and sampling rounds.

General chemistry results indicated that total dissolved solids (TDS) and total phosphorus concentrations were above Basin Plan benchmarks across all sites during each of the sampling rounds (Table 2-5). TDS ranged from 7 to more than 28 times the Basin Plan benchmark of 500 mg/L, whereas total phosphorus ranged from 1.2 to 5.6 times the Basin Plan benchmark of 0.1 mg/L. TDS followed the same pattern as salinity and conductivity results and was highest at Sites 4, 5, and 7 during each sampling round. The largest jump in TDS concentrations was observed at Site 4, where concentrations were nearly twice those found at Site 3 (Figure 2-2).

TDS concentrations then decreased markedly from Site 4 to Site 5. In the upper watershed (Sites 2, 3, and 4), TDS concentrations showed a very consistent pattern of relatively low concentrations in the morning, which increased from the noon to the 6 p.m. samples. At Sites 5, 6, and 7 in the lower portion of the watershed, this daily temporal trend was not present. Concentrations at these sites showed no consistent trends over the course of the day.

Total phosphorus concentrations varied considerably throughout the watershed, with no consistent pattern evident by either site or by time of day. Total orthophosphate also varied among sites and among sampling rounds, ranging from 0.08 mg/L at Site 3 (Round 2) to 0.54 mg/L at Site 6 (Round 3). Total suspended solids (TSS) were well below the Basin Plan benchmark of 58 mg/L at all sites, and ranged from less than 20 mg/L at several sites to 32 mg/L at Site 5. Nitrite and ammonia levels were highest in the upper watershed, whereas nitrate levels peaked at Site 4, and TKN levels varied little between sites or sampling rounds.

Table 2-5. Water Quality and Bacterial Results from the December 14, 2010 Monitoring Event and Comparison to Benchmark Values

Parameter	Units	Water Quality Benchmarks	Benchmark References	Sanitary Survey 1																	
				Site 2 Round 1	Site 2 Round 2	Site 2 Round 3	Site 3 Round 1	Site 3 Round 2	Site 3 Round 3	Site 4 Round 1	Site 4 Round 2	Site 4 Round 3	Site 5 Round 1	Site 5 Round 2	Site 5 Round 3	Site 6 Round 1	Site 6 Round 2	Site 6 Round 3	Site 7 Round 1	Site 7 Round 2	Site 7 Round 3
				6:00	12:00	19:30	6:30	12:35	18:40	7:45	13:20	19:45	8:15	14:00	20:30	8:25	14:15	20:45	9:00	14:30	21:10
				12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10	12/14/10
Physical Chemistry																					
Conductivity	µS/cm	NA		4,712	6,738	6,488	5,792	8,707	8,892	10,215	12,800	13,145	9,489	10,453	10,658	8,789	8,991	9,016	10,091	10,016	10,148
Dissolved Oxygen	mg/L	<5	Basin Plan	8.8	8.51	8.71	8.92	14.41	8.83	9.94	10.12	7.8	12.36	14.1	8.96	10.81	11.19	9.95	10.48	10.5	10.55
pH	pH units	6.5-9.0	Basin Plan	8.02	7.96	8.03	7.76	7.96	7.9	7.96	8.17	7.64	7.93	8.5	7.93	7.98	8.36	8.04	8.09	8.14	7.95
Salinity	ppt	NA		2.54	3.72	3.56	3.16	4.86	4.99	5.78	7.36	7.62	5.35	5.94	6.07	4.93	5.06	5.09	8.72	5.68	5.75
Turbidity	mg/L	20	Basin Plan	4.7	2.8	3.9	3.1	4.7	1.5	9	11.1	5.8	7.1	3.3	1.7	5.4	1.7	0.7	2.4	1.5	4.6
Water Temperature	Celsius	NA		16.05	16.44	16.5	14.58	16.99	14.38	13.18	13.88	13.96	13.23	14.79	13.49	12.03	13.41	12.8	13.48	13.92	13.7
General Chemistry																					
Ammonia-N	mg/L	(a)	U.S. EPA Water Quality Criteria (Freshwater)	0.55	0.51	0.82	0.22	0.27	0.34	0.39	0.32	0.15	0.11	<0.1	0.11	<0.1	<0.1	0.18	0.14	0.15	<0.1
Nitrate-N	mg/L	10	Basin Plan	1.52	1.2	1.51	1.73	1.89	2.08	2.98	3.49	3.22	1.88	1.62	2.21	0.88	0.83	0.78	1.23	1.52	1.61
Nitrite-N	mg/L	1	Basin Plan	0.34	0.95	0.64	0.19	0.2	0.14	0.1	0.07	0.06	0.06	<0.05	0.06	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
TDS	mg/L	500	Basin Plan	**3,520**	**5,940**	**6,470**	**5,350**	**8,190**	**8,910**	**11,100**	**13,800**	**14,300**	**8,650**	**9,850**	**9,990**	**8,600**	**8,790**	**8,870**	**10,900**	**9,830**	**9,620**
TKN	mg/L	NA		2.1	3.2	2.1	2.4	3.6	3.2	0.6	2	3	2	2.2	2	3	2.1	3.2	2.1	3.3	3
Total Orthophosphate	mg/L	NA		0.29	0.42	0.25	0.26	0.08	0.49	0.22	0.53	0.09	0.3	0.28	0.43	0.38	0.29	0.54	0.29	0.3	0.14
Total Phosphorus	mg/L	0.1	Basin Plan	**0.32**	**0.45**	**0.25**	**0.28**	**0.14**	**0.51**	**0.24**	**0.56**	**0.12**	**0.32**	**0.3**	**0.44**	**0.41**	**0.33**	**0.55**	**0.32**	**0.34**	**0.16**
TSS	mg/L	58	NSQD, 2000	<20	<20	<20	<20	<20	<20	<20	20	23	23	32	<20	<20	<20	<20	<20	<20	<20
Bacteriological																					
Enterococci	MPN/100 mL	104	Basin Plan	**10,860**	**2,247**	**480**	**4,165**	**733**	**183**	**2,909**	**393**	**359**	**650**	**323**	52	**213**	**743**	**315**	20	20	<10
Fecal Coliform	MPN/100 mL	400	Basin Plan (REC-1)	**50,000**	**900**	170	**3,000**	300	300	**3,000**	**500**	170	**800**	300	40	**5,000**	**800**	300	80	<20	20
Bacteroides																					
General *Bacteroides*	-	NA		Pos			Pos			Pos			Pos			Pos			Pos		
Human *Bacteroides*	-	Pos		Neg			Neg			Neg			Neg			Neg			Neg		
Total Metals																					
Nickel (Ni)	mg/L	NA		0.021	0.051	0.06	0.119	0.195	0.235	0.415	0.636	0.652	0.267	0.105	0.146	0.104	0.1	0.07	0.092	0.084	0.086
Cadmium (Cd)	mg/L	NA		0.003	0.004	0.004	0.014	0.023	0.031	0.054	0.081	0.084	0.018	0.014	0.022	0.006	0.006	0.004	0.009	0.008	0.014
Dissolved Metals																					
Cadmium (Cd)	mg/L	NA		0.002	0.004	0.004	0.013	0.021	0.023	0.053	0.072	0.076	0.016	0.013	0.022	<0.005	0.005	0.006	0.009	0.008	0.007
Nickel (Ni)	mg/L	NA		0.036	0.05	0.06	0.114	0.186	0.2	0.446	0.618	0.612	0.117	0.103	0.149	0.103	0.098	0.115	0.082	0.079	0.075

< = results less than the reporting limit.
(a) Water Quality Benchmark is based on CMC (salmonids absent) and CCC (early life stages present) using water temperature and pH described in the EPA, 1999 Update of Ambient Water Quality Criteria for Ammonia, EPA-822-R-99-014, 12/99.
NA = Indicates no criteria or published value was available or applicable to the matrix or program.
Pos = Positive
Neg = Negative
Shaded text = exceeds water quality benchmarks.

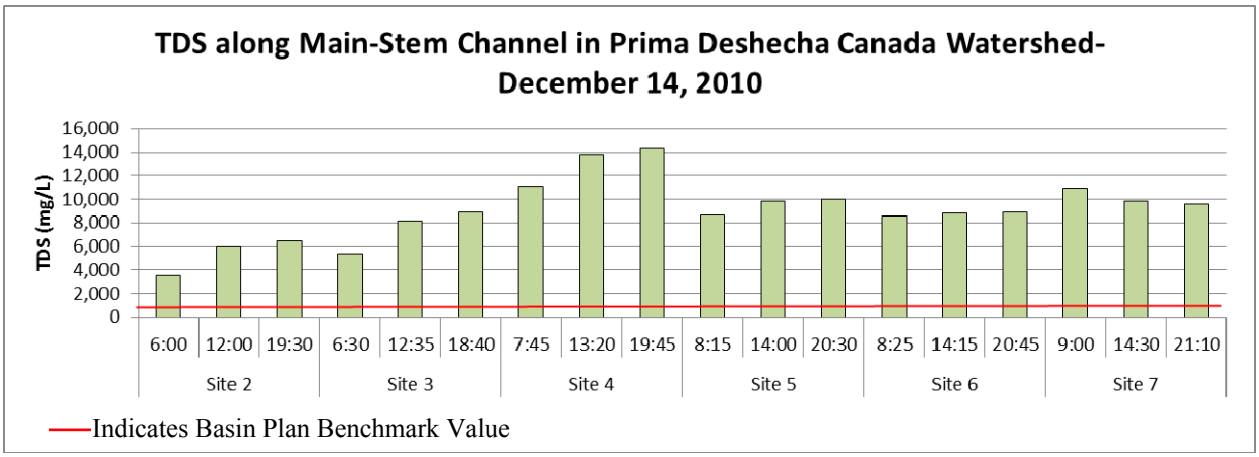


Figure 2-2. TDS Concentrations during December 14, 2010 Sampling Event and Comparison to Basin Plan Benchmark Value

Metal concentrations were substantially higher at Site 4 during all sampling rounds than at any other site for both total and dissolved nickel and total and dissolved cadmium (Figure 2-3 and Figure 2-4). Total and dissolved nickel concentrations gradually increased in magnitude in the upper watershed, peaking at Site 4, and then dropping to low concentrations in the lower watershed. These spatial and temporal trends were very similar to those observed for TDS concentrations (Figure 2-2). Total nickel concentrations ranged from 0.021 mg/L at Site 2 to 0.656 mg/L at Site 4, whereas dissolved nickel concentrations ranged from 0.050 mg/L at Site 2 to 0.618 mg/L at Site 4. The temporal pattern mirrored that of TDS, with low concentrations in the morning and high concentrations in the early evening. Total and dissolved cadmium concentrations followed the same pattern as nickel concentrations, gradually increasing from Site 2 through Site 4 before dropping off and remaining relatively static from Site 5 through Site 7. Total and dissolved cadmium ranged from 0.003 mg/L at Site 2 to 0.084 mg/L at Site 4 and from 0.002 mg/L at Site 2 to 0.076 mg/L at Site 4.

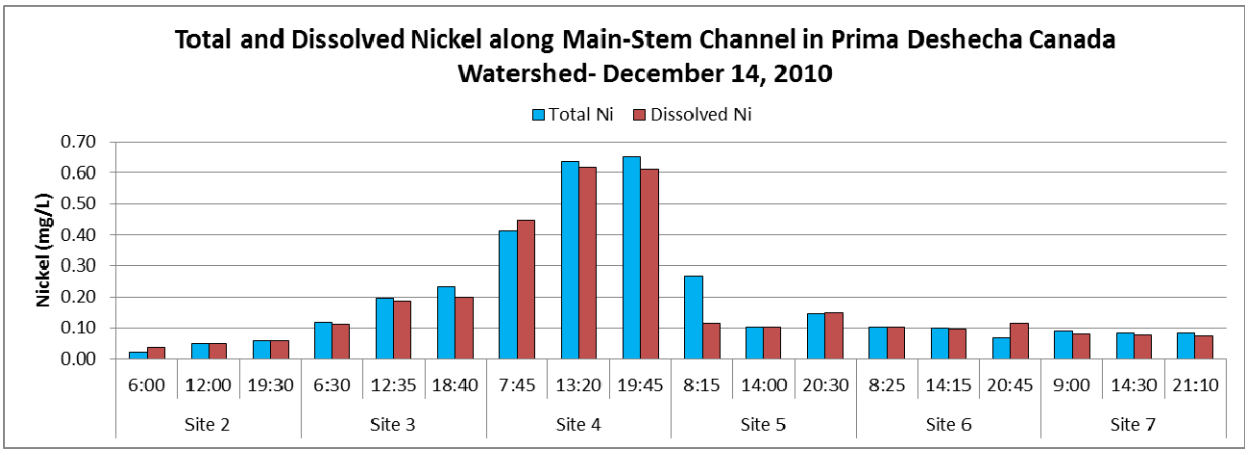


Figure 2-3. Nickel Concentrations during December 14, 2010 Sampling Event

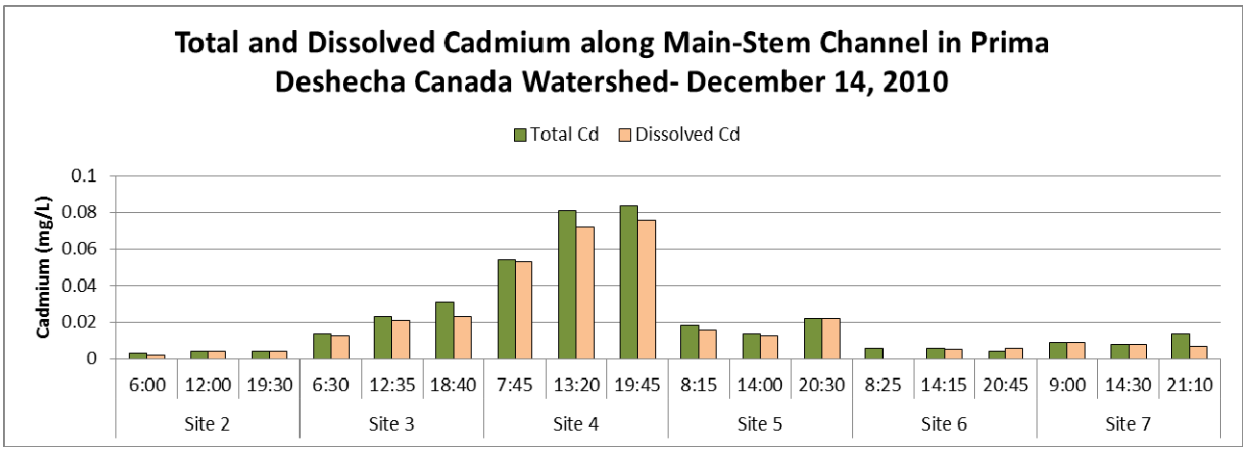


Figure 2-4. Cadmium Concentration during December 14, 2010 Sampling Event

Bacterial results from the December 14, 2010 sampling event indicated REC-1 Basin Plan benchmarks were exceeded for enterococci and fecal coliform bacteria at all sites, with the exception of Site 7 (Figure 2-5 and Figure 2-6). Enterococci benchmarks were exceeded during all three sampling rounds at Sites 2, 3, 4, and 6, and during rounds 1 and 2 at Site 5. Fecal coliform benchmarks were exceeded during Round 1 at all sites except Site 7, and during Round 2 at Sites 2, 4, and 6. No exceedances of fecal coliform benchmarks occurred during evening sampling (Round 3) at any of the sites. Concentrations of both fecal coliforms and enterococci were greatest in the upper watershed at Sites 2, 3, and 4 and decreased consistently from upstream to downstream. The lowest concentrations during all sampling rounds were found at Site 7 at the base of the watershed (downstream of the confluence of the Mainstem and Cascadita Channels). Temporal patterns of both indicator bacteria suggested a decrease in concentrations from the early morning to the early evening periods. This pattern was consistent across all sites and was inversely related to that observed for TDS and metals concentrations, which increased over the course of the day in the upper and middle watershed sites.

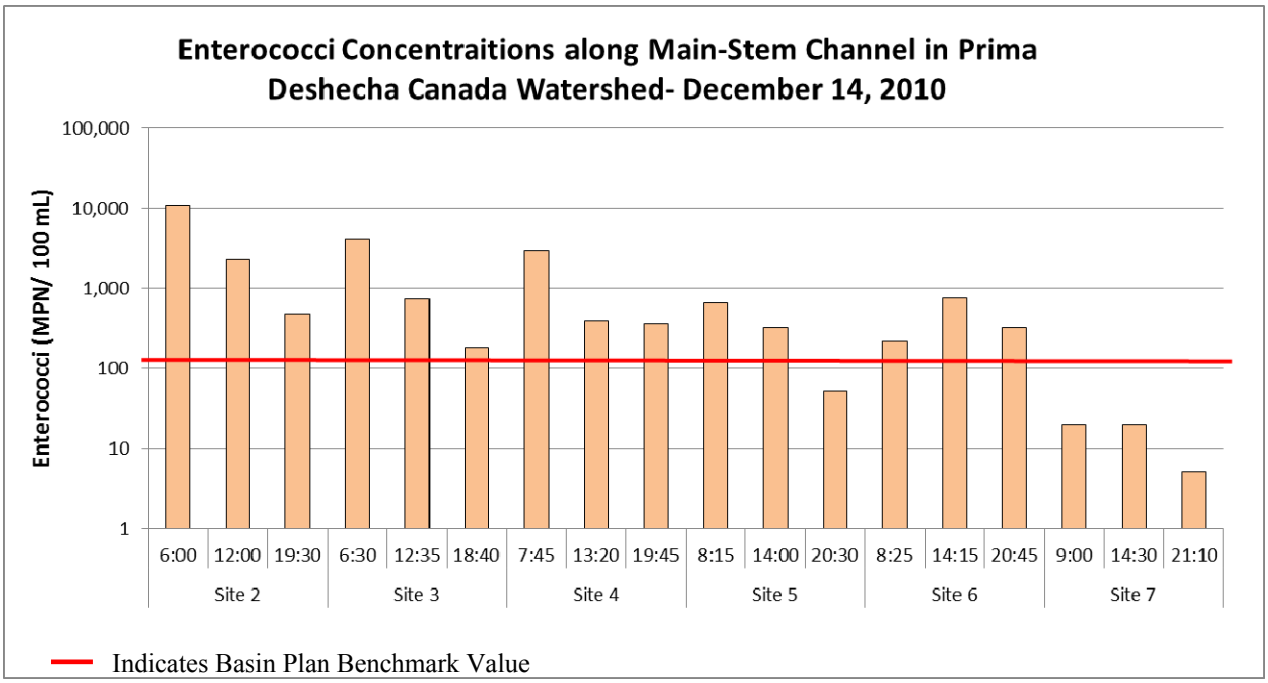


Figure 2-5. Enterococci Concentrations during December 14, 2010 Sampling Event and Comparison to Basin Plan Benchmark Value

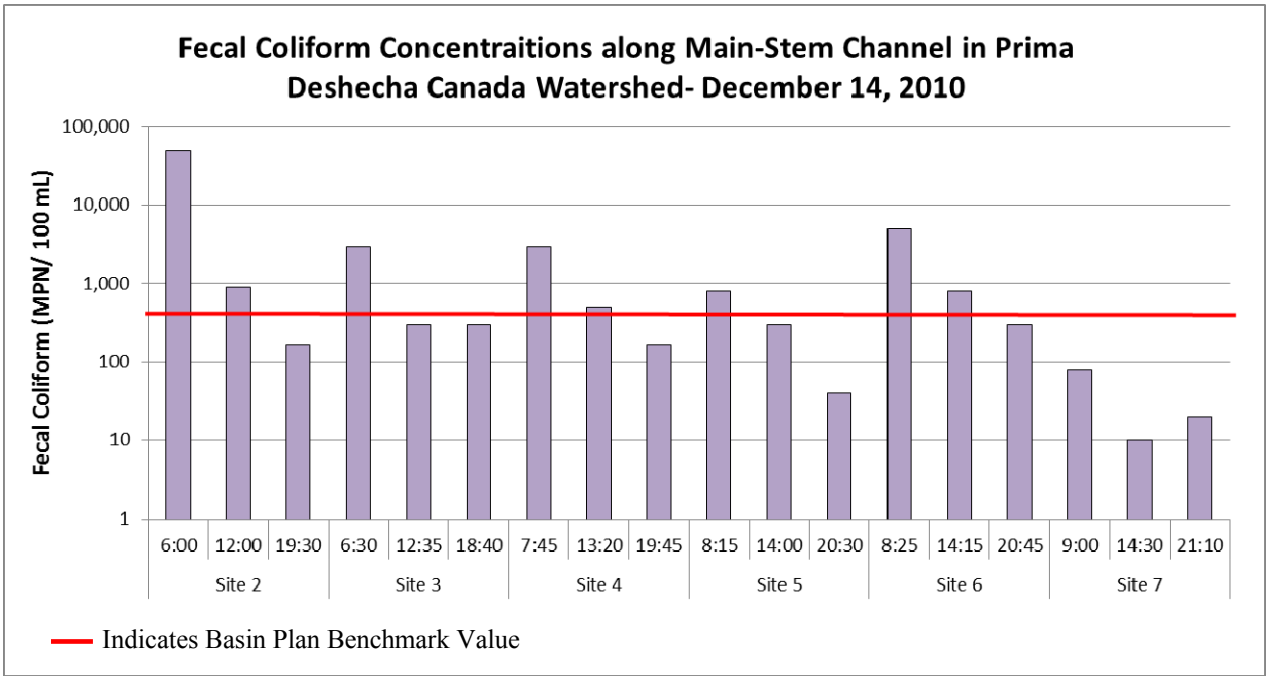


Figure 2-6. Fecal Coliform Concentrations during December 14, 2010 Sampling Event and Comparison to Basin Plan Benchmark Value

In addition to measuring enterococci and fecal coliform concentrations at each site, MST by real-time PCR was conducted on samples. All samples were tested for both the *Bacteroides*-General and the HF183 with melt assay (human-associated *Bacteroides*) assays (Table 2-3). The results indicated that each of the samples tested positive for the general marker and negative for the human-associated marker (Table 2-5). The results suggested that the high levels of indicator bacteria found in the watershed did not originate from human sources.

2.3.2 Survey 2

Water chemistry and bacteria results from the July 21, 2011 monitoring event (Survey 2) are shown in Table 2-6. In general, all physical water quality parameters, with the exception of turbidity at Site 2, were within San Diego Basin Plan benchmarks. In contrast to the three sampling rounds performed on December 14, 2010, four rounds of sampling were performed on July 21, 2011 (the fourth round was collected at midnight). Conductivity and salinity were generally lower in the upper watershed during the early morning and late night hours (Round 1 and Round 4) than in the early afternoon and evening hours (Rounds 2 and 3). In the lower water shed, conductivity and salinity were less variable, remaining relatively static across all sampling rounds. Similar to the December 14, 2010 sampling event, conductivity and salinity were highest at Site 4, followed by Site 5 and Site 7, and were lowest at Site 2. DO varied little across sites and between sampling times, ranging from 6.89 mg/L at Site 5 to 11.49 mg/L at Site 4, whereas water temperature tended to be slightly higher during the early afternoon hours than at any other time of day across all sites. With the exception of Site 2 and Site 4, turbidity was below 5 NTU across nearly all sites and sampling rounds. The Basin Plan benchmark of 20 NTU was exceeded during the midnight sampling round at Site 2 but was not exceeded at any other time.

Table 2-6. Water Quality and Bacterial Results from the July 22, 2011 Monitoring Event and Comparison to Benchmark Values

Parameter	Units	Water Quality Benchmarks	Benchmark References	Poche Beach Sanitary Survey 2																						
				Site 2 Round 1	Site 2 Round 2	Site 2 Round 3	Site 2 Round 4	Site 3 Round 1	Site 3 Round 2	Site 3 Round 3	Site 3 Round 4	Site 4 Round 1	Site 4 Round 2	Site 4 Round 3	Site 4 Round 4	Site 5 Round 1	Site 5 Round 2	Site 5 Round 3	Site 5 Round	Site 6 Round 1	Site 6 Round 2	Site 6 Round 3	Site 6 Round 4	Site 7 Round 1	Site 7 Round 2	Site 7 Round 3	Site 7 Round 4
				6:00	12:00	18:00	0:00	6:15	12:20	18;25	0:20	6:45	12:45	19:00	0:45	7:15	12:55	19:35	1:05	7:30	13:10	20:00	1:25	7:55	13:30	20:20	1:45
				7/21/11	7/21/11	7/21/11	7/22/11	7/21/11	7/21/11	7/21/11	7/22/11	7/21/11	7/21/11	7/21/11	7/22/11	7/21/11	7/21/11	7/21/11	7/22/11	7/21/11	7/21/11	7/21/11	7/22/11	7/21/11	7/21/11	7/21/11	7/22/11
Physical Chemistry																										
Conductivity	µS/cm	NA		4,097	5,884	6,261	3,169	6,573	7,727	8,941	4,749	13,890	13,170	17,620	5,283	12,780	9,551	11,150	9,435	5,594	5,789	6,335	5,644	9,190	9,293	8,881	9,125
Dissolved Oxygen	mg/L	<5	Basin Plan	7.7	7.51	7.91	8.23	7.55	9.24	8.2	7.73	7.59	11.49	8.14	8.68	7.78	8.33	6.89	5.65	8.34	9.6	7.99	7.99	7.62	8.37	8.34	7.9
pH	pH units	6.5-9.0	Basin Plan	7.49	7.52	7.66	7.83	7.45	7.54	7.41	7.4	7.64	7.82	7.6	7.79	7.68	8.54	7.7	7.66	7.8	8.2	7.71	7.71	7.87	7.9	7.91	7.94
Salinity	ppt			2.18	3.19	3.41	1.66	3.61	4.26	5.02	2.55	8.07	7.55	10.44	2.86	7.37	5.32	6.35	5.32	3.04	3.13	3.47	3.06	5.16	5.19	4.97	5.12
Turbidity	mg/L	20	Basin Plan	2.9	0.8	0.5	32.5	2.9	1.7	2	7.6	6.4	4.6	10.6	6	1.2	0.7	1.1	1.4	1.1	0.1	0.4	1.5	0.4	0.3	0.2	0.8
Water Temperature	Celsius	NA		21.12	22.71	21.65	20.7	19.67	22.75	19.17	19.68	19.02	24.65	19.02	18.85	19.27	28.12	19.93	19.22	19.55	25.03	20.72	19.82	21.11	24.54	22.46	21.82
General Chemistry																										
Ammonia-N	mg/L	(a)	U.S. EPA Water	<0.1	<0.1	0.58	0.46	<0.1	0.21	0.15	0.37	<0.1	0.19	0.14	0.44	<0.1	0.21	0.13	0.26	0.1	0.22	0.15	0.3	<0.1	0.23	0.16	0.16
Nitrate-N	mg/L	10	Basin Plan	1.59	1.74	1.67	1.26	2.06	2.14	2.19	1.68	4.32	4.02	7.21	4.4	2.66	2.06	1.66	2.59	0.43	0.28	0.38	0.22	1.46	2.07	1.37	1.38
Nitrite-N	mg/L	1	Basin Plan	0.09	0.13	0.16	0.08	0.06	0.07	0.07	0.07	0.08	0.06	0.08	0.08	0.14	0.08	0.09	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Total Dissolved Solid	mg/L	500	Basin Plan	3,220	4,770	5,250	2,320	5,150	6,220	7,540	3,490	11,100	12,300	18,700	8,760	11,200	7,690	9,580	8,770	4,640	4,700	5,360	4,630	7,780	7,300	7,740	7,720
Total Kjeldahl Nitrog	mg/L	NA		2.9	3.5	4.8	2.9	3	4.1	3.5	2	3.3	2.5	2.4	2.4	2.8	8.2	3.2	2.8	2.8	3.8	3	2.7	3.2	2.5	2.6	3.2
Total Orthophosphat	mg/L	NA		0.38	0.32	0.29	0.43	0.29	0.2	0.2	0.47	0.3	0.11	0.07	0.12	0.2	0.12	0.17	0.15	0.06	0.09	0.05	0.06	0.09	0.12	0.12	0.12
Total Phosphorus	mg/L	0.1	Basin Plan	0.38	0.34	0.34	0.92	0.3	0.24	0.27	0.51	0.32	0.18	0.26	0.19	0.22	0.18	0.21	0.21	0.11	0.11	0.1	0.13	0.16	0.16	0.17	0.17
Total Suspended Soli	mg/L	58	NSQD, 2000	<20	<20	<20	122	<20	<20	<20	30	<20	23	61	24	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
Bacteriological																										
Enterococcus	MPN/100 mL	104	Basin Plan	11,123	2,820	81,641	4,725	7,589	5,794	24,809	5,806	27,551	10,168	670	2,086	5,475	4,106	6,766	2,976	1,421	1,014	1,483	2,851	691	404	457	495
Fecal Coliform	MPN/100 mL	400	sin Plan (REC	2,200	1,100	170,000	17,000	5,000	5,000	50,000	7,000	17,000	13,000	230	8,000	1,700	22,000	17,000	2,300	110	1,100	80	260	600	1,100	1,300	230
Bacteroides																										
General *Bacteroides*	-	NA		Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
Human *Bacteroides*	-	Pos		Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
Dissolved Metals																										
Cadmium (Cd)	mg/L	NA		0.001	0.001	0.002	<0.001	0.012	0.014	0.021	0.009	0.045	0.042	0.147	0.037	0.025	0.006	0.015	0.015	0.001	0.001	<0.005	0.001	0.003	0.004	0.005	<0.005
Nickel (Ni)	mg/L	NA		0.02	0.026	0.026	0.014	0.111	0.129	0.167	0.071	0.468	0.417	1.16	0.372	0.235	0.068	0.11	0.143	0.031	0.027	0.054	0.033	0.044	0.042	0.036	0.039
Total Metals																										
Cadmium (Cd)	mg/L	NA		0.002	0.003	0.003	0.004	0.014	0.016	0.02	0.011	0.055	0.055	0.157	0.055	0.028	0.01	0.018	0.017	0.003	0.003	0.006	0.004	0.007	0.007	0.006	0.007
Nickel (Ni)	mg/L	NA		0.021	0.034	0.029	0.03	0.111	0.138	0.165	0.086	0.472	0.474	1.21	0.45	0.246	0.086	0.114	0.15	0.035	0.033	0.061	0.045	0.046	0.046	0.042	0.043

< = results less than the reporting limit.

(a) Water Quality Benchmark is based on CMC (salmonids absent) and CCC (early life stages present) using water temperature and pH described in the U.S. EPA, 1999 Update of Ambient Water Quality Criteria for Ammonia, EPA-822-R-99-014, December 1999.

NA = Indicates no criteria or published value was available or applicable to the matrix or program.

Pos = Positive

Neg = Negative

Shaded text – exceeds water quality benchmarks.□

General chemistry results indicated that TDS and total phosphorus concentrations were above Basin Plan benchmarks across all sites and during each of the sampling rounds, with the exception of Round 3 at Site 6 for total phosphorus (Figure 2-7 and Figure 2-8). TDS concentrations ranged from four to more than 37 times the Basin Plan benchmark of 500 mg/L, whereas total phosphorus ranged from 1.1 to 9.2 times the Basin Plan benchmark of 0.1 mg/L. TDS concentrations followed the same pattern as salinity and conductivity and were highest at Sites 4, 5, and 7 during each sampling round. Similar to the December 2010 sanitary survey, TDS concentrations in July peaked sharply at Site 4 during the 6 p.m. sampling round. Temporal patterns in July also were similar to those observed in December with concentrations increasing from morning (6 a.m.) to early evening (6 p.m.). The additional sampling round at midnight in the July survey demonstrates that TDS concentrations decrease markedly from 6 p.m. to midnight at Sites 2, 3, and 4. In the lower watershed (Site 5 and 7) and the Cascadita Channel (Site 6), these temporal patterns were not apparent.

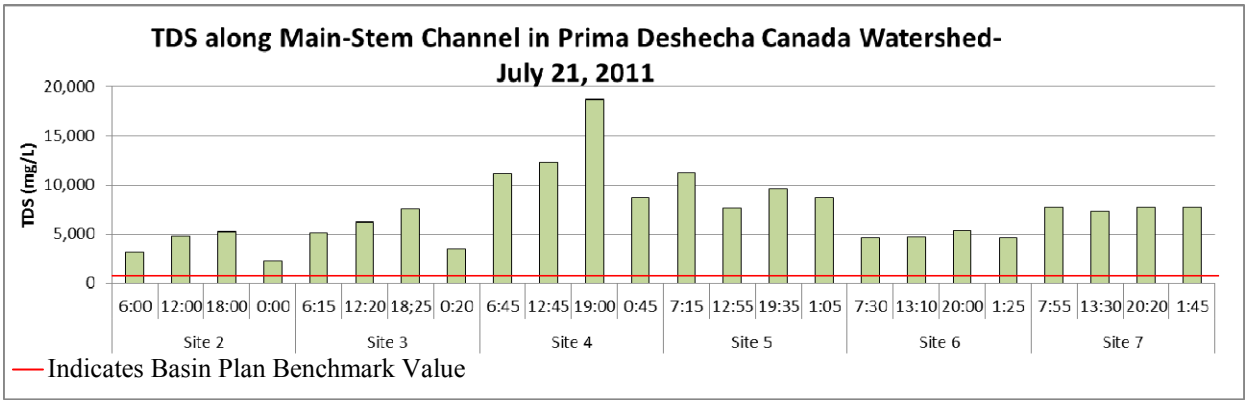


Figure 2-7. TDS Concentrations during July 21, 2011 Sampling Event and Comparison to Basin Plan Benchmark Value

Total phosphorus concentrations were slightly higher in the upper watershed than in the lower watershed and were greatest at Sites 2 and 3 around midnight. In the lower watershed, total phosphorus concentrations remained relatively static between sites and across sampling times. Total orthophosphate was approximately two to three times higher in the upper watershed than in the lower watershed. TSS was well below the Basin Plan benchmark of 58 mg/L at all sites, with the exception of Site 2, and ranged from less than 20 mg/L at several sites to 122 mg/L at Site 2. Nitrite, ammonia, and TKN levels were relatively consistent across sites and among sampling times, whereas nitrate levels peaked at Site 4. Nitrate levels at Site 4 were two to three times greater than at any other site.

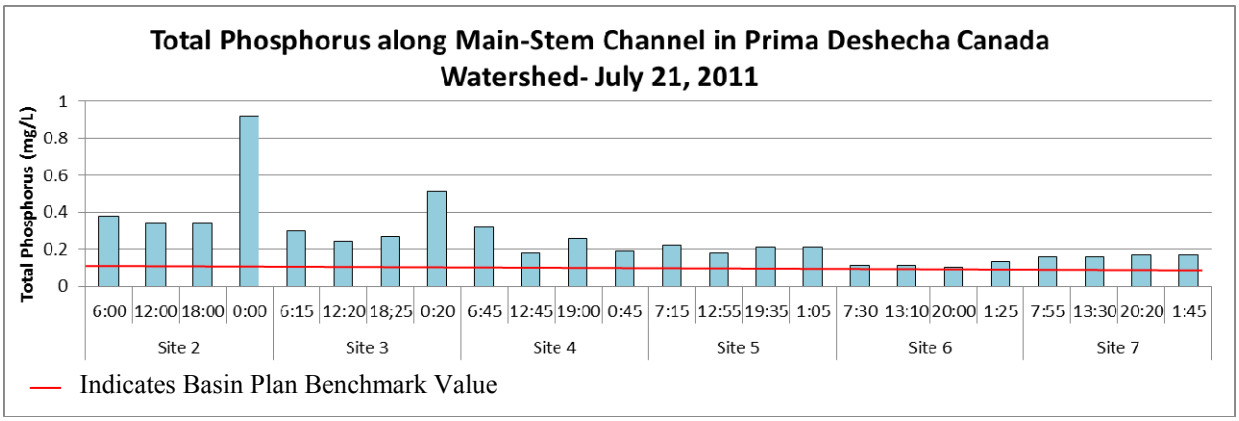


Figure 2-8. Total Phosphorus Concentrations during July 21, 2011 Sampling Event and Comparison to Basin Plan Benchmark Value

Metal concentrations were substantially higher at Site 4 during all sampling rounds than at any other site for both total and dissolved nickel and total and dissolved cadmium (Figure 2-9 and Figure 2-10). Total and dissolved nickel concentrations gradually increased in magnitude in the upper watershed, peaking at Site 4, and then dropped to lower concentrations in the lower watershed. This pattern was identical to that observed for metals concentrations during the December 14, 2010 sampling event. Total nickel concentrations ranged from 0.021 mg/L at Site 2 to 0.474 mg/L at Site 4, whereas dissolved nickel concentrations ranged from 0.020 mg/L at Site 2 to 0.468 mg/L at Site 4. Total and dissolved cadmium concentrations followed the same pattern as nickel concentrations, gradually increasing from Site 2 through Site 4 before decreasing to much lower levels at Site 5 and showing ever further reductions at Sites 6 and 7. Total and dissolved cadmium ranged from 0.002 mg/L at Site 2 to 0.157 mg/L at Site 4 and from 0.001 mg/L at Site 2 to 0.147 mg/L at Site 4.

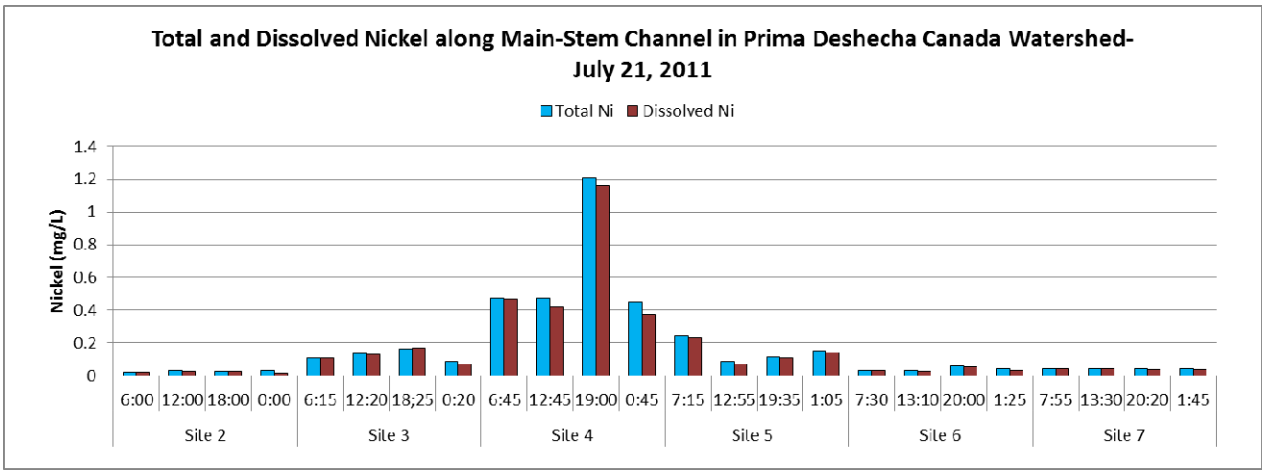


Figure 2-9. Nickel Concentrations during July 21, 2011 Sampling Event

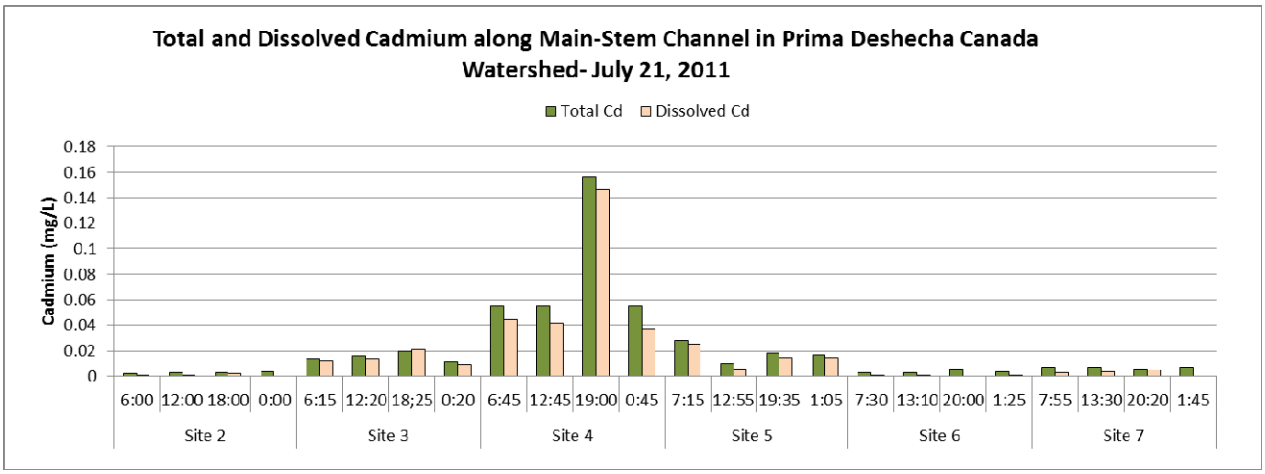


Figure 2-10. Cadmium Concentrations during July 21, 2011 Sampling Event

Bacterial results from the July 21, 2011 sampling event indicated that Basin Plan REC-1 benchmarks were exceeded for enterococci and fecal coliform bacteria at all sites (Figure 2-11 and Figure 2-12). Enterococci benchmarks were exceeded during every sampling round at all sites, whereas fecal coliform benchmarks were exceeded during nearly every round at all sites (Round 3 at Site 4, Rounds 1, 3, and 4 at Site 6, and Round 4 at Site 7 were below benchmark values). Enterococci concentrations were greatest in the upper watershed at Sites 2, 3, and 4 and were highest in the early evening hours (6 p.m.) at Sites 2 and 3, and the early morning hours (6 a.m.) at Site 4. Concentrations of fecal coliform also were generally higher in the upper watershed than in the lower watershed and tended to peak during the early evening hours, especially at Sites 2 and 3.

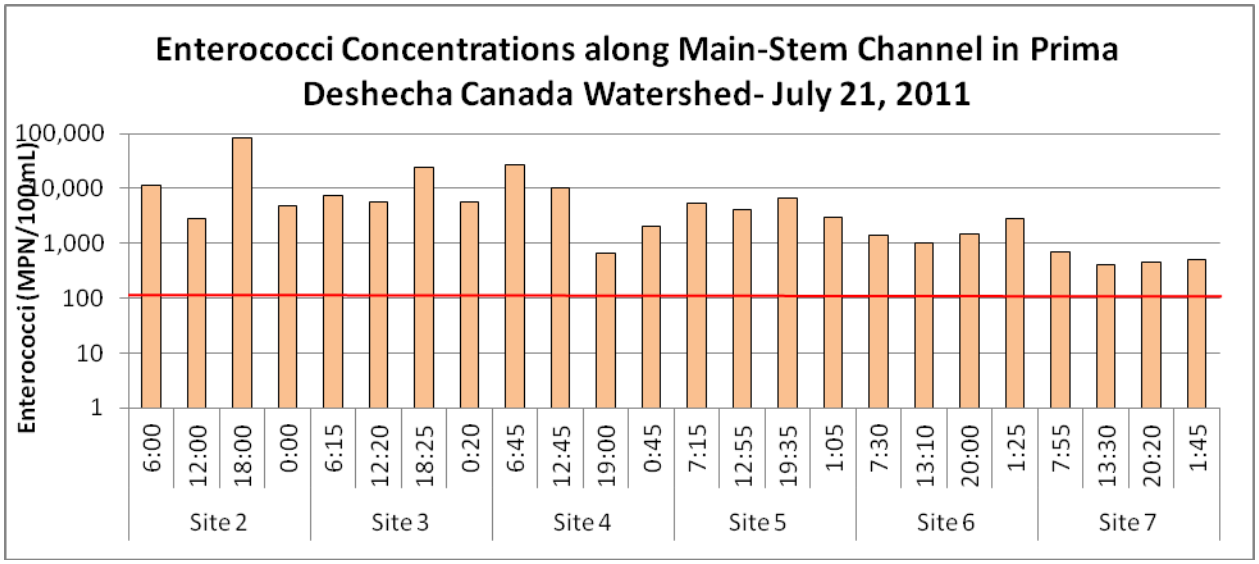


Figure 2-11. Enterococci Concentrations during July 21, 2011 Sampling Event and Comparison to Basin Plan Benchmark Value

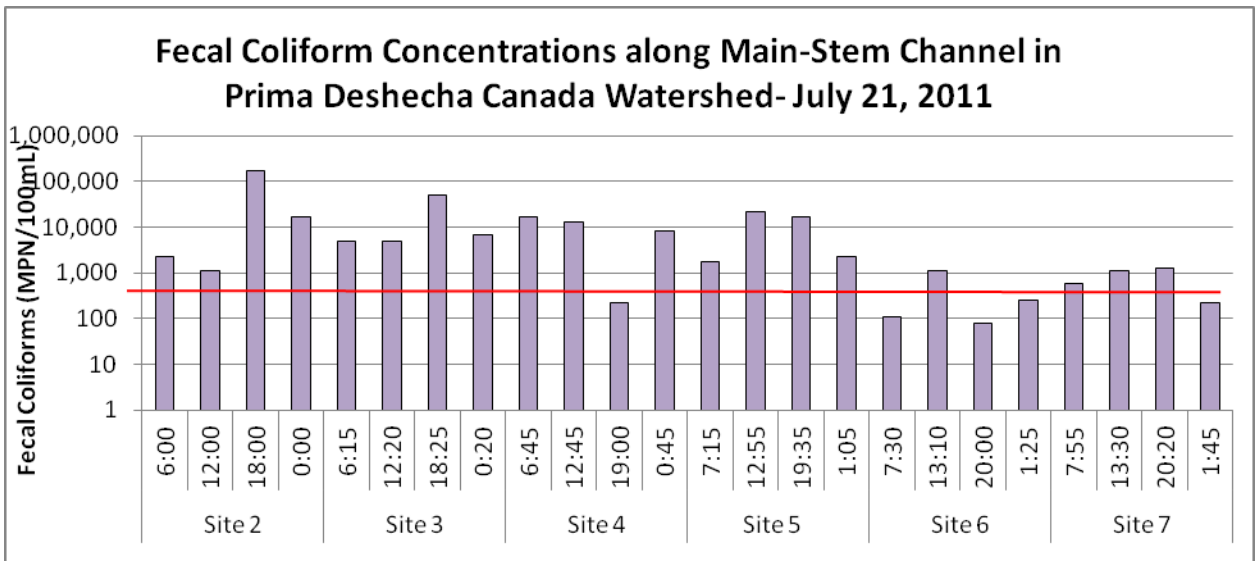


Figure 2-12. Fecal Coliform Concentrations during July 21, 2011 Sampling Event and Comparison to Basin Plan Benchmark Value

In addition to measuring enterococci and fecal coliform concentrations at each site, MST by real-time PCR was conducted on samples. All samples were tested for both the *Bacteroides*-General and the HF183 with melt assay (human-associated *Bacteroides*) assays (Table 2-6). Results from the PCR analyses indicated that each of the samples tested positive for the general marker and negative for the human-associated marker, except that one sample taken at Site 6 during Round 3 was a putative positive for the HF183 with melt assay (human-associated *Bacteroides*). This sample was re-analyzed using the HF183Taqman Assay (Table 2-3) and confirmed positive. In general, the results suggested that human sources were not a significant contributor to the high levels of indicator bacteria found in the watershed.

2.3.3 Flow

Average monthly flows were calculated for each of the monitoring sites along the Mainstem Channel at which flow meters were installed on a year-round basis (Sites 3, 4, 5, 6, and 7) (Figure 2-13). During 2011, flow was greatest at Sites 5 and 7 near the base of the watershed across all months and was particularly elevated from February through May relative to the other monitored sites. It should be noted that 2011 was an unusually wet year with several large storm events occurring during the winter months so that flow at Sites 5 and 7 may not be representative of flow during a typical year. The increased flow from February through May at Sites 5 and 7 likely is the result of a high groundwater table that is slowly receding toward base flow conditions as the rainy season ends. Groundwater springs, which are known to occur between Site 4 and Site 5, may be the predominant source of increased flow at these sites during the winter and early spring.

The average flow pattern at sites in the upper watershed (Sites 3 and 4) and at the base of the Cascadita Channel (Site 6) varied little over the course of the year compared to sites in the lower

watershed. The apparent influence of groundwater influx to the Mainstem Channel at Sites 5 and 7 was not apparent at the upper watershed sites and the Cascadita Channel.

A secondary peak in flow at Sites 5 and 7 occurred during the month of September 2011. The increased flow during this time period is believed to be a combination of irrigation runoff and a seasonal decrease in temperature typical of late summer in coastal Orange County. Peaks in dry weather flow during late summer are also typically seen in other urbanized watersheds in the region.

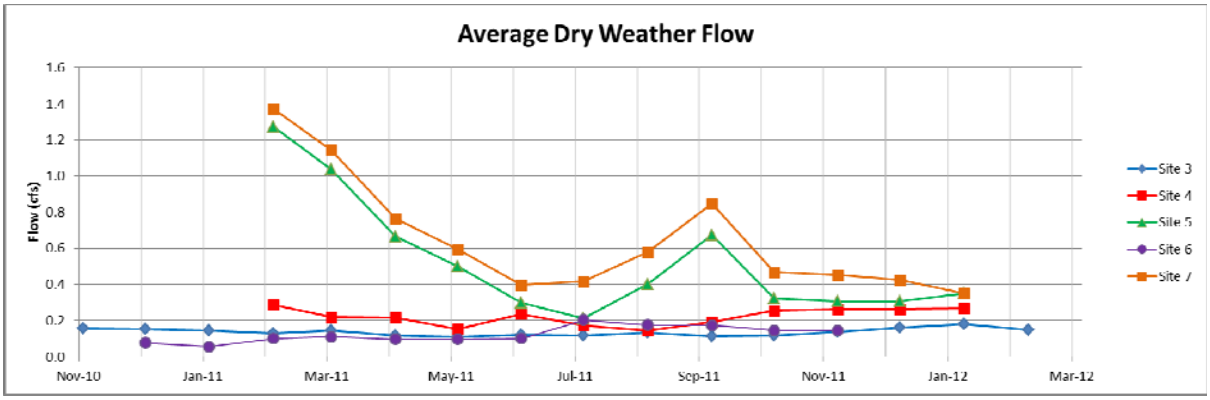


Figure 2-13. Average Monthly Dry Weather Flows at Monitoring Sites on the Mainstem and Cascadita Channels

Cumulative flow over the course of the July 21, 2011 monitoring event is shown in Figure 2-14. The largest increase in flow occurs between Site 4 and Site 5 (Figure 2-14A). The sub-watershed area between Site 4 and Site 5 contributed approximately 57% of the total flow that reached Poche Beach on the day of sampling, whereas the Cascadita Channel, which flows into the Mainstem Channel at Site 6, contributed 23% of the total flow (Figure 2-14B). In the upper watershed, the area above Site 3 contributed a total of 14% of the flow into the Mainstem Channel, whereas the area between Site 3 and Site 4 contributed 6% of the total flow. Flow from the monitoring event on December 14, 2010 is not depicted because flow data for that event was not available for all sites (three flow meters were lost during a large storm that followed the monitoring event on December 14, 2010).

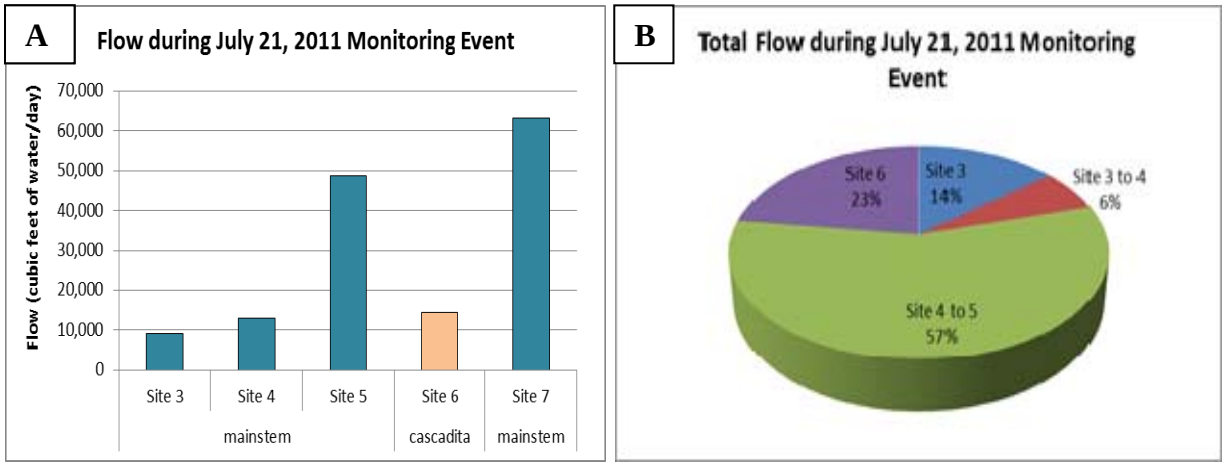


Figure 2-14. Flow rate and Total Flow at Monitoring Sites on July 21, 2011

Because the monitoring event in July 2011 spanned only a single day, it was important to verify the flow data over a longer time period to reduce flow variability and gain a better understanding of the typical flow during the summer months. The bar chart in Figure 2-15A represents the average cumulative flow for each of the monitored sites in August 2011. The pie chart shown in Figure 2-15B represents the average percentage of total flow contributed by each sub-watershed during the month of August 2011. Over this time period, flow from the uppermost portion of the watershed (Site 3) represented 24% of the total flow, whereas the sub-watershed between Site 3 and Site 4 contributed only 2% of the total flow, and the sub-watershed between Site 4 and Site 5 contributed 42% of the total flow. The southern portion of the watershed contributed 32% of the flow into the Mainstem Channel from Cascadita Channel (Site 6). Although the data during the month of August represent up to a 15% change in total flow from the data during the monitoring event on July 21, 2011, in general, the August data confirm that the greatest percentage of flow entering the Mainstem Channel during this period occurred in the sub-watershed between Site 4 and Site 5.

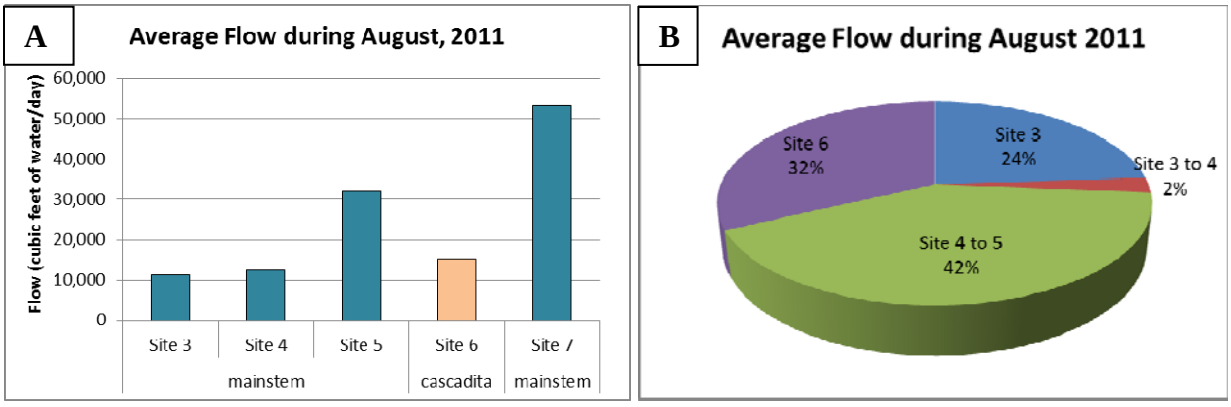


Figure 2-15. Average Flow Rate and Average Total Flow Contribution during August 2011

As shown in Figure 2-16, Daily flow during the week of July 21, 2011 was generally highest in the upper watershed (Site 3) during the early morning hours, between midnight and 6 a.m. This

is the period of time that is generally recommended for landscape irrigation as the water dries less quickly and has more time to soak into the soil, conserving water usage. Because the flow at lower sites includes flow from the upper watershed as well, there is generally a bit of a lag time for the peak flow from the upper sites to arrive. Thus at the bottom of the watershed, flow at Site 6 and Site 7 North, flow is typically highest between 6 a.m. and noon. It should be noted that Site 7 splits into two streams, and the flow represented in Figure 2-16 includes only the northern stream (assumed to be approximately 25% of the flow).

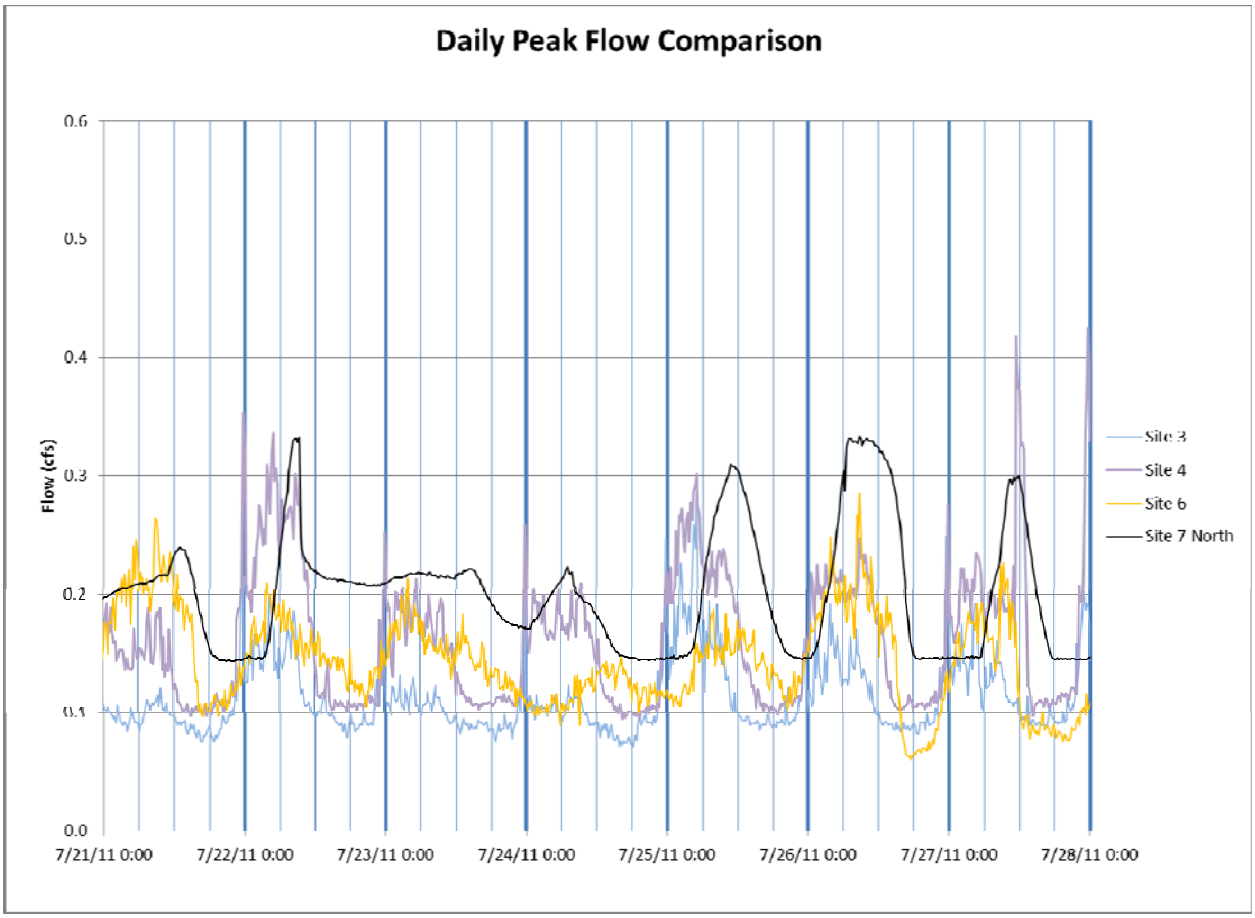


Figure 2-16. Daily Flow Rates across the Watershed

A comparison of the flows calculated for the 2005 to 2006 study and the flows calculated for the 2010 to 2011 was performed to determine whether flow rates had changed significantly over time. Flow meter measurements from the 2005 to 2006 study were averaged for the month of November 2005 and are presented in Figure 2-17. Because only three flow meters were used for this study, only data from Sites 3, 4, and 6 are presented. Flow measurements from the 2010 to 2011 study from Sites 3, 4, and 6 are presented in Figure 2-18. Because data from November 2010 were not collected, data from December 1 through December 17, 2010 were used for Sites 3 and 6 (only 17 days were used as the average due to a series of large storms rolling through southern Orange County beginning on December 18, 2010). Data from February 1 through

February 17, 2011 were used for Site 4 because a new flow meter for this site had not yet been re-installed following the December storm event that swept away the previous flow meter.

In November 2005, the period of peak flow occurred during the early morning hours at approximately 6:30 a.m. at Sites 4 and 6 and at approximately 8:30 a.m. at Site 3. Peak flow during winter 2010 to 2011 was between 8 p.m. and midnight at Site 3, whereas at Site 4 and Site 6 flow only slightly increased during the early morning hours over the rest of the day and evening. In general, within this period of time identified in Figure 2-17 and Figure 2-18, average flow in 2010 to 2011 appears to be similar to that in 2005 to 2006 at Sites 3 and 4, whereas at Site 6, there appears to have been a significant decrease in dry weather flow from 2005 to 2010. Site 6 averaged greater than 0.3 cfs throughout the day in November 2005, whereas in December 2010, Site 6 averaged less than 0.1 cfs.

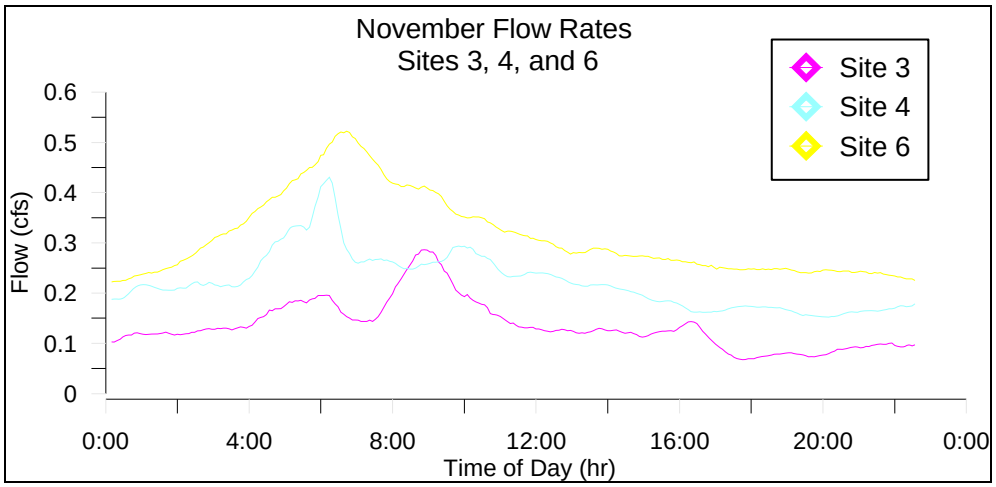


Figure 2-17. Average Flow during the Month of November 2005 at Sites 3, 4, and 6

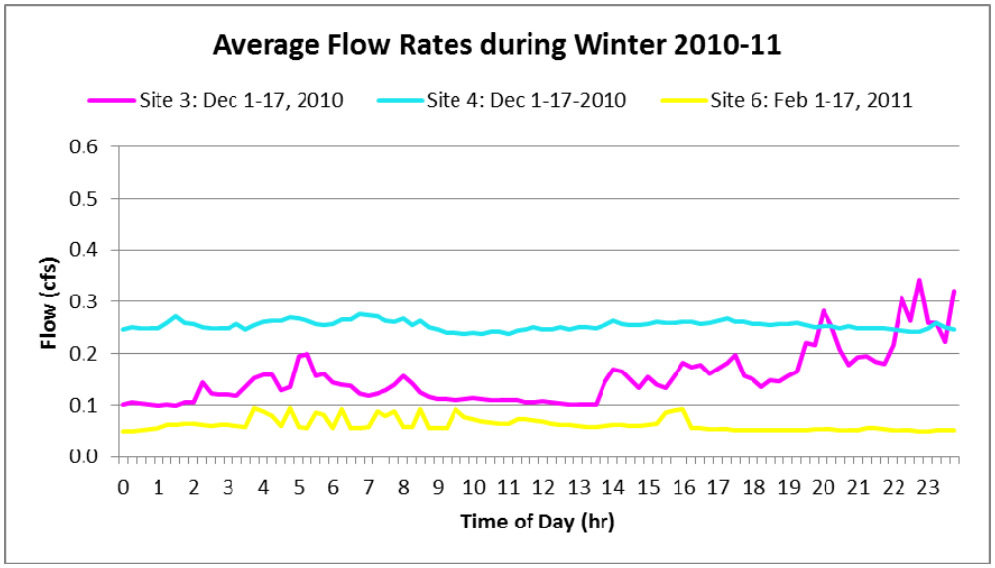


Figure 2-18. Average Flow during Winter 2010-2011 at Sites 3, 4, and 6

2.3.4 Bacterial Loads

Bacterial loads were calculated based on the geometric mean of measured bacterial concentrations collected at each site three times throughout the day on December 14, 2010 and four times throughout the day on July 21, 2011, as well as both measured and modeled flow data from the entire year. Modeled flow data were used to fill data gaps for periods in which equipment failures occurred or when data appeared to be inaccurate due to electronic drift, debris/algal buildup around the sensor, or any unexplainable fluctuations. Annualized loads of enterococci and fecal coliforms for each of the monitored sub-watersheds are presented in Table 2-7.

Based on the bacterial concentrations during the two sanitary surveys, the greatest annual loads of enterococci occurred at Site 5 (4.49×10^{12} MPN) and Site 4 (4.41×10^{12} MPN), whereas the lowest annual loads occurred at Site 7 and Site 6 (4.85×10^{11} and 7.51×10^{11} MPN, respectively). Annual fecal coliform loads also were greatest at Site 5 and Site 4 (6.77×10^{12} and 4.06×10^{12} MPN, respectively) and lower by one order of magnitude at Site 6 and Site 7 (6.31×10^{11} and 8.64×10^{11} MPN, respectively). It appears that the loads for both types of fecal indicator bacteria are greatest in areas in which the Mainstem Channel is largely underground and are lowest where the channel is exposed to the ultraviolet rays from the sun (i.e., upstream of Sites 6 and 7). This trend also was evident in the previous study in 2005 to 2006.

Table 2-7. Annual Bacteria Loads for Sub-watersheds

Parameter		Units	Monitoring Station				
			Sites 2 and 3	Site 4	Site 5	Site 6	Site 7
Annual Dry Weather Flow Volume		cubic feet	3.67×10^{12}	5.70×10^{12}	1.53×10^{13}	3.39×10^{12}	1.89×10^{13}
Sanitary Survey 1	Mean Enterococci Concentration	MPN/ 100 mL	824	743	222	368	16
	Mean Fecal Coliform concentration		10,044	10,066	4,844	1,665	517
Sanitary Survey 2	Mean Enterococci Concentration	MPN/ 100 mL	646	634	213	1,063	32
	Mean Fecal Coliform Concentration		14,715	9,972	11,479	408	818
Annual Load	Enterococci	MPN	2.99×10^{12}	4.41×10^{12}	4.49×10^{12}	7.51×10^{11}	4.85×10^{11}
	Fecal Coliforms		3.21×10^{12}	4.06×10^{12}	6.77×10^{12}	6.31×10^{11}	8.64×10^{11}
Developed Watershed		Acres	478	632	1,326	491	1,853
Annual Load/ Acre	Enterococci	MPN/ Acre	6.26×10^{9}	6.98×10^{9}	3.39×10^{9}	1.53×10^{9}	2.62×10^{8}
	Fecal Coliform		6.72×10^{9}	6.42×10^{9}	5.11×10^{9}	1.29×10^{9}	4.66×10^{8}

2.4 Summary, Sanitary Survey

Water chemistry, bacterial concentrations, flow, and bacterial loads were examined in the Prima Deshecha Cañada Watershed over the course of a year, from November 2010 through December 2011. This sanitary study consisted of two 24-hour monitoring events conducted on December 14, 2010 and July 21, 2011, and continuous flow monitoring throughout the year at six sample locations along the length of the watershed. A summary of each component of the study is discussed below.

2.4.1 Monitoring Event Water Chemistry and Bacteria

TDS and total phosphorus concentrations were greater than Basin Plan benchmarks across all sites and during nearly every sampling round for the December 14, 2010 and July 21, 2011 monitoring events. TDS concentrations ranged from four to more than 37 times the Basin Plan benchmark, whereas total phosphorus concentrations ranged from 1.1 to 9.2 times the Basin Plan benchmark. TDS, salinity, and conductivity concentrations were generally greatest at Sites 4, 5, and 7 across all sampling times. Metal concentrations were substantially greater at Site 4 during all sampling rounds than at any other site for both total and dissolved nickel and total and dissolved cadmium. Total and dissolved nickel concentrations gradually increased in magnitude in the upper watershed, peaking at Site 4, and then dropping to low concentrations in the lower watershed.

Enterococci and fecal coliform bacterial concentrations were above REC-1 Basin Plan benchmarks at all sites, with the exception of Site 7 during the December 14, 2010 sampling event and during nearly all sampling rounds. During both sampling events, enterococci concentrations were greatest in the upper watershed at Sites 2, 3, and 4 and were highest in the early morning hours. Although concentrations of fecal coliforms also were highest during the early morning hours across all sites during the first sampling event, they peaked in the early evening hours during the second sampling event. Fecal coliform concentrations at Site 2 during both sampling events were approximately one order of magnitude greater than the fecal coliform concentrations at any other site. One sample collected from the Cascadita Channel drainage (Site 6-3; Table 2-6) was identified as positive using the human-associated *Bacteroides* HF183 with melt assay and the HF183Taqman assay. Although this result indicates the presence of human sources of bacteria in this drainage, the overall low frequency of occurrence of human-associated MST marker suggests that human sources were not the primary contributor to the high levels of indicator bacteria measured in the watershed.

2.4.2 Flow

Flow meters placed at regular intervals along the Mainstem Channel in November, 2010 monitored flow for a period of one year. Over the course of the year, flow was found to be greatest at Sites 5 and 7 near the base of the watershed across all months, and was particularly elevated in February and March relative to the other monitored sites. Because the 2010 to 2011 wet weather season contained several unusually large storm events, the high flow at Sites 5 and 7 may be somewhat unrepresentative of flow during a more typical year. Site 6 likely did not have elevated flow, despite being of similar size and containing similar land use as the sub-watersheds of Sites 4 and 5 because the Cascadita Channel watershed that drains to Site 6 contains no known groundwater springs. A secondary peak in flow occurred during the month of September at Sites

5 and 7 and is believed to be the result of irrigation runoff and a shift in temperature typical of late summer in coastal Orange County.

During the July 21, 2011 monitoring event, the sub-watershed area between Site 4 and Site 5 contributed approximately 57% of the total flow that reaches Poche Beach, whereas the Cascadita Channel, which flows into the Mainstem Channel at Site 6, contributed 23% of the total flow. In the upper watershed, the area above Site 3 contributed a total of 14% of the flow into the Mainstem Channel, whereas the area between Site 3 and Site 4 contributed 6% of the total flow.

Flow data over a one-month period in August 2011 indicated that flow from the uppermost portion of the watershed (Site 3) represented 24% of the total flow, whereas the sub-watershed between Site 3 and Site 4 contributed only 2% of the total flow, and the sub-watershed between Site 4 and Site 5 contributed 42% of the total flow. The southern portion of the watershed contributed 32% of the flow into the Mainstem Channel from Cascadita Channel (Site 6). Flow data from the July 21, 2011 monitoring event differed somewhat from these percentages but also found that the greatest percentage of flow entering the Mainstem Channel occurs in the sub-watershed between Site 4 and Site 5, and only a minimal amount of the flow enters the Mainstem Channel between Site 3 and Site 4. Flow from the Cascadita Channel sub-watershed represented approximately one third of the total flow entering Poche Beach.

Daily flow at each site was tracked during the week of July 21, 2011 and was generally highest in the upper watershed during the early morning hours between midnight and 6 a.m. This is the period of time that is generally recommended for landscape irrigation as the water dries less quickly and has more time to soak into the soil, conserving water usage. Flow at sites near the base of the watershed generally peaked between 6 a.m. and 6 p.m. The lag of peak flow is likely the result of the time it takes for water from the upper watershed to reach the lower watershed rather than irrigation occurring at a later point in the day in the lower watershed.

A comparison of the flows calculated for the 2005 to 2006 study and the flows calculated for the 2010 to 2011 was performed to determine whether flow rates had changed significantly over time. Flow meter measurements from the 2005 to 2006 study were averaged over one month during winter whereas flow meter measurements were averaged over 17 days in winter (due to the arrival of winter storms). In general, average flow appears to be similar at Sites 3 and 4 between the two studies, whereas at Site 6 there appears to have been a significant decrease in dry weather runoff from 2005 (0.3 cfs) to 2010 (0.1 cfs). In November 2005, the period of peak flow occurred during the early morning hours at Sites 3, 4, and 6, whereas peak flow during 2010 to 2011 was between 8 p.m. and midnight at Site 3, and at Site 4 and Site 6, flow was only slightly higher during the morning compared to the rest of the day.

The relative percentages of flow in the watershed are highly variable and change over time. These variances are depicted in Figure 2-13, which shows the average monthly flows at all sites monitored over the course of the study. The very high flows recorded from February through May 2011 at the bottom of the watershed are the most interesting component of the figure. During February of that period, flows at Sites 5 and 7 were six times greater than those in the upper watershed and the Cascadita Channel. The relative contribution to flow at the bottom of

the Mainstem Channel during this time period is dominated by flows coming from the sub-drainage between Sites 4 and 5. A similar pattern would be observed if a flow comparison were made from August or September 2011 when flows at Sites 5 and 7 peaked again (although to a lesser extent than those observed from February to May). In contrast, the relative contribution to the overall flow from the sub-drainage between Sites 4 and 5 is much lower in December 2011 and January 2012. Much of this variability in flows and the discrepancies between the relative contributions of the sub-drainages over time is likely due to changes in groundwater influx in the lower portion of the watershed. The period from December 2010 through March 2011 brought record rainfall to southern California. Groundwater levels were likely very high during this time period, which is the most likely explanation for the elevated flows at the bottom of the watershed (Sites 5 and 7).

2.4.3 Bacterial Loads

Bacterial loads are a product of both concentration and flow. In general, bacterial concentrations were greatest in the upper and middle portion of the watershed (Sites 2, 3, and 4) and flows were highest in the lower portion of the watershed (Sites 5 and 7) (Table 2-7). As a result, the data indicate that the annual fecal coliform load was greatest at Sites 4 and 5 and the annual enterococci load was greatest at Site 5. Loads for both indicator bacteria were an order of magnitude lower at the base of the Mainstem Channel (Site 7) and Cascadita Channel (Site 6). Although flows are greatest at the bottom of the channel, relatively low bacterial concentrations at Site 6 and Site 7 are the driving factors behind the reduced loads at these sites. The reasons for this may be primarily due to the physical environment of the channel itself. It appears that the loads for both types of fecal indicator bacteria are greatest in areas in which the Mainstem Channel storm drain is largely underground. At Site 6, the entire Cascadita Channel is open to the sun's UV rays that act to degrade bacteria. Similarly, the Mainstem Channel above Site 7 is also open to the sun, although for a shorter reach. Exposure to UV radiation would likely constrain regrowth (compared to protected sites), which may reduce bacterial concentrations in the water column.

3.0 BIOFILM STUDY

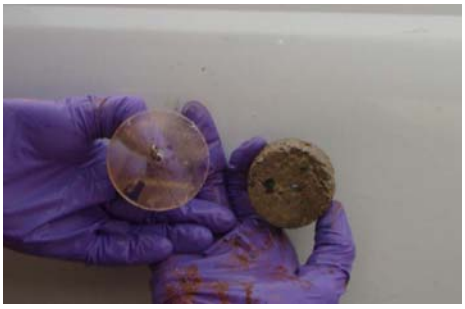
3.1 Overview, Biofilm Study

The Mainstem Channel has a mainly underground configuration; thus it has only short sections that allow the water to be exposed to the sun's UV radiation. There is continual year-round flow in the channel for nearly the entire 3-mile distance from top to bottom. Moreover, water quality data suggest that the flow in the channel is high in nutrients (see Section 2), which are known to stimulate bacterial growth and reproduction. These conditions of continual flow, elevated nutrient concentrations, and limited exposure to UV radiation are ideal for the growth of biofilms on the wetted surface of the channel. To determine the extent to which biofilms exist in the Mainstem Channel and to assess the potential for re-growth of enterococci and fecal coliforms in this environment, a biofilm study was conducted.

The biofilm study described in this section was designed to answer the following questions:

1. **Does the MS4 act as a reservoir for fecal indicator bacteria?**
2. **If so, what are the concentrations per square inch?**

In order to answer these questions, the growth of biofilms was assessed on 'coupons' (*i.e.,* concrete stubs) installed in the Mainstem Channel (shown in photograph to the right) and harvested for bacterial analyses over a period of several months. The results were used to determine whether biofilms act as a reservoir for bacteria and whether the channels provide a favorable environment for bacterial regrowth.



Concrete Coupon for Regrowth Studies.

3.2 Methods, Biofilm Study

3.2.1 Field Methods

3.2.1.1 Concrete Coupons

The coupons were made by pouring approximately two inches of cement into a Styrofoam cup. A stainless steel bolt was secured in the middle of the cement and the cement was allowed to cure. The resulting concrete coupon was approximately 1.5 inches thick with a diameter of 2 inches. The coupons were sterilized and then secured to the bottom of the channel using the stainless steel bolt. At each site, a set of 8 coupons was installed. Two coupons were removed from each site for each of the four sampling events (see below).

3.2.1.2 Site Locations

Coupons were deployed at four of the locations used in the sanitary surveys (Sites 3, 4, 6, and 7) (Table 3-1 and Figure 2-1). Coupons were initially installed on November 23, 2010 (Survey 1) and harvested after 9 days of growth for enumeration of enterococci and total and fecal coliform bacteria associated with the biofilms. These initial coupons were lost during a storm event following the first collection and new coupons were installed at the same locations on March 10, 2011 (Survey 2).

Table 3-1. Location and Description of Biofilm Study Sites

Site	Sampling	Latitude	Longitude
3	✓	33.464701	-117.639541
4	✓	33.457830	-117.642437
6	✓	33.442420	-117.644127
7N	✓	33.441701	-117.644751
7S	✓	33.441641	-117.644673

3.2.1.3 Sampling Frequency

Biofilm samples for bacterial analyses were collected during five events from the locations listed above. Sampling occurred on December 2, 2010 for the first survey, but the remaining coupons were lost during the December 20, 2010 storm event, precluding subsequent collection. Coupons for the second survey were deployed on March 10, 2011 and collected after 8 days (Event 1), 7 weeks (Event 2), 10 weeks (Event 3), and 6 months (Event 4) as shown in Table 3-2.

Table 3-2. Sampling Frequency for Survey 2 of the Biofilm Study

Sampling Event	Date of Collection	Duration of Deployment
Event 1	3/18/2011	8 days
Event 2	4/26/2011	7 weeks
Event 3	5/14/2011	10 weeks
Event 4	9/7/2011	6 months

3.2.1.4 Sample Collection

Coupons were harvested by field scientists wearing sterile, disposable gloves. In the field, the entire coupon was removed from the substrate and placed into a 250-mL sterile plastic jar containing 50 mL of sterile phosphate buffer solution. The coupons were then transported on ice to WESTON's Microbiology Laboratory in Carlsbad, CA. The sample was sonicated for approximately 2 minutes in order to remove the biofilm as described in the QAPP (WESTON, 2010). The coupon was then removed from the jar and the resultant suspension was poured into a 100-mL bacteria sample bottle. A second aliquot of 50 mL of 1X phosphate buffer solution was poured into the jar, swirled, and added to the bacteria sample bottle to increase the sample

volume to a total of 100 mL for enumeration of total and fecal coliforms and enterococci. Samples were handled and processed using the methods presented in Section 1.4.2.1.

3.2.1.5 Field Measurements

At each sampling station, temperature, conductivity, and pH were measured in the field with a YSI 6920 water quality data sonde. In addition, a description of the site, including meteorological characteristics, water quality appearance, flow estimates, and potential sources of fecal material, was recorded. All data were recorded on field data sheets (Appendix A).

3.2.2 Analytical Methods

The methods used in microbiological analyses of biofilm samples are presented in Section 1.4.2.1 and are summarized in Table 3-3. Concentrations were expressed as most probable number (MPN)/100 mL then converted to MPN/in^2 based on the surface area of the coupon.

Table 3-3. Bacteriological Parameters and Corresponding Analytical Methods

Analytical Parameter	Analytical Method	Units	Method Detection Limit	Laboratory Reporting Limit	Sample Volume	Container (#, size, type)	Preservation	Holding Time
Total Coliforms	SM 9221 B	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Fecal Coliforms	SM 9221 E	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Enterococci	Enterolert	MPN/ 100 mL	1 MPN	<10 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours

3.2.3 Quality Assurance/Quality Control Procedures

QA/QC procedures outlined in Section 1.4.3 and detailed in the QAPP (WESTON, 2010) were followed for the biofilm study. Samples were collected in duplicate at each site and sampling event. Blank samples were not applicable to this study.

3.2.4 Chain-of-Custody Procedures

COC procedures outlined in Section 1.4.4 were used for all samples throughout the collection, transport, and analytical processes.

3.3 Results, Biofilm Study

3.3.1 Total Coliforms

Total coliform concentrations measured in biofilm suspension samples for Survey 1 are presented in Table 3-4. Bacterial concentrations after 9 days of deployment ranged from 412 to

4,440 MPN/in^2, with the highest concentration occurring at Site 4. The lowest concentrations in the first survey were found at Site 6 at the base of the Cascadita Channel.

Table 3-4. Survey 1 Total Coliform Concentrations by Site and Event in Storm Drain Biofilms

Sampling Event / Time Since Coupon Installation	Site			
	3	4	6	7S
	MPN/ in^2	MPN/ in^2	MPN/ in^2	MPN/ in^2
Event 1 – 9 days	1,586	4,440	412	951

Coupons for Survey 2 were deployed on March 10, 2011 and harvested after 8 days, 7 weeks, 10 weeks, and 6 months. Total coliform concentrations for Events 1 through 4 are presented in Table 3-5 and in Figure 3-1. At Site 3, concentrations ranged from 349 MPN/in^2 after 8 days of growth to 11,100 MPN/in^2 after 6 months. Concentrations rose between 8 days and 7 weeks of growth, decreased between 7 and 10 weeks, and rose again sharply between 10 weeks and 6 months. At Site 4, total coliform concentrations ranged from 3,489 MPN/in^2 after 8 days of growth to 95,152 MPN/in^2 after 6 months. A similar pattern to that described for Site 3 was observed, with concentrations rising between Events 1 and 2, dropping between Events 2 and 3, and rising again sharply between Events 3 and 4. At Site 6, concentrations were relatively low (73 to 349 MPN/in^2) and decreased with each event. At Site 7S, concentrations also were relatively low (54 to 5,391 MPN/in^2) when compared to Sites 3 and 4 and increased with each event. The total coliform concentration at Site 7N was 41 MPN/in^2 but the coupons at that location were lost before additional sampling occurred.

Table 3-5. Survey 2 Total Coliform Concentrations by Site and Event in Storm Drain Biofilms

Sampling Event / Time Since Coupon Installation	Site				
	3	4	6	7N	7S
	MPN/ in^2	MPN/ in^2	MPN/ in^2	MPN/ in^2	MPN/ in^2
Event 1 – 8 days	349	3,489	349	41	54
Event 2 – 7 weeks	8,880	15,857	254	ns	729
Event 3 – 10 weeks	951	5,391	222	ns	2,537
Event 4 – 6 months	11,100	95,142	73	ns	5,391

ns = not sampled (A large storm event removed these coupons after Event 1.)

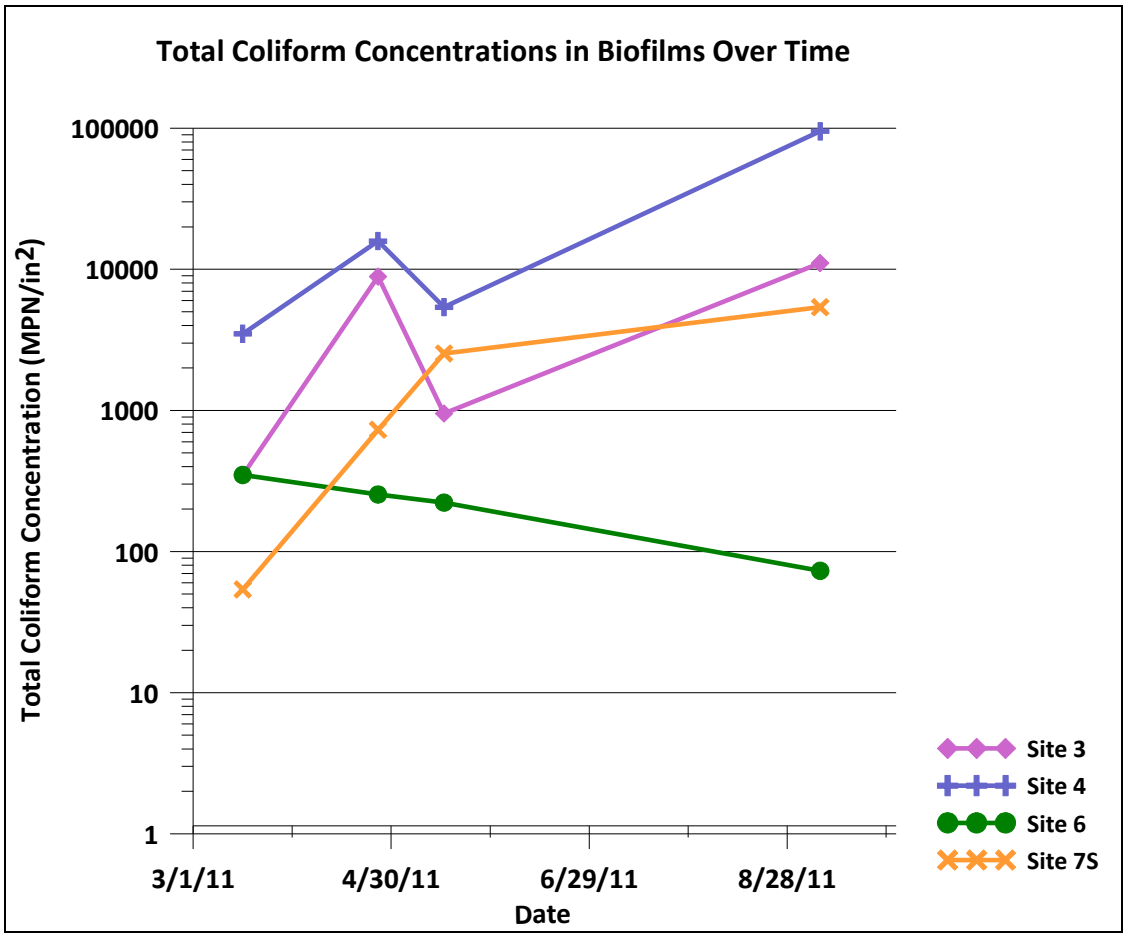


Figure 3-1. Total Coliform Concentrations over Time in Storm Drain Biofilms

3.3.2 Fecal Coliforms

Fecal coliform concentrations measured in biofilm suspension samples for Survey 1 are presented in Table 3-6.

Table 3-6. Survey 1 Fecal Coliform Concentrations by Site and Event in Storm Drain Biofilms

Sampling Event / Time Since Coupon Installation	Site			
	3	4	6	7
	MPN/ in^2	MPN/ in^2	MPN/ in^2	MPN/ in^2
Event 1 – 9 days	698	1,586	6	25

Fecal coliform concentrations for Survey 2 are presented in Table 3-7 and in Figure 3-2. At Site 3, concentrations ranged from 126 MPN/in^2 during Event 1 to 7,222 MPN/in^2 during Event 3. Concentrations rose between 8 days and 10 weeks of growth and then decreased between 10 weeks and 6 months. At Site 4, fecal coliform concentrations ranged from 942 MPN/in^2 to

34,540 MPN/in^2. Concentrations dropped between 8 days and 7 weeks of growth, remained low through 10 weeks, and then rose sharply between 10 weeks and 6 months. At Sites 6, 7N, and 7S, concentrations were relatively low (less than the reporting limit to 722 MPN/in^2) when compared to Sites 3 and 4. The coupons at Site 7N were lost following Event 2.

Table 3-7. Survey 2 Fecal Coliform Concentrations by Site and Event in Storm Drain Biofilms

Sampling Event / Time Since Coupon Installation	Site				
	3	4	6	7N	7S
	MPN/ in^2	MPN/ in^2	MPN/ in^2	MPN/ in^2	MPN/ in^2
Event 1 – 8 days	13	951	25	<6	<6
Event 2 – 7 weeks	349	95	<6	*	54
Event 3 – 10 weeks	729	95	<6	*	41
Event 4 – 6 months	412	3,489	13	*	73

* No data available, coupons removed during storm events.

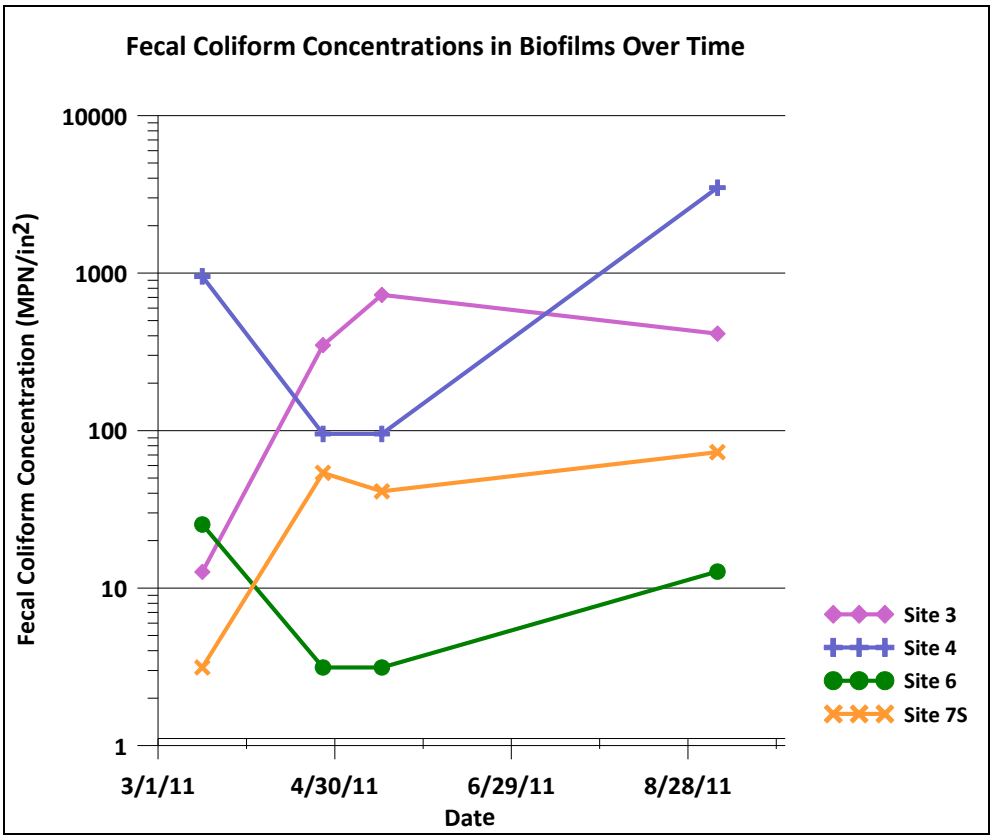


Figure 3-2. Fecal Coliform Concentrations over Time in Storm Drain Biofilms

3.3.3 Enterococci

Enterococci concentrations measured in biofilm suspension samples for Survey 1 are presented in Table 3-8. Concentrations ranged from less than the reporting limit to 854 MPN/in^2, with the highest concentration occurring at Site 4.

Table 3-8. Survey 1 Enterococci Concentrations by Site and Event in Storm Drain Biofilms

Sampling Event / Time Since Coupon Installation	Site			
	3	4	6	7
	MPN/ in^2	MPN/ in^2	MPN/ in^2	MPN/ in^2
Event 1 – 9 days	716	854	<3	198

Enterococci concentrations for Survey 2 are presented in Table 3-9 and in Figure 3-3. At Site 3, concentrations concentrations rose between 8 days and 10 weeks of growth and then decreased to a concentration similar to that at 8 days for the remainder of the study. At Site 4, concentrations rose between 8 days and 7 weeks of growth, dropped between 7 and 10 weeks, and rose sharply between 10 weeks and 6 months. At Sites 6, 7N, and 7S, concentrations were generally low (less than the reporting limit to 543 MPN/in^2) when compared to Sites 3 and 4. The coupons at Site 7N were lost following Event 2.

Table 3-9. Survey 2 Enterococci Concentrations by Site and Event in Storm Drain Biofilms

Sampling Event / Time Since Coupon Installation	Site				
	3	4	6	7N	7S
	MPN/ in^2	MPN/ in^2	MPN/ in^2	MPN/ in^2	MPN/ in^2
Event 1 – 8 days	120	134	6	<3	3
Event 2 – 7 weeks	1,549	246	10	*	27
Event 3 – 10 weeks	200	54	6	*	55
Event 4 – 6 months	162	380	<3	*	3

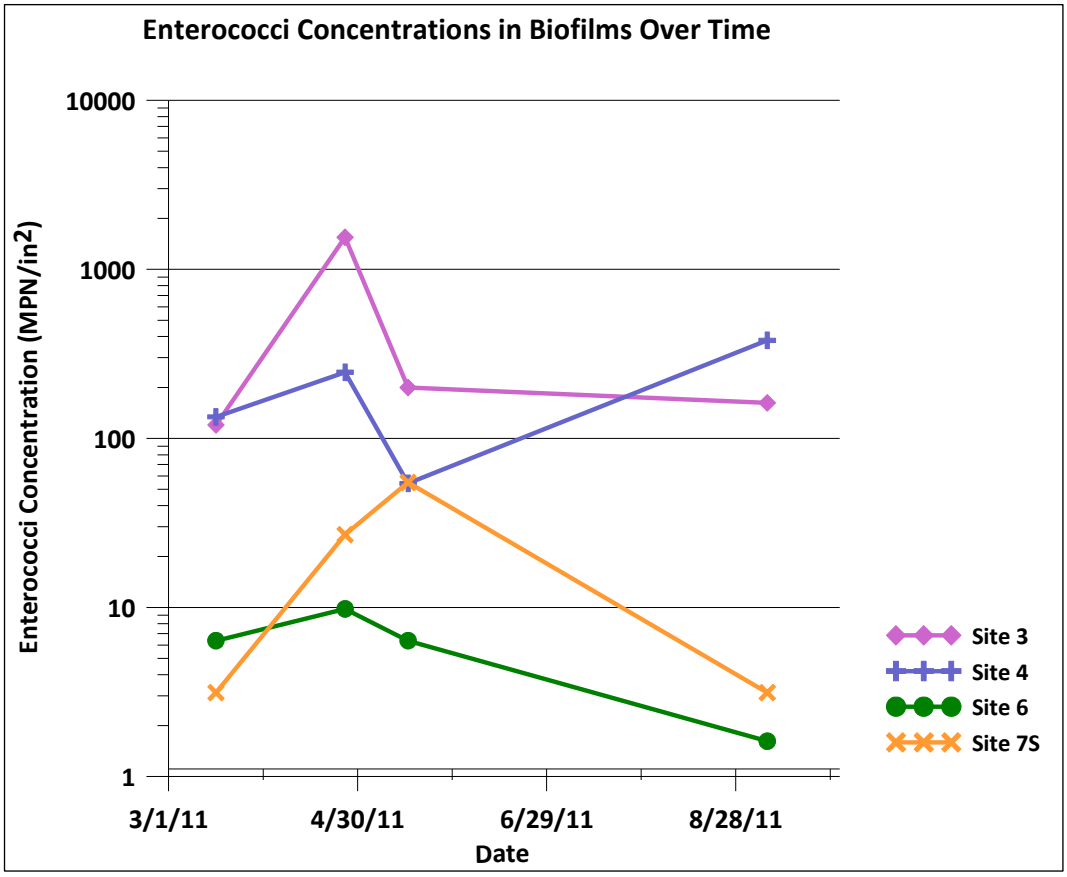


Figure 3-3. Enterococci Concentrations over Time in Storm Drain Biofilms

3.4 Summary, Biofilm Study

The biofilm study was designed to address the following questions:

1. **Does the MS4 act as a reservoir for fecal indicator bacteria?**
2. **If so, what are the concentrations per square inch?**

The results of the study demonstrate that regrowth of total coliform, fecal coliform, and enterococci is occurring at all sites within the Mainstem and Cascadita Channels. The results of both surveys suggest that colonization of the concrete substrate of the coupons occurs rapidly (within 8 or 9 days of deployment) and that microbial communities containing the three indicator bacteria are maintained over time under conditions found in the storm drain system. In general, concentrations remained close to those observed initially after 8 days of growth or increased over time (by one to two orders of magnitude at some sites). The exception was Site 6, where concentrations after 6 months of deployment (Event 4) were less than those observed after 8 days of deployment (Event 1) for all three indicators. Moreover, concentrations of all three indicators were lowest at Site 6 in nearly all sampling events. These results are consistent with the results of the sanitary surveys. During both sanitary surveys, water column bacterial concentrations at Site 6 were typically the lowest among the sites sampled during all sampling rounds (see Section 2).

Water chemistry results from the sanitary surveys indicate that nutrient concentrations (nitrate, total orthophosphate, and total phosphorus) also were lowest at Site 6. Lower concentrations of nutrients in the Cascadita Channel may limit biofilm growth, resulting in lower concentrations in the water column.

Overall, the results of the study suggest that regrowth of indicator bacteria within the Mainstem Channel and, to a lesser extent, the Cascadita Channel, can provide a source of indicator bacteria that can be delivered downstream. Given the continuous dry weather flows, high nutrient concentrations, and the lack of a significant human fecal contamination (Sanitary Surveys 1 and 2), the results suggest that the biofilm within the Cascadita and Mainstem Channels can serve as a reservoir of indicator bacteria in the Prima Deshecha Cañada Watershed.

4.0 GROUNDWATER STUDY

4.1 Overview, Groundwater Study

The 2005 to 2006 bacterial source identification study described in Section 1 investigated dry weather bacterial concentrations within the Mainstem Channel discharging from the Prima Deshecha Cañada Watershed to Poche Beach. Results suggest that the majority of bacterial loads and concentrations in the channel originated at the top and mid points of the watershed from side inlets downstream of the Prima Deshecha Landfill, possibly as a result of groundwater intrusion and over-irrigation. Groundwater was not assessed in terms of bacterial reservoir potential during the 2005 to 2006 study.

The ultimate goal of the groundwater study described in this section is to answer the following question:

Is groundwater a source of bacteria to the MS4?

The study was designed and implemented to determine whether groundwater is a source of bacteria in the watershed or acts as a transport mechanism for bacteria via infiltration into the MS4.

4.2 Methods, Groundwater Study

4.2.1 Field Methods

4.2.1.1 Site Locations

Temporary groundwater monitoring wells were installed on October 20 to 22, 2010. The well locations are shown in Table 4-1 and Figure 4-1 and briefly described below.

- A1 – The top of the watershed at the origin of the MS4 to assess potential bacterial inputs from the Prima Deshecha Landfill.
- B1 – Just above Shorecliffs Golf Course at Calle Nuevo to assess potential inputs from the greenbelt at the top of the watershed.
- C1 – In the middle of Shorecliffs Golf Course at Avenida Vaquero to assess the upper portion of the golf course.
- D1 – In the bottom portion of the watershed near Calle Grande Vista to assess characteristics in the lower portion of the watershed.

Table 4-1. Location and Description of Groundwater Study Sites

Site	Sampling	Latitude	Longitude
A1	✓	33.478605	-117.628045
B1	✓	33.465368	-117.639473
C1	✓	33.457486	-117.64257
D1	✓	33.445408	-117.644385

Groundwater Study

A utility sweep was conducted prior to the initiation of groundwater well drilling. Wells were then installed with direct-push technology, which uses a hydraulic hammer, stainless steel rods, and dedicated sampling equipment to collect undisturbed samples for laboratory analyses. Polyvinyl chloride (PVC) casings were installed to maintain the integrity of the bore-holes and the casings were cut flush with the surface of the ground and secured with PVC caps.

All samples were collected with little to no disturbance to the areas surrounding the wells. After all the samples were collected, each well area was returned to its original condition by removing the well casings and back-filling the bore-holes.

Figure 4-1. Groundwater Monitoring Well Locations

4.2.1.2 Sampling Frequency

Groundwater samples were collected during five events from each of the temporary groundwater monitoring wells between November 2010 and September 2011. Sampling occurred on November 4, 2010 (Event 1), January 26 to 27, 2011 (Event 2), February 23 to 24, 2011 (Event 3), July 25, 2011 (Event 4), and August 31 to September 1, 2011 (Event 5). As there was not sufficient water present, a sample was not collected at Site A during the November 2010 event. As a result of elevated bacteria levels measured in the November 2010 event, a sample for bacterial analyses at Site C was re-collected on December 2, 2010.

4.2.1.3 Sample Collection

Groundwater samples were collected using a GeoPump peristaltic pump (Model 5750) powered by a 12-volt DC battery. For each sampling event, depth to groundwater was measured with a sterilized water level meter (Heron dipper-T), several casing volumes were purged using the peristaltic pump, and groundwater was collected. Dedicated, sterile tubing was used for each event; therefore, no decontamination process was necessary. Purging was accomplished by using the pump to remove groundwater from the well at a low flow rate in order to minimize the impact of the purging process on groundwater chemistry and to minimize the volume of water purged and disposed. Initial depth data were used to verify that purging rates did not exceed the recharge capacity of the well. During purging, water level measurements were obtained to assess hydraulic effects of the purging. The process was considered complete when water quality parameters and monitoring water levels stabilized. If stabilization did not occur, sample collection took place following a minimum of three volume purges. The flow rate was then adjusted to a steady stream at 100 to 300 mL/min and samples for each set of analyses were collected as described below. Alternatively, a bailer was used at Site A and Site C on occasions when there was not sufficient volume in the monitoring well to allow for a three-volume purge.

Groundwater samples were collected from four locations during five events Samples were collected for analysis of enterococci, total and fecal coliforms, and general and human-associated *Bacteroides* MST as well as nutrients, metals, and general chemistry. Field water quality measurements (temperature, pH, DO, and conductivity) also were measured and recorded on field data sheets.

Sample Collection for Analysis of Bacteria by Culture
Bacteria grab samples were collected during five sampling events at Site A, six events at Sites B and D, and seven events at Site C, due to the December 2010 re-sample. Samples were analyzed for total and fecal coliforms and enterococci bacteria. Samples were handled and processed using the methods presented in Section 1.4.1.1

Sample Collection for MST Analysis by PCR
Grab samples of water for *Bacteroides*-General and human-associated *Bacteroides* analyses by real-time PCR were collected during four sampling events at Site A and five events at Sites B, C, and D. Samples were analyzed using the *Bacteroides*-General and the HF183 with melt assay (for human-associated *Bacteroides*) (Table 4-3). Samples were handled and processed using the methods presented in Section 1.4.1.2.

Sample Collection for Analysis of Water Chemistry
Chemistry grab samples were collected at each of the four sampling locations and were analyzed for physical and general chemistry and metals. At Site A, due to insufficient volume in the monitoring well, general chemistry and metals were not analyzed and physical chemistry was only analyzed for Events 4 and 5. Physical chemistry was analyzed for all five sampling events at Site B. General chemistry and metals were analyzed for Events 1 and 2. For Site C, physical chemistry was analyzed for all sampling events with the exception of the December 2010 resample, which was for bacteriological analyses only. General chemistry and metals were

analyzed for the Events 1 and 2. For Site D, samples from all five sampling events were analyzed for physical chemistry. General chemistry and metals were analyzed for Events 1 and 2.

Samples were handled and processed using the methods presented in Section 2.2.1.5.

4.2.1.4 Field Measurements

At each sampling station, field water quality measurements were recorded using a YSI 6920 water quality data sonde. Field measurements included temperature, pH, conductivity, and DO. In addition, the appearances of the purged and collected samples were described and static water levels and purge volumes were recorded. All data were recorded on field data sheets. Completed field data sheets for each sampling event are presented in Appendix A.

4.2.2 Analytical Methods

The methods used in microbiological and chemical analyses of groundwater samples were identical to those used in the sanitary survey. These methods are presented in Section 2.2.2 and are summarized below in Table 4-2, Table 4-3, and Table 4-4.

Table 4-2. Bacterial Parameters and Corresponding Analytical Methods for Groundwater Samples

Analytical Parameter	Analytical Method	Units	Method Detection Limit	Laboratory Reporting Limit	Sample Volume	Container (#, size, type)	Preservation	Holding Time
Total Coliform	SM 9221 B	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Fecal Coliform	SM 9221 E	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Enterococci	Enterolert	MPN/ 100 mL	1 MPN	<10 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours

Table 4-3. Microbial Source Tracking (MST) by Real-Time Polymerase Chain Reaction (PCR) Parameters for Groundwater Samples

Target	Assay	Sequence 5'-3' (Final Conc, µM)	References	Conditions
General *Bacteroides*	*Bacteroides* -General	Bac32F: AACGCTAGCTACAGGCTT (0.4) Bac708R: CAATCGGAGTTCTTCGTG (0.4) GenProbe: 6-FAM-CAATATTCCTCACT GCTGCCTCCCGTA-BHQ1 (0.2)	Bernhard and Field, 2000; Dick and Field, 2004	95°C, 30s; 40 cycles: 95°C, 15s; 60°C, 30s
Human *Bacteroides*	HF183 with melt	HF183F: ATCATGAGTTCACATGTCCG (0.4) Bac708R: CAATCGGAGTTCTTCGTG (0.4)	Bernhard and Field, 2000: Layton et. al., 2013	95°C, 15min; 50 cycles: 94°C, 30s; 54°C, 30s, 72°C, 45s; Melt: 60°C to 95°C at 0.2°/s

[a] Master Mix and thermocycler conditions typically consisted of Quanta-Perfecta QPCR Fastmix w/UNG (#84077) used on a BioRad CFX 96 thermocycler except for paired *Bacteroides*-General/ HF183 with melt assays, which

were run on a Cepheid Smart Cycler. The master mix for the *Bacteroides*-General assay was Qiagen Quantitect Sybr Green (Cepheid #1017340). Reaction volumes were 25 µL.

Table 4-4. Chemistry Parameters and Corresponding Analytical Methods for Groundwater Samples

Analytical Parameter	Analytical Method	Units	Method Detection Limit	Laboratory Reporting Limit	Sample Volume	Container (#, size, type)	Preservation	Holding Time
Total and Dissolved Cadmium	EPA 200.8	µg/L	0.4	0.8	500 mL	1, 500 mL plastic	Cool to 4ºC	6 Months
Total and Dissolved Nickel	EPA 200.8	µg/L	0.2	0.4	500 mL	1, 500 mL plastic	Cool to 4ºC	6 Months
Ammonia - N	SM 4500-NH3 F	mg/L	0.01	0.05	250mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
Nitrate – N	SM 4500 NO3 E	mg/L	0.01	0.05	250mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
Nitrite - N	SM 4500 NO2 B	mg/L	0.01	0.05	250mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
TKN	SM 4500 N C	mg/L	0.456	0.0	250mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	28 days
Total Orthophosphate	SM 4500-P C	mg/L	0.01	0.01	250mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
Total Phosphorus	SM 4500 P E	mg/L	0.016	0.05	250mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours

4.2.3 Quality Assurance/Quality Control Procedures

QA/QC procedures outlined in Section 1.4.3 and detailed in the QAPP (WESTON, 2010) were followed for the groundwater study. For chemistry analyses, a duplicate and field blank were collected for each sampling event. In the laboratory, EMA employed replicate spikes to determine the precision and accuracy of an analysis when some or all of the parameters being determined were below the detection limit. One set of duplicate samples or spike duplicates, a Laboratory Control Material or Certified Reference Material sample, and a method blank also were analyzed with each batch of samples.

4.2.4 Chain-of-Custody Procedures

COC procedures outlined in Section 1.4.4 were used for all samples throughout the collection, transport, and analytical processes.

4.3 Results, Groundwater Study

The Poche Beach Bacterial Source Identification Groundwater Study consisted of five sampling events at four locations in the Prima Deshecha Cañada Watershed. From these investigations, bacterial densities were calculated and water quality and water chemistry were analyzed. This portion of the overall investigation helped to determine whether groundwater intrusion may be contributing bacteria or providing a transport mechanism for bacteria in the watershed. The results from these analyses are presented below.

4.3.1 Indicator Bacteria

4.3.1.1 Total Coliforms

Total coliform concentrations measured in groundwater samples are presented in Table 4-5 and in Figure 4-2. At Site A, total coliform concentrations were low throughout the study up to the final event, when a concentration of 17,000 MPN/100 mL was measured. At Site C, the concentration of total coliforms during the first event was ≥16,000 MPN/100 mL. The site was re-sampled due to the elevated bacterial concentrations and a concentration of 13,000 MPN/100 mL was measured. Concentrations dropped markedly during the second event and remained low throughout the remainder of the study. At Site D, the concentration of total coliforms also was highest during the first event and was measured at 9,000 MPN/100 mL. As was observed at Site C, concentrations dropped during the second event and remained low throughout the remainder of the study.

Table 4-5. Total Coliform Concentrations (MPN/100 mL) in Groundwater

Sampling Event/Date	Site			
	A	B	C	D
1 – 11/04/10	NS	350	≥16,000	9,000
1 (re-sample) – 12/02/10	NS	NS	13,000	NS
2 – 01/26-27/11	270	<20	40	80
3 – 02/23-24/11	110	<20	<20	<20
4 – 07/25/11	170	<20	<20	<20
5 – 08/31-09/01/11	17,000	<20	199	<20

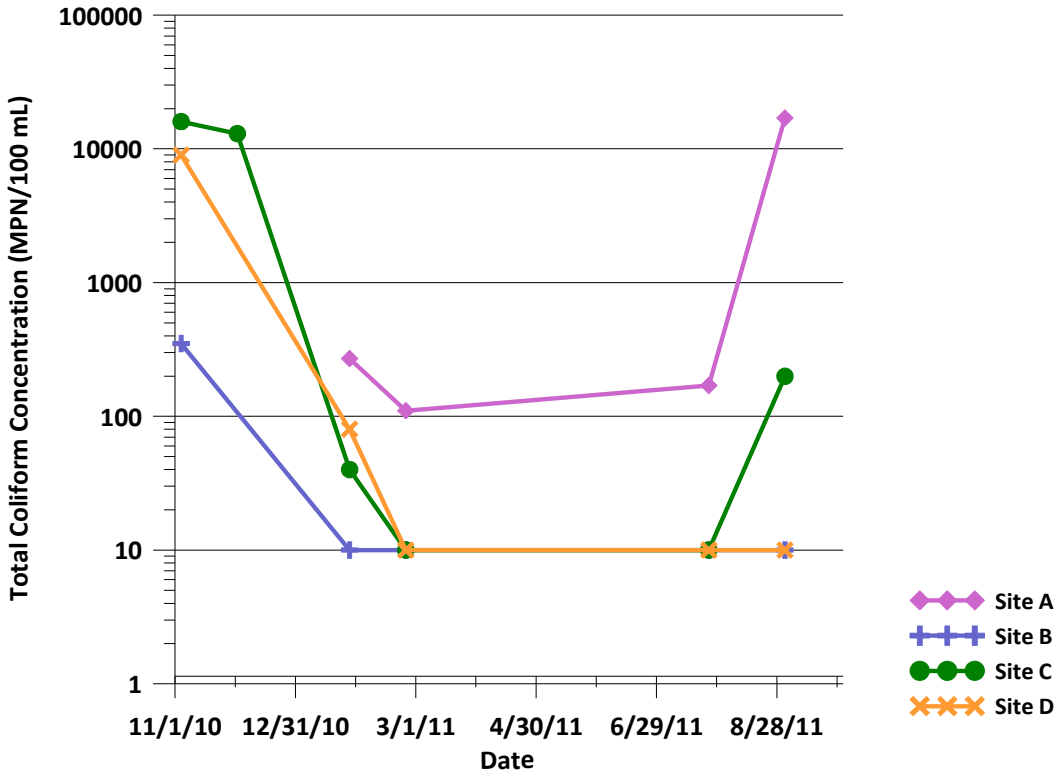


Figure 4-2. Total Coliform Concentrations over Time in Groundwater

4.3.1.2 Fecal Coliforms

Fecal coliform concentrations measured in groundwater samples are presented in Table 4-6 and in Figure 4-3. Fecal coliform concentrations were at or below the laboratory reporting limit with the exceptions of Event 1 (and the associated re-sampling event) at Site C and Event 1 at Site D. At Site C, the concentration of fecal coliforms during the first event was 1,700 MPN/100 mL. The site was re-sampled due to the elevated bacterial concentrations and a concentration of 300 MPN/100 mL was measured. Concentrations returned to below the laboratory reporting limit during the second event and remained low throughout the remainder of the study. At Site D, the

concentration of fecal coliforms during the first event was 500 MPN/100 mL. Concentrations were below the laboratory reporting limit for the remainder of the study.

Table 4-6. Fecal Coliform Concentrations (MPN/100 mL) in Groundwater

Sampling Event/Date	Site			
	A	B	C	D
1 – 11/04/10	NS	4	1,700	500
1 (re-sample) – 12/02/10	NS	NS	300	NS
2 – 01/26-27/11	<20	<20	<20	<20
3 – 02/23-24/11	<20	<20	<20	<20
4 – 07/25/11	<20	<20	<20	<20
5 – 08/31-09/01/11	20	<20	20	<20

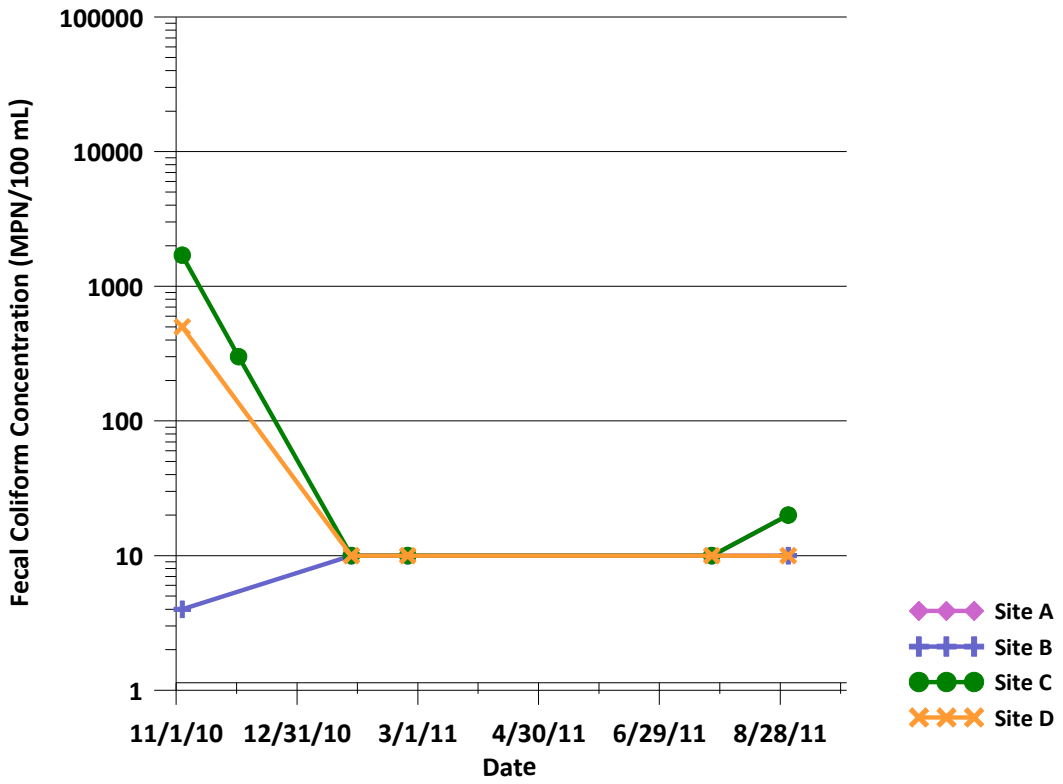


Figure 4-3. Fecal Coliform Concentrations over Time in Groundwater

4.3.1.3 Enterococci

Enterococci concentrations measured in groundwater samples are presented in Table 4-7 and in Figure 4-4. Concentrations were near or below the laboratory reporting limit with the exceptions of Event 1 (and the associated re-sampling event) at Site C and Event 1 at Site D. At Site C, the concentration of enterococci during the first event was ≥16,000 MPN/100 mL. The site was re-sampled due to the elevated bacterial concentrations and a concentration of 14,209 MPN/100 mL

was measured. Concentrations were below the laboratory reporting limit for the remainder of the study. At Site D, the concentration of enterococci during the first event was 1,100 MPN/100 mL. Concentrations were below the laboratory reporting limit for the remainder of the study.

Table 4-7. Enterococci Concentrations (MPN/100 mL) in Groundwater

Sampling Event/Date	Site			
	A	B	C	D
1 – 11/04/10	NS	8	≥16,000	1,100
1 (re-sample) – 12/02/10	NS	NS	14,209	NS
2 – 01/26-27/11	30	<10	<10	<10
3 – 02/23-24/11	10	<10	<10	<10
4 – 07/25/11	<10	<10	<10	<10
5 – 08/31-09/01/11	<10	<10	<10	<10

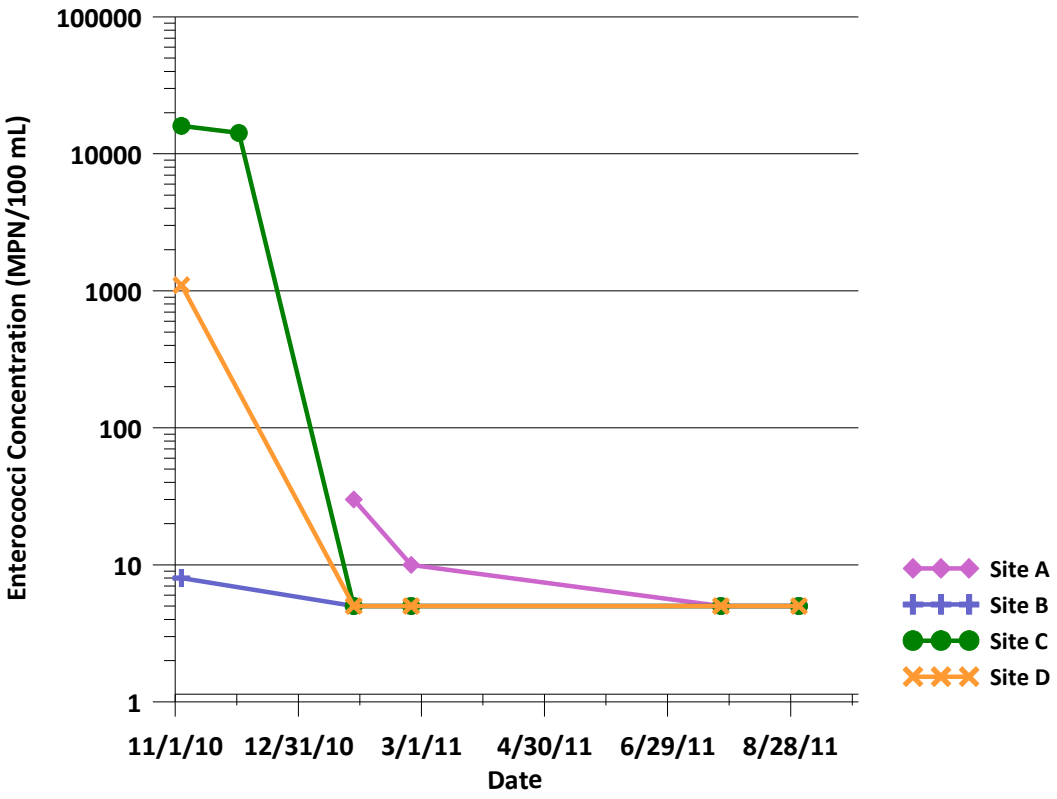


Figure 4-4. Enterococci Concentrations over Time in Groundwater

4.3.2 Microbial Source Tracking

In addition to measuring enterococci and fecal coliform concentrations at each site, MST by real-time PCR was conducted on samples. All samples were tested for both the *Bacteroides*-General

and the HF183 with melt assay (human-associated *Bacteroides*) assays (Table 1-3). All samples tested negative for both general and *Bacteroides*-human assays (Table 4-8). All controls, including inhibition controls (see Section 1.4.3), were deemed acceptable.

Table 4-8. Real-time polymerase Chain Reaction (PCR) Results for the General-*Bacteroides* and Human *Bacteroides* Assays (Presence/Absence) in Groundwater

Sampling Event/Date	Site							
	A		B		C		D	
	General	Human	General	Human	General	Human	General	Human
1 – 11/04/10	ns	ns	Neg	Neg	Neg	Neg	Neg	Neg
1 (re-sample) – 12/02/10	ns	ns	ns	ns	ns	ns	ns	ns
2 – 01/26-27/11	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
3 – 02/23-24/11	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
4 – 07/25/11	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
5 – 08/31-09/01/11	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

ns – Not sampled.
Neg – Negative

4.3.3 Water Chemistry

The grab samples collected at each of the four groundwater study site locations were analyzed for physical and general chemistry and metals. The high bacterial concentrations in groundwater in the first sampling event resulted in the sampling frequency being increased from the original design described in the QAPP. As a consequence, general chemistry parameters and metals analyses were performed in the first three sampling events, but not in Events 4 and 5. At Site A, due to insufficient volume in the monitoring well, general chemistry and metals analyses were not performed for any sample, and physical chemistry analysis was only performed for Events 4 and 5. The results of the chemical analyses are presented in Table 4-9.

In order to compare the chemistry results among sites and sampling events, those results that were greater than Basin Plan water quality benchmarks were highlighted in green in Table 4-9. These benchmarks are for receiving waters and are not directly applicable to groundwater samples. They were used in this case to allow a relative comparison of the groundwater chemistry results. Chemical analyses of groundwater samples revealed that TDS, TSS, and total phosphorus concentrations in groundwater were consistently high relative to the receiving water benchmarks. In addition, TKN and ammonia appeared to be elevated in most sampling events.

Table 4-9. Water Chemistry Results in Groundwater

Parameter	Units	Site A					Site B					Site C					Site D				
		Event 1	Event 2	Event 3	Event 4	Event 5	Event 1	Event 2	Event 3	Event 4	Event 5	Event 1	Event 2	Event 3	Event 4	Event 5	Event 1	Event 2	Event 3	Event 4	Event 5
Physical Chemistry																					
Conductivity	µS/cm	ns	ns	ns	14,510	13,990	17,050	18,490	17,830	17,530	17,340	25,050	24,340	24,580	24,720	24,240	25,290	26,650	25,730	24,850	24,420
Dissolved Oxygen	mg/L	ns	ns	ns	5.49	6.58	**2.65**	**3.51**	**2.67**	**2.39**	**2.57**	**4.84**	6.06	6.24	**2.77**	5.72	**3.02**	**3.01**	**2.97**	**2.18**	**2.74**
pH	pH units	ns	ns	ns	6.55	7.19	6.50	6.84	6.81	6.68	6.76	7.28	6.72	6.90	**6.29**	6.46	6.92	6.62	6.70	6.58	6.66
Salinity	ppt	ns	ns	ns	9.20	8.11	10.05	ns	10.55	10.36	10.24	ns	ns	15.07	15.00	14.77	15.42	16.43	15.71	15.15	14.86
Turbidity	NTU	ns	ns	ns	**45.3**	**86.1**	11.2	6.1	6.6	2.8	11.9	**831**	**1,758.9**	**99.2**	**85.1**	**72.2**	**281.4**	2.2	**26.6**	3.5	0.9
Water Temperature	Celsius	ns	ns	ns	21.00	22.25	20.59	20.27	19.94	21.46	20.94	29.30	19.60	18.38	21.23	21.56	25.27	21.85	21.08	21.72	22.25
General Chemistry																					
Ammonia-N	mg/L	ns	ns	ns	ns	ns	0.52	1.20	0.34	ns	ns	2.85	3.56	1.55	ns	ns	2.17	4.88	3.08	ns	ns
Nitrate-N	mg/L	ns	ns	ns	ns	ns	0.07	<0.05	<0.05	ns	ns	<0.05	<0.05	<0.05	ns	ns	<0.05	0.05	<0.05	ns	ns
Nitrite-N	mg/L	ns	ns	ns	ns	ns	0.05	<0.05	<0.05	ns	ns	<0.05	<0.05	<0.05	ns	ns	<0.05	<0.05	<0.05	ns	ns
TDS	mg/L	ns	ns	ns	ns	ns	**14,900**	**14,600**	**15,400**	ns	ns	**17,200**	**17,200**	**16,600**	ns	ns	**22,200**	**23,100**	**23,500**	ns	ns
TKN	mg/L	ns	ns	ns	ns	ns	2.6	3.0	2.8	ns	ns	6.8	4.5	4.0	ns	ns	5.3	4.9	4.8	ns	ns
TOC	mg/L	ns	ns	ns	ns	ns	11	ns	36	ns	ns	37	ns	46	ns	ns	34	ns	56	ns	ns
Total Orthophosphate	mg/L	ns	ns	ns	ns	ns	0.49	0.35	0.15	ns	ns	0.22	0.39	0.10	ns	ns	0.52	0.62	0.49	ns	ns
Total Phosphorus	mg/L	ns	ns	ns	ns	ns	**0.5**	**0.4**	**0.8**	ns	ns	**0.8**	**0.5**	**1.6**	ns	ns	**0.6**	**0.7**	**0.7**	ns	ns
TSS	mg/L	ns	ns	ns	ns	ns	ns	**147**	**102**	ns	ns	ns	**19,000**	**18,300**	ns	ns	ns	**99**	**115**	ns	ns
Total Metals																					
Cadmium (Cd)	mg/L	ns	ns	ns	ns	ns	0.007	<0.1	0.010	ns	ns	0.012	<0.1	0.082	ns	ns	0.022	<0.1	0.013	ns	ns
Nickel (Ni)	mg/L	ns	ns	ns	ns	ns	0.040	<0.5	0.050	ns	ns	0.163	<0.5	0.429	ns	ns	0.435	<0.5	0.290	ns	ns
Dissolved Metals																					
Cadmium (Cd)	mg/L	ns	ns	ns	ns	ns	0.006	0.004	<0.005	ns	ns	0.010	0.004	0.008	ns	ns	0.012	0.008	0.006	ns	ns
Nickel (Ni)	mg/L	ns	ns	ns	ns	ns	0.038	0.055	0.046	ns	ns	0.091	0.255	0.198	ns	ns	0.228	0.231	0.263	ns	ns

ns = not sampled
Values in green are outside of water quality benchmarks.

4.4 Summary, Groundwater Study

The results from the 2005 to 2006 Poche Beach bacterial source identification study suggest that the majority of bacterial loads and concentrations in Mainstem Channel discharging to Poche Beach originated at the top and mid points of the watershed from side inlets downstream of the Prima Deshecha Landfill, possibly as a result of groundwater intrusion and over-irrigation (WESTON, 2006). Groundwater was not assessed in the 2005 to 2006 study, but surface water samples were negative for the human marker, suggesting that leaking sewage infrastructure (or other groundwater sources of anthropogenic bacteria) was not a likely source of bacteria to the surface waters. The current study was designed to determine whether groundwater intrusion may be contributing bacteria or providing a transport mechanism for bacteria within the Prima Deshecha Cañada Watershed by infiltrating into the Mainstem channel. The main question posed in this study is addressed below.

Is groundwater a source of bacteria to the MS4?

In general, the results indicated that groundwater in the watershed is not a significant direct source of bacteria to the MS4. Concentrations of all three indicator bacteria were largely at or below detection limits in the majority of samples collected. The major exception to this trend was the first survey in November 2010, in which concentrations of total coliform, fecal coliform, and enterococci were very high at Sites C and D. The reason for high concentrations at these sites is unclear; however, concentrations of all three indicators were very low in nearly all subsequent samples. Overall, the results suggest that groundwater at the sites monitored does not typically contain elevated levels of indicator bacteria and does not appear to be a direct source of bacteria to the watershed.

However, the results of the groundwater monitoring did suggest that groundwater likely acts as a transport mechanism for bacteria in the watershed. In wells B, C, and D, the water level readings indicated that the surface of the groundwater table was higher than the bottom of the MS4 channel during all five surveys, which allows for the potential of groundwater influx into the MS4. Although quantifying groundwater influx was beyond the scope of this study, it is readily apparent from visual observations that groundwater intrusion is occurring. This is particularly true at Site 4 and between Sites 4 and 5, where groundwater intrusion through the seams of the MS4 channel is obvious. Thus, the groundwater contribution to the overall flow in the Mainstem Channel may be substantial, particularly in the lower portion of the watershed (below Site 4). Groundwater influx likely plays an important role in maintaining continual flows in the Mainstem Channel, thereby contributing to regrowth of indicator bacteria as discussed in Section 3. Regrowth may be further enhanced by groundwater influx, because it contains elevated levels of nutrients (total orthophosphate and total phosphorus) and ammonia, which is readily converted to nitrate under aerobic conditions. High concentrations of nutrients in the Mainstem Channel (see Section 2) will tend to enhance the biofilm microbial community, including indicator bacteria.

Thus, the results of the study suggest that groundwater does not appear to be a direct source of indicator bacteria in the watershed, but likely contributes to bacterial regrowth through influx of high-nutrient groundwater into the MS4.

5.0 BIOSWALE BMP EFFECTIVENESS STUDY

5.1 Overview, Bioswale Study

Shorecliffs Golf Course is located within the middle and lower portion of the Prima Deshecha Cañada Watershed and covers approximately 20% of the drainage. In the late 1990s, a bioswale was installed as a BMP in order to assess whether the diversion of dry weather flows from the Mainstem Channel through the bioswale would reduce nutrient and bacterial loads to the lower portion of the watershed and ultimately to Poche Beach. The bioswale was created through a dry weather diversion just below Calle Nuevo at the top of the golf course (labeled Site A in Figure 5-1). It carries water diverted from the Mainstem Channel a distance of approximately 1.5 miles before discharging to the channel just upstream of Calle Grande Vista. The bioswale consists of three discrete sections. Section 1 extends from the dry weather diversion at Site A where it becomes a surface water creek with a fairly well-grown riparian corridor that parallels the Mainstem Channel for a distance of approximately 0.5 mile to Site B, just upstream of Avenida Vaquero. The second section of the bioswale extends from Site B to C, where surface flows in the first section of the bioswale are diverted to a 10-inch diameter PVC pipe that is strapped to the inside of the Mainstem Channel. The pipe directs the flows from Site B to Site C where it daylights at the Shorecliffs Golf Course Clubhouse, downstream of Interstate 5. The third section of the bioswale is a surface water stream that courses through the lower section of the golf course before discharging to the Mainstem Channel at Site D (just above Calle Grande Vista).

The goal of the Bioswale BMP Effectiveness Study described in this section is to answer the following Study Questions:

1. **Is the bioswale effective in reducing bacterial concentrations and flow?**
2. **Is the bioswale effective in reducing concentrations other constituents, such as metals and nutrients?**

Two effectiveness assessment surveys were conducted in order to answer these questions and to assess the effectiveness of the bioswale in reducing flow and the levels of indicator bacteria and other constituents. The first survey was conducted on December 14, 2010, during the first sanitary survey. Water samples were collected three times over the course of the survey at Site A and Site B (Figure 5-1). The second survey was conducted on June 21, 2012. A total of six samples were collected at each of the four sites identified on Figure 5-1. In each survey, stream flow and bacterial concentrations from samples taken at the top of the bioswale were compared to those at the bottom of the bioswale to determine the effectiveness of the BMP in reducing constituent concentrations and flows.

Figure 5-1. Map of Prima Deshecha Cañada Watershed Showing Bioswale and Monitoring Sites

5.2 Methods, Bioswale Study

5.2.1 Field Methods

5.2.1.1 Site Locations

Bioswale samples were collected from four locations in the watershed as listed in Table 5-1 and shown on Figure 5-1. Samples were collected at Site A and B in Survey 1 and at all four sites in Survey 2.

Table 5-1. Location and Description of Bioswale Effectiveness Monitoring Sites

Site	Latitude	Longitude	Description
A	33.464350	-117.640042	Top of the bioswale, downstream of Calle Nuevo where the diverted water from the Mainstem Channel enters the bioswale.
B	33.457849	-117.642547	Bottom of first section of bioswale just upstream of Avenida Vaquero. Flows from the first section of the bioswale discharge to a 12-inch PVC pipe at this location.
C	33.451642	-117.646351	Downstream of Interstate 5 where the PVC pipe daylights just downstream of the Shorecliffs Golf Course Clubhouse.
D	33.446041	-117.644361	Bottom of the bioswale in surface water creek before it discharges back to the Mainstem Channel just upstream of Calle Grande Vista.

5.2.1.2 Sampling Frequency

In Survey 1, grab samples were collected from Site A and Site B (Figure 5-1) a total of three times at each site over the course of the day at 6 a.m., noon, and 6 p.m. Samples were collected for analysis of enterococci, fecal coliforms, and general and human-associated *Bacteroides* assays, as well as nutrients and general chemistry, following the same methods and QA/QC procedures discussed in Section 2. Field water quality measurements also were taken and recorded on field data sheets (Appendix A).

In Survey 2, grab samples were collected from Sites A, B, C, and D (Figure 5-1) a total of six times at each site. Samples were collected between 2:40 a.m. and 6:50 a.m. on June 21, 2012. This morning period was chosen because flows in the Mainstem Channel typically peak during the evening and early morning hours.

5.2.1.3 Sample Collection

During Survey 1, three grab samples were collected at each site using the procedures described in Section 2.2.1.5 for indicator bacteria, PCR for presence/absence using the *Bacteroides*-General assay, the HF183 with melt assay for human-associated *Bacteroides*, and water

chemistry. During Survey 2, six samples were collected at each site and analyzed for indicator bacteria and the HF183 with melt assay for human-associated *Bacteroides*.

5.2.1.4 Field Measurements

At each sampling location, field water quality measurements were recorded using a YSI 6920 water quality data sonde and recorded on field data sheets. Field measurements included temperature, conductivity, dissolved oxygen, pH, salinity, and turbidity. In addition, a description of the site, including water quality appearance, flow estimates, and potential sources of fecal material, was recorded. All data were recorded on field data sheets (Appendix A).

5.2.1.5 Flow Measurements

During Survey 1, flow was monitored at Site A and Site B (Figure 5-1) for a 24-hour period using the methods discussed in Section 2. Flow was monitored using the methods discussed in Section 2. During Survey 2, flow was monitored at Site A and Site D over a period of 7 days between July 30 and August 6, 2012 using the same methods as those used in Survey 1.

5.2.2 Analytical Methods, Bioswale Study

The methods used in microbiological and chemical analyses of bioswale samples are summarized below for bacterial parameters (Table 5-2), microbial parameters (Table 5-3), and analytical chemistry parameters (Table 5-4). Bacterial and molecular samples were collected and analyzed in Survey 1, but analytical samples were not collected during Survey 2.

Table 5-2. Bacterial Parameters and Corresponding Analytical Methods in the Bioswale BMP Effectiveness Study

Analytical Parameter	Analytical Method	Units	Method Detection Limit	Laboratory Reporting Limit	Sample Volume	Container (#, size, type)	Preservation	Holding Time
Fecal Coliform	SM 9221 E	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Enterococci	Enterolert	MPN/ 100 mL	1 MPN	<10 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours

Table 5-3. Microbial Source Tracking (MST) by Real-Time Polymerase Chain Reaction (PCR) in the Bioswale BMP Effectiveness Study

Target	Assay	Sequence 5'-3' (Final Conc, µM)	References	Conditions
General *Bacteroides*	*Bacteroides* -General	Bac32F: AACGCTAGCTACAGGCTT (0.4) Bac708R: CAATCGGAGTTCTTCGTG (0.4) GenProbe: 6-FAM-CAATATTCCTCACT GCTGCCTCCCGTA-BHQ1 (0.2)	Bernhard and Field, 2000; Dick and Field 2004	95°C, 2 min; 40 cycles: 95°C, 15s; 60°C, 30s
Human *Bacteroides*	HF183 with melt	HF183F: ATCATGAGTTCACATGTCCG (0.4) Bac708R: CAATCGGAGTTCTTCGTG (0.4)	Bernhard and Field, 2000; Layton et al., 2013	95°C, 15 min; 50 cycles: 94°C, 30s; 54°C, 30s, 72°C, 45s; Melt: 60°C to 95°C at 0.2°/s

[a] Master Mix and thermocycler conditions typically consisted of Quanta-Perfecta QPCR Fastmix w/UNG (#84077) used on a BioRad CFX 96 thermocycler except for paired *Bacteroides*-General/ HF183 with melt assays, which were run on a Cepheid Smart Cycler. The master mix for the *Bacteroides*-General assay was Qiagen Quantitect Sybr Green (Cepheid #1017340). Reaction volumes were 25 µL.

Table 5-4. Chemistry Parameters and Corresponding Analytical Methods (Survey 1 only) in the Bioswale BMP Effectiveness Study

Analytical Parameter	Analytical Method	Units	Method Detection Limit	Laboratory Reporting Limit	Sample Volume	Container (#, size, type)	Preservation	Holding Time
Total and Dissolved Cadmium	EPA 200.8	µg/L	0.4	0.8	500 mL	1, 500-mL plastic	Cool to 4ºC	6 Months
Total and Dissolved Nickel	EPA 200.8	µg/L	0.2	0.4	500 mL	1, 500-mL plastic	Cool to 4ºC	6 Months
Ammonia - N	SM 4500-NH3 F	mg/L	0.01	0.05	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
Nitrate – N	SM 4500 NO3 E	mg/L	0.01	0.05	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
Nitrate - N	SM 4500 NO2 B	mg/L	0.01	0.05	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
TKN	SM 4500 N C	mg/L	0.456	0.0	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	28 days
Total Orthophosphate	SM 4500-P C	mg/L	0.01	0.01	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours
Total Phosphorus	SM 4500 P E	mg/L	0.016	0.05	250 mL	1, 250-mL HDPE plastic	Cool to 4ºC; H_2SO_4 to pH<2	48 Hours

5.2.3 Quality Assurance/Quality Control

QA/QC procedures outlined in Section 2.2.3 and detailed in the QAPP (WESTON, 2010) were followed for both surveys in the bioswale BMP effectiveness study.

5.2.4 Chain-of-Custody Procedures

COC procedures outlined in Section 1.4.4 were used for all samples throughout the collection, transport, and analytical processes employed in both surveys.

5.3 Results, Bioswale Study

The results of the Bioswale BMP Effectiveness Study are separated into Survey 1 results and Survey 2 results below.

5.3.1 Survey 1

5.3.1.1 Bacteriological Analyses

Fecal Coliforms

Fecal coliform concentrations measured in bioswale samples during Survey 1 are presented in Table 5-5 and on Figure 5-2. Concentrations were below the Basin Plan benchmark with the exceptions of the 6 a.m. samples taken at Sites A and B (3,000 and 500 MPN/100 mL, respectively). Fecal coliform concentrations were greater at the upstream site (Site A) compared to the downstream site (Site B) during the 6 a.m. sampling period, but all other samples had low concentrations of similar magnitude. The results suggest that based on this limited data set in Survey 1, the bioswale had limited, if any, effect on reducing fecal coliform concentrations between Site A and Site B.

Table 5-5. Fecal Coliform Concentrations (MPN/100 mL) in the Bioswale BMP Effectiveness Study – Survey 1

Sampling Date	Site/Time					
	A-1 6 a.m.	A-2 noon	A-3 6 p.m.	B-1 6 a.m.	B-2 noon	B-3 6 p.m.
12/14/10	**3,000**	300	230	**500**	230	230
Values shaded purple were above the Basin Plan benchmark of 400 MPN/100 mL for fecal coliforms.						

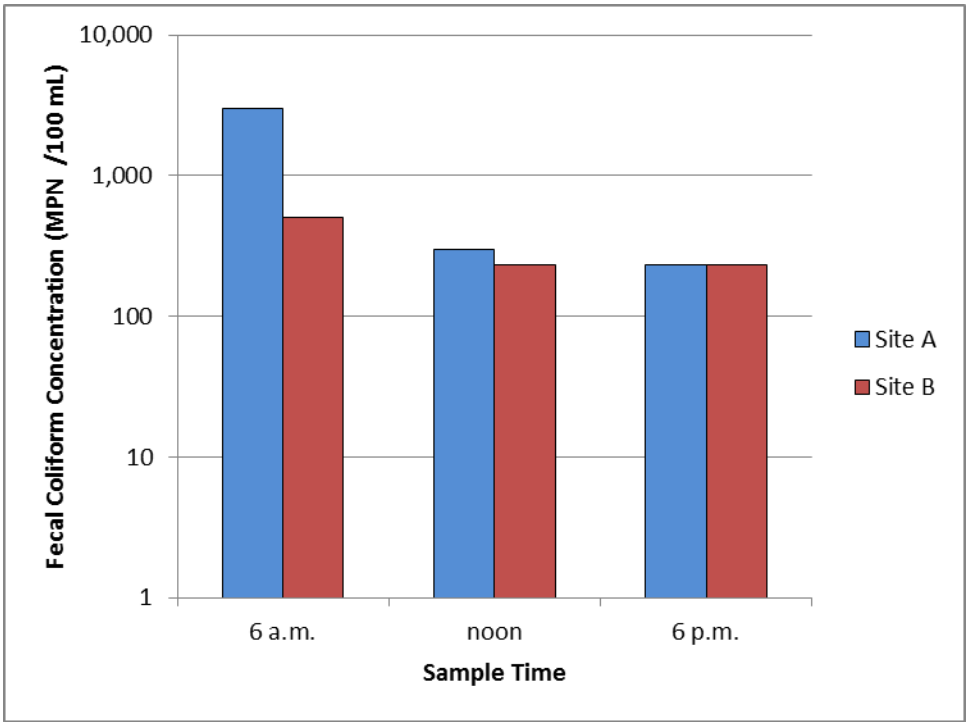


Figure 5-2. Fecal Coliform Concentrations in the Bioswale BMP Effectiveness Study – Survey 1

Enterococci

Enterococci concentrations measured in bioswale samples are presented in Table 5-6 and on Figure 5-3. Concentrations were greater than the Basin Plan benchmark at both sites during all three sampling events. Similar to the fecal coliform results, the enterococci concentrations were greater at the upstream site (Site A) compared to the downstream site (Site B) during the 6 a.m. sampling period, but all other samples had low concentrations of similar magnitude. The results suggest that based on this limited data set in Survey 1, the bioswale had limited, if any, effect on reducing enterococcus concentrations between Site A and Site B.

Table 5-6. Enterococci Concentrations (MPN/100 mL) in the Bioswale BMP Effectiveness Study – Survey 1

Sampling Date	Site/Time					
	A-1 6 a.m.	A-2 noon	A-3 6 p.m.	B-1 6 a.m.	B-2 noon	B-3 6 p.m.
12/14/10	**4,352**	**727**	**530**	**1,314**	**689**	**657**

Values shaded purple were above the Basin Plan benchmark of 104 MPN/100 mL for enterococci.

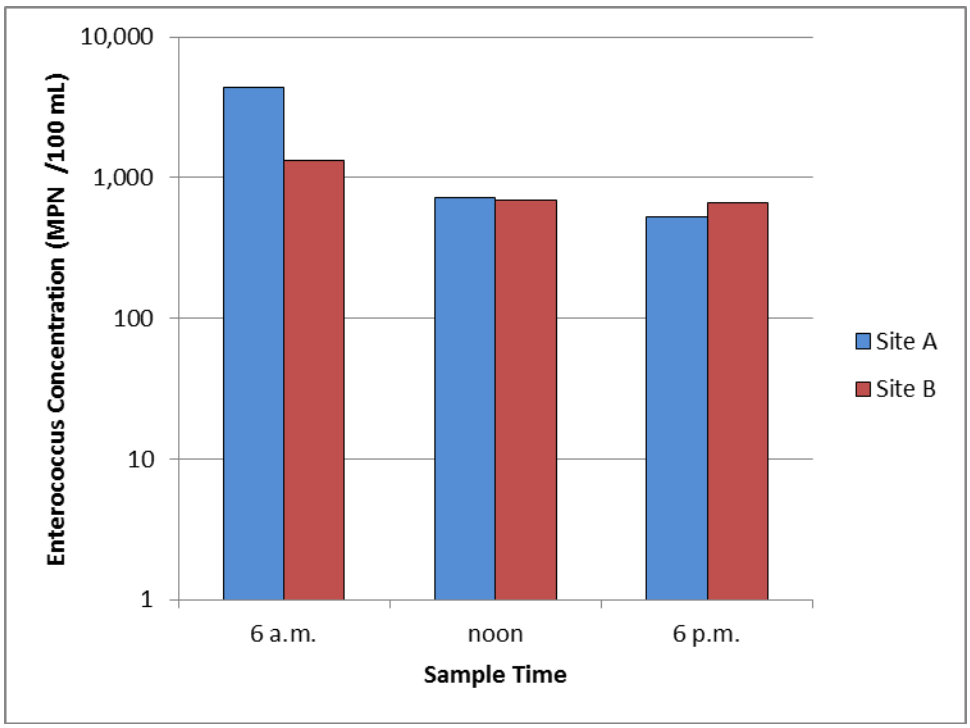


Figure 5-3. Enterococci Concentrations in the Bioswale BMP Effectiveness Study – Survey 1

5.3.1.2 Microbial Source Tracking

The results of the MST analyses for the bioswale study samples are provided below in Table 5-7. All samples tested positive for the general *Bacteroides* marker and negative for the human-associated *Bacteroides* marker. These results are consistent with those obtained for the other sites monitored in the first sanitary survey and overall suggest a lack of significant human sources of fecal bacteria in the watershed. All controls (see Section 1.4.3) were deemed acceptable.

Table 5-7. Real-Time PCR Results for General *Bacteroides* and Human-Associated *Bacteroides* (Presence/Absence) for Bioswale BMP Effectiveness Study – Survey 1

Sampling Date	Site/Time					
	A-1 6 a.m.	A-2 noon	A-3 6 p.m.	B-1 6 a.m.	B-2 noon	B-3 6 p.m.
Assay:						
Bacteroides-General	Pos	Pos	Pos	Pos	Pos	Pos
Bacteroides-Human	Neg	Neg	Neg	Neg	Neg	Neg

Pos = Positive
Neg = Negative

5.3.1.3 Water Chemistry

Grab samples collected at both of the bioswale study site locations were analyzed for physical and general chemistry and metals. The results of the chemical analyses are presented below in

Table 5-8. The results were compared to the Basin Plan water quality benchmark for each constituent where a benchmark is available. Concentrations of all analytes measured were less than Basin Plan benchmarks with the exception of TDS and total phosphorus, for which values were above the respective benchmarks for all samples collected.

Concentrations of the majority of the constituents did not change markedly from the upstream to downstream location. However, concentrations of ammonia, nitrite, cadmium, and nickel (total and dissolved) decreased from upstream to downstream during all three sampling periods.

Table 5-8. Water Chemistry Results for the Bioswale BMP Effectiveness Study – Survey 1

Parameter	Units	Site/Time					
		A-1 6 a.m.	A-2 noon	A-3\ 6 p.m.	B-1 6 a.m.	B-2 noon	B-3 6 p.m.
Physical Chemistry							
Conductivity	µS/cm	6,069	8,779	8,929	6,995	6,892	8,340
Dissolved Oxygen	mg/L	8.80	13.24	8.83	9.59	12.13	9.14
pH	pH units	7.80	8.00	7.81	7.92	8.23	7.99
Salinity	ppt	3.32	4.93	5.02	3.86	3.80	4.66
Turbidity	mg/L	3.5	3.5	2.1	3.3	13.3	2.2
Water Temperature	Celsius	14.41	16.13	14.39	12.62	15.41	13.55
General Chemistry							
Ammonia-N	mg/L	0.37	0.33	0.26	<0.1	<0.1	0.15
Nitrate-N	mg/L	1.77	2.02	1.91	1.87	1.44	1.60
Nitrite-N	mg/L	0.18	0.17	0.16	<0.05	<0.05	<0.05
TDS	mg/L	**2,620**	**8,280**	**8,270**	**6,690**	**6,240**	**7,990**
TKN	mg/L	3.0	2.6	3.0	2.8	1.5	2.0
Total Orthophosphate	mg/L	0.66	0.29	0.31	1.52	0.22	0.47
Total Phosphorus	mg/L	**0.69**	**0.3**	**0.35**	**1.55**	**0.23**	**0.52**
TSS	mg/L	<20	<20	<20	<20	<20	<20
Total Metals							
Nickel (Ni)	mg/L	0.133	0.212	0.238	0.077	0.058	0.078
Cadmium (Cd)	mg/L	0.016	0.027	0.031	0.008	0.006	0.008
Dissolved Metals							
Cadmium (Cd)	mg/L	0.015	0.021	0.026	0.007	0.005	0.007
Nickel (Ni)	mg/L	0.129	0.191	0.194	0.069	0.057	0.075
Values in green are outside water quality benchmarks.							

5.3.2 Survey 2

During Survey 2 of the Bioswale BMP Effectiveness Study, samples were collected from Site A and Site B (as in Survey 1), but also from Site C (near the Shorecliffs Golf Course Clubhouse) and Site D (just above Calle Grande Vista) (Figure 5-1). Photographs of the top and bottom of the bioswale (Site A and Site D, respectively) are shown in Figure 5-4. Six samples were collected at each site between 2:40 a.m. and 6:50 a.m.

Figure 5-4. Stream Rating and Flow Monitoring at Site A (upstream) (A) and Site D (downstream) (B) in the Bioswale Effectiveness Assessment

5.3.2.1 Bacteriological Analyses

Fecal Coliforms
Fecal coliform concentrations measured in bioswale samples during Survey 2 are presented in Table 5-9 and on Figure 5-5. Concentrations were greater than the Basin Plan benchmark of 400 MPN/100 mL in all samples collected except those collected at Site D, which were all less than the Basin Plan benchmark. The mean fecal coliform concentration at the top of the bioswale (Site A) was 6,708 MPN/100 mL, which was an order of magnitude greater than the mean concentration at the bottom of the bioswale (Site D), which was 267 MPN/100 mL. Mean concentrations were greatest at Sites B and C (5,833 and 7,500 MPN/100 mL, respectively) in the middle of the bioswale.

Enterococci
Enterococci concentrations measured in bioswale samples during Survey 2 are presented in Table 5-9 and on Figure 5-5. Concentrations were greater than the Basin Plan benchmark of 104 MPN/100 mL in all samples collected during Survey 2. The mean enterococcus concentration at the top of bioswale (6,719 MPN/100 mL) was nearly five times greater than the mean concentration at the bottom of the bioswale (1,494 MPN/100 mL). Intermediate concentrations were measured in the middle of the bioswale (Sites B and C).

Table 5-9. Bacterial Concentrations in the Bioswale BMP Effectiveness Study – Survey 2

Site	Sample ID	Collection Time	Fecal Coliform (MPN/100 mL)	Enterococcus (MPN/100 mL)
A	A1	0240	2,300	4,500
	A2	0330	1,100	6,504
	A3	0420	3,000	6,314
	A4	0510	800	8,126
	A5	0600	2,800	6,504
	A6	0630	2,200	8,361
A Geometric Mean			**1,829**	**6,587**
B	**B1**	0251	7,000	4,352
	B2	0340	2,200	4,353
	B3	0430	8,000	4,352
	B4	0520	7,000	3,255
	B5	0608	2,800	4,352
	B6	0635	8,000	4,884
B Geometric Mean			**5,180**	**4,227**
C	**C1**	0255	8,000	4,106
	C2	0345	3,000	4,106
	C3	0445	8,000	2,613
	C4	0530	5,000	3,873
	C5	0620	13,000	3,448
	C6	0640	8,000	3,448
C Geometric Mean			**6,811**	**3,558**
D	**D1**	0300	300	1,607
	D2	0400	170	1,658
	D3	0455	300	1,607
	D4	0535	300	1,291
	D5	0625	300	1,529
	D6	0650	230	1,274
D Geometric Mean			**261**	**1,486**

Values shaded purple were above the Basin Plan benchmark of 400 MPN/100 mL for fecal coliforms and 104 MPN/100 mL for enterococci.

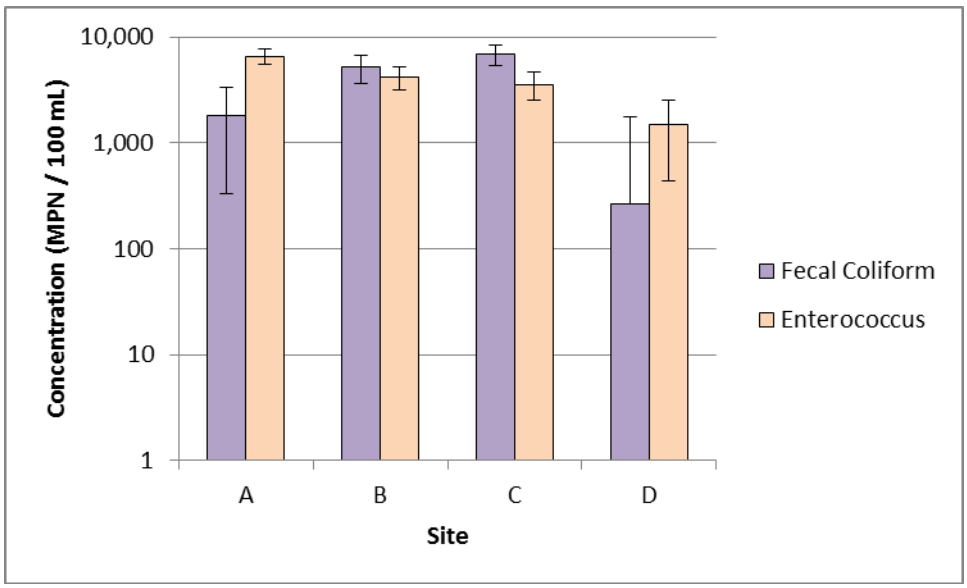


Figure 5-5. Geometric Mean Concentrations (± 1 Standard Error) of Fecal Coliform and Enterococci Concentrations by Site in the Bioswale Effectiveness Assessment – Survey 2

Flow

Flow was measured at the top (Site A) and bottom (Site D) of the bioswale for 7 days during Survey 2 (Figure 5-6). Flow at Site A varied between 0.1 and 0.3 cfs with a clear diurnal pattern of baseflow during mid-day and peaks in the evening and early morning when flow approximately doubled. Flow at Site D was greater than that at Site A, ranging between 0.3 and 0.5 cfs. In contrast to Site A, flow at Site D peaked just after 12 p.m. on each of the 7 days that were monitored. The daily average flow at the downstream site was 36,102 cubic feet per day (Table 5-10), which was over 70% greater than flow at the upstream site (20,913 cubic feet per day).

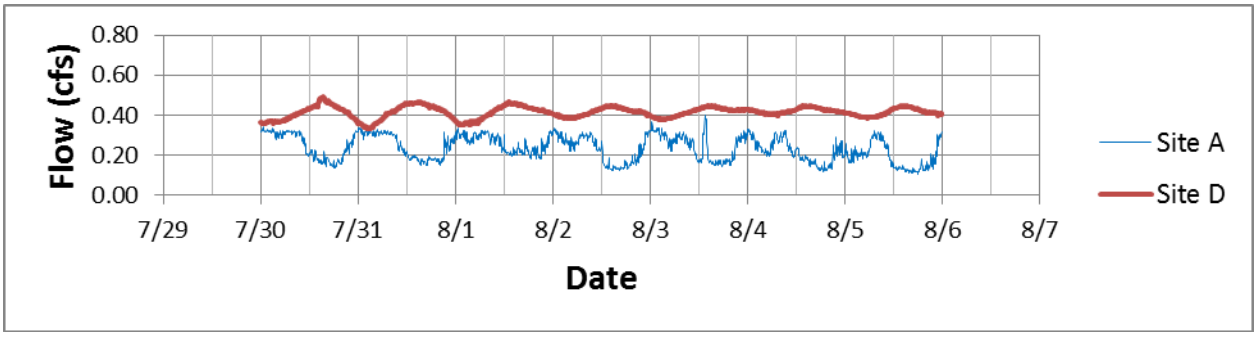


Figure 5-6. Example of Flow Results from Site A (upstream) and Site D (downstream) in the Bioswale BMP Effectiveness Assessment – Survey 2

Bacterial Loads

Bacterial loads were calculated for Site A and Site D during Survey 2 by multiplying site-specific flow by the geometric mean concentrations of fecal coliforms and enterococci. The data used to calculate the loads are presented in Table 5-10, and the loads are represented graphically in Figure 5-7.

Table 5-10. Fecal Coliform and Enterococci Loads in the Bioswale BMP Effectiveness Assessment – Survey 2

Parameter	Units	Site A (upstream)	Site D (downstream)
Daily Average Flow	Cubic feet per day	20,913	36,102
Fecal Coliform Geometric mean	MPN/100 mL	1,829	261
Enterococci Geometric Mean	MPN/100 mL	6,587	1,486
Fecal Coliform Daily Average Load	MPN/day (in millions)	10,831	2,668
Enterococci Daily Average Load	MPN/day (in millions)	39,007	15,191

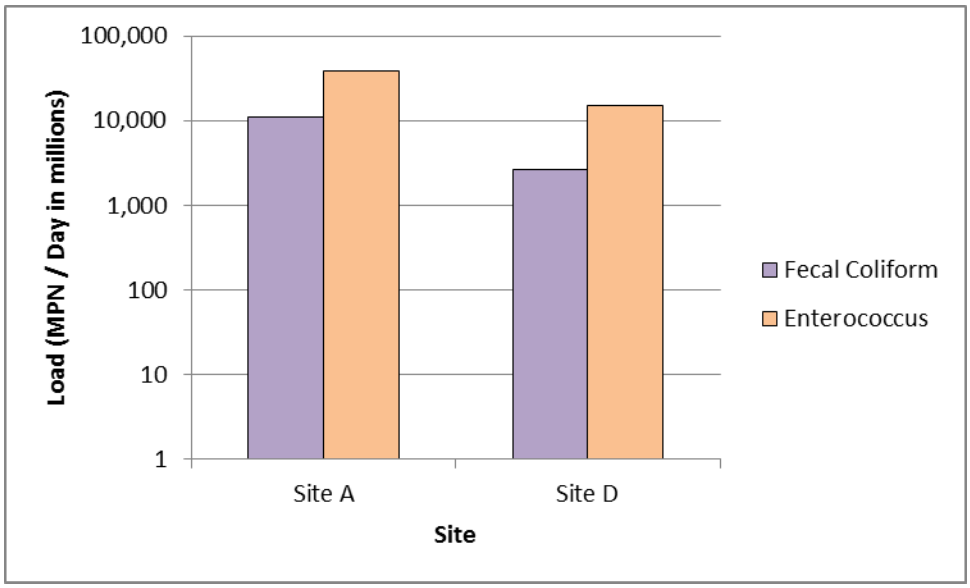


Figure 5-7. Fecal Coliform and Enterococci Loads at Site A (upstream) and Site D (downstream) in the Bioswale BMP Effectiveness Assessment – Survey 2

5.4 Summary, Bioswale Study

The Bioswale BMP Effectiveness Study was designed to determine whether the bioswale was effective in reducing bacterial concentrations in the watershed. The first survey was a small-scale assessment of the first of three sections of the bioswale in the upper part of the watershed (between Site A and Site B) where samples were taken three times over the course of a day (6 a.m., noon, and 6 p.m.). The results of Survey 1 suggest that bacterial concentrations at the entrance to the bioswale (Site A) were similar to those ½ mile downstream (Site B). Although fecal coliform and enterococcus concentrations were greater upstream than downstream during the first sampling event of the sanitary survey (6 a.m.), concentrations during subsequent rounds were similar at both sites. These results suggest that the bioswale has a limited effect, if any, on reducing bacterial concentrations in the watershed.

MST analyses of samples collected during the first bioswale survey were negative for the human-associated *Bacteroides* marker, which is consistent with the overall results of the other sampling events conducted over the course of this project as well as those obtained in the 2005-2006 bacterial source identification study (WESTON, 2006).

The results of the chemical analyses conducted as part of Survey 1 suggest that the bioswale BMP may have an effect in reducing concentrations of nickel and cadmium. Concentrations of these metals (both dissolved and total) at the downstream end of the bioswale were two to four times less than concentrations entering the bioswale. Although the study was designed to assess the effectiveness of the bioswale in reducing bacterial concentrations, the results suggest that it may have the potential for reducing levels of cadmium and nickel, both of which are on the SWRCB 303(d) List of impaired waterbodies for the Prima Deshecha Cañada Watershed. Concentrations of some nitrogenous compounds (ammonia and nitrite) may have also been reduced by the first section of the bioswale, but these compounds can be ephemeral in the environment and further studies would be needed to assess the extent to which true reductions occur as a result of the BMP.

The second bioswale BMP effectiveness survey was designed to provide a more focused assessment of flow and bacterial concentrations throughout the entire course of the bioswale. In Survey 2, bacterial samples were collected at four sites along the bioswale in the early morning to capture the peak in flow presumably caused by excess irrigation. The bacterial results of Survey 2 were somewhat mixed. Enterococci concentrations appeared to have decreased gradually from the top of the bioswale to the bottom, whereas fecal coliform concentrations appeared to have peaked at the two middle sites (Sites B and C) before falling at the bottom of the BMP. These results suggest that the bioswale may have a limited ability to reduce bacterial concentrations in the watershed.

Flow monitoring conducted at the top and bottom of the watershed indicates that flow increases at the bottom of the watershed. Flows at Site D were approximately two times greater than flows at Site A. These results suggest an input of surface water in the upper section of the bioswale (between Sites A and B), the lower section of the bioswale (between Sites C and D, or both. Water in the middle portion of the bioswale (between Sites B and C) is conveyed via a PVC pipe, and external contributions in this reach are presumed to be negligible. It is unclear whether

the increase in flow at the bottom of the watershed is due to increases in surface flow from irrigation practices or from surfacing groundwater, which has been identified in the lower portion of the watershed. One of the design features of the bioswale was that the soft-bottom upper and lower sections would provide some infiltration of surface flows from the Mainstem Channel, thereby reducing bacterial loads. However, the increase in flow at the bottom of the watershed suggests that substantial infiltration may not be occurring in the bioswale.

Bacterial load reduction is the primary objective of the bioswale. The results of Survey 2 indicate that both fecal coliform and enterococci loads decreased from the top of the bioswale to the bottom, suggesting that the bioswale may have had a limited positive effect in reducing bacterial levels in the watershed. However, WESTON believes that these results should be interpreted with some caution. The load values are the product of the mean bacterial concentration at a site and the daily average flow at that site. Although flow was approximately two times greater at the bottom of the bioswale than at the top, the mean bacterial concentrations were four to seven times lower at the bottom of the bioswale. Thus, the decrease in load estimates at the bottom of the bioswale is driven by the lower bacterial concentrations. Because of the inherent variability of indicator bacteria concentrations in urban drainages, additional studies would be needed to confirm the effectiveness of the bioswale before pursuing enhancements or changes to the design as a bacterial reduction BMP.

6.0 SCOUR POND AND BEACH ENVIRONMENT STUDY

6.1 Overview, Scour Pond and Beach Study

The scour pond located at the terminus of the Prima Deshecha Cañada Mainstem Channel is thought to attract daily congregations of birds to Poche Beach. The birds defecate on the beach sands and are considered a significant source of indicator bacteria at Poche Beach. Although the scour pond is predominantly fresh water, it is influenced by seawater through wave and tidal actions that can over-ride the berm during high spring tides. In general, the scour pond remains roughly the same size throughout the year unless the County of Orange reshapes it during periodic maintenance practices or the configuration is changed during major storm events.

The purpose of the scour pond and beach environment study described in this section was to answer the following questions:

1. **What is the impact of the scour pond on bacterial concentrations in the ocean receiving waters at Poche Beach?**
2. **What is the impact of the sand in conjunction with the scour pond and the bird population on bacterial concentrations in the ocean receiving waters at Poche Beach?**
3. **What is the impact of sand only on bacterial concentrations in the ocean receiving waters at Poche Beach?**

In order to answer these questions, the Scour Pond and Beach Environment Study was designed to measure the bacterial concentrations at the base of the watershed, within the scour pond environment, in the beach sands adjacent to the scour pond, and in the ocean receiving waters.

The Scour Pond and Beach Environment Study consisted of three separate surveys conducted on the following dates:

- Survey 1 – January 20, 2011
- Survey 2 – September 20, 2011
- Survey 3 – October 19, 2011

The studies were conducted in adaptive fashion, such that the results from each survey were used to design the subsequent surveys. A brief description of each survey is given below, followed by the methods used for each survey and the corresponding results.

Scour Pond Survey 1. The first scour pond survey was conducted on January 20, 2011. The study was designed to assess the influence of the scour pond and beach sand on bacterial concentrations in the ocean receiving waters. To address this question, samples were collected from several locations in the scour pond and from the surf zone in front of the scour pond as well as sand from several locations on the beach adjacent to the scour pond discharge.

Scour Pond Survey 2. The second scour pond survey was conducted on September 20, 2011. The purpose of this study was to verify the results of the first study and to contribute to the understanding of the effectiveness of the ultraviolet (UV) treatment system at the base of the watershed. Samples were collected at four different times throughout the day from several sites: the base of the watershed (upstream of the scout pond), the scour pond, the tidal creek leaving the scour pond, the ocean receiving waters, and effluent from the UV discharge.

Scour Pond Survey 3. The third scour pond study was conducted on October 19, 2011, to verify the results of the second survey and to determine the spatial extent of the scour pond discharge on bacterial concentrations along the beach. The design was the same as that used in Scour Pond Survey 2, but additional samples were collected from the surf zone upcoast and downcoast of the scour pond discharge.

6.2 Methods, Scour Pond/Beach Study

6.2.1 Field Methods

6.2.1.1 Site Locations

Scour Pond Survey 1

For this investigation, the following types of samples were collected (Figure 6-1): 1) sand samples collected from the beach adjacent to or in the scour pond, and 2) water samples collected from the scour pond and the ocean receiving waters. Sand samples (a-e) were collected directly from the beach along four transects (TR1-4) that ran perpendicular to the surf zone as shown in Figure 6-1 in red and as described as follows:

- **Transect 1** (TR1) was positioned 30 meters (m) north of the tidal creek outlet to the ocean.
- **Transect 2** (TR2) was positioned directly adjacent to the tidal creek.
- **Transect 3** (TR3) was positioned 30 m south of the tidal creek outlet.
- **Transect 4** (TR4) was positioned 75 m south of the tidal creek and was used as a control site, thought to be outside of the direct influence of the scour pond effluent.

Water samples were taken from the surface of the scour pond (SCOUR) at four locations as shown in Figure 6-1 in yellow. The sites were chosen to provide spatial characterization of the scour pond as it flows from the upstream input to the downstream tidal creek that discharges to the ocean. In addition, sediment samples were collected from the bottom of the scour pond. The ocean receiving water samples (WAT) were taken from the surf zone at the end of the sand monitoring transects as shown in Figure 6-1 in blue. All samples were collected between 3 p.m. and 5 p.m. on January 20, 2011.

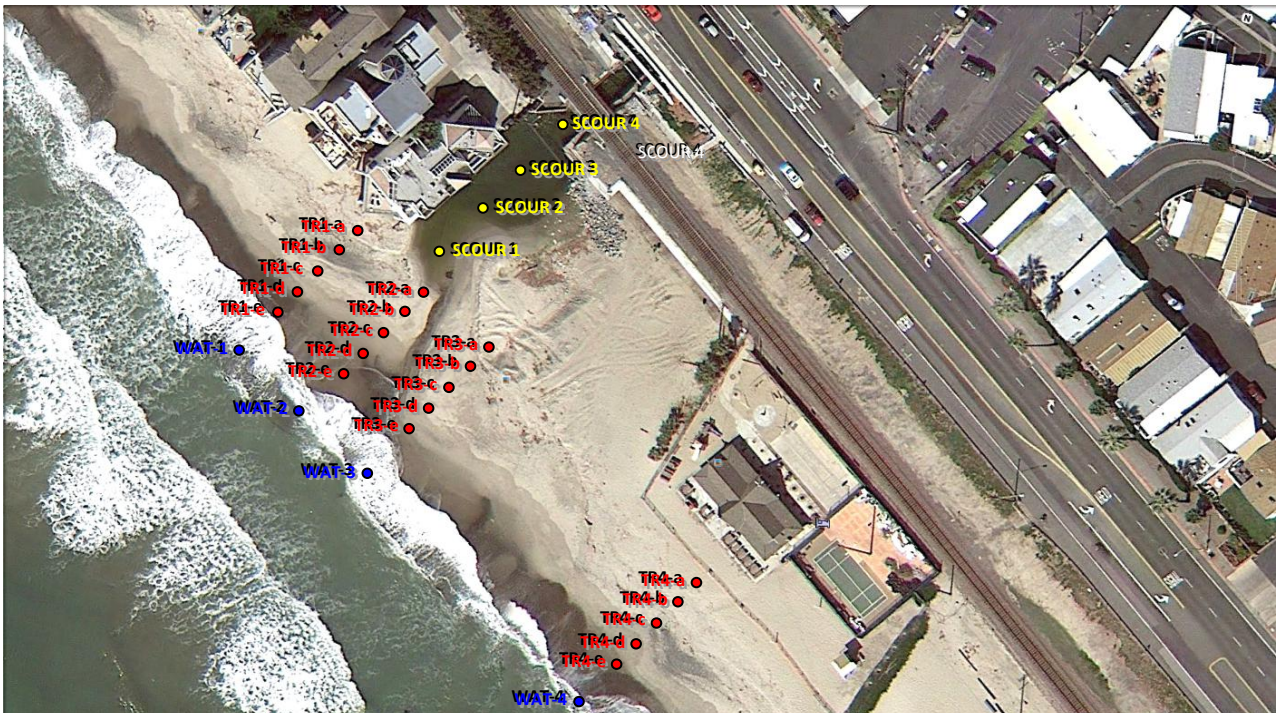


Figure 6-1. Sites Monitored for Surface Water and Sediment in the Scour Pond during Scour Pond Survey 1 – January 20, 2011

Scour Pond Survey 2

Historically, the UV treatment system at the base of the Prima Deshecha Cañada Watershed discharged UV-treated water to the upstream end of the scour pond across from the beach access stairway (Figure 6-2A). In early September 2011, the terminus of the discharge was moved to downstream end of the scour pond adjacent to the point where the scour pond discharges to the ocean (end of arrow in Figure 6-2B). Scour Pond Survey 2 was conducted on September 20, 2011, during a 3-month period when the UV treatment effluent was being discharged to the distal end of the scour pond. During the survey, the terminus of the discharge was approximately 4 inches below the surface of the scour pond.

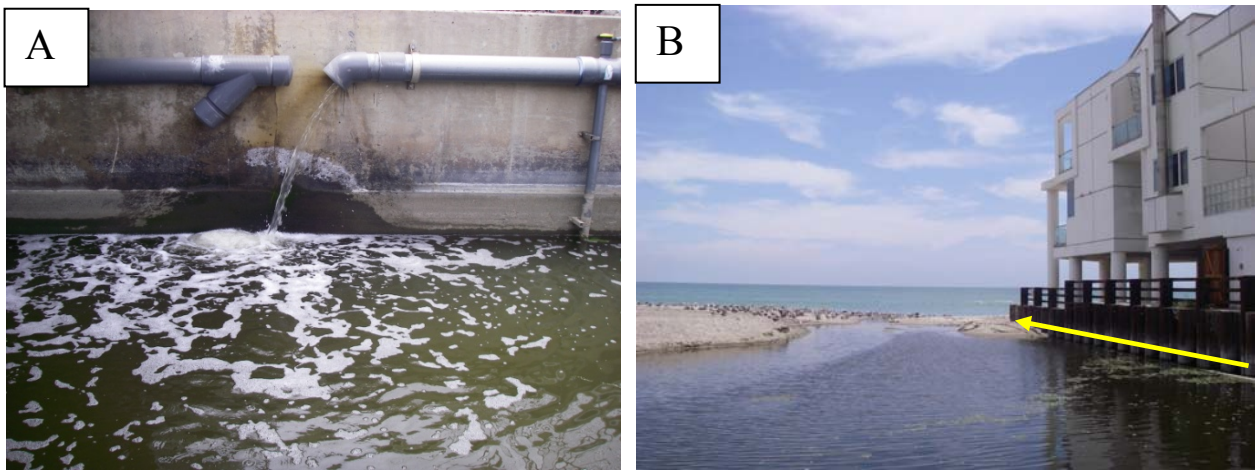


Figure 6-2. Location of UV Treatment System Discharge A) before Scour Pond Survey 2 and B) during Scour Pond Survey 2 (represented by end of yellow arrow)

Scour Pond Survey 2 was designed to assess whether the new discharge location affected water quality in the scour pond and to evaluate the extent to which the scour pond contributes to elevated bacterial concentrations in the ocean receiving waters at Poche Beach. The following five sites were monitored during the survey, as shown in Figure 6-3:

- **Site 7** was located at the base of the Mainstem Channel below the confluence with the Cascadita Channel and upstream of the scour pond. This is the same site that was monitored in the Sanitary Surveys (Section 2).
- **Site SP** represents the scour pond sample, which was a composite of three samples taken along the surface of the pond.
- **Site UV** was positioned directly in front of the UV treatment system's discharge as shown by the yellow arrow in Figure 6-2B. During the survey, the terminus of the 10-inch PVC discharge pipe was below the surface of the scour pond. Samples were collected directly below the discharge, where treated water was co-mingled with water from the scour pond.
- **Site TC** represents the tidal creek, which is the fresh water discharge from the scour pond that enters the ocean at Poche Beach. During the survey, this site was monitored at the distal end of the creek, just before the scour pond effluent mixed with the ocean receiving waters.
- **Site PO-1** was located in the surf zone directly in front of the scour pond discharge at the tidal creek. Samples at this site were collected in ankle to knee-deep water in the mixing zone.

Figure 6-3. Sites Monitored in Scour Pond Survey 2 – September 20, 2011

Scour Pond Survey 3

The monitoring design for Scour Pond Survey 3 was the same as that for Survey 2 with additional sites added along the beach to determine the spatial extent of any impact from the scour pond on the ocean receiving waters. During the survey, the five sites identified in Figure 6-3 were monitored in addition to four ocean receiving water sites shown on Figure 6-4 and described in Table 6-1.

Table 6-1. Location and Description of Ocean Monitoring Sites in Scour Pond Survey 3 – October 19, 2011

Site Name	Site Description	Longitude	Latitude
300-N	On the beach 300 m north of the tidal creek discharge	-117.647649	33.442048
150-N	On the beach 150 m north of the tidal creek discharge	-117.646595	33.441220
150-S	On the beach 150 m south of the tidal creek discharge	-117.644093	33.439837
300-S	On the beach 300 m south of the tidal creek discharge	-117.642856	33.439147

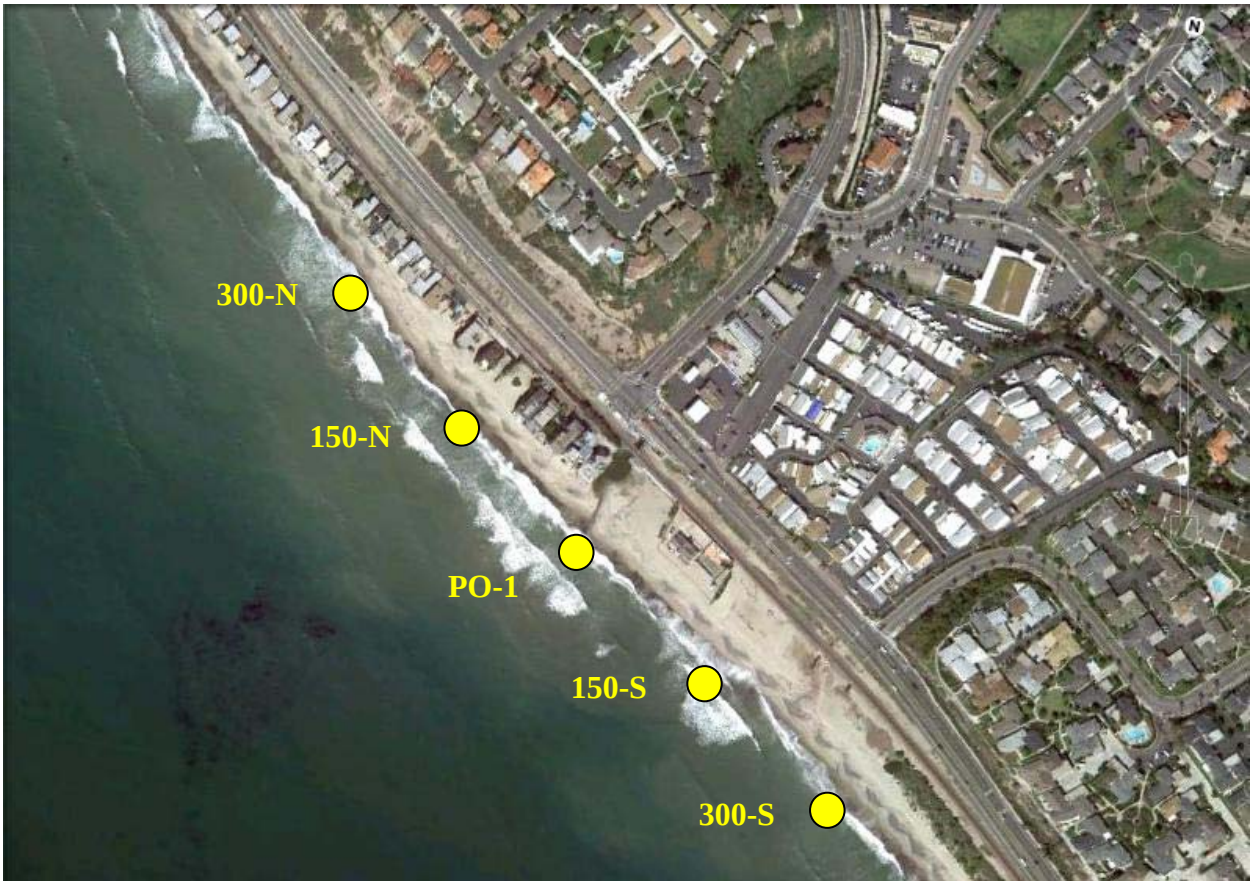


Figure 6-4. Beach Sites Monitored in Scour Pond Survey 3 – October 19, 2011

6.2.1.2 Sampling Frequency

During Scour Pond Survey 1, samples were collected by three two-person teams within a 2-hour period to minimize the temporal differences among sites. Samples were collected once from each of the sites identified in Figure 6-1 between 3 p.m. and 5 p.m. on January 20, 2011.

Samples for Scour Pond Surveys 2 and 3 were collected during four discrete sampling periods: 4 a.m., 7 a.m., 11 a.m., and 1 p.m. These times were chosen to bracket the range in flow observed at Site 7, as shown in Figure 6-5. In Scour Pond Survey 2, three two-person teams were used to collect the samples as close to the scheduled time as possible from all sites. During Scour Pond Survey 3, a fourth two-person team was added to collect the additional receiving water samples along the beach.

High spring tides at Poche Beach frequently breach the sand berm at the distal end of the scour pond, flooding it with seawater. However, during both Scour Pond Surveys 2 and 3, tidal height ranged from +3 to +5 feet above mean lower low water (MLLW) over the course of the monitoring period. The tide never breached the sand berm and did not influence water quality in the scour pond during the surveys.

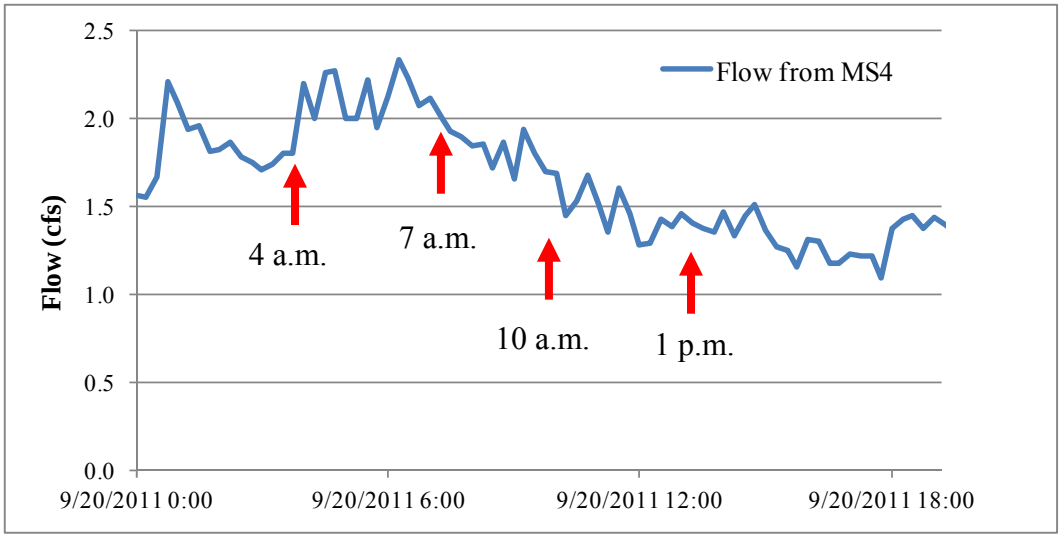


Figure 6-5. Flow at Site 7 on September 20, 2011 during Scour Pond Survey 2

6.2.1.3 Sample Collection

Scour Pond Water

Water samples were collected from the surface of the scour pond by a technician in an inflatable watercraft as shown in Figure 6-6A. Prior to sample collection, sites within the scour pond were identified and marked with a small buoy attached by a rope to a mushroom anchor secured in the sediment. This site marker assured that the same site was sampled during each round of sample collection. The latitude and longitude of the location was recorded on field data sheets, and each site was photographed. An aseptic technique, as described in Section 1.4 for the collection of samples for bacterial and MST analyses, was used to collect the samples.

Figure 6-6. Sampling Surface Water in the A) Scour Pond and B) Tidal Creek

Ocean Water

Samples for bacterial analyses were collected from ocean water by wading into the surf zone to a depth of approximately 2 feet. Samples were collected using the protocol described in Section 1.4.

Scour Pond Sediment

Sediment was collected from the scour pond during Scour Pond Survey 1 using a push core sampler. The push core consisted of a 20-foot long fiberglass rod with a large neoprene stopper secured to the end. An 18-inch length by 2-inch diameter sterile plastic tube was secured to the end of the stopper. The tube was pushed into the sediment with the fiberglass rod to a depth of approximately 1 to 2 inches. The apparatus was then pulled to the surface; and the sediment was placed into pre-labeled 250-mL sterile plastic jar, sealed in a Ziploc™ bag, and stored on ice in a cooler on the inflatable. The plastic tubing was then discarded, and the rubber neoprene stopper and end of the fiberglass rod were cleaned with a mild detergent, followed by an ethanol rinse. A new sterile plastic tube was used for each subsequent site.

Beach Sand

During Scour Pond Survey 1, field scientists wearing clean, disposable gloves collected sand samples in sterile, plastic containers. At each site, the sampler removed the surficial sand with a sterile plastic bottle. A new sterile bottle was then used to collect a sample from a depth of 2 to 3 inches below the surface of the sand. Sampling containers were kept in clear Ziploc™ bags until they were used and then placed into a new Ziploc™ bag after the sample had been collected. Samples were transported on ice to the WESTON microbiology laboratory.

6.2.1.4 Field Measurements

During all three surveys, field measurements were taken at all sites and during all sampling rounds. Field water quality measurements were recorded with a YSI 6920 water quality data sonde and recorded on field data sheets (Appendix A). These measurements included temperature, conductivity, dissolved oxygen, pH, salinity, and turbidity. In addition, water quality appearance (odor, color, floating materials, and turbidity); meteorological characteristics (wind, temperature, cloud cover); and physical conditions at the time of collection also were recorded on field data sheets.

6.2.2 Analytical Methods, Scour Pond/Beach Study

6.2.2.1 Total and Fecal Coliforms/Enterococci

Samples were analyzed for total and fecal coliforms and enterococci by WESTON's in-house microbiology laboratory using methods described in Section 1.4.2.1 and Table 6-2.

Table 6-2. Bacterial Parameters and Corresponding Analytical Methods in the Scour Pond/Beach Study

Analytical Parameter	Analytical Method	Units	Method Detection Limit	Laboratory Reporting Limit	Sample Volume	Container (#, size, type)	Preservation	Holding Time
Total Coliform	SM 9221 B	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Fecal Coliform	SM 9221 E	MPN/ 100 mL	2 MPN	<20 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours
Enterococci	Enterolert	MPN/ 100 mL	1 MPN	<10 MPN	100 mL	1, Sterile, 100 mL plastic	$Na_2S_2O_3$ >0 to 10°C	6 Hours

6.2.2.2 Microbial Source Tracking

Samples collected for PCR analyses by real-time PCR were analyzed in WESTON's in-house Molecular Biology Laboratory as described in Section 1.4.2.2 and Table 6-3. Samples were analyzed for General *Bacteroides*, human-associated and gull-associated MST markers. In addition, Scour Pond/Beach Survey 3 samples were analyzed for a canine MST assay (Table 6-3). Laboratory controls included the following: laboratory blanks, no-template controls, positive controls, and inhibition controls. See Section 1.4.3 for more information.

6.2.3 Quality Assurance/Quality Control

QA/QC procedures as outlined in Section 1.4.3 and detailed in the QAPP (WESTON, 2010) were followed for the Scour Pond Surveys.

6.2.4 Chain-of-Custody Procedures

COC procedures outlined in Section 1.4.4 were used for all samples throughout the collection, transport, and analytical processes.

Table 6-3. Microbial Source Tracking (MST) by Real-Time Polymerase Chain Reaction (PCR) Analyses in the Scour Pond Survey

Target	Assay	Sequence 5'-3' (Final Conc, µM)	References	Conditions
General *Bacteroides*	*Bacteroides* -General	Bac32F: AACGCTAGCTACAGGCTT (0.4) Bac708R: CAATCGGAGTTCTTCGTG (0.4) GenProbe: 6-FAM-CAATATTCCTCACT GCTGCCTCCCGTA-BHQ1 (0.2)	Bernhard and Field, 2000; Dick and Field, 2004	95°C, 2 min; 40 cycles: 95°C, 15s; 60°C, 30s
Human *Bacteroides*	HF183 with melt	HF183F: ATCATGAGTTCACATGTCCG (0.4) Bac708R: CAATCGGAGTTCTTCGTG (0.4)	Bernhard and Field, 2000; Layton et al., 2013	95°C, 15 min; 50 cycles: 94°C, 30s; 54°C, 30s, 72°C, 45s; Melt: 60°C to 95°C at 0.2°/s
Human *Bacteroides*	HF183 Taqman	HF183F: ATCATGAGTTCACATGTCCG (1.2) BthetR1: CGTAGGAGTTTGGACCGTGT (1.2) BthetP1: 6FAM-CTGAGAGGAAGGTCC CCCACATTGGA-TAMRA (0.09)	Haugland et al., 2010; Layton et al., 2013	95°C, 20s; 40 cycles: 95°C, 1s; 60°C, 20s
Gull[b] *Catellicoccus marimammalium*	Gull2 TaqMan	Gull2forward: TGCATCGACCTAAAGTTTTGAG (0.9) Gull2reverse: GTCAAAGAGCGAGCAGTTACTA (0.9) Gull2probe: 6FAM-CTGAGAGGGTGATCGGCC ACATTGGGACT-BHQ1 (0.3)	Sinigalliano et al., 2013	95°C, 15 min; 40 cycles: 95°C, 15s; 62°C, 1min
Canine *Bacteroides*	CanineBac	DF475F: CGCTTGTATGTACCGGTACG Bac708R: CAATCGGAGTTCTTCGTG CanineBact: 6FAM-ATTCGTGGTGTAGCG GTGAAATGCTTAG-BHQ1 (0.3)	Schriewer et al., 2013	95°C, 15 min; 40 cycles: 95°C, 15s; 60°C, 30s

[a] Master Mix and thermocycler conditions typically consisted of Quanta-Perfecta QPCR Fastmix w/UNG (#84077) used on a BioRad CFX 96 thermocycler except for paired *Bacteroides*-General/ HF183 with melt assays, which were run on a Cepheid Smart Cycler. The master mix for the *Bacteroides*-General assay was Qiagen Quantitect Sybr Green (Cepheid #1017340). Reaction volumes were 25 µL.
[b] Also detected pigeon feces for samples collected from S. CA (Sinigalliano et al., 2013).

6.3 Results, Scour Pond/Beach Study

6.3.1 Survey 1

The results of the first Scour Pond/Beach Study conducted on January 20, 2011 are presented in Table 6-4 and Table 6-5 for water samples and sand samples, respectively. Surface water samples were collected at four locations in the scour pond and analyzed for total coliforms, fecal coliforms, and enterococci. Total coliform concentrations ranged from 1,700 to 8,000 MPN/100 mL, with a geometric mean for all samples of 4,294 MPN/100 mL. Fecal coliform concentrations ranged from 500 to 1,700 MPN/100 mL, with a geometric mean of 902 MPN/100 mL. Enterococci concentrations ranged from 110 to 185 MPN/100 mL, with a geometric mean of 141 MPN/100 mL.

Table 6-4. Indicator Bacteria Concentrations (MPN/100 mL) in Ocean Water and Scour Pond Water Samples during Scour Pond/Beach Survey 1 – January 20, 2011

Location	Sample Number	Total Coliform	Fecal Coliform	Enterococci
Scour Pond	1	5,000	1,700	185
	2	5,000	500	145
	3	1,700	1,300	110
	4	8,000	600	134
Geometric Mean		4,294	902	141
Ocean	1	40	20	31
	2	80	80	30
	3	20	< 20	10
	4	220	70	266
Geometric Mean		61	39	40

Shaded text – exceeds water quality benchmarks.

The single sample concentrations for samples collected from the scour pond were greater than AB411 criteria (Table 6-4) for all three indicators. In contrast, ocean water samples collected in the ocean receiving waters were low (less than AB411 criteria), except the sample from Site 4, which exceeded AB411 criteria for enterococci (Table 6-4). Site 4 was the farthest site from the scour pond discharge among the sites assessed (Figure 6-1).

Indicator bacteria concentrations in samples collected from the beach sand and scour pond sediment are presented in Table 6-5. Bacterial concentrations in beach sands were at or below the detection limit for nearly all of the samples collected. Indicator bacteria concentrations also were low in the samples collected from the bottom of the scour pond (Table 6-5). All of the fecal coliform and enterococci concentrations were below or close to the detection limit. Total coliform concentrations were above the detection limit, but also low.

Table 6-5. Indicator Bacteria Concentrations (MPN/dry gram) in Beach Sand and Scour Pond Sediment Samples during Scour Pond/Beach Survey 1 – January 20, 2011

Transect	Station	Total Coliform	Fecal Coliform	Enterococci
Beach Sand				
Transect 1	**A**	2	<2	<1
	B	2	<2	<1
	C	2	<2	<1
	D	2	<2	<1
	E	2	<2	<1
Transect 2	**A**	2	<2	<1
	B	2	<2	1
	C	2	<2	<1
	D	2	<2	<1
	E	2	<2	<1
Transect 3	**A**	<2	<2	<1
	B	2	<2	3
	C	2	<2	1
	D	2	<2	<1
	E	2	<2	1
Transect 4	**A**	11	2	<1
	B	2	<2	3
	C	<2	<2	1
	D	<2	<2	<1
	E	<2	<2	1
Scour Pond Sediment				
Scour Pond	**1**	10	<2	1
	2	26	2	2
	3	9	<2	<1
	4	26	<2	5

6.3.2 Survey 2

6.3.2.1 Indicator Bacteria, Survey 2

The indicator bacteria concentrations obtained during Scour Pond/Beach Survey 2 are presented in Figure 6-7. The four graphs depict the concentrations of fecal coliforms and enterococci during the four monitoring periods (4 a.m., 7 a.m., 10 a.m., and 1 p.m.). During the 4 a.m. monitoring period, concentrations were lowest at Site 7 and the UV site. It should be noted that the treated water from the UV treatment system was discharging below the surface of the water in the scour pond. The sample was collected directly underneath the point of discharge, but the sample contained both treated water and water from the scour pond. Bacterial concentrations in samples collected from the scour pond, tidal creek, and ocean receiving waters were relatively similar in magnitude (generally between 1,500 and 2,000 MPN/100 mL), and all exceeded AB411 criteria.

Indicator bacteria concentrations during the 7 a.m. and 10 a.m. monitoring periods tended to be greatest in the tidal creek and scour pond. By 1 p.m., concentrations at these sites had decreased slightly, whereas concentrations in the ocean had increased.

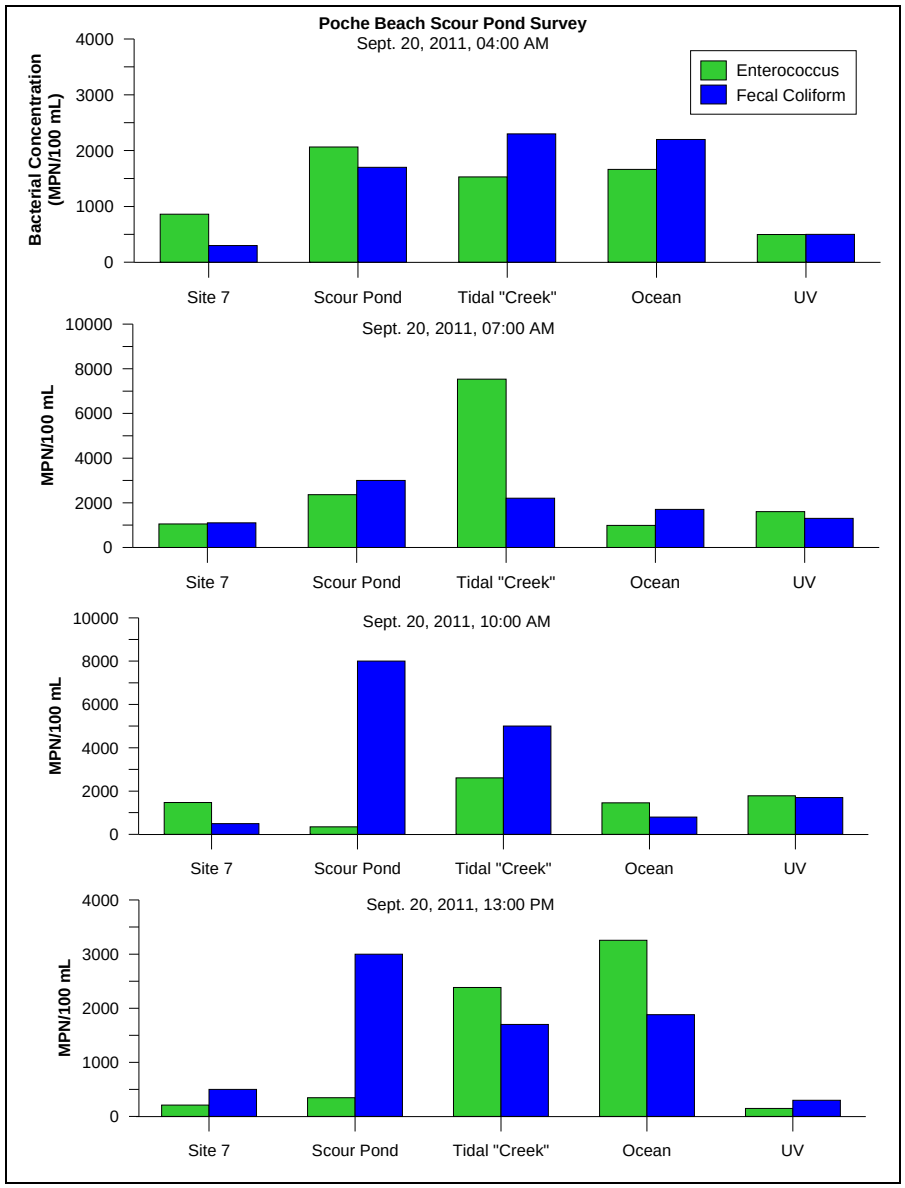


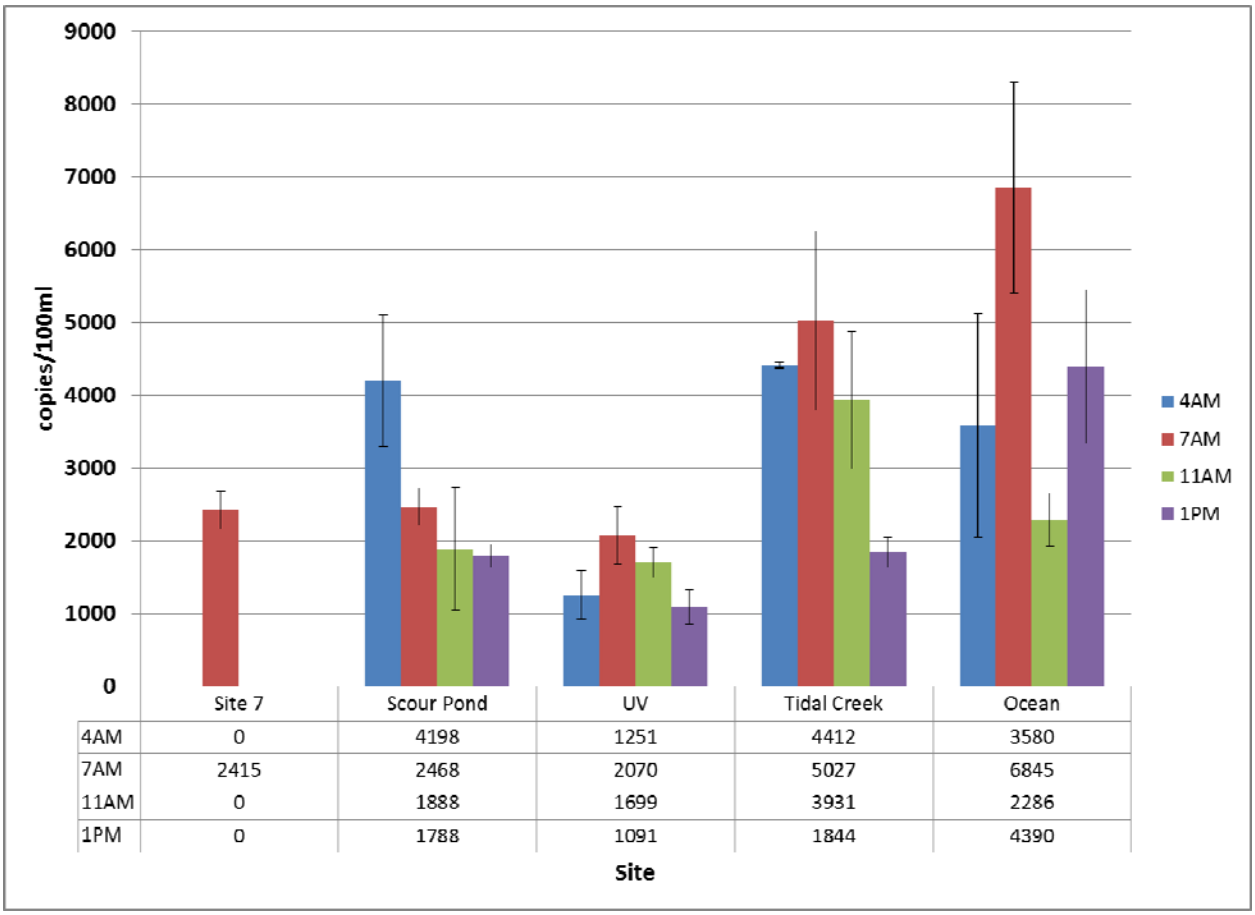
Figure 6-7. Enterococci and Fecal Coliform Concentrations in Water Samples at Sites Monitored in Scour Pond/Beach Survey 2 – September 20, 2011

6.3.2.2 Gull MST, Survey 2

Water samples collected in the Scour Pond/Beach Survey 2 were analyzed for a gull MST marker (Table 6-3). Quantifiable levels of the gull marker were present in all samples for all sampling periods except Site 7, located at the base of the watershed (Figure 6-3), where the marker was found only in the sample collected at 7 a.m. In comparison, the ocean site exhibited

the highest concentration during the 7 a.m. sampling (Figure 6-8). Concentrations were fairly consistent across sites with a tendency to be higher at the tidal creek and adjacent ocean site.

The concentration units are expressed in terms of the number of copies of the assay target per 100 mL of sample. MST data are useful in determining the presence and magnitude of bacteria originating from a particular host and are used to demonstrate the relative spatial and temporal differences among monitoring sites. Although correlations to culturable bacteria may be found, the gull MST assay targets a genetic signature. Furthermore, the gull MST assay targets a bacterial species different from those measured by culture, thus the metrics for the two types of assays are not equivalent.



	Site 7	Scour Pond	UV	Tidal Creek	Ocean
4AM	0	4198	1251	4412	3580
7AM	2415	2468	2070	5027	6845
11AM	0	1888	1699	3931	2286
1PM	0	1788	1091	1844	4390

Figure 6-8. Gull Marker Target Sequence Concentrations in Water Samples at Sites Monitored in Scour Pond/Beach Survey 2 – September 20, 2011.

Error bars represent ± range of PCR reactions from one sample.

6.3.2.3 Human MST, Survey 2

Water samples collected in the Scour Pond/Beach Survey 2 on September 20, 2011 were analyzed for General *Bacteroides* spp. and for a MST marker for human fecal contamination. All samples were positive by the *Bacteroides*-General assay, indicating that the samples were not

inhibited. In addition, samples passed all inhibition controls (see Section 1.4.3). Putative positives with the HF183 melt assay were tested by the HF183 Taqman assay (Table 6-3).

Among the 16 samples collected over the course of this survey (not counting QA/QC samples), one was potentially positive for the human marker. The results are presented in Table 6-6. The positive sample was collected from Site 7 at 1 p.m. (sample PB-SP-7-4). Site 7 was located at the base of the watershed, upstream of the scour pond and prior to the UV treatment system (Figure 6-3). In addition to verification by the HF183 Taqman assay (presence/ absence), this sample was rerun in triplicate by the HF183 Taqman assay with a full calibration curve; 2/3 reactions were amplified, but the results were deemed equivocal (EQV) because although the Ct value was > 0, it fell below the limit of detection (see Section 1.4.2.2). The limit of detection (LOD) was 3.8 copies/reaction (271 copies/100 mL sample), and the lower limit of quantification (LLOQ) was 9.9 copies/reaction (707 copies/ 100 mL sample).

Table 6-6. Real-Time Polymerase Chain Reaction (PCR) Results for the Human-Associated *Bacteroides* Assays (Presence/Absence) in Scour Pond/Beach Survey 2– September 20, 2011

Site	Sample ID	Time Collected	Result
7 (base of watershed)	PB-SP-7-1	4 a.m.	Neg
	PB-SP-7-2	7 a.m.	Neg
	PB-SP-7-3	10 a.m.	Neg
	PB-SP-7-4	1 p.m.	Equivocal[a]
SP (Scour Pond)	PB-SP-SP-1	4 a.m.	Neg
	PB-SP-SP-2	7 a.m.	Neg
	PB-SP-SP-3	10 a.m.	Neg
	PB-SP-SP-4	1 p.m.	Neg
TC (Tidal Creek)	PB-SP-TC-1	4 a.m.	Neg
	PB-SP-TC-2	7 a.m.	Neg
	PB-SP-TC-3	10 a.m.	Neg
	PB-SP-TC-4	1 p.m.	Neg
PO (Pacific Ocean)	PB-SP-PO-1	4 a.m.	Neg
	PB-SP-PO-2	7 a.m.	Neg
	PB-SP-PO-3	10 a.m.	Neg
	PB-SP-PO-4	1 p.m.	Neg

Neg = Negative
Pos – Positive
[a] Quantified by HF183 Taqman: Value was below the limit of detection, see text.

6.3.3 Survey 3

6.3.3.1 Indicator Bacteria, Survey 3

The third scour pond survey was conducted in October 2011, one month after Scour Pond/Beach Survey 2. The UV treatment system was still discharging to the distal end of the scour pond during the time of the survey. Samples were collected in Scour Pond Survey 3 at the same locations and frequency as those collected in the second survey, but additional surf zone sites were added north and south of the scour pond discharge. All samples were analyzed for indicator

bacteria (fecal coliforms and enterococci), as well as human, gull, and canine MST markers (Table 6-2 and Table 6-3). The results of the fecal coliform and enterococci analyses are presented in Figure 6-9 for the 4 a.m., 7 a.m., 10 a.m., and 1 p.m. monitoring periods.

In general, the results of Scour Pond/Beach Survey 3 were similar to those observed during the previous survey. Bacterial concentrations were typically low at Site 7 (located at the base of the water shed, Figure 6-3). In this survey (Figure 6-9), concentrations in the scour pond did not appear to increase compared to those at Site 7, as was observed during the previous survey (Figure 6-7). However, concentrations were generally higher in the tidal creek compared to those at Site 7 and the scour pond.

The most obvious pattern in this data set is the high concentrations of fecal indicator bacteria in the ocean receiving waters (Figure 6-9). Enterococci concentrations were greatest in the ocean samples during all monitoring periods, typically by an order of magnitude or more. Fecal coliform concentrations followed a similar pattern except during the 10 a.m. monitoring period, when concentrations were similar across most sites, except for Site 7, which tended to have lower concentrations.

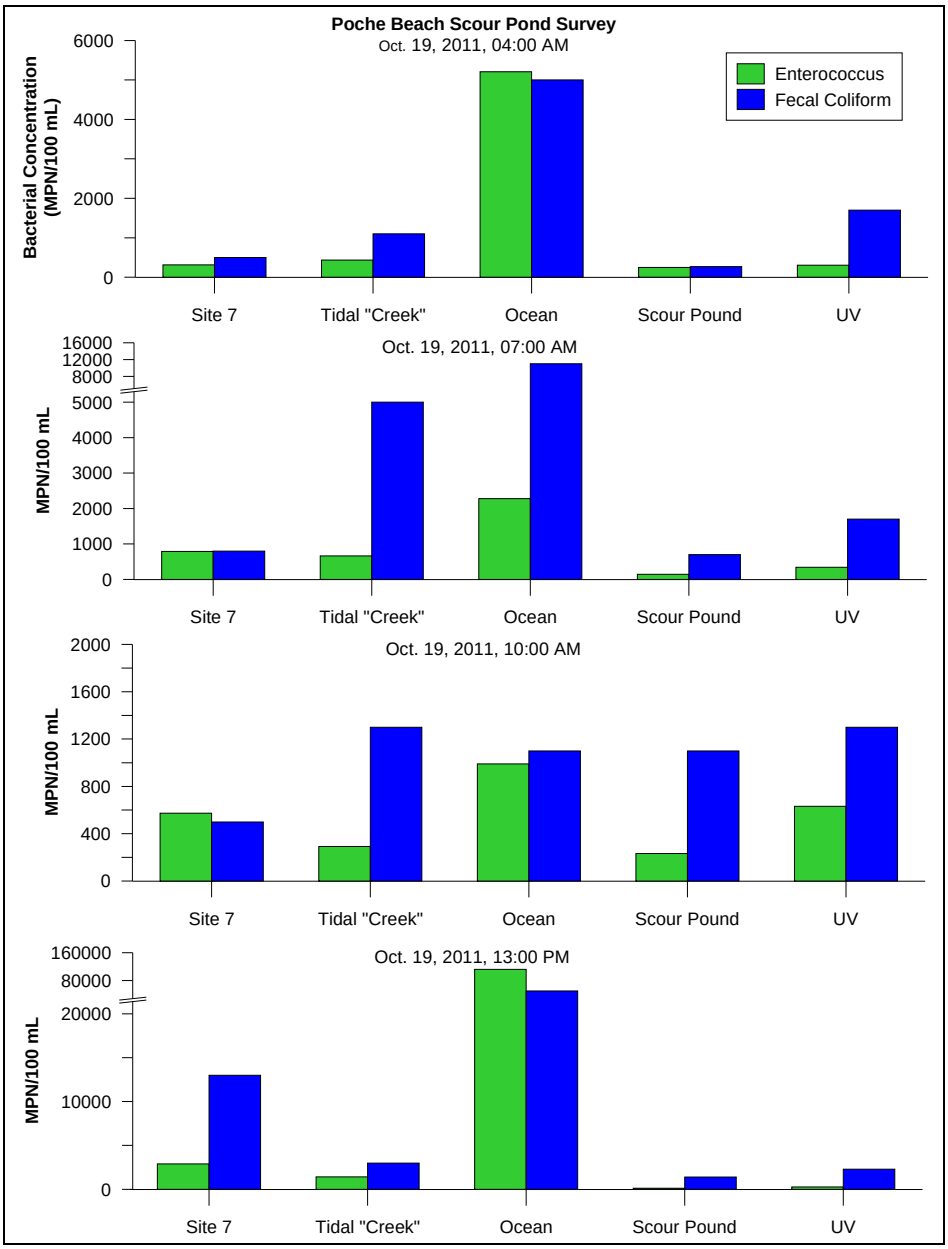


Figure 6-9. Enterococci and Fecal Coliform Concentrations in Water Samples at Sites Monitored in Scour Pond/Beach Survey 3 – October 19, 2011

Fecal coliform and enterococci concentrations at the beach sites monitored in Survey 3 are presented in Figure 6-10 and Figure 6-11, respectively. In these graphs, the ocean receiving water monitoring site directly in front of the tidal creek that drains the scour pond is designated as PO-1. Sites north and south of the discharge are designated with an N or S and the distance (in meters) from the discharge point. The results shown in Figure 6-10 suggest that fecal coliform concentrations were elevated throughout the day and all along the coastal sites surveyed on that day. The majority of the samples had fecal coliform concentrations greater than the AB411 criteria of 400 MPN/100 mL. In addition, for each monitoring period, fecal coliform

concentrations were greatest at Site PO-1, directly in front of the tidal creek that drains the scour pond (except for the 11 a.m. monitoring period when concentrations also were high at Site 150-S). In general, concentrations tended to decrease with distance from the scour pond discharge.

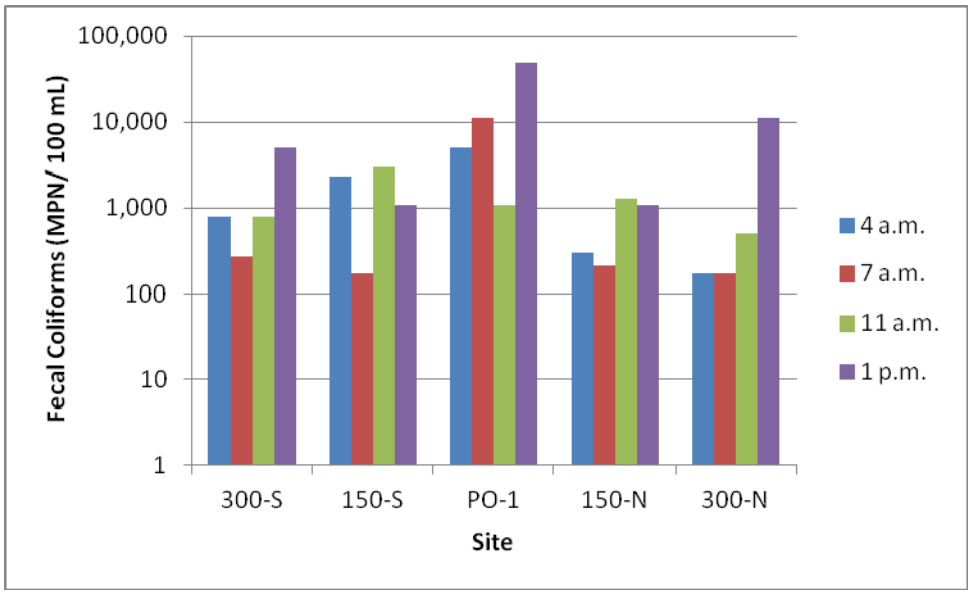


Figure 6-10. Fecal Coliform Concentrations at Beach Sites Monitored in Scour Pond/Beach Survey 3 – October 19, 2011

Enterococci concentrations showed a similar pattern to that observed for fecal coliforms. Enterococci concentrations at all sites monitored during all four surveys were above the single sample AB411 water quality objective for enterococci of 104 MPN/100 mL, many by an order of magnitude (Figure 6-11). Similar to the pattern observed for coliforms, enterococci concentrations were greatest at Site PO-1 in front of the scour pond tidal creek discharge during all four monitoring events (except the 10 a.m. period when concentrations also were high at Site 150-S). Concentrations of both fecal coliforms and enterococci were greatest during the 1 p.m. monitoring period.

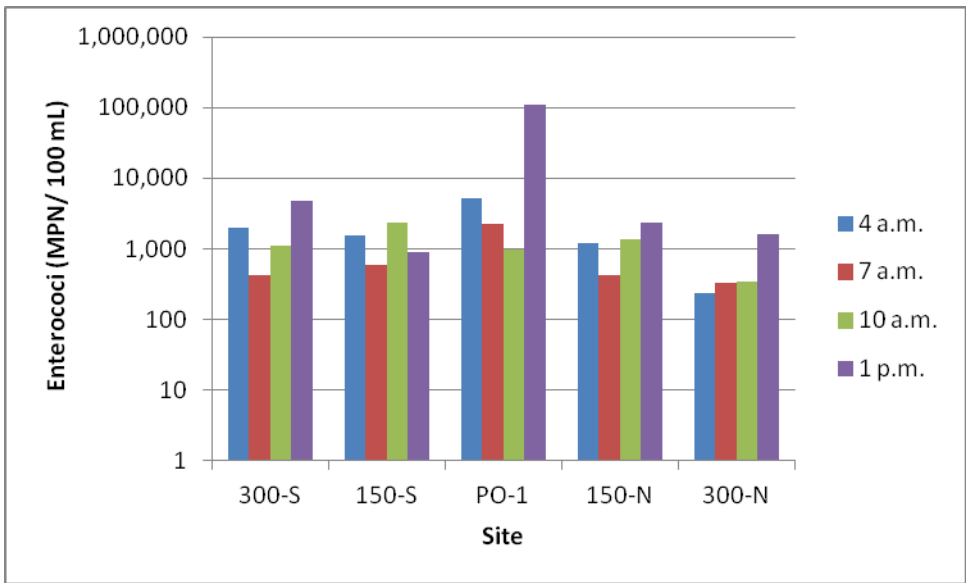
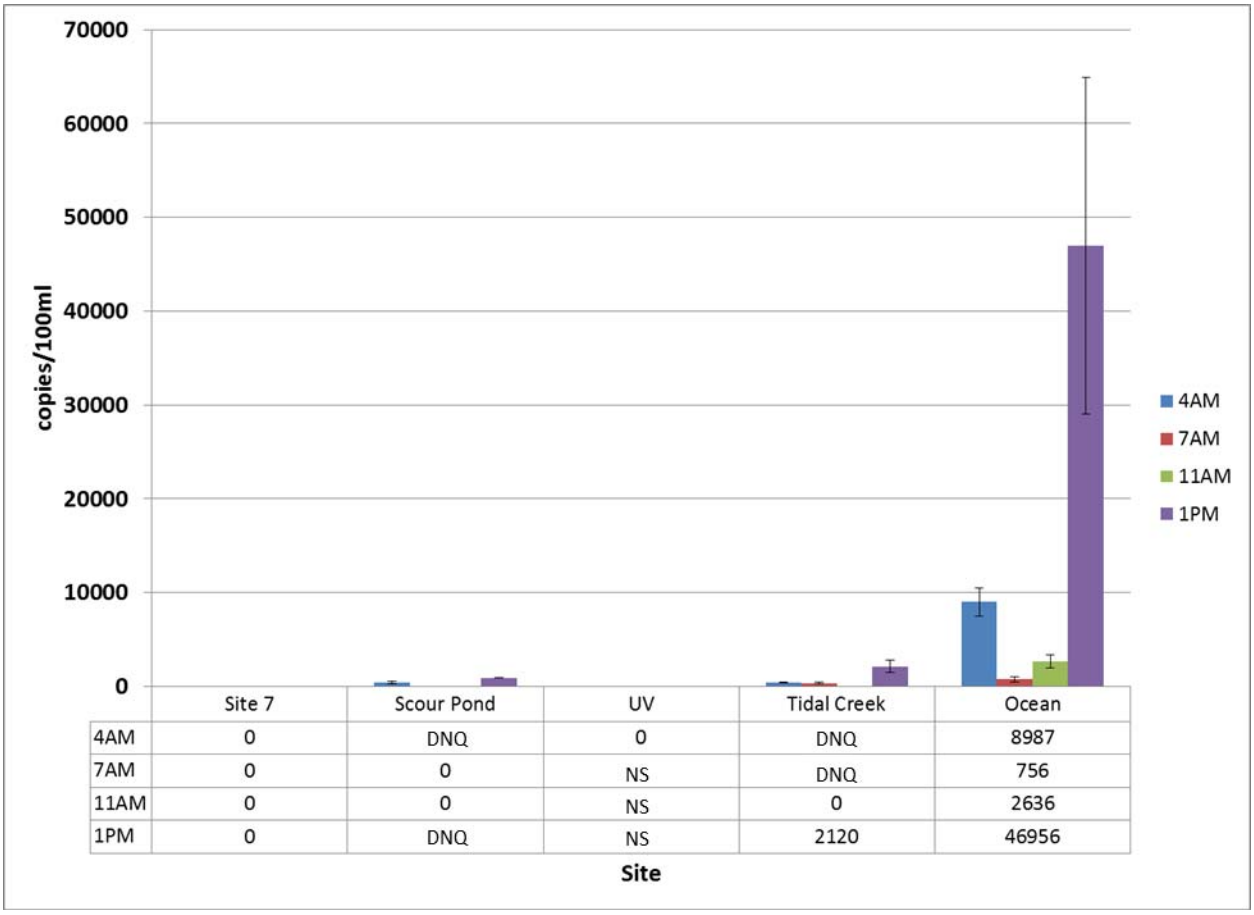


Figure 6-11. Enterococci Concentrations at Beach Sites Monitored in Scour Pond/Beach Survey 3 – October 19, 2011

6.3.3.2 Gull MST, Survey 3

Water samples collected during Scour Pond/Beach Survey 3 were analyzed for a gull MST marker (Table 6-3). Gull MST results for sites in a transect from the base of the watershed (Site 7) to the ocean (PO-1) are presented in Figure 6-12. The gull MST results for the beach sites in a transect north and south of the scour pond are presented in Figure 6-13. For the transect perpendicular to the coast, concentrations of the gull marker were highest at ocean site PO-1. Concentrations in the ocean were greater than those in the scour pond and tidal creek and peaked during the 1 p.m. monitoring round in a similar pattern to that observed for fecal coliform and enterococci concentrations on the same day. Unlike the results observed during Survey 2, the gull marker concentrations in the tidal creek and scour pond samples were not notable.

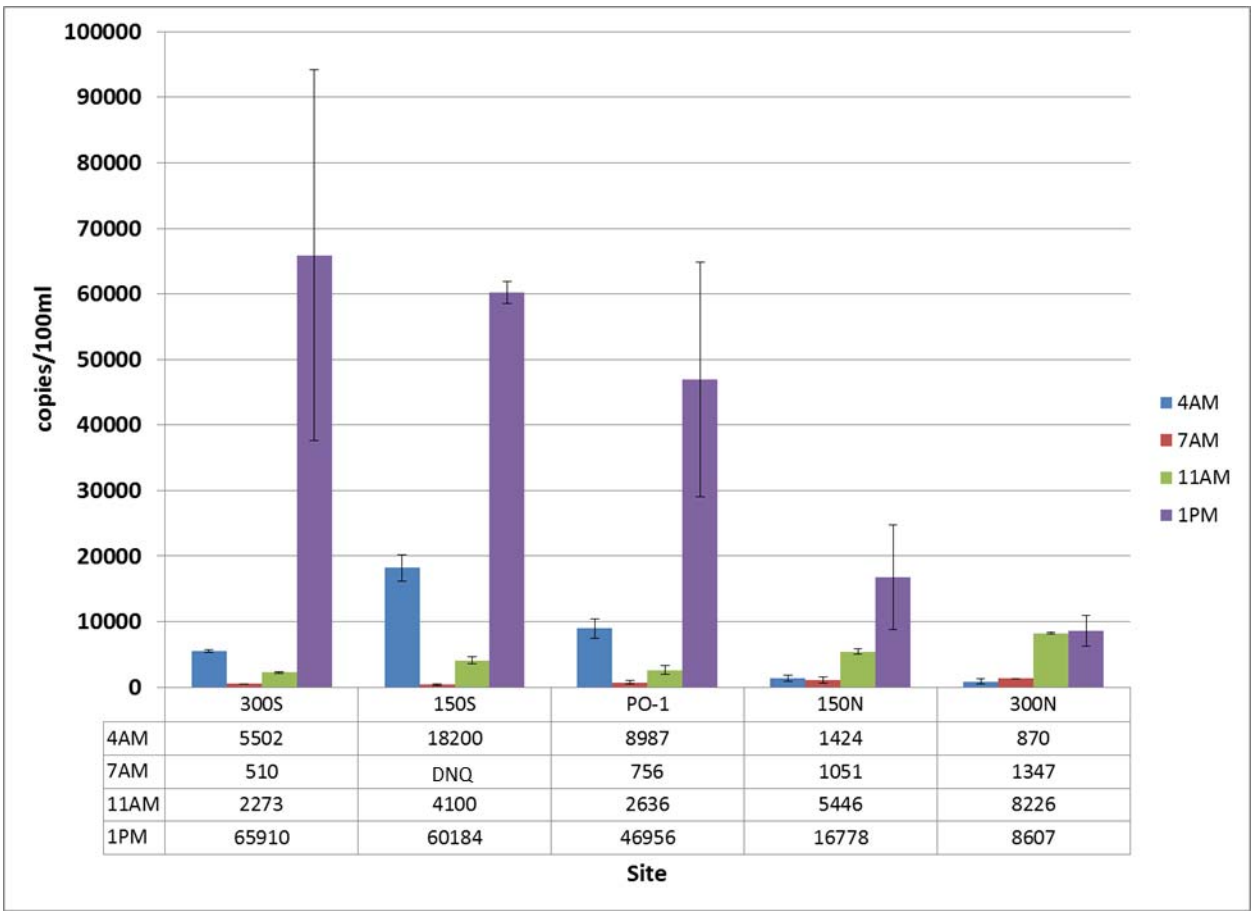
Quantifiable concentrations of the gull marker were observed at all of the beach sites during every monitoring period except for the 7 a.m. sample at Site 150-S. The concentration for that sample was detectable but not quantifiable (DNQ) because the concentration was below the lower limit of quantification (Figure 6-13). Similar to the fecal coliform and enterococci concentrations, the gull marker concentrations were greatest during the 1 p.m. monitoring round at all five ocean receiving water sites. The 1 p.m. gull marker concentrations tended to show a linear trend from north to south.



	Site 7	Scour Pond	UV	Tidal Creek	Ocean
4AM	0	DNQ	0	DNQ	8987
7AM	0	0	NS	DNQ	756
11AM	0	0	NS	0	2636
1PM	0	DNQ	NS	2120	46956

Figure 6-12. Gull Marker Concentrations in Water Samples at Sites Monitored in Scour Pond/Beach Survey 3 – October 19, 2011

DNQ = Detectable but Not Quantifiable, NS = Not Sampled., Error bars represent range of 2 PCR reactions from 1 sample.



	300S	150S	PO-1	150N	300N
4AM	5502	18200	8987	1424	870
7AM	510	DNQ	756	1051	1347
11AM	2273	4100	2636	5446	8226
1PM	65910	60184	46956	16778	8607

Figure 6-13. Gull Marker Concentrations at Beach Sites Monitored in Scour Pond/Beach Survey 3 – October 19, 2011

DNQ = Detectable but Not Quantifiable, Error bars represent range of 2 PCR reactions from 1 sample.

6.3.3.3 Canine MST, Survey 3

In addition to assessing the presence of bacteria associated with gull fecal matter, the samples also were assessed for host origin using a canine-associated *Bacteroides* marker to detect fecal contamination associated with canines. The presence and concentration of this genetic marker was assessed in all samples collected in Scour Pond Survey 3, and the results are presented in Figure 6-14. A total of 12 samples were positive for the canine marker: four at Site 7, three each in the scour pond and tidal creek, and one each in the ocean and at the UV treatment discharge. Of these, only the following two samples had the marker in high enough concentrations to be quantifiable: Site 7 and the ocean site (PO-1) during the 1 p.m. monitoring period. The other sites shown in Figure 6-14 with a cross symbol showed the presence of bacteria originating from canines, but at concentrations that were below the lowest standard used on the calibration curve.

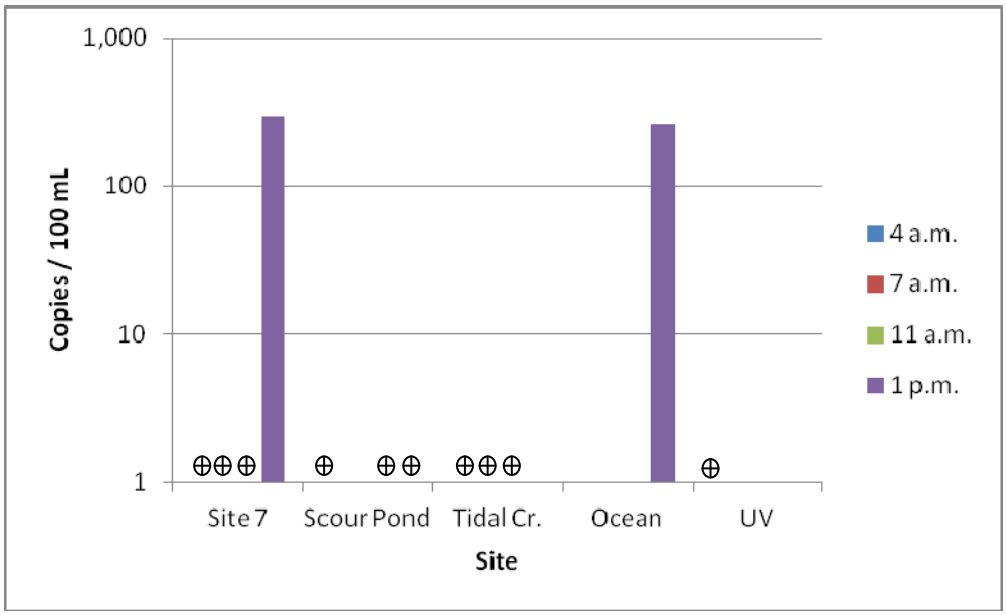


Figure 6-14. Canine Marker Concentrations in Water Samples at Sites Monitored in Scour Pond/Beach Survey 3 – October 19, 2011 (samples with concentrations detected but not quantified are represented with a cross symbol)

6.3.3.4 Human MST, Survey 3

Water samples collected in the Scour Pond/Beach Survey 3 on October 19, 2011 were analyzed for General *Bacteroides* spp. and for an MST marker for human fecal contamination. All samples were positive by the *Bacteroides*-General assay, indicating that the samples were not inhibited. In addition, samples passed all inhibition controls (see Section 1.4.3). Putative positives with the HF183 melt assay were tested by the HF183 Taqman assay (Table 6-3).

Among the 32 samples collected over the course this survey (not counting QA/QC samples), one was verified positive for the human marker and one was a potential positive via the HF183 Taqman assay. Both samples were collected from Site 7, located at the base of the watershed, upstream from the scour pond and prior to the UV treatment facility (Figure 6-3). The positive sample was collected from Site 7 at 1 p.m. (PB-SP-7-4); it was positive for 8/8 samples by endpoint analysis. The Site 7 sample collected at 7 a.m. (PB-SP-7-2) was deemed equivocal (EQV) because 3/8 samples were positive by the HF183 Taqman endpoint analysis. Three putative positive ocean samples (PO-1 at 7 a.m., 11 a.m., 1 p.m.) by the HF183 with melt assay were found negative when tested by the HF183 Taqman assay. There was no evidence of the human marker in the other samples. The results are presented in Table 6-7.

In addition to endpoint analysis, the 1 p.m. and 7 a.m. samples at Site 7 were tested by the HF183 Taqman assay with a full calibration curve. The LOD was 0.5 copies/reaction (102 copies/100 mL sample), with the LLOQ varying depending on the run (range: 1.49-1.95 copies/ reaction; 297-391 copies/100 mL sample). The concentration of the target in the Site 7 sample from 1 p.m. (PB-SP-7-4) was within the range of quantification (ROQ) for five out of eight reactions and DNQ for three out of eight. Out of three separate qPCR runs, the highest value was

811 ± 380 genome copies/100 mL (n=3 reactions). By qPCR analysis, the Site 7 sample from 7 a.m. (PB-SP-7-2) had five out of eight reactions that did not amplify, and out of the three positive reactions, two were below the limit of detection and one was below the limit of quantification, consistent with the "equivocal" definition determined by endpoint analysis.

Table 6-7. Real-Time Polymerase Chain Reaction (PCR) Results for the Human-Associated *Bacteroides* Assays (Presence/Absence) in Scour Pond/Beach Survey 3

Site	Sample ID	Time Collected	Result	Comments[a]
7 (base of watershed)	PB-SP-7-1	4 a.m.	Neg	
	PB-SP-7-2	7 a.m.	Equivocal[b]	Endpoint: 3/8 positive reactions
	PB-SP-7-3	10 a.m.	Neg	
	PB-SP-7-4	1 p.m.	**Pos**[b]	Endpoint: 8/8 positive reactions
SP (Scour Pond)	PB-SP-SP-1	4 a.m.	Neg	
	PB-SP-SP-2	7 a.m.	Neg	
	PB-SP-SP-3	10 a.m.	Neg	
	PB-SP-SP-4	1 p.m.	Neg	
TC (Tidal Creek)	PB-SP-TC-1	4 a.m.	Neg	
	PB-SP-TC-2	7 a.m.	Neg	
	PB-SP-TC-3	10 a.m.	Neg	
	PB-SP-TC-4	1 p.m.	Neg	
PO -1 (Pacific Ocean in front of scour pond)	PB-SP-PO-1(0)	4 a.m.	Neg	
	PB-SP-PO-2(0)	7 a.m.	Neg[c]	
	PB-SP-PO-3(0)	10 a.m.	Neg[c]	
	PB-SP-PO-4(0)	1 p.m.	Neg[c]	
PO (150N) (Pacific Ocean 150 m north of the scour pond discharge)	PB-SP-PO-1(150N)	4 a.m.	Neg	
	PB-SP-PO-2(150N)	7 a.m.	Neg	
	PB-SP-PO-3(150N)	10 a.m.	Neg	
	PB-SP-PO-4(150N)	1 p.m.	Neg	
PO (300N) (Pacific Ocean 300 m north of the scour pond discharge)	PB-SP-PO-1(300N)	4 a.m.	Neg	
	PB-SP-PO-2(300N)	7 a.m.	Neg	
	PB-SP-PO-3(300N)	10 a.m.	Neg	
	PB-SP-PO-4(300N)	1 p.m.	Neg	
PO (150S) (Pacific Ocean 150 m south of the scour pond discharge)	PB-SP-PO-1(150S)	4 a.m.	Neg	
	PB-SP-PO-2(150S)	7 a.m.	Neg	
	PB-SP-PO-3(150S)	10 a.m.	Neg	
	PB-SP-PO-4(150S)	1 p.m.	Neg	
PO (300S) (Pacific Ocean 300 m south of the scour pond discharge)	PB-SP-PO-1(300S)	4 a.m.	Neg	
	PB-SP-PO-2(300S)	7 a.m.	Neg	
	PB-SP-PO-3(300S)	10 a.m.	Neg	
	PB-SP-PO-4(300S)	1 p.m.	Neg	

Neg = Negative
Pos = Positive
[a] Samples were run in duplicate except for putative positive samples, which received additional analysis.
[b] Quantified by HF183 Taqman, see text.
[c] Suspected positives by HF183 with melt assay checked by HF183 Taqman assay and were negative.

6.4 Summary, Scour Pond/Beach Study

Three scour pond and beach assessments were conducted in 2011: January 20, September 20, and October 19. The overall objective of the study was to determine the potential impacts from the scour pond and beach sands on bacterial concentrations in the receiving waters at Poche Beach.

6.4.1 Summary Scour Pond/Beach Survey 1

In the first survey, both water and sand samples were collected from the scour pond and adjacent beach and ocean receiving waters. The scour pond surface waters contained elevated bacterial concentrations relative to those measured at the ocean site during this survey and relative to Site 7 measured during other elements of the study, suggesting that the scour pond provides an environment conducive to regrowth of indicator bacteria and/or has sources other than the watershed.

However, the sediments at the bottom of the scour pond had very low indicator bacteria concentrations (mostly nondetects), suggesting that the sand and sediment were not serving as a significant reservoir for indicator bacteria during this sampling event. The apparent difference between the surface waters and the sediment may be due to a strong halocline that has been measured in the scour pond. Because the pond is influenced by both saline waters from spring tides and fresh water from the watershed, a strong salinity gradient may be established wherein high saline water is trapped at the bottom of the pond and fresh water from the watershed moves over the top of the pond and out to the tidal creek. Fecal coliform and enterococci bacteria tend to prefer brackish environments and do not survive as well under higher saline conditions (MEC-WESTON, 2005). Salinity profiles taken during Scour Pond Surveys 2 and 3 suggest that a halocline was established at the time of the surveys, which may have limited the capacity of the scour pond sediments to act as a bacterial reservoir.

Beach sands also contained very low concentrations of indicator bacteria among the four transects established perpendicular to the surf zone. Concentrations of fecal coliforms, total coliforms, and enterococci were all at or close to non-detect values among the 20 samples collected along the transects. Samples were collected from a depth of 2 to 3 inches for this assessment and did not include surficial sediments. The low concentrations in beach sands just below the surface suggest that high bacterial concentrations in the ocean receiving waters at sites away from the scour pond were likely due to surficial deposits of fecal material (such as bird droppings) rather than from deeper sources, such as groundwater exfiltration on the beach face. Alternatively, the low concentrations observed in beach sand may have been due to the lack of birds on the day of the survey. Field data sheets indicate that relatively few birds (particularly gulls) were present on the beach on January 20. This is in marked contrast to Scour Pond Surveys conducted in September and October when gulls were extremely abundant on the beach and numerous bird droppings were identified on the surface of the sand. In addition, Survey 1 was conducted in January when air and ocean temperatures are typically at their lowest, which may have helped limit bacterial concentrations in beach sands.

6.4.2 Summary Scour Pond/Beach Survey 2

The second survey conducted on September 20, 2011 focused on assessing the extent to which the scour pond may act to increase bacterial concentrations received from the watershed via bacterial regrowth in the scour pond. The study also was designed to assess the potential impact from gull fecal matter on beach water quality. Monitoring sites were established at the base of the watershed (Site 7), in the scour pond, in the tidal creek that discharges from the scour pond, and from the ocean receiving waters in front of the scour pond. A fifth site was located at the terminus of UV treatment plant that discharged to the scour pond. These sites were sampled four times throughout the day to bracket diurnal flow patterns resulting from irrigation in the watershed. Overall, bacterial concentrations were lowest at Site 7, which is consistent with other surveys conducted in the study. Concentrations of indicator bacteria at other sites appeared to be somewhat variable with the greatest concentrations in the scour pond, tidal creek, and ocean receiving waters.

An MST marker for bacteria associated with gull feces was found in all samples collected in the ocean receiving waters during all sampling periods, except Site 7 (located at the base of the watershed) where the marker was found only in the 7 a.m. sample. Low indicator bacteria concentrations at Site 7 and infrequent positive results for the gull marker there suggest that gull fecal matter may be an unlikely source of bacteria within the watershed. In contrast, the high frequency of the gull marker in the ocean samples suggests that gull fecal matter on the beach face (between the scour pond and the ocean) is a likely source of indicator bacteria in the Poche Beach receiving waters. The gulls may be attracted to the fresh water flowing from the scour pond and were observed to be abundant on the beach during the day of the survey.

6.4.3 Summary Scour Pond/Beach Survey 3

The third scour pond survey was conducted on October 19, 2011. The study design was the same as that for the second survey, but four sites were added along the beach at distances of 150 and 300 m north and south of the scour pond discharge. The purpose of the additional sites was to assess the spatial extent of the potential impact from birds on the bacterial concentrations in the ocean receiving waters at Poche Beach. In this survey, indicator bacteria concentrations were again higher in the ocean receiving waters than the tidal creek, scour pond, or Site 7, suggesting a source of bacteria on the beach.

During Survey 3, the frequency of detection and the concentrations of the gull marker were greatest for samples collected from beach sites compared to the other sites. The gull marker was detected at all beach sites during every monitoring period. The gull marker concentrations in the 1 p.m. samples tended to decrease from north to south. The gull marker was detected in scour pond and tidal creek samples, but concentrations were low, with all but one tidal creek sample returning DNQ results (detectable but not quantifiable). No samples were positive for the gull marker in samples collected from Site 7 located at the base of the watershed.

The results from the additional beach monitoring sites in Survey 3 indicated that the gulls on the beach were a source of bacteria in the ocean on the day of the survey. High concentrations of indicator bacteria were found at all of the beach sites during all four monitoring periods, most of which were greater than AB411 criteria. Salinity readings taken during the study at the sites 150 and 300 m north and south of the scour pond discharge did not indicate that effluent from the

scour pond had influenced these areas of the beach. These results, along with field observations that recorded large numbers of gulls on the beach at all sites during the survey, indicate that gull fecal matter was a likely source of the elevated bacterial concentrations in the ocean. This assertion was supported by gull-associated molecular assays that showed elevated levels of the gull marker at all beach sites during all four sampling periods. In contrast, only one sample contained a quantifiable concentration of the gull marker in samples collected from the scour pond, tidal creek, and Site 7. The paucity of positive gull marker results in the watershed and scour pond, coupled with the high frequency of occurrence at the beach sites, suggests that gulls on the beach were likely a prominent source of bacteria to the ocean receiving waters at Poche Beach.

An MST marker associated with fecal bacteria originating from canine sources also was applied to the samples collected during the third survey. The results of this assay suggested that bacteria originating from canine sources were present at most sites during the survey, but quantifiable concentrations were found only at Site 7 and the ocean receiving waters. The results suggest that bacteria originating from canines (coyotes cannot be ruled out) are present in the watershed.

6.4.4 Overall Summary Scour Pond/Beach Survey

6.4.4.1 Gull MST

Overall, the scour pond/ beach survey results suggest that birds on the beach are a source of fecal bacteria in the receiving waters at Poche Beach. This conclusion is supported by linear regression results showing a correlation between concentrations of the gull marker and enterococci and between concentrations of the gull marker and fecal coliform for the combination of all beach samples collected adjacent to the scour pond during Surveys 1 and 2 (r^2 = 0.98 for enterococci; r^2 = 0.92 for fecal coliform, Figure 6-15 and Figure 6-16, respectively). This relationship was weaker when all the ocean samples were used in the analysis (r^2 = 0.29 for enterococci; r^2 = 0.27 for fecal coliform), lending support to the theory that the scour pond may act to congregate birds.

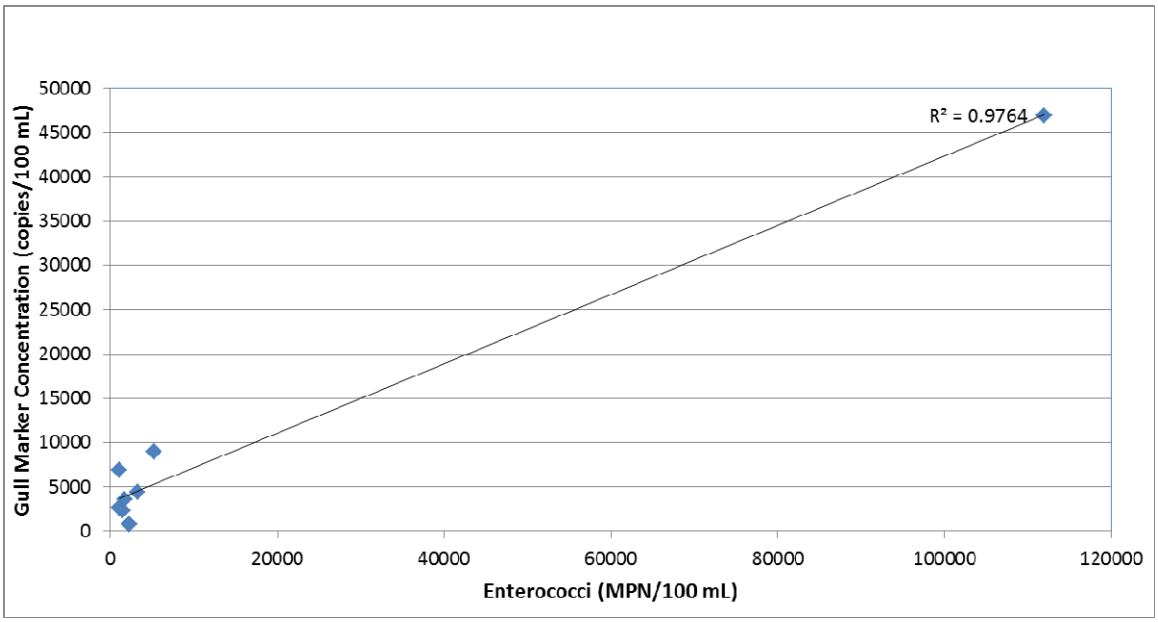


Figure 6-15. Linear Relationship between Concentrations of Enterococci and Gull MST Marker for Samples Collected from the Ocean Adjacent to the Scour Pond

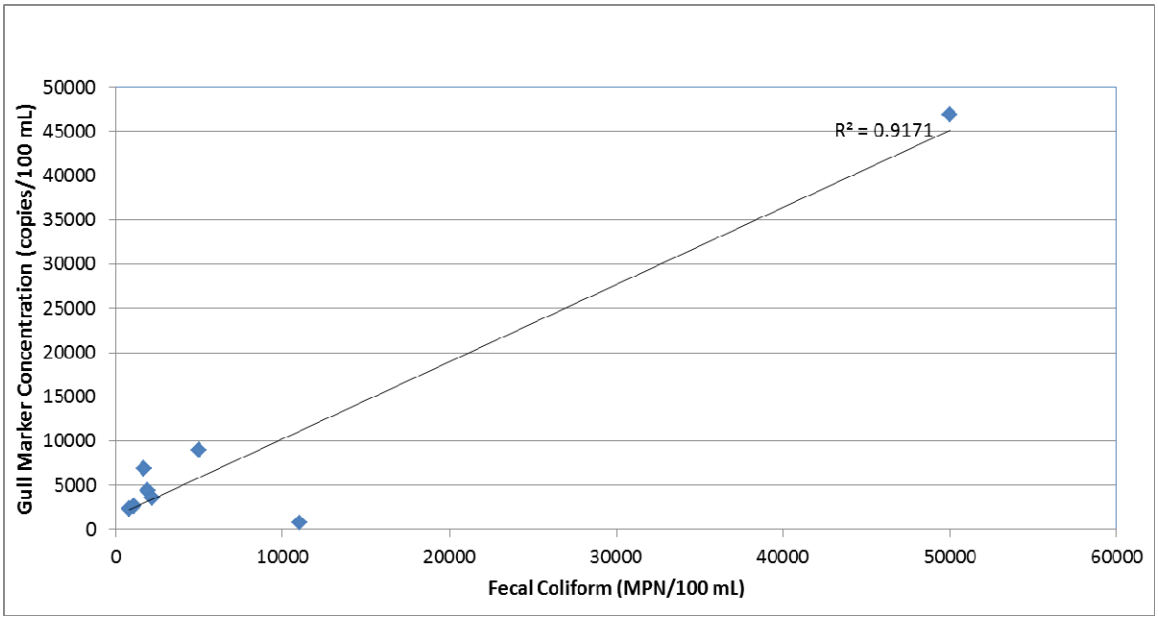


Figure 6-16. Linear Relationship between Concentrations of Fecal Coliforms and Gull MST Marker for Samples Collected from the Ocean adjacent to the Scour Pond

6.4.4.2 Human MST

The MST assay for the human-associated *Bacteroides* assay also was applied to all samples collected in Surveys 2 and 3. Over the course of both surveys, the human marker was detected in a total of three samples, all of which were collected from Site 7 at the base of the watershed (upstream of the scour pond). These samples were quantified by qPCR, and only one sample

(Survey 3, PB-SP-7-4) contained quantifiable concentrations of the marker. The other two samples were deemed "equivocal" because although there was amplification, the concentration was below the limit of detection (note that an equivocal result represents a lower concentration than a DNQ result).

The presence of the human MST marker suggests that bacteria originating from human sources were present in the watershed at the time of the surveys. However, the low frequency of occurrence (3 positive samples among 48 samples over both surveys) and the lack of positive results for the human marker in the scour pond, tidal creek, or ocean samples (in contrast to gull MST results) suggest that bacteria originating from humans had a minimal impact on indicator bacteria levels in the ocean receiving waters at Poche Beach.

6.4.4.3 Indicator Bacteria at the UV Site

Samples collected at the UV site in Scour Pond Surveys 2 and 3 showed, in general, relatively low concentrations of indicator bacteria. During both sampling events, the terminus of the discharge was approximately 4 inches below the surface of the scour pond. Thus, treated effluent was co-mingled with water from the scour pond and the effectiveness of the treatment facility could not be accurately assessed. Indicator bacteria concentrations were generally low in the UV samples, but it was not apparent that the UV discharge had any positive effect on reducing bacterial concentrations in the scour pond or ocean receiving waters.

In summary, the results of the scour pond surveys illustrate an interesting pattern of the sources and mechanisms of bacterial dynamics in the lower portion of the watershed and Poche Beach. Indicator bacteria concentrations were generally low at the base of the Mainstem and Cascadita Channels. However, flow from these drainages provides a continual source of water to the scour pond, maintains high concentrations of nutrients, and promotes the regrowth of bacteria, which likely contributes to elevated bacterial levels in the scour pond. Effluent from the scour pond does impact the receiving waters, as evidenced by higher bacterial ocean water concentrations in front of the scour pond effluent compared to other sites along the beach. However, when large numbers of birds are found on the beach, bacteria originating from avian sources tend to increase bacterial concentrations in the ocean, both in front of the scour pond discharge and at least as far as 300 m north and south.

7.0 HUMAN BACTERIAL SOURCE IDENTIFICATION SURVEY

7.1 Overview, Human Bacterial Source Identification Survey

This survey was conducted in the summer of 2012 as part of the larger Poche Beach Bacterial Source Identification Study to identify potential human contamination in the Prima Deshecha Cañada Watershed. One of the major goals of the survey was to determine the extent to which indicator bacteria (total coliforms, fecal coliforms, and enterococci) originating from human origin (e.g., sewage, homeless population) may be impacting water quality at Poche Beach. This study used real-time PCR to analyze samples for a human-associated *Bacteroides* MST marker.

A number of MST methods have been developed for discriminating between human and non-human sources of fecal contamination. Many MST methods take advantage of host-specific genetic differences in the 16S rRNA gene of *Bacteroides* spp., anaerobic bacteria that are predominant in the feces of warm-blooded animals. Analysis for *Bacteroides* is thought to have advantages over standard enumeration of fecal indicator bacteria. *Bacteroides* are obligate anaerobes and thus should be unable to survive long outside of the intestinal tract and thus are thought to provide a good indicator of recent fecal pollution (Dick and Field, 2004). They are abundant in the feces of warm-blooded animals; *Bacteroides* comprise approximately one-third of human fecal microflora (Noble et al., 2005). In this study, a real-time PCR assay was used to detect *Bacteroides* associated with the human gut.

The Poche Beach Human Bacterial Source Identification Survey was initiated in response to positive results in the human-associated *Bacteroides* samples collected as part of the Poche Beach Bacterial Source Identification Study (WESTON, 2012). These positive results were infrequent throughout the study and did not show a regular pattern suggestive of a consistent source, such as leaking sewage infrastructure. However, the City of San Clemente felt it was important to pursue an investigation of potential sources to assure the protection of human health. In addition, the County of Orange (County), an active stakeholder in the project, had identified a potential positive human-associated *Bacteroides* result from a sample collected from a storm drain that discharges to the Mainstem Channel in the Prima Deshecha Cañada Watershed (also known as the MO1 Channel). The storm drain is located approximately 100 meters upstream of Site 5, a primary monitoring site of the Source Identification Study (Figure 7-1).

Figure 7-1. Historical Sites Monitored in the Human Bacterial Source Identification Survey Conducted on June 21 and July 25, 2012

7.2 Methods, Human Bacterial Source Identification Survey

The Poche Beach Human Bacterial Source Identification Survey was conducted on June 21, 2012 and July 25, 2012. The purpose of the initial survey was to provide a screening level assessment of locations in the watershed that had the potential to be impacted by anthropogenic sources of indicator bacteria. Several sites (Figure 7-1, Figure 7-2, and Figure 7-3) within the Mainstem Channel, scour pond, ocean receiving waters, and suspect storm drain outfall (identified by the County as a putative source of bacteria originating from human sources) were sampled and analyzed for a human MST marker (Table 7-2).

In addition to water samples, bird fecal matter was collected from the beach and analyzed for the human MST marker. The second survey was designed to identify the potential sources of human-associated *Bacteroides* in a tributary drainage that discharges via a storm drain outfall to the Mainstem Channel upstream of Site 5.

7.2.1 Field Methods

7.2.1.1 Site Locations

Maps depicting monitoring sites are presented in Figure 7-2 for the survey conducted on June 21, 2012, and Figure 7-3 for the survey conducted July 25, 2012. Physical descriptions and site locations are presented in Table 7-1.

Figure 7-2. Map of Human Bacterial Source Identification Survey Sites Sampled on June 21, 2012

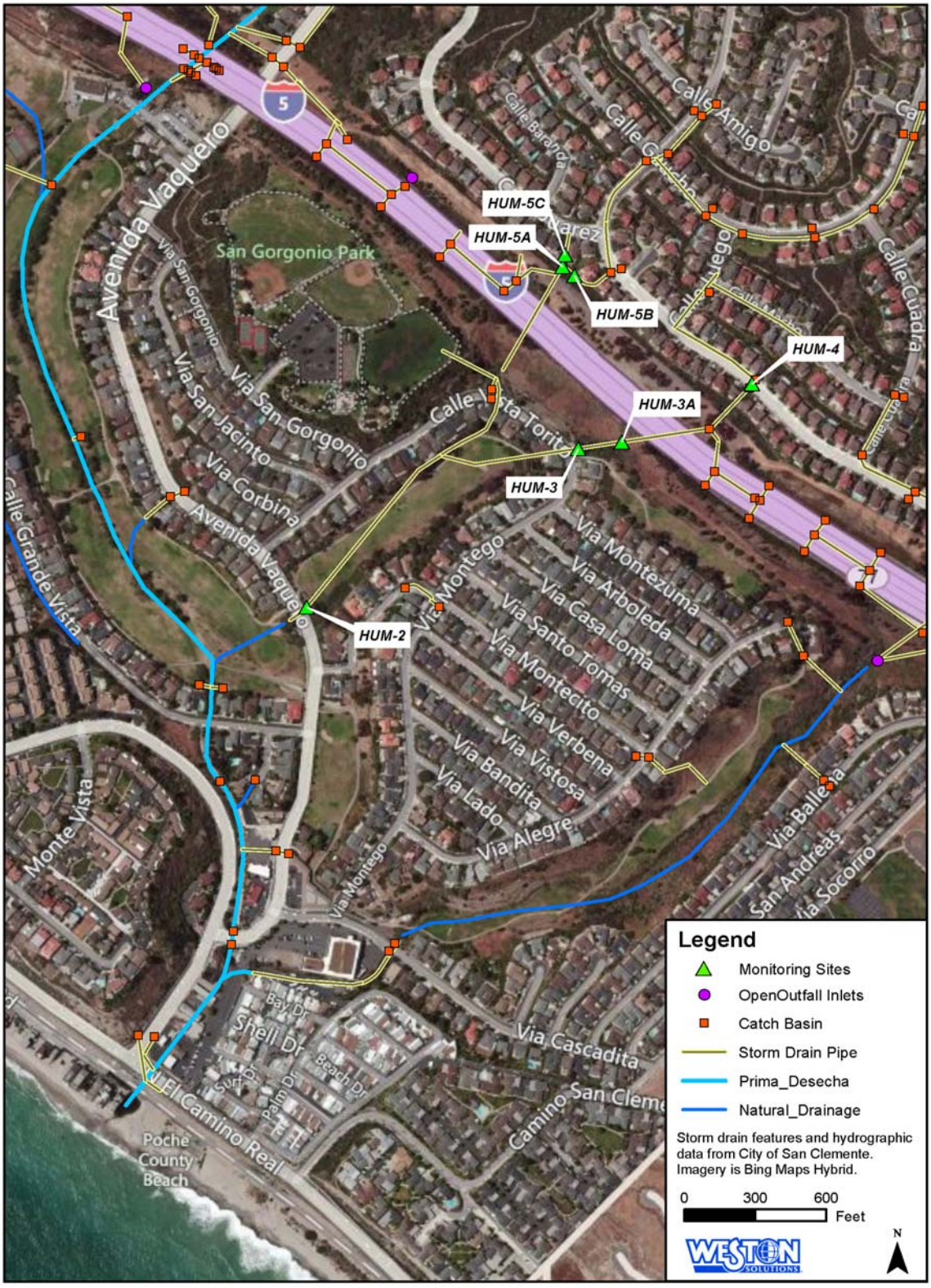


Figure 7-3. Map of Human Bacterial Source Identification Survey Sites Sampled on July 25, 2012

Table 7-1. Location and Description of Sites for the Human Bacterial Source Identification Survey Conducted on June 21, 2012 and July 25, 2012

Sample ID	Site Description	Latitude	Longitude
PB-5UP	In M01 Channel Upstream of Site 5, bioswale return, and outfall of storm drain PB-5UP-SDS	33.445935	-117.644166
PB-BS2-D7	Upstream of Site 5, taken from flow in bioswale return, prior to mixing with flow from the Mainstem Channel	33.445829	-117.644216
PB-5UP-SDS	Outfall of storm drain entering from the southeast side of the Mainstem Channel, across from bioswale return	33.445812	-117.644169
PB-6-1	Historical Site 6 at base of Cascadita drainage	33.442588	-117.643989
PB-7-1	Historical Site 7 downstream of the MO1 Channel and the Cascadita Channel	33.442475	-117.643944
PB-SP-1	Surface sample taken from middle of scour pond	33.440681	-117.645361
PB-TC-1	Tidal Creek leaving scour pond, prior to entering the ocean	33.440681	-117.645361
PB-PO-1	Pacific Ocean receiving waters directly in front of scour pond discharge	33.440284	-117.644451
PB-HUM-2	At Avenida Vaquero, just north of Via Montecito	33.446775	-117.642889
PB-HUM-3	At Calle Vista Torito, just north of Via Montezuma	33.448691	-117.639197
PB-HUM-3A	Upstream of PB-HUM-3A, as drainage passes under Interstate 5	33.448719	-117.638770
PB-HUM-4	East of Interstate 5, on Calle Frontera between Calle Luego and Calle Cuadra	33.449521	-117.636691
PB-HUM-5A	Manhole between Interstate 5 and Calle Juarez – flow from southeast drain	33.450660	-117.639506
PB-HUM-5B	Manhole between Interstate 5 and Calle Juarez – pooled water below Sites 5A and 5B	33.450692	-117.639529
PB-HUM-5C	Manhole between Interstate 5 and Calle Juarez – flow from northwest drain	33.450667	-117.639527
PB-HUM-MO1-5	Historical Site 5 on Mainstem Channel	33.445055	-117.644221

7.2.2 Sample Collection

Water samples were collected from the sites listed in Table 7-1 according to the methods described in Section 1.4.1.2. In addition, samples of bird fecal matter were collected during the June 21, 2012 survey. Samples of gull (*Laurus* spp.) feces were collected from fecal matter found on the surface of the sand within the swash zone at Poche Beach, south of the scour pond. Each sample was collected with a sterile stainless steel spatula. For each sample, the fecal pellet was split and placed into two sterile 250-mL, irradiated nuclease-free plastic containers. Each container held approximately 0.25 gram of fecal matter. One of the split samples was analyzed by WESTON using the methods described below, and the other was analyzed by the County of Orange Microbiological Laboratory using the same procedures.

7.2.3 Analytical Methods

Samples were processed and DNA was extracted as described in Section 1.4.2.2. Extracted DNA was analyzed for MST by real-time PCR for a human-associated marker, as detailed in Table 7-2 (Boehm et al., 2013; Layton et al., 2013). All samples were tested with at least duplicate PCR reactions. A full calibration curve was not utilized; therefore, results were analyzed in endpoint analysis mode and results are presented as presence/absence. Laboratory controls were as described in Section 1.4.3.

Table 7-2. Analytical Methods for Microbial Source Tracking (MST) by Real-Time Polymerase Chain Reaction (PCR) for the Human Bacterial Source Identification Survey

Target	Assay	Sequence 5'-3' (Final Conc, µM)	References	Conditions[a]
Human *Bacteroides*	HF183 Taqman	HF183F: ATCATGAGTTCACATGTCCG (1.2) BthetR1: CGTAGGAGTTTGGACCGTGT (1.2) BthetP1: 6FAM-CTGAGAGGAAGGTCC CCCACATTGGA-TAMRA (0.09)	Haugland et al., 2010; Layton et al., 2013	95°C, 20s; 40 cycles: 95°C, 1s; 60°C, 20s

[a] Master Mix and thermocycler conditions consisted of Quanta-Perfecta QPCR Fastmix w/UNG (#84077) used on a BioRad CFX 96 thermocycler. Reaction volumes were 25 µL.

7.2.4 Quality Assurance/Quality Control Procedures

QA/QC procedures for this study were as outlined in Section 1.4.3.

7.2.5 Chain-of-Custody Procedures

COC procedures outlined in Section 1.4.4 were used for all samples throughout the collection, transport, and analytical processes.

7.3 Results, Human Bacterial Source Identification Survey

Water and bird fecal samples collected in the Human Bacterial Source Identification Survey were analyzed for a MST marker for human fecal contamination (Table 7-2). During the survey conducted on June 21, 2012, eight sites and six bird fecal samples were tested. Site PB-5UP-SDS, a storm drain that discharges to the Mainstem Channel just upstream of Site 5, was positive for the human marker; 6/6 reactions were positive by endpoint analysis. Site PB-BS2-D7 returned results that were deemed equivocal because only 2/6 reactions were positive by endpoint analysis. Samples from this site represent the return flow from the bioswale before it discharges to the Mainstem Channel, upstream of Site 5. In addition, one of the six bird fecal samples tested (PB-Bird-2) was positive for the human marker (4/6 reactions). All of the other samples were negative for the human-associated *Bacteroides* marker. The results are presented in Table 7-3.

In addition to the endpoint analysis, the samples from PB-5UP-SDS, PB-BS2-D7, and bird fecal sample PB-Bird-2 were tested by the HF183 Taqman assay with a full calibration curve. The

LOD was 0.5 copies/reaction (102 copies/100 mL sample), with an LLOQ of 0.75 copies/reaction (149 copies/100 mL sample). The concentration of the target in the PB-5UP-SDS sample was within the ROQ for 6 out of 6 reactions. Out of two separate qPCR runs, the highest value was 399 ± 161 genome copies/100 mL (n=3 reactions). By qPCR analysis, the PB-BS2-D7 sample had five out of six reactions that did not amplify, qualifying the overall analysis as negative (Sinigalliano et al., 2013; Schriewer et al., 2013). Sample PB-Bird2, a bird fecal sample, was classified as detectable but not quantifiable (DNQ) by qPCR analysis.

Based on the results of the first survey, a follow-up survey was conducted on July 25, 2012, to determine whether a source of the human-associated *Bacteroides* found at Site PB-5UP-SDS could be identified within the drainage for this storm drain outfall. A total of eight sites were sampled within the drainage (as well as the Mainstem Channel at Site 5 and the Cascadita Channel at Site 6), all of which were negative for human MST marker (HF183 Taqman). The results are presented in Table 7-3.

In concert with the water quality monitoring investigation described above, the City also conducted an investigation of the integrity of the sewer system within the drainage area of the PB-5UP-SDS outfall. All sewer pipes within the area that may have cross-connectivity with the storm drain system in this drainage were inspected using closed circuit television (CCTV). The integrity of the sewer system was found to be in good condition with no suggestion of leaks that may have the potential to contaminate the storm drain system within this drainage area.

Table 7-3. Real-Time Polymerase Chain Reaction (PCR) Results for the Human-Associated *Bacteroides* Assays (Presence/Absence) in the Human Bacterial Source Identification Study

Site	Sample ID	Time Collected	Result	Comments[a]
Initial Survey – June 21, 2012				
In M01 Channel Upstream of Site 5, bioswale return, and outfall of storm drain PB-5UP-SDS	PB-5UP	0715	Neg	
Upstream of Site 5, taken from flow in bioswale return, prior to mixing with flow from the Mainstem Channel	PB-BS2-D7	0740	Equivocal[b]	Endpoint: 2/6 positive reactions
Outfall of storm drain entering from the southeast side of the Mainstem Channel, across from bioswale return	PB-5UP-SDS	0730	**Pos**[b]	Endpoint: 6/6 positive reactions
Historical Site 6 at base of Cascadita drainage	PB-6-1	0750	Neg	
Historical Site 7 downstream of the MO1 Channel and the Cascadita Channel	PB-7-1	0800	Neg	
Surface sample taken from middle of scour pond	PB-SP-1	0810	Neg	
Tidal Creek leaving scour pond, prior to entering the ocean	PB-TC-1	0820	Neg	
Pacific Ocean receiving waters directly in front of scour pond discharge	PB-PO-1	0815	Neg	
Bird fecal sample	PB-Bird-1	0904	Neg	
Bird fecal sample	PB-Bird-2	0910	**Pos**[b]	Endpoint: 4/6 positive reactions
Bird fecal sample	PB-Bird-3	0915	Neg	
Bird fecal sample	PB-Bird-4	0920	Neg	
Bird fecal sample	PB-Bird-5	0925	Neg	
Bird fecal sample	PB-Bird-6	0933	Neg	
Follow-up Survey – July 25, 2012				
Outfall of storm drain entering from the southeast side of the Mainstem Channel, across from bioswale return	PB-5UP-SDS	1130	Neg	
At Avenida Vaquero, just north of Via Montecito	PB-HUM-2	0945	Neg	
At Calle Vista Torito, just north of Via Montezuma	PB-HUM-3	1000	Neg	
Upstream of PB-HUM-3A, as drainage passes under Interstate 5	PB-HUM-3A	1005	Neg	
East of Interstate 5, on Calle Frontera between Calle Luego and Calle Cuadra	PB-HUM-4	1050	Neg	
Manhole between Interstate 5 and Calle Juarez – flow from southeast drain	PB-HUM-5A	1110	Neg	
Manhole between Interstate 5 and Calle Juarez – pooled water below Sites 5A and 5B	PB-HUM-5B	1112	Neg	
Manhole between Interstate 5 and Calle Juarez – flow from northwest drain	PB-HUM-5C	1114	Neg	
Historical Site 5 on Mainstem Channel	PB-5-1	1150	Neg	
Historical Site 6 at base of Cascadita drainage	PB-6-1	1145	Neg	

[a] Samples were run in duplicate except for putative positive samples.
[b] Quantified by HF183 Taqman, see text.

7.4 Summary, Human Bacterial Source Identification Study

The Poche Beach Human Bacterial Source Identification Survey was a limited survey of a small drainage within the Prima Deshecha Cañada Watershed that was designed to complement the larger scale effort of the Poche Beach Bacterial Source Identification Study. The conclusions presented below are based on the data collected as part of the overall investigation with the knowledge that additional data may need to be collected to verify the study conclusions.

- The results of the Poche Beach Human Bacterial Source Identification Survey indicated that flow from the PB-5UP-SDS storm drain was positive for the human MST marker, suggesting that bacteria originating from anthropogenic sources were present at the time the sample was collected. However, negative results were observed at Mainstem Channel sites upstream and downstream of the storm drain, the scour pond, and ocean receiving waters at Poche Beach, which suggests that potential sources originating from the storm drain outfall did not have a measurable impact downstream on the day the samples were collected.

- The lack of positive results at any of the sites in the second survey suggests that any potential source of bacteria from human origin that may have been present in the drainage of PB-5UP-SDS during the first survey was ephemeral in nature. Overall, the data did not provide evidence of a chronic source, such as leaking sewage infrastructure.

- The CCTV investigation showed no evidence of leaking sewage infrastructure within the drainage. This result, in concert with a lack of a strong and consistent human MST signal, suggests that leaking sewage infrastructure was not a likely source of indicator bacteria in the receiving waters of the Mainstem Channel and Poche Beach.

- The presence of a weak positive signal for a human MST marker in bird feces deserves further investigation.

- Additional surveys would be needed to categorically rule out human sources of indicator bacteria in the PB-5UP-SDS drainage or other areas within the Prima Deshecha Cañada Watershed.

8.0 CONCLUSIONS

The Poche Beach Bacterial Source Identification Study consisted of several independent, but interlinked, studies to determine the sources of bacteria in the watershed and beach environment, understand the dynamics of bacterial transport and regrowth, and assess Best Management Practice (BMP) effectiveness. The study consisted of the following elements:

1. Sanitary Survey Investigation
2. Biofilm Study
3. Groundwater Study
4. Bioswale BMP Effectiveness Study
5. Scour Pond and Beach Environment Study
6. Human Bacterial Source Identification Survey

The major conclusions based on the results of each of these studies are summarized in the following subsections.

8.1 Sanitary Survey Investigation

- A strong diurnal pattern was observed in flow; flows at night and in the early morning were two to three times greater than flows during the day. This is consistent with residential and commercial irrigation, which typically peaks at night in urbanized watersheds.
- Flow was found to be greatest at Sites 5 and 7 near the base of the watershed across all months, and was particularly elevated in February and March relative to the other monitored sites.
- The relative contribution of flow from the upper watershed appears to have decreased since 2006, but the results were highly seasonal.
- Flow in the Cascadita Channel appears to have decreased since 2006.
- Fecal coliform and enterococci concentrations were greatest in the upper and middle watershed and were highest in the early morning hours. These results are similar to those in the 2006 study.
- Fecal coliform and enterococci concentrations were lowest at Sites 6 and 7 at the bottom of the watershed.

8.2 Biofilm Study

- Regrowth of total coliform, fecal coliform, and enterococci occurred at all sites within the Mainstem and Cascadita Channels.
- Colonization of the concrete substrate of the coupons occurred rapidly (within 8 or 9 days of deployment).
- The microbial communities that contained the three types of indicator bacteria were maintained over time (the 6-month time frame of the study) under the conditions found in the storm drain system.
- Biofilm concentrations of all three indicators were highest in the upper Mainstem Channel sites.

- Biofilm concentrations of all three indicators were lowest at the Cascadita Channel (Site 6) in nearly all sampling events.
- The results indicated that the biofilm within the Cascadita and Mainstem Channels could serve as a reservoir of indicator bacteria and a source of indicator bacteria to the ocean receiving waters at Poche Beach.

8.3 Groundwater Study

- Groundwater at the sites monitored did not contain elevated levels of indicator bacteria and did not appear to be a direct source of bacteria to the watershed. Concentrations of all three indicator bacteria were largely at or below detection limits in the majority of samples collected.
- Although bacterial levels in groundwater were low, groundwater infiltration into the storm drain network likely helps maintain an atmosphere conducive to bacterial regrowth inside the channel.
- Total phosphorous (TP) concentrations were greater than the water quality benchmark in all groundwater samples collected, suggesting that groundwater influx contributes to elevated TP levels in the channel, which may enhance regrowth of indicator bacteria.

8.4 Bioswale BMP Effectiveness Study

- Indicator bacteria concentrations and loads were lower at the bottom of the bioswale than the top, suggesting that the bioswale may have a limited positive effect in reducing bacterial levels in the watershed.
- Concentrations of ammonia, nitrite, cadmium, and nickel (total and dissolved) decreased from upstream to downstream in the first section of the bioswale, suggesting that the bioswale had been effective in reducing concentrations of these constituents.
- Flow was greater at the bottom of the bioswale than the top, suggesting an increase in surface flow from irrigation practices or surfacing groundwater in the lower portion of the bioswale.

8.5 Scour Pond and Beach Environment Study

- Both beach and scour pond sands had low concentrations of indicator bacteria during Survey 1 (at or below detection limit in most samples), suggesting that they did not serve as a reservoir of bacteria during the time of the survey (January 2011).
- During Survey 2, the concentrations of indicator bacteria at Site 7 were lower than those in the scour pond, tidal creek draining the scour pond, and ocean receiving waters, suggesting the presence of regrowth and/or bacterial contributions from sources other than the watershed.
- During Survey 3, indicator bacteria concentrations in the ocean receiving waters were greater than those at the other sites, suggesting a source of bacteria in the ocean receiving waters other than the watershed.
- The results of the quantitative MST gull assay during Survey 2 showed that bacteria originating from birds were found in all samples collected during all of the sampling periods, except Site 7 where the marker was found only once (7 a.m.). Quantifiable levels

of the gull marker were measured at all five sites, with the ocean samples having the most frequent occurrence and the highest concentration (1 p.m.).

- During Survey 3, the gull marker concentrations and the frequency of detection were highest in samples collected from beach sites. The gull marker was detected during all four sampling periods in the ocean receiving waters. The gull marker concentrations in the 1.p.m. ocean samples tended to decrease from north to south. The gull marker was detected in scour pond and tidal creek samples, but concentrations were low, with all but one tidal creek sample returning DNQ results (detectable but not quantifiable). No samples were positive for the gull marker in samples collected from Site 7 at the base of the watershed.
- The results of Surveys 1 and 2 suggest that birds on the beach were a source of indicator bacteria in the receiving waters at Poche Beach. Furthermore, enterococci and fecal coliform concentrations were correlated to the gull marker concentrations for ocean samples collected adjacent to the scour pond. This relationship was weak when all ocean samples were used, lending support to the theory that the scour pond may act to congregate birds.
- A canine-associated *Bacteroides* marker was detected from all sites during Survey 3, suggesting that bacteria originating from canines (coyotes cannot be ruled out) are present in the watershed. A total of 12 samples were positive for the canine marker: four at Site 7, three each in the scour pond and tidal creek, and one each in the ocean and at the UV treatment discharge. Of these, only Site 7 and the ocean sample during the 1 p.m. monitoring period had concentrations high enough for quantification.
- The human-associated *Bacteroides* marker was found at Site 7 (bottom of the watershed), suggesting that bacteria originating from human sources were present in the watershed during Surveys 1 and 2. However, the low frequency of occurrence (3 positive samples among 48 samples over both surveys) and the lack of positive results for the human marker in the scour pond, tidal creek, or ocean samples (in contrast to gull MST results) suggest bacteria originating from humans had, at most, a minimal impact on indicator bacteria levels in the ocean receiving waters at Poche Beach.

The Poche Beach Bacterial Source Identification Study illustrates an interesting pattern of the sources and mechanisms of bacterial dynamics in the Prima Deshecha Cañada Watershed, the scour pond, and the beach environment. The very upper portion of the watershed by the landfill appears to have no impact on dry weather bacterial loads via surface or groundwater flows. Bacterial concentrations dramatically increase in sub-drainages 2 and 3 where excess water from irrigation in the urbanized area creates substantial regrowth of indicator bacteria in the Mainstem Channel. The major feature affecting Site 4 is a groundwater spring that flows into a tributary storm drain just upstream of the monitoring site. In this region, water chemistry changes dramatically due to the influx of water from the spring, which is a likely source of 303(d) listed metals (nickel and cadmium). In sub-drainage 5, the major feature observed in the study is the influx of groundwater. Flows at this site show dramatic seasonal changes, which likely represent groundwater influx in the winter and spring.

In the lower portion of the watershed (Sites 6 and 7), bacterial concentrations are the lowest of any site monitored in the study and loads decrease in this area as a result. The channels directly above both these sites are open to the sunlight, and UV radiation may play a positive role in

reducing bacterial concentrations at the bottom of the watershed. However, flow from these drainages provides a continual source of water to the scour pond, maintains high concentrations of nutrients, and promotes the regrowth of bacteria, which likely contributes to elevated bacterial levels in the scour pond. Elevated bacterial concentrations in the scour pond compared to those at Site 7 suggest that regrowth may occur in the scour pond water column, but the data did not suggest that this was an issue in the sediments or beach sand. Effluent from the scour pond does impact the receiving waters, as evidenced by higher bacterial ocean water concentrations in front of the scour pond effluent compared to other sites along the beach. When large numbers of birds are found on the beach, it appears that bacteria originating from avian sources tend to increase bacterial concentrations in the ocean, both in front of the scour pond discharge and at least as far as 300 m north and south along the beach.

8.6 Human Bacterial Source Identification Survey

One of the major goals of the study was to determine the extent to which indicator bacteria (total coliforms, fecal coliforms, and enterococci) originating from human origin (e.g., sewage, homeless population) may be impacting water quality at Poche Beach. This study used real-time PCR to analyze samples for a human-associated *Bacteroides* Microbial Source Tracking (MST) marker.

The Poche Beach Human Bacterial Source Identification Survey was a limited survey of a small drainage within the Prima Deshecha Cañada Watershed that was designed to complement the larger scale effort of the Poche Beach Bacterial Source Identification Study. The major findings presented below are based on the data collected as part of the overall investigation with the knowledge that additional data may need to be collected to verify the study conclusions.

- The results of the Poche Beach Human Bacterial Source Identification Survey indicated that flow from the PB-5UP-SDS storm drain was positive for the human-associated *Bacteroides* marker, suggesting that bacteria originating from anthropogenic sources were present at the time the sample was collected.
- The negative results at Mainstem Channel sites upstream and downstream of the storm drain, the scour pond, and ocean receiving waters at Poche Beach suggest that any source originating from the storm drain outfall did not appear to have a measurable impact downstream on the day the samples were collected.
- The lack of positive results at any of the sites in the second survey suggests that any potential source of bacteria from human origin that may have been present in the drainage of PB-5UP-SDS during the first survey was ephemeral in nature. Overall, the data did not provide evidence of a chronic source, such as leaking sewage infrastructure.
- The results of the CCTV investigation conducted by the City showed no evidence of leaking sewage infrastructure within the drainage. This result was consistent with the lack of positive results for the human MST analyses conducted by WESTON and suggested that leaking sewage infrastructure within the drainage was not a likely source of indicator bacteria in the receiving waters of the Mainstem Channel and Poche Beach.
- The presence of a weak positive signal for the human-associated *Bacteroides* marker in bird feces needs further investigation to identify the potential sources or causes.

- Additional surveys would be needed to categorically rule out human sources of indicator bacteria in the PB-5UP-SDS drainage or other areas within the Prima Deshecha Cañada Watershed.

9.0 RECOMMENDATIONS

Based on the findings of the various studies conducted over the course of this project, some basic recommendations can be made. There are three major areas that should be considered in order to reduce bacterial concentrations in the receiving waters of Poche Beach:

- **Reduce flows from excess irrigation.** The results of the sanitary surveys and biofilm studies indicate that excess irrigation is likely a major source of flow in the Prima Deshecha Cañada Watershed. A constant flow of water can help maintain a well-developed biofilm in the Mainstem Channel, which is likely a source of indicator bacteria to the ocean receiving waters at Poche Beach. Therefore, reducing over-irrigation in the watershed will likely reduce bacterial levels at Poche Beach.

- **Address the scour pond configuration.** The scour pond surveys revealed that the scour pond at Poche Beach is at least 15 feet deep. This large depression at the base of the watershed provides an environment that may be conducive to growth of indicator bacteria and provides a fresh water source that attracts birds. Additional studies should be considered to address the configuration of the scour pond, to address the limited public access to Poche Beach, and to reduce the flows to the ocean that appear to attract gulls.

- **Reduce the impact of birds at the beach.** The scour pond studies revealed that fecal material from gulls is a likely source of indicator bacteria in the receiving waters. Management plans to reduce the impact of gulls on indicator bacteria in the receiving waters should be considered.

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