James V. Fitzgerald Area of Special Biological Significance Pollution Reduction Program

Microbial Source Tracking Study Summary Reports

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Executive Summary & Recommendations

The James V. Fitzgerald Area of Special Biological Significance (ASBS) is located to the north of the City of Half Moon Bay in unincorporated San Mateo County and extends from 4th Street in Montara south to the Pillar Point Harbor breakwater. The Fitzgerald Marine Reserve (Reserve) is located within the boundary of the ASBS. The Reserve is recognized by many as one of the richest, most biodiverse intertidal environments on the California coast and is a popular recreational area as well. Three main creeks drain to the Reserve – Montara, Dean, and San Vicente Creek. Kanoff and Martini Creeks are located immediately to the north of the ASBS boundary.

The Pacific Ocean at the Reserve and San Vicente Creek are on the 303 (d) List of Impaired Water Bodies due to elevated coliform bacteria, and Total Maximum Daily Loads are scheduled to be completed by 2019. This microbial source tracking (MST) study was undertaken due to the 303d listing, numerous exceedances of water quality objectives, and the frequency of needed beach and creek postings warning visitors that San Vicente Creek and the Reserve may not be suitable for contact recreation. This study was conducted as part of the James V. Fitzgerald ASBS Pollution Reduction Program, a multi-faceted program designed to reduce pollutant loading and protect natural resources and beneficial uses of the ASBS. The main goal of this MST study was to provide information about the primary sources of fecal contamination within the ASBS watershed and to assist with the selection of appropriate Best Management Practices (BMPs) to reduce fecal pollution.

MST monitoring was conducted from January 2012 through October 2012 within Martini, Kanoff, Montara, Dean, and San Vicente Creeks. The San Francisco Estuary Institute (SFEI) monitored fecal indicator bacteria (FIB), including total coliform, *Escherichia coli*, and *Enterococcus* spp., at multiple locations within each creek during two rainy season events (January 20, 2012 and March 14, 2012) and two dry season events (July 9, 2012 and August 15, 2012). The purpose of the FIB monitoring was to determine FIB levels throughout the watersheds and investigate seasonal and land use-related spatial trends. Researchers from the University of California, Davis (UCD) collected samples from the same five creeks at sites located immediately upstream of the confluences with the Pacific Ocean during a rainy season event (March 2012), dry season event (July 2012), and during first flush (October 2012) and conducted genetic analysis of host-associated *Bacteroidales* to determine the contribution of human, bovine, dog, and horse sources to fecal contamination. SFEI collected a total of 78 water samples for FIB analysis, and UCD collected a total of 58 samples from water, sediment, and biofilm matrices for genetic analysis.

The sites were located within the unincorporated communities of Montara and Moss Beach. The watersheds are rural in nature, although there are small commercial and medium to high density residential areas located within all of the watersheds, except for Martini Creek. A large portion of the watersheds is open space including McNee Ranch State Park and Rancho Corral de Tierra, part of the Golden Gate National Recreation Area managed by the National Park Service. Potential sources of fecal contamination within the five watersheds include wildlife, recreation (i.e., dog walking, beach and park use), equestrian facilities, other confined animal

facilities/livestock, agriculture, leaking pipes or overflows from septic and/or sanitary sewer systems, and other residential-related sources (i.e., pets, compost).

The results of FIB monitoring, conducted by SFEI, showed that FIB concentrations in the five drainages were elevated during both the dry and wet seasons and often exceeded water quality objectives for contact recreation. FIB concentrations were generally lower in the dry season than in the rainy season. In Martini and Kanoff Creeks, the less urbanized watersheds, FIB levels were lower than the more urbanized watersheds of Montara, Dean, and San Vicente Creeks. Due to the study design and limited timeframe, consistent spatial trends in FIB concentrations related to specific land use types were not detected. One notable increase in FIB occurred in Montara Creek during the January 2012 rainy season event when *E. coli* and *Enterococcus* levels were approximately six times higher at the Pacific Ocean confluence site in comparison to the next site upstream. Increases were also observed between these sites during the two dry season events. Within this reach of Montara Creek, there are several land use types; therefore, the primary contributing source could not be identified.

The results of the genetic analysis, conducted by UCD, showed that concentrations of the universal *Bacteroidales* marker, derived from all warm-blooded animals, increased during rain and was generally lower in the dry season. Increased levels of *Bacteroidales* were significantly higher in the wet season event in comparison to the first flush event. Differences in first flush and the rainy season events could be due to differences in microorganism survival related to environmental conditions such as temperature, differences in source loading related to the degree of ground saturation, groundwater levels, resulting runoff, and observed streamflow at the beginning of the storm season versus the end of the storm season, and/or the resuspension of sediments and release of microorganisms from sediment and biofilms as the result of higher streamflow and turbulence.

Of the four host-specific markers that were analyzed (dog, horse, bovine, and human), dog-associated *Bacteroidales* was the most frequently detected host marker in the water, as well as in sediments and biofilms at all sites in the wet season. On the contrary, the dog-associated marker was less frequently detected during the dry season and first flush event. The bovine-associated marker was detected in water, sediments, or biofilms at all sites during the rainy season, most notably from Kanoff and San Vicente Creeks, but was not detected during the dry season or first flush events. Horse-associated *Bacteroidales* were found at high concentrations in water at Dean and San Vicente Creeks during rain in the wet season. The horse marker was also detected at all sites during the dry season, but did not appear to be a predominant source of fecal contamination. Human-associated *Bacteroidales* were detected in water at all sites during the first flush event, but were not present during the dry season and were only detected in two samples during the rainy season event. During the dry season, at all sites except Montara Creek, less than 5% of the universal *Bacteroidales* concentrations were made up of the tested host-specific markers, which indicates that uncharacterized fecal sources, such as wildlife or other domestic animals, likely contributed a large amount of fecal pollution.

The results of this MST study provide good insight and a first glimpse into the understanding and control of fecal contamination sources in watersheds draining into the Pacific Ocean in the vicinity of the ASBS. FIB levels were highest during the rainy season. This study confirms

fecal contamination from human, dog, bovine, and horse sources, and of these, dog appears to be the most prevalent source during the rainy season. While there may be other more significant sources of fecal pollution that were not characterized as part of this study, such as wildlife or other domestic animals, this study provides useful information to guide the selection of BMPs to reduce fecal pollution.

Recommendations for further work and BMPs include:

- Continued MST within the five watersheds including:
    - Additional monitoring for the existing host-associated *Bacteroidales* markers used in this study (increased sites and sampling frequency)
    - Genetic analysis using new host-associated markers or improved MST techniques (i.e., Phylochip, techniques to distinguish between intact and impaired cells allowing for determination of the age of fecal pollution), as available
    - Implementation of a bacterial tracer experiment
- Implementation of an education and outreach program to address dog waste
- Implementation of BMPs to address horse waste
- Investigation of potential sources of bovine contamination including research on:
    - Presence of cows within the watersheds
    - Contribution potential from applied manure compost and level of use within the watersheds
    - Specificity of the bovine-associated marker
- Development of a project to investigate the condition of septic systems and provide education and outreach on proper maintenance
- Development of a plan to investigate the potential for source contributions related to the sanitary sewer system. If needed, a future project could include a system condition assessment, enhanced program for maintenance and leak/overflow prevention, and public education and outreach on proper sewer lateral maintenance

Fecal Indicator Bacteria In Creeks Draining Into The Pacific Ocean In Montara and Moss Beach, CA

As Part Of The

Monitoring Program for the

James V. Fitzgerald Area of Special Biological Significance

Pollution Prevention Program

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Summary

Bacteria, such as coliform*, Enterococcus*, and *E. coli*, can be used as indicators for fecal contamination in surface waters. The goal of this study was to identify potential sources of fecal indicator bacteria in different reaches of creeks draining from Montara Mountain to the Pacific Ocean in the vicinity of the James V. Fitzgerald Area of Special Biological Significance and to inform future bacterial loading reduction efforts in those creeks. Fecal indicator bacteria concentrations exceeded U.S. EPA recommended standards at most sites during the dry and the wet season. However, concentrations were generally lower in the dry season. In addition to the high total coliform concentrations at all creek sites during the wet season, some counterintuitive observations were noted for Martini and Kanoff Creek for *E. coli* and *Enterococcus*. Bacteria concentrations in the upper reaches of the creeks were generally higher than downstream locations even though likely urban sources of fecal contamination (e.g., pet waste, sewer and septic system effluent) would be expected to be concentrated in the central and lower reaches of the creeks. Similar observations to the ones made in this study have been made in other watersheds. As expected, Martini and Kanoff Creek showed lower bacteria concentrations than the more urbanized watersheds of Dean, San Vicente, and Montara Creek. Montara Creek was the only creek out of the five monitored creeks where increased bacterial loading was observed from the upstream to the downstream reaches. However, the understanding of the complex bacteria loading in mountainous creeks and the transport and fate of the indicator bacteria downstream are not, at this point, well understood despite multiple decades of use of these indicators in state and federally promulgated water quality standards.

Introduction

Impairment of the James V. Fitzgerald Marine Reserve (Reserve) and Area of Special Biological Significance (ASBS) due to fecal bacteria is of concern to the State of California, County of San Mateo, and local stakeholders. The Pacific Ocean at the Reserve and San Vicente Creek are listed on the 303 (d) List of impaired water bodies due to elevated coliform bacteria, and total maximum daily loads are scheduled to be completed by 2019. The original 2002 listings were based on three years of fecal indicator bacteria (FIB) monitoring data collected by the San Mateo County Environmental Health Department and associated exceedances of Basin Plan and Ocean Plan objectives for water contact recreation. The 303d listing, numerous exceedances of water quality objectives, frequency of needed beach and creek postings at the Reserve, and the potentially associated consequences for public and

ecosystem health led to the monitoring of fecal contamination, using FIB in five creeks draining watersheds in Montara and Moss Beach, CA. Fecal contamination can lead to acute gastrointestinal illnesses in humans after contact with the water (U.S. EPA 1997). High levels of FIB in water can indicate the potential presence of pathogens known to cause diseases like cholera, typhoid, dysentery, cryptosporidiosis, and hepatitis. Indicator bacteria are usually harmless but are easy to measure, more numerous than, and strongly correlated to waterborne pathogens and fecal matter. However, since some of the indicators are also common in nature, e.g., some coliform species of the total coliform group, it is not certain that their presence necessarily indicates fecal contamination (U.S. EPA 2004).

Total coliform is the indicator that was originally recommended in 1968 by the Federal Water Pollution Control Administration of the Department of the Interior as a screening indicator for waterborne illness. The two other indicators that were monitored during this study, *E. coli* and *Enterococcus*, were actually indicators that showed stronger correlation to contact-associated gastroenteritis than total coliform (U.S. EPA 2004). Also, *Enterococcus* has the ability to survive in salt water, and therefore more closely resembles many pathogens. Additionally, it can point to a more human-related source than other subgroups of the fecal streptococcus group that it belongs to (U.S. EPA 1997). Since the primary source of waterborne pathogens are thought to be fecal pollution from humans and other warm-blooded animals (National Research Council 2000), fecal contamination is often associated with an increase in nutrient concentrations, predominantly nitrate and phosphorus, that can cause excessive algae growth and diminish the vital amount of oxygen in the water (U.S. EPA 2000).

Conceptually, source models for bacteria indicate higher bacterial loading from impervious land segments and urban areas, usually located in the central and downstream reaches of creeks. Potential sources within these reaches include equestrian facilities, other confined animal facilities, pet waste, wildlife, homeless encampments, and failing or leaking septic and sewer systems. The upper reaches have the potential for bacterial contributions from wildlife, recreational uses (i.e., equestrian and dog walking), and where present, livestock and pastures. Shorter residence times due to steeper slopes and higher precipitation usually do not allow for any degradation in the upper reaches while die-off can influence the amount of bacteria in the lower watersheds (Kim et al. 2007).

Often, the source for dry weather bacterial loading can be attributed to human sources, e.g., septic system and sewer leaks (Jensen et al. 2003), since there is very little runoff facilitated loading during the dry season. There could be a greater potential risk for septic system leaks in the studied area due to tectonic activity that could potentially lead to fractures in septic tanks or pipes.

Several factors contribute to the elimination of pathogen indicators in water. These factors include pH, temperature, solar radiation, nutrients, pesticides, and organic matter (Moore et al. 1988). We assume creek water pH to be near, or more likely above, neutral and to not affect the breakdown of bacteria but none of the influencing factors were measured in this study. Additionally, during larger runoff events, when the hydraulic retention time in the studied creeks is around 1-2 days, it is not likely that a depletion of bacteria occurred due to nutrient enrichment, pesticides, organic matter, or sunlight (Easton et al. 2005).

Methods

Site Description

The five studied creeks are located in unincorporated San Mateo County, traversing a rural to urban land use gradient and draining into the Pacific Ocean in Montara and Moss Beach (Figure 1). The coastal communities of Montara and Moss Beach border the Reserve and ASBS. Their population in the 2010 census was 2,909 and 3,103, respectively. The communities are situated approximately 20 miles (32 km) south of San Francisco and 50 miles (80 km) north of Santa Cruz. Montara and Moss Beach cover an area of 3.9 square miles (10.0 km^2) and 2.3 square miles (5.8 km^2), respectively. Montara and Moss Beach have mild weather throughout the year. January average maximum temperature (56.9°F or 13.8°C) and September average maximum temperature (73.1°F or 22.8°C) span a narrow range based on the long-term record (www.weather.com). Typical of central California, most of the rainfall occurs from November through April, normally totaling more than 27 inches (69 cm). Nearby Montara Mountain, part of the Santa Cruz Mountains, rises to an elevation of 1,898 feet (578 m) above sea level. The area is characterized by the Franciscan Complex, a geological term for an accreted terrain of heterogeneous rocks (altered volcanic rocks (greenstones), deep-sea cherts, sandstone, limestone, serpentinies, shales, and high-pressure metamorphic rock) found on and near the San Francisco Peninsula (Conradson et al. 1999). All five creeks originate on Montara Mountain. Martini and Kanoff Creek reach the ocean at

Montara State Beach, just north of the Reserve and ASBS boundary. Montara, Dean, and San Vicente creeks reach the ocean within the ASBS and Reserve boundary.

The monitored creeks are between two and four miles (3.2 – 6.4 km) long with an elevation drop of up to 1,500 ft (457 m) down to sea level. The sampling locations were selected to span from the creek mouths at the ocean to between 0.05 (100 m) and 2 (3,220 m) miles upstream depending on accessibility of the sites (Figure 1, Table 1). The monitored creek reaches span rural and urban land use. All five watersheds have confined animal facilities, estuarine habitat, public recreation, and open space (i.e., Fitzgerald Marine Reserve, McNee Ranch State Park, Golden Gate National Recreation Area Rancho Corral de Tierra, public beach access, trails), rare or endangered species habitat, rural/dirt roads, a state highway, wetlands, and septic and sewer systems. Montara, Dean, and San Vicente watersheds also have equestrian facilities and equestrian uses. With the exception of Dean Creek, all watersheds also have agriculture (row crops or flower farms). Medium-density residential land use is present in all watersheds but Martini Creek, and medium to high-density residential use occurs in Montara Creek and San Vicente Creek watersheds. Very low-density residential land use is seen in all watersheds but Dean Creek. The Montara State Marine Reserve and the Fitzgerald Marine Reserve are located at the bottom of the Montara, Dean, and San Vicente Creek watersheds. Municipal water sources or reservoirs are located in Montara Creek and San Vicente Creek watersheds. Neighborhood commercial use occurs in Kanoff Creek and Dean Creek watersheds.

Figure 1. Map of creeks and sampling locations within the study area. The lower number sampling sites are near the beach, and the higher numbers are upstream. Latitudes and longitude are listed in Table 1.

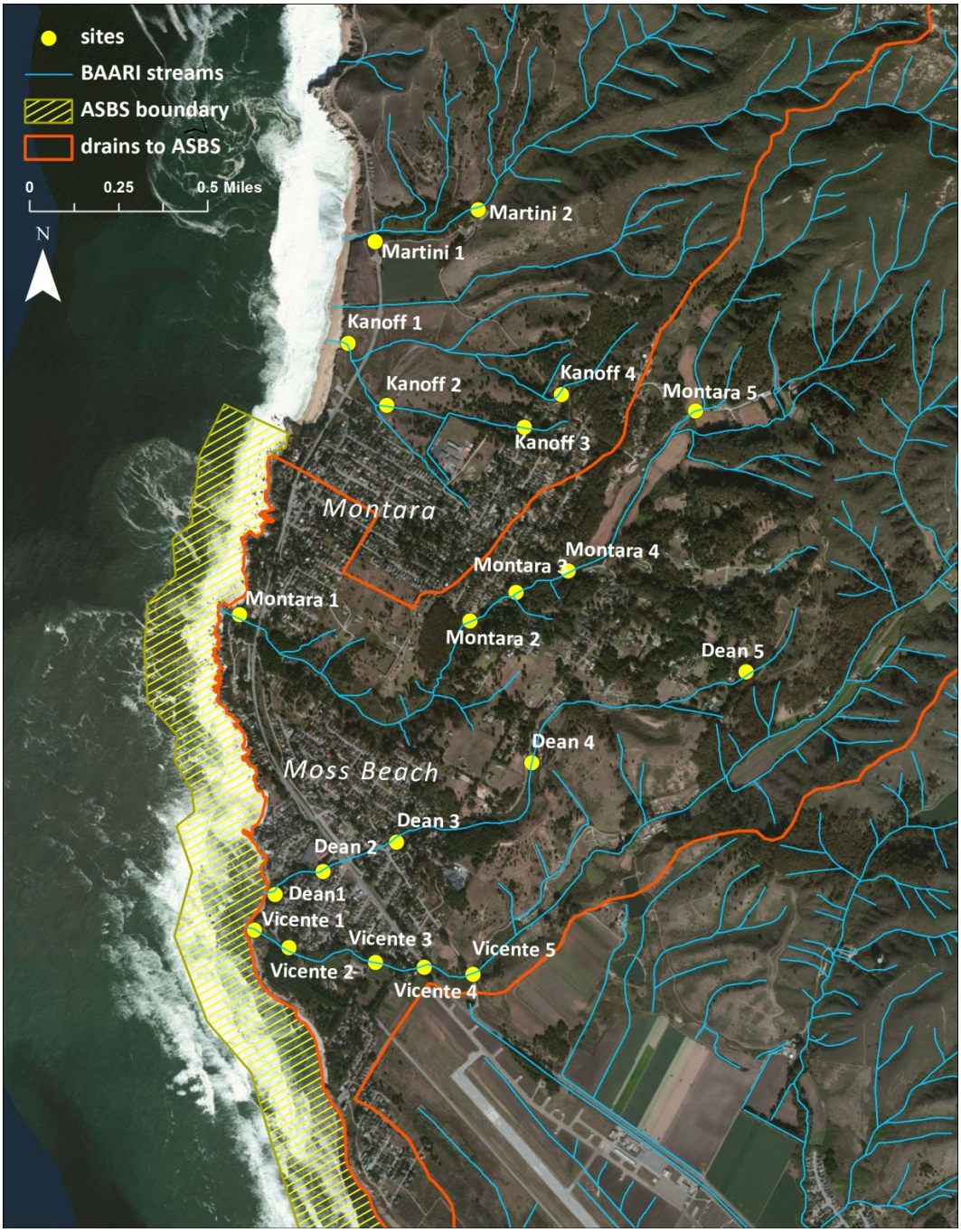

Table 1. Coordinates of the sampling locations.

Name	Type	Lat	Long
Montara 1	creeks	37.53702965100	-122.51870163100
Montara 2	creeks	37.53702159000	-122.50690645000
Montara 3	creeks	37.53824482600	-122.50458058400
Montara 4	creeks	37.53917476400	-122.50193763200
Montara 5	creeks	37.54588612500	-122.49564108500
Dean1	creeks	37.52559298300	-122.51649793800
Dean 2	creeks	37.52657534100	-122.51407348700
Dean 3	creeks	37.52786750200	-122.51036398900
Dean 4	creeks	37.53126339000	-122.50354886200
Dean 5	creeks	37.53522603900	-122.49270097100
Kanoff 1	creeks	37.54827006100	-122.51351154200
Kanoff 2	creeks	37.54577145000	-122.51145700200
Kanoff 3	creeks	37.54502965600	-122.50438831900
Kanoff 4	creeks	37.54641298000	-122.50252041900
Vicente 1	creeks	37.52409674000	-122.51749720200
Vicente 2	creeks	37.52342099500	-122.51572146100
Vicente 3	creeks	37.52290874600	-122.51125722200
Vicente 4	creeks	37.52276008600	-122.50876680900
Vicente 5	creeks	37.52252634900	-122.50626229600
Martini 1	creeks	37.55248063700	-122.51227370900
Martini 2	creeks	37.55390466800	-122.50704581500

Field Methods

Three fecal indicators, total coliform, *E. coli*, and *Enterococcus*, were monitored in five creeks that drain the Montara and Moss Beach areas (Figure 1, Table 1). Two sampling events were conducted during the wet season coinciding with rainfall (January 20, 2012 and March 14, 2012), and two sampling events were conducted during the dry season (July 9, 2012 and August 15, 2012).

Approximately 100 mL of creek water were collected into plastic sampling containers at all sites. Water samples were collected mid-column at equal distance from both creek banks. Water samples were only collected when the creek had flowing water. Ponded water was not sampled and marked as dry conditions on the field data sheet. The sampling containers were pre-preserved with sodium

hydrochlorite and after filling were kept on ice until delivery to the lab. All samples were delivered to the San Mateo County Public Health Laboratory within six hours of collection.

Parallel to the collection of water samples, researchers from the Civil and Environmental Engineering Laboratory at UC Davis conducted genetic analysis of water samples from the beach sites during one of the rain events (pre-, during, and post-rainfall) and one of the dry season events that were monitored for this study. UC Davis' creek samples were assessed to determine the occurrence of genetic markers for *Bacteroidales*. This additional microbial source tracking will provide information on the likelihood of fecal contamination from human, bovine, dog, and horse sources.

Analytical Methods

For the analysis of *Enterococcus*, 10 ml of the sample was pipetted to a sterile container of 90 ml de-ionized water. A packet of the Enterolert™ test kit (IDEXX Laboratories, Westbrook, Maine, USA) was mixed into the dilution. The sample was poured into an Idexx Quanti-Tray and then into a 41 °C incubator. Results were read after 24 hours. Reported counts were obtained from the Idexx Quanti-Tray 2000 MPN Table. The test method employed to detect *Enterococcus* is called Enterolert from Idexx. It uses the Defined Substrate Technology (DST). When B-glucosidase enzyme from the *Enterococcus* is mixed with 4-methyl umbellifery B-D-glucoside from the Enterolert test kit, the sample fluoresces. It can detect *Enterococcus* at 10 colony-forming units (cfu) per 100 mL. The reporting limit is 24,196 most probable number (MPN) per 100 mL.

For the analysis of total coliform and *E. coli*, a pouch of the Colilert® 18 test kit (IDEXX Laboratories, Westbrook, Maine, USA) was mixed into a 10 to 1 dilution sample. The sample was poured into a Quanti-Tray and was incubated at 35 °C. Results were read between 18 to 22 hours after incubation. Reported counts were obtained from the Idexx Quanti-Tray 2000 MPN Table. Colilert® 18 test kit uses the DST to detect total coliform and *E. coli*. Ortho-nitrophenyl-B-D-galactopyranoside (ONPG) from the Colilert® 18 test kit detects B-D-galactosidase enzyme from the total coliform bacteria by turning the sample to yellow. 4-methylumbelliferyl-B-D-glucuronide (MUG) from the test kit detects the enzyme B-glucuronidase produced by *E. coli* when the sample fluoresces. It can detect total coliform and *E. coli* at 10 cfu per 100/mL. The reporting limit is 24,196 MPN per 100 mL.

Results

FIB concentrations were generally elevated above guidelines at all sites during the wet and the dry season sampling events. Out of 78 samples collected, 73 (94%) exceeded the U.S. Environmental Protection Agency's (EPA) freshwater criterion for contact recreation for *Enterococcus* (U.S. EPA 2003) of 33 MPN/100 mL. Exceedances ranged from 52 to 24,196 MPN/100mL. *E. coli* concentrations exceeded the EPA criterion of 126 MPN/100 mL in 63 out of 78 samples (81%). Exceedances for *E. coli* ranged from 175 to 24,196 MPN/100 mL. Even though the EPA criterion is derived from the geometric mean of at least five samples collected over the course of 30 days, the exceedances at most sites in this study were so far above the criterion that potential adverse impact due to the high concentrations in the studied watersheds should remain a concern especially in areas where there is high potential for human exposure.

The five *Enterococcus* samples that did not exceed the criterion were all collected in the dry season (four samples on July 9, 2012 and one sample on August 15, 2012). Out of those five samples, two samples were collected from the central reach of Dean Creek, two samples were from the lower reach of Kanoff Creek (including the beach site), and one sample was from the upstream portion of Montara Creek. However, at these locations the low concentrations were not observed during both dry season events.

Out of the 15 *E. coli* samples that did not exceed the criterion, four were collected during the wet season (all four on January 20, 2012) and 11 were collected during the dry season (six in July and five in August). The lower reach of Martini Creek (including the beach site) and the lower and central reaches of Kanoff Creek showed lower concentrations in January. In July and August, Dean Creek seemed to have lower bacterial concentrations with four samples below the suggested criterion and D3 (central reach) repeating the low July concentration in August. Also, Ma2 (lower reach) repeated the low bacterial concentrations from January and July in August. Furthermore, the upper Montara Creek watershed showed bacterial concentrations below the EPA criterion in July and August.

Wet weather samples (high flow conditions) were usually higher in FIB concentrations than the dry weather samples (low flow conditions). This trend has been observed in previous studies (Giddings and Oblinger 2004, Kistemann et al. 2002). FIB concentrations were also higher during the second rain event of the wet season that was monitored. Precipitation later in the wet season that often concurs with

saturated soils will facilitate bacterial transport from the surrounding areas to the creeks. Additionally in this study, the second monitored storm was of higher intensity, with increased streamflow and higher creek stages, also aiding the transport of bacteria.

Martini Creek

Martini Creek had only two sampling sites due to the rural nature of the upper watershed and the difficulty for access. Upstream of site Ma2 the land use includes some open space, for example McNee Ranch State Park and Corral de Tierra, managed by the Golden Gate National Recreation Association. Pets are allowed on all trails and horses are permitted on designated trails. An equestrian facility is located immediately upstream and downstream of Ma2. Additionally, a small agricultural field is located downstream of Ma 2. Bacterial input into Martini Creek seemed to be less than in the other studied watersheds probably due to the lack of urbanization (Figure 2). The dry season samples (July 9 and August 15, 2012) showed a pattern that was expected to be more visible throughout this study, with lower bacterial concentrations in the upper watershed (*E. coli*: 10 and 20 MPN/100 mL, respectively and *Enterococcus*: 96 and 52 MPN/100 mL, respectively) and higher concentrations in the lower reaches and at the beach sites (*E. coli*: 259 and 41 MPN/100 mL and *Enterococcus*: 292 and 842 MPN/100 mL, respectively). This expectation is caused by the hypothesis that more urban bacterial sources in the downstream reaches of the creeks would lead to higher bacterial input. Even though there is no urban development in the Martini Creek watershed, the beach site of Martini Creek includes a popular parking lot with beach access that does not have any restroom facilities.

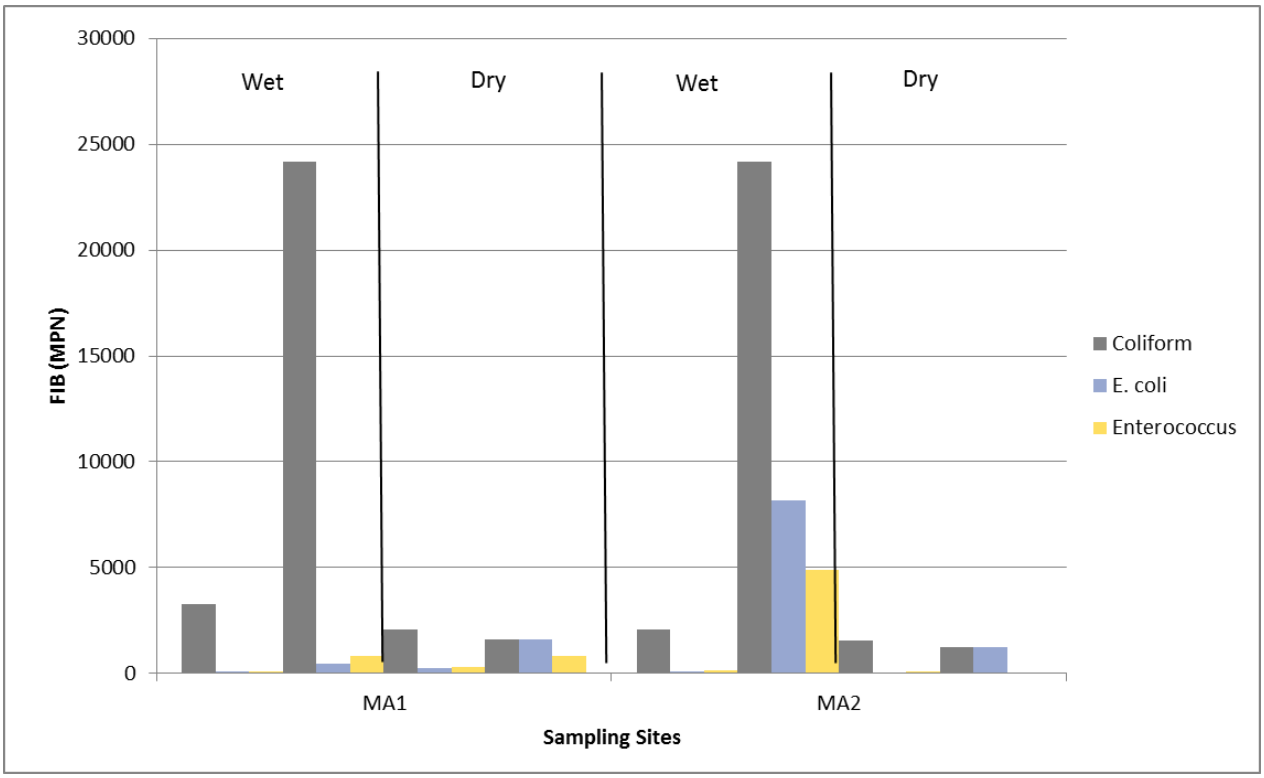

Figure 2. Fecal Indicator Bacteria in Martini Creek, displayed as most probable numbers. Ma1 indicates the beach site. The x-axis shows the sampling sites, the y-axis shows FIB concentrations in MPN/100 mL.

However, decreasing concentrations of *E. coli* and *Enterococcus* at the beach site of Martini Creek and the central reaches of other monitored creeks during the second rainy season event could be due to relatively clean subsurface runoff from agricultural irrigation that dilutes creek water in the vicinity of the sampling point or possibly relatively clean groundwater flows. Therefore, a conclusion to spatial and/or temporal bacterial loading in this watershed cannot be made.

Kanoff Creek

Four sites were sampled along Kanoff Creek with the central and upstream reaches of the creek touching the northern boundary of the community of Montara and draining urbanized areas. K1 is located at Montara State Beach, K2 and K3 include some open space to the north and residential areas immediately south of the sampling locations. K4 is located downstream of Mc Nee Ranch State Park, and confined animals are immediately located downstream of K4 and between K2 and K3. *E. coli* concentrations at the beach site (K1) were relatively low compared to K2 (approximately 0.25 mile

upstream) during the wet season (Figure 3). E. coli concentrations were also low at K3 compared to K4. These lower concentrations could be related to die off or reduced bacterial survival in water without any significant bacterial input into the creek upstream of the beach site K1 or K3. Meanwhile, concentrations for *Enterococcus* showed almost the opposite distribution. The upper watershed sites were dry during the dry season sampling events and the lower sites were low in comparison to wet season concentrations, with 50% of the samples being below EPA guidelines. Martini and Kanoff Creek in their entirety and Montara Creek in the uppermost watershed had lower bacterial concentrations than the other creek sites that were located within the urban boundaries of Montara and Moss Beach (Figure 7).

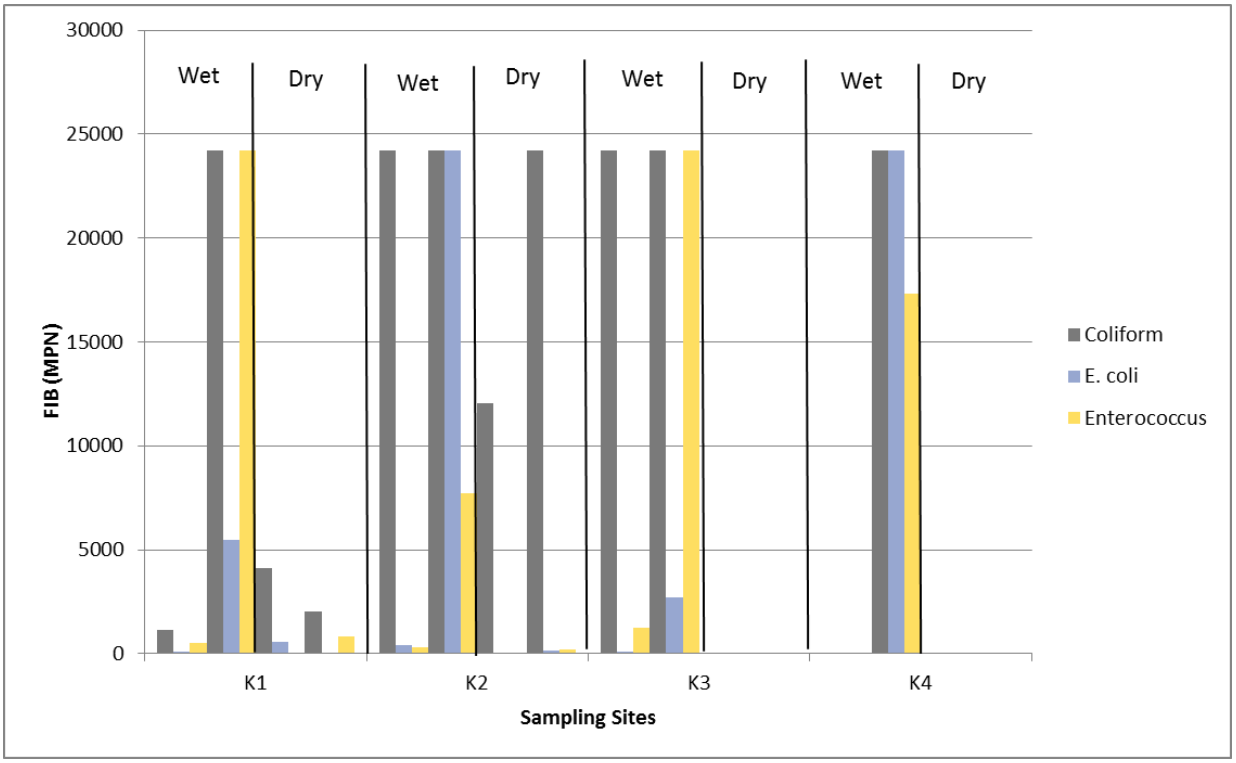

Figure 3. Fecal Indicator Bacteria in Kanoff Creek, displayed as most probable numbers. K1 indicates the beach site. Sites with no data were not collected during the dry season due to no flow in the creek bed. The x-axis shows the sampling sites, the y-axis shows FIB concentrations in MPN/100 mL. Note low *E. coli* concentrations at beach site during the wet season sampling.

Montara Creek

Montara Creek is one of the longer creeks monitored for this study. Samples were collected at five sites along the creek with the upstream site being located above the urban influence. Sites Mo1 through Mo4 drain residential areas in the community of Montara and the creek flows into the Pacific Ocean at the Montara Lighthouse. Open space is the dominant land use of the upper watershed, with limited agricultural fields and floriculture present in the upper watershed as well. Equestrian facilities are located between Mo1 and Mo2 and between Mo4 and Mo5. The sampling results from the Montara Creek sites showed a bacterial concentration pattern that indicates low bacterial presence in the upstream reaches, elevated bacterial presence in the central reaches, and high bacterial concentrations at the downstream sites (Figure 4). This is the pattern that was expected to show for most creeks due to higher bacterial loading in the urban areas from potential pet waste, leaking sewer lines, etc. (Jensen et al. 2003).

A six-times increase in *Enterococcus* and *E. coli* (Figure 8) concentrations was observed in Montara Creek between Mo2 (downstream reach) and Mo1 (beach site) during the first rainy season event. Additionally, a two to three-time increase was observed during both dry season sampling events. This was a longer stretch of the creek (about 0.5 miles long, between the south end of Cedar Street and the Montara Lighthouse) that should be investigated further for bacterial loading. This increase may be attributed to the increase in horse/confined animal facilities that are within this stretch between Mo2 and Mo1. These could be a potential source for bacteria but the source may also be related to pets, homeless encampments, or sewer/septic systems, which also occur in this reach.

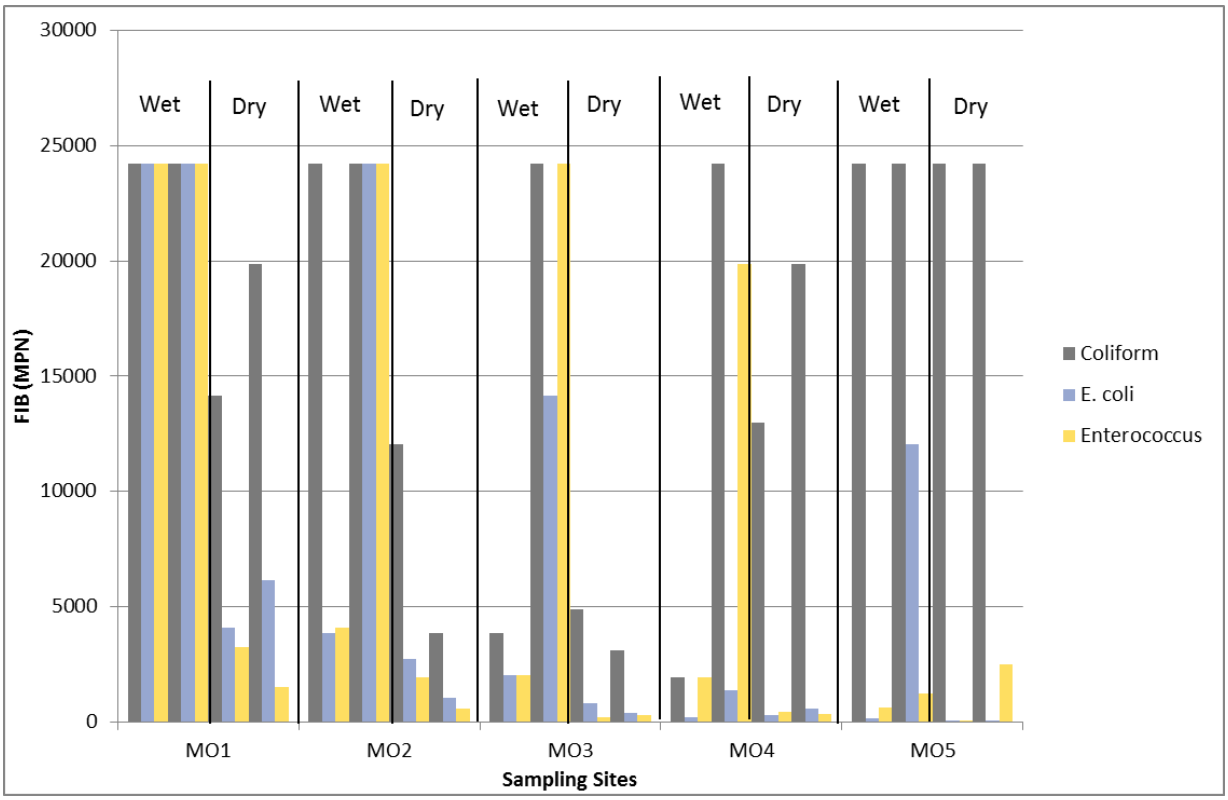

Figure 4. Fecal Indicator Bacteria in Montara Creek, displayed as most probable numbers. Mo1 indicates the beach site. The x-axis shows the sampling sites, the y-axis shows FIB concentrations in MPN/100 mL.

Dean Creek

Dean Creek parallels Sunshine Valley Road almost through its entire length with medium-density residential use between D4 and D1. Upstream of D4 very low-density residential use occurs. A small commercial area is located in the lower reaches of the creek (between D3 and D1). Equestrian facilities exist in the upper reach of the creek. High concentrations (greater than 24,196 MPN per 100 mL) of all three bacterial indicators were observed during the March wet season sampling at all but the upstream sampling site (Figure 5). The primary change in land use from site D5 to D4 is the increase in equestrian facilities along Sunshine Valley Road. However, trails and horse facilities are also present upstream of D5. Dry season sampling showed high concentrations of total coliform but not of *Enterococcus* and *E. coli*, except for the most upstream site that had high concentrations (greater than 24,196 MPN per 100 mL) for all three indicators during the July sampling event. Sites D4 and D5 in the upstream reach of the creek did not have any flow during the August sampling event, therefore the July peak in concentrations could not be confirmed.

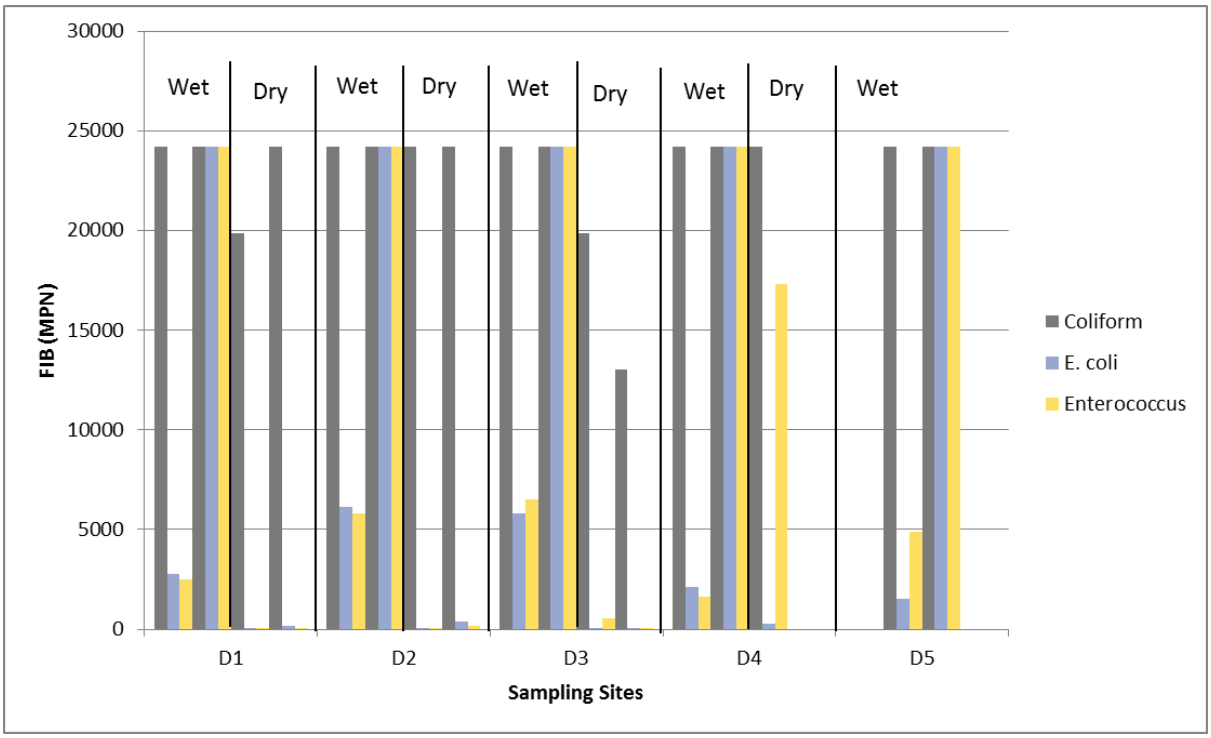

Figure 5. Fecal Indicator Bacteria in Dean Creek, displayed as most probable numbers. D1 indicates the beach site. Sites with no data were not collected during the dry season due to no flow in the creek bed. The x-axis shows the sampling sites, the y-axis shows FIB concentrations in MPN/100 mL.

San Vicente Creek

San Vicente Creek is, together with Montara Creek, one of the longer creeks monitored in this study. The upper watershed of San Vicente Creek is mostly open space. Agricultural use and row crops are present in the watershed upstream of V5. Like Dean Creek, San Vicente Creek also has equestrian facilities upstream of V5 and downstream of V4, in addition to trails and reservoirs. Similar to Dean Creek, San Vicente Creek showed high concentrations of all three indicator bacteria during the March sampling event except for the most upstream location (Figure 6). For all other sampling events *Enterococcus* input seemed to be elevated at V2 and V3 and decreasing toward the beach site (V1) again. However, the beach site was still far above the federal standard (between 45 to 690 times higher) recommended for safe water contact for all sampling events.

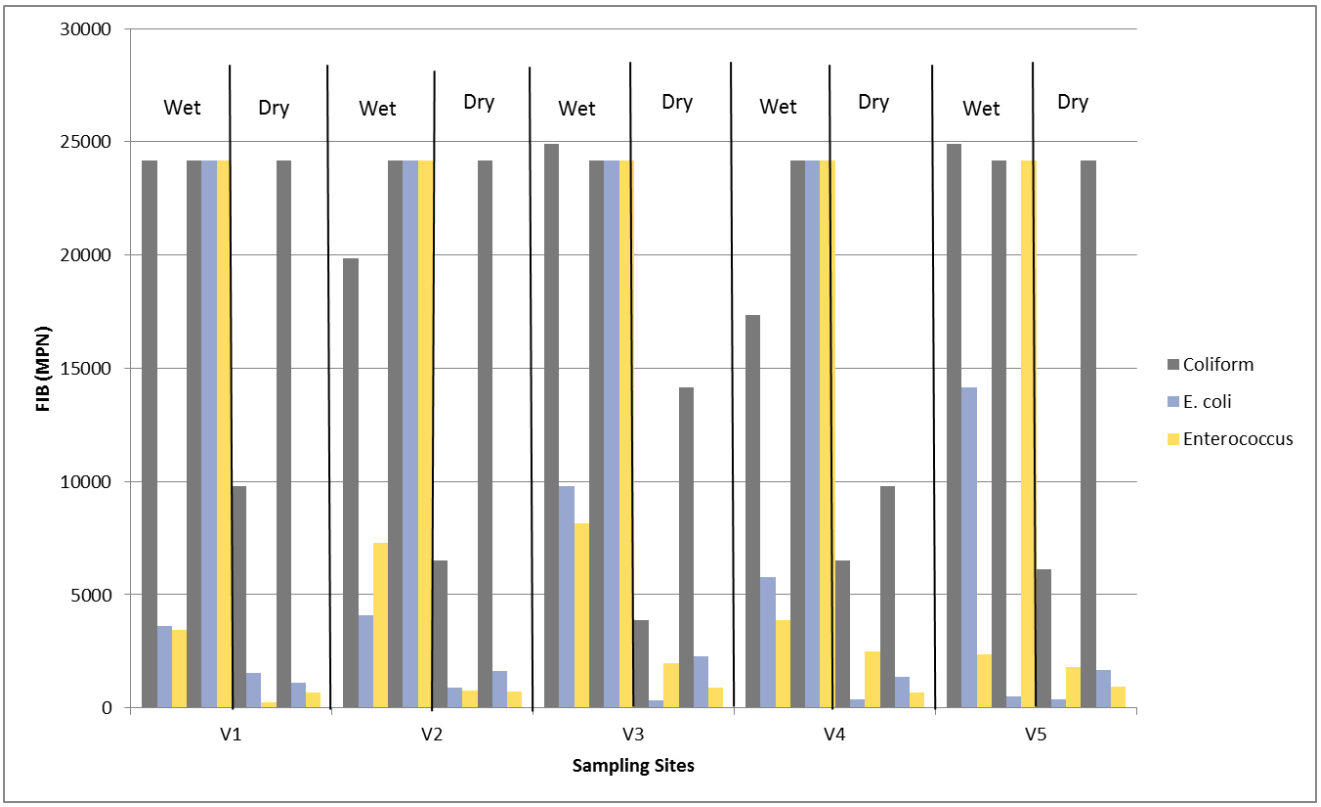

Figure 6. Fecal Indicator Bacteria in San Vicente Creek, displayed as most probable numbers. V1 indicates the beach site. The x-axis shows the sampling sites, the y-axis shows FIB concentrations in MPN/100 mL.

Another notable observation was that during the January sampling event the *E. coli* concentration at V5 was much higher than the *Enterococcus* concentration (14,136:2,382) (Figure 7 and 8), suggesting that the bacteriological contamination originates rather from an animal source than a human source. However, the recognition that the number of fecal streptococci (which include *Enterococcus*) in animal feces is considerably higher than fecal coliform numbers (which include *E. coli*) is highly debated. The second wet season event in March showed very high concentration for *E. coli* and *Enterococcus* at almost all sites along San Vicente Creek.

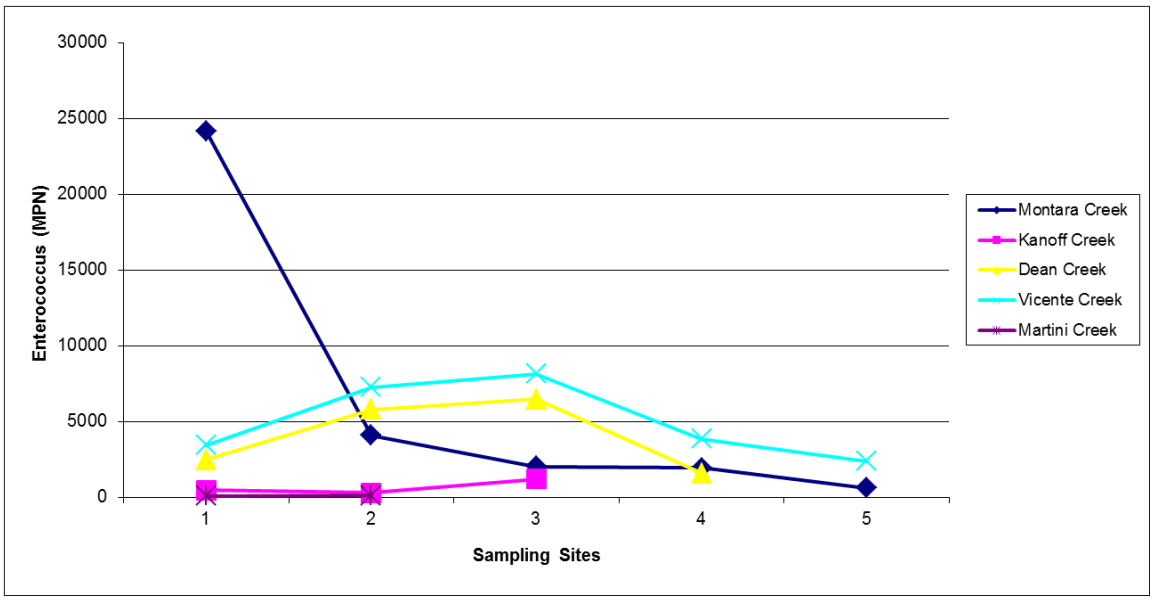

Figure 7a. *Enterococcus* concentrations on January 20, 2012. Creeks sites ranging from 5 (upstream) to 1 (beach sites).

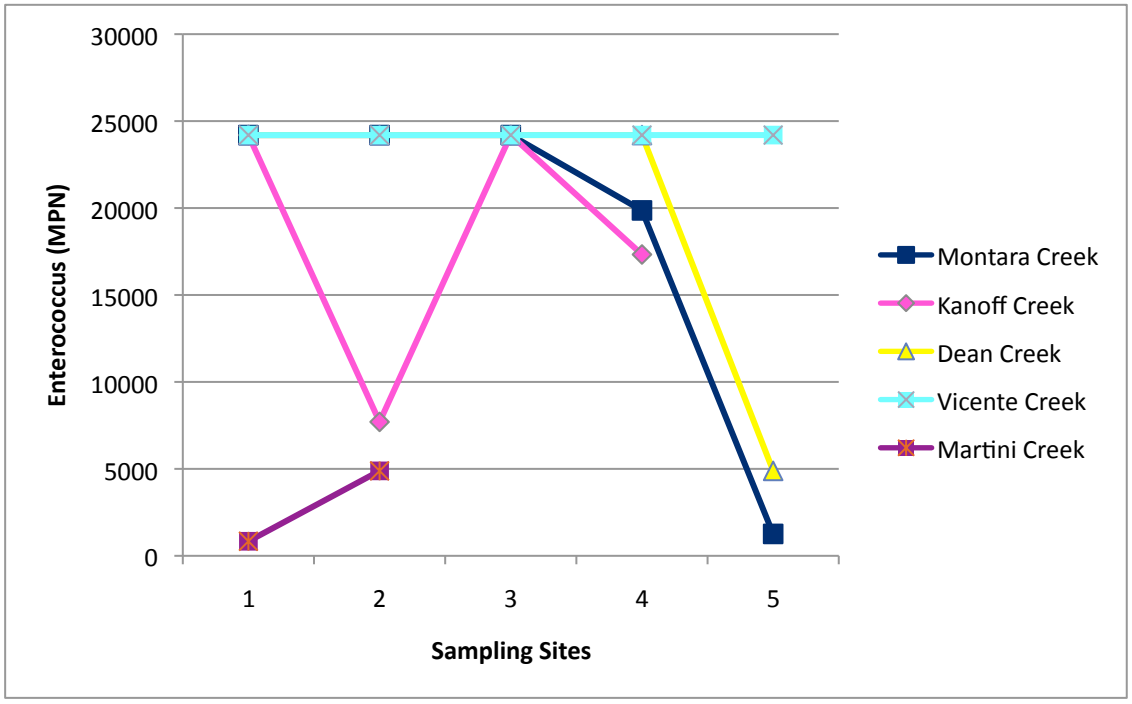

Figure 7b. *Enterococcus* concentrations on March 14, 2012. Creeks sites ranging from 5 (upstream) to 1 (beach sites). San Vicente Creek data for sites 1 and 2 overlap results from Montara Creek.

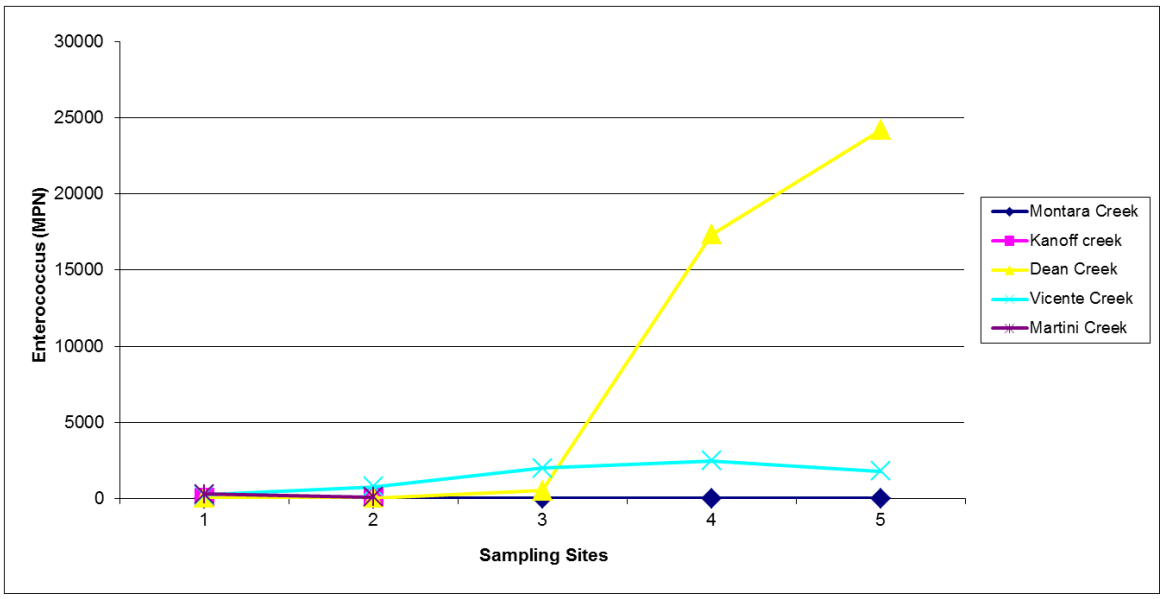

Figure 7c. *Enterococcus* concentrations on July 9, 2012. Creeks sites ranging from 5 (upstream) to 1 (beach sites).

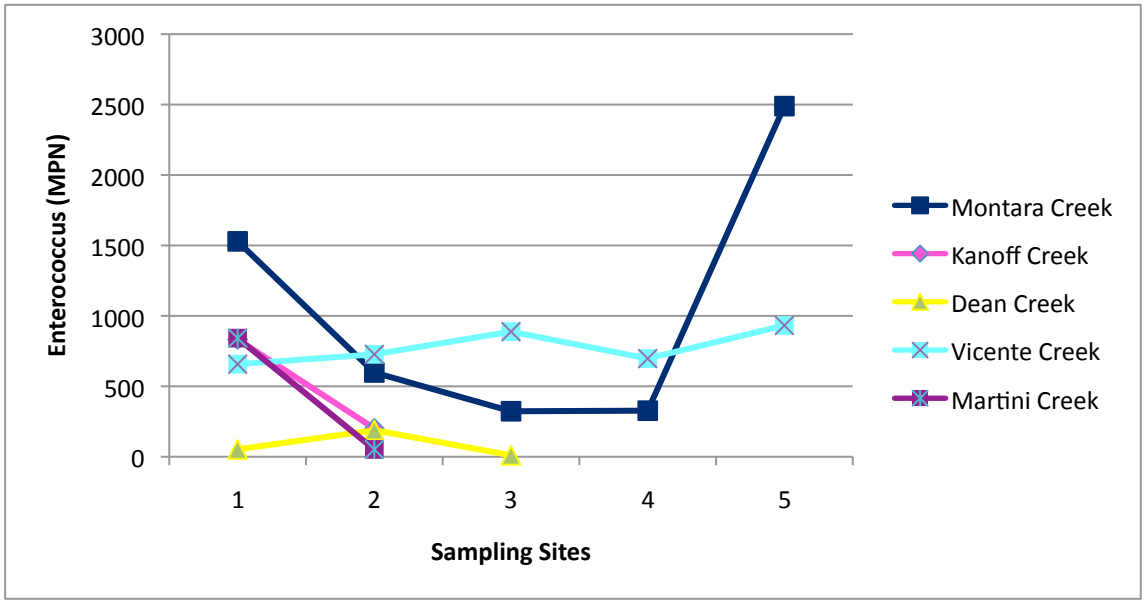

Figure 7d. *Enterococcus* concentrations on August 15, 2012. Creeks sites ranging from 5 (upstream) to 1 (beach sites).

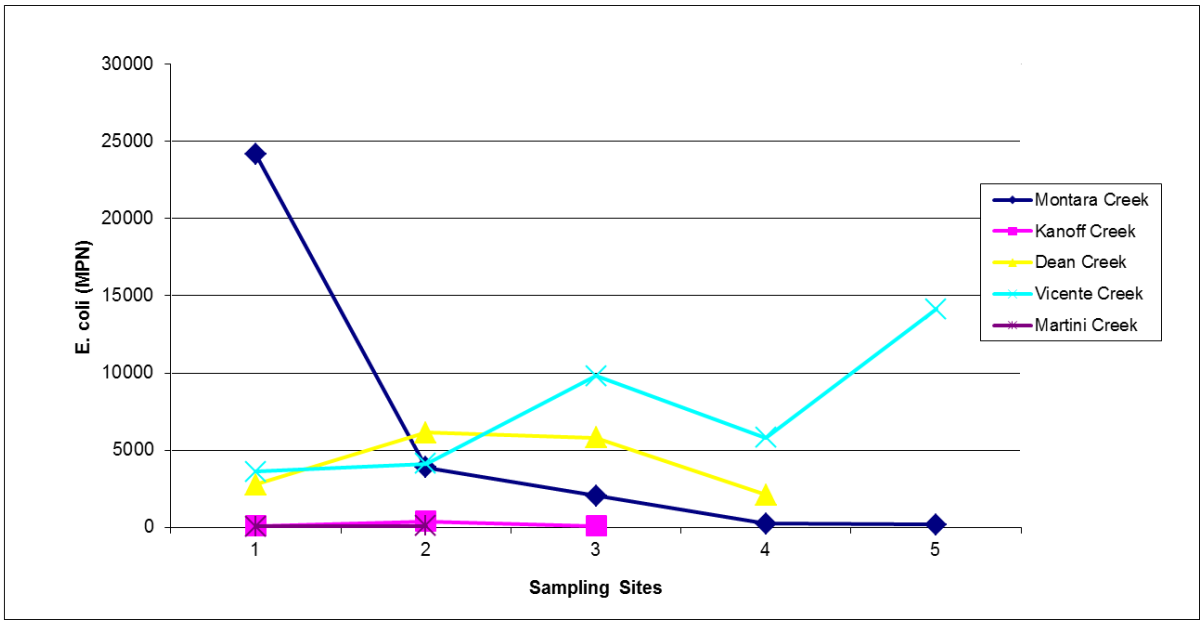

Figure 8a. *E. coli* concentrations on January 12, 2012. Creeks sites ranging from 5 (upstream) to 1 (beach sites).

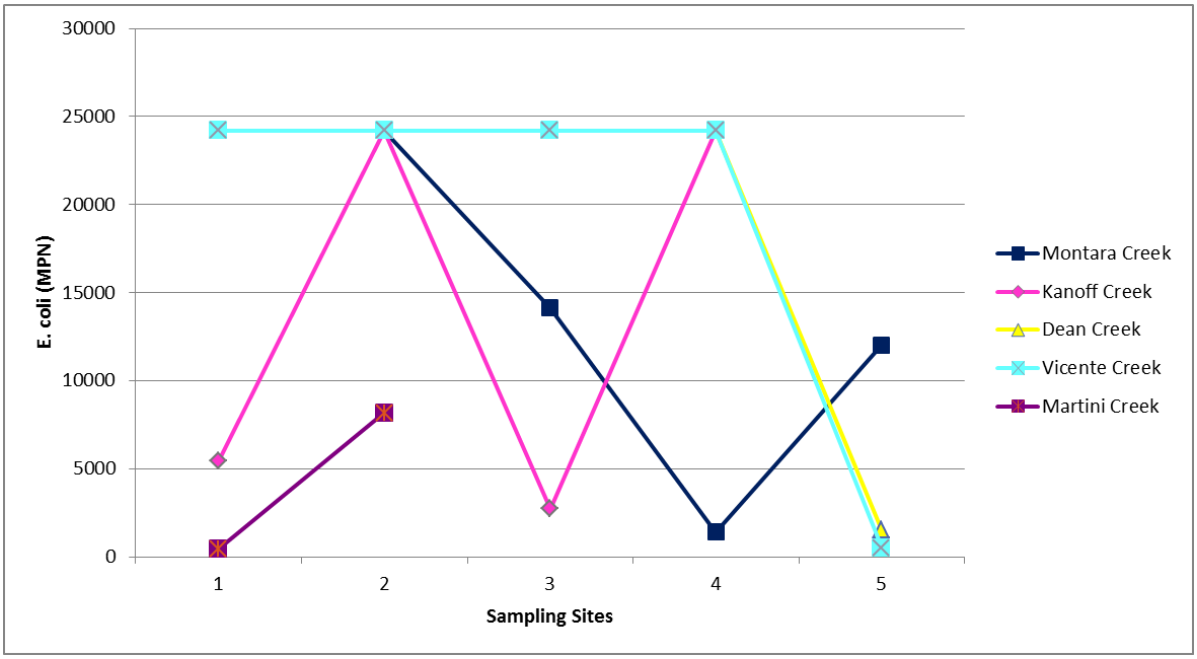

Figure 8b. *E. coli* concentrations on March 14, 2012. Creeks sites ranging from 5 (upstream) to 1 (beach sites). San Vicente Creek data for sites 1 and 2 overlap results from Montara Creek.

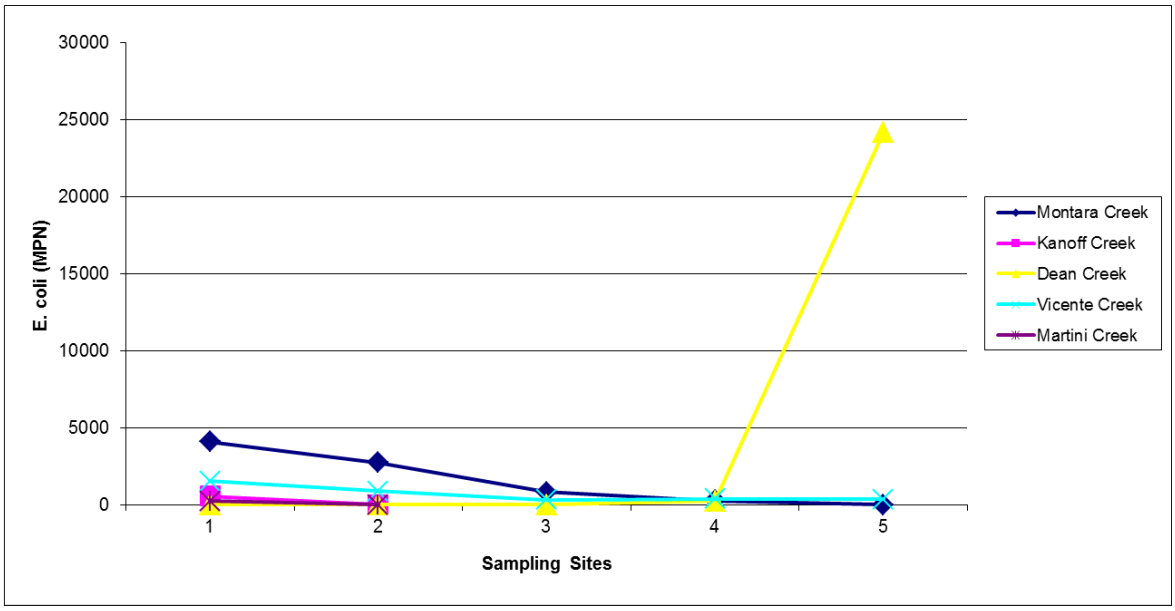

Figure 8c. *E. coli* concentrations on July 9, 2012. Creeks sites ranging from 5 (upstream) to 1 (beach sites).

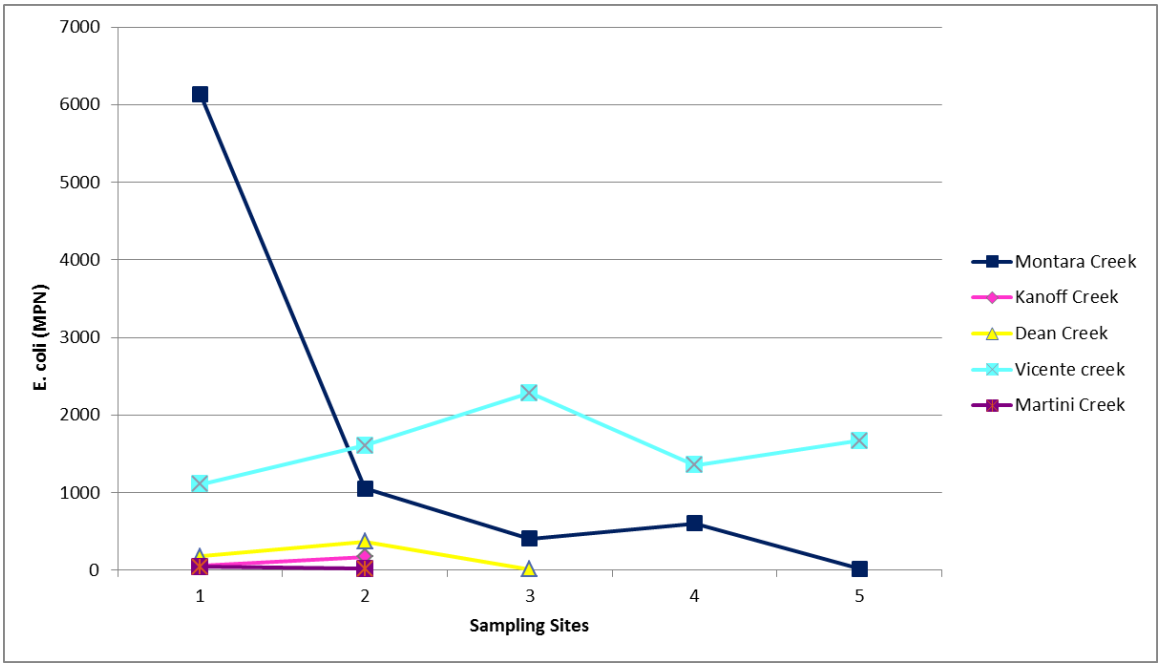

Figure 8d. *E. coli* concentrations on August 15, 2012. Creeks sites ranging from 5 (upstream) to 1 (beach sites).

Conclusion

FIB concentrations exceeded EPA recommended standards at most sites during both the dry and the wet season. However, the concentrations were generally lower in the dry season. The bacterial concentrations in Martini and Kanoff Creek, the less urbanized watersheds, were generally lower than in the more urbanized watersheds.

The dynamic of bacterial loading in creeks traversing a rural to urban gradient is very complex and cannot be fully understood using the sampling design employed during this study. However, the study results presented demonstrate that concerns about possible impacts to humans and wildlife exposed may be warranted if the mechanisms for exposure or sources are not controlled. The results suggest that a bacterial tracer experiment would be beneficial in identifying possible sources of spatial and temporal bacteria loading. By introducing a bacteria tracer, using a unique soil bacteria at a specific concentration, attenuation of bacteria and spatial distribution could be quantified. In combination with stream gauges, loads of bacterial contamination could be calculated canceling out the effects of dilution from subsurface drainage, tributary influent, and groundwater interactions. A tracer experiment including flow data would cancel out some of the variation and noise that the data exhibited during this study.

One notable change in bacterial loading potentially occurred in Montara Creek between the south end of Cedar Street and the Montara Lighthouse. During the January sampling event concentrations for *Enterococcus* and *E. coli* were at least six times higher when creek water reached the beach at the Lighthouse. A two to three-time increase was observed between those sites during both dry season sampling events. Further investigation to address the source of this bacterial loading is recommended, especially since this is a stretch of Montara Creek between the communities of Montara and Moss Beach that is not densely populated with probably less than 5% impervious surfaces.

Genetic source tracking of bacteria at the farthest downstream sites for all five creeks was conducted by UC Davis. The samples for the UC Davis study and this study were collected concurrently during one rainy season and one dry season event. Additional information may be provided by combining the results of both studies.

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JAMES V. FITZGERALD AREA OF SPECIAL BIOLOGICAL SIGNIFICANCE POLLUTION REDUCTION PROGRAM

MICROBIAL SOURCE TRACKING STUDY SUMMARY REPORT

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February 2013

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of variance
ASBS	Area of special biological significance
BMP	Best management practice
cm	Centimeter
FIB	Fecal indicator bacteria
g	Gram
gc	Gene copies
L	Liter
µ	Micro
m	Milli or meter
MST	Microbial source tracking
PMA	Propidium monoazide
QA/QC	Quality assurance/quality control
qPCR	Quantitative polymerase chain reaction
RNA	Ribonucleic acid
RPD	Replicate percent difference
S	Siemens
SFEI	San Francisco Estuary Institute
S_{LOD}	Sample limit of detection
UCD	University of California, Davis

Abstract

The James V. Fitzgerald Marine Reserve (Reserve) is a biologically significant habitat for diverse species as well as a popular recreational area. However, impaired water quality due to fecal contamination has been reported at the Reserve and San Vicente Creek. We conducted a microbial source tracking (MST) study based on genetic analysis of host-associated *Bacteroidales* to determine the contribution of human, bovine, dog, and horse sources to fecal contamination in the five watersheds draining to the Pacific Ocean in the vicinity of the Reserve. A total of 58 samples from water, sediment, and biofilm matrices were collected during 7 monitoring events in 2012.

Universal *Bacteroidales* derived from all warm-blooded animals were detected at all sites in 7 sampling events. Concentrations of universal marker in water were elevated during rain in the wet season and first flush events compared to concentrations before and after rain. In the dry season, universal *Bacteroidales* concentrations in water at all sites were generally lower than those in the wet season and first flush sampling events. Biofilm samples usually contained higher universal marker concentrations compared to sediment samples collected at the same sites. Human-associated *Bacteroidales* were not often detected in the wet and dry seasons but were prevalent in water throughout the first flush events. Bovine-associated *Bacteroidales* were found in water at Kanoff and San Vicente Creek during rain in the wet season. Bovine marker was frequently found in sediments or biofilm at all sites throughout the wet season. Dog-associated *Bacteroidales* were the most frequently detected host marker in water as well as in sediments and biofilm at all sites in the wet season. On the contrary, dog marker was less frequently detected in dry and first flush events. Horse-associated *Bacteroidales* were found at high concentrations in water at Dean and San Vicente Creek during rain in the wet season. During the dry season, the horse marker was detected at all sites, but it was not a predominant fecal source in water. The horse marker was more often detected in sediment and biofilm samples than in water samples.

The monitoring study provides good insights into the prevalence of host-associated *Bacteroidales* at five creeks draining into the Reserve. Future monitoring studies that can distinguish host-associated *Bacteroidales* DNA from intact and impaired cells could allow

determination of the age of fecal pollution and may provide more information about potential health risks.

1. Introduction

The James V. Fitzgerald Marine Reserve (Reserve) is a biologically significant habitat for diverse species as well as a popular recreational area. However, impaired water quality due to fecal contamination has been reported at the Reserve and San Vicente Creek. As part of the monitoring plan of this Area of Special Biological Significance (ASBS) Pollution Reduction Program, we conducted a microbial source tracking (MST) study based on genetic analysis of *Bacteroidales.* The main goal was to provide information about the primary sources of fecal contamination in the five watersheds draining to the Pacific Ocean in the vicinity of the Reserve and to assist in the selection of the appropriate best management practices (BMPs) to reduce fecal pollution. A total of 58 samples (excluding QA/QC samples) from water, sediment, and biofilm matrices were collected at the five creeks during 7 monitoring events in 2012. All samples were analyzed to determine the prevalence of universal, human-, bovine-, dog-, and horse-associated *Bacteroidales*. In addition, the probabilistic model developed at University of California, Davis (UCD) was applied to estimate the true value of host-associated *Bacteroidales*. Fecal indicator bacteria (FIB) including total coliforms, *Escherichia coli*, and *Enterococcus* spp. were monitored concurrently with MST by the San Francisco Estuary Institute (SFEI).

2. Materials and methods

2.1 Sample collection and processing

Water samples were collected from the five creeks draining to the Pacific Ocean in the vicinity of the Reserve including Martini, Kanoff, Montara, Dean, and San Vicente Creeks (Figure 1 and Table 1). In order to capture all potential sources, samples were collected immediately upstream of the creek confluences with the Pacific Ocean. Water samples were collected 7 times through the wet season (March), dry season (July), and first flush events (October) in 2012. In the wet season and first flush sampling events, water samples were collected at three stages based on rainfall conditions (pre-, during-, and post-rain). At every sampling event, one field blank and one field duplicate sample were produced for quality assurance/quality control (QA/QC) purposes. Water grab samples (10 to 20 L) were obtained by directly submerging sample carboys or by using sterile scoops just below the water surface after pre-rinsing in the creek. Sediment and biofilm (submerged aquatic vegetation) samples were collected using sterile spoons and placed in sterile bottles (Figure 2). Additional surface water was filled into the bottle to minimize oxygen contact and dehydration of sediment and biofilm samples. Water quality parameters including temperature, pH, conductivity, salinity, and dissolved oxygen concentration were measured *in situ*. Total suspended solids (TSS) were determined using a Standard Method 2540D (APHA 1998). After collection, samples were kept chilled with ice packs and transported to the laboratory within 6 hours. Upon arrival, the samples were kept at 4ºC in a temperature-controlled room followed by processing within 48 hours. For better quantification of nucleic acids, 10 to 20 L water samples were concentrated to approximately 150 mL using a Fresenius hollow fiber ultrafiltration (HFF) system (Rajal et al. 2007). The surrogate *Acinetobacter baylyi* ADP1 was added into all water samples to calculate filtration recoveries by comparing concentrations of *A. baylyi* in subsamples of pre-filtration (feed) and post-filtration (retentate) samples.

Sediment samples were processed by adding 50 g of sediments and 50 mL of 1% Tween 80/NaOH, pH 7.0 solution in a 250-mL sterile bottle followed by vigorous hand-shaking of the mixture for 2 min to elute microorganisms attached to sediment surfaces. After 10 min of deposition of suspended particles in water, the supernatants were collected and used for quantitative polymerase chain reaction (qPCR). The DNA concentrations in supernatants were

converted to those of dry weight of sediments. Biofilm samples were processed in the same manner. Dry weights of sediments and biofilm were determined after drying at 105ºC for 24 hours.

Figure 1. A map of the James V. Fitzgerald ASBS and watersheds. Numbers denote MST sampling locations.

Table 1. Latitude and longitude of MST sampling sites monitored in 2012

Site number	Site name	Latitude	Longitude
1	Dean	37.52559298300	-122.51649793800
2	Kanoff	37.54827006100	-122.51351154200
3	Martini	37.55248063700	-122.51227370900
4	Montara	37.53702965100	-122.51870163100
5	Vicente	37.52409674000	-122.51749720200

Figure 2. Photos of collected biofilm samples (submerged aquatic vegetation) at Kanoff Creek (A) and Montara Creek (B)

2.2. Nucleic acid extraction

Nucleic acids of a 500-µL aliquot from feed and retentate water samples were extracted using the Invitrogen PureLink Viral RNA/DNA extraction Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Final eluted volume of DNA was 100 µL. For sediments and biofilm samples, DNA in an aliquot of supernatant ranging from 10 to 40 mL was extracted using the UltraClean Water DNA isolation kit (Mo Bio Laboratories INC. Carlsbad, CA) with a 0.22-µm sterile membrane filter according to the manufacturer's protocols. Phenol-Chloroform DNA purification was applied to all sediment and biofilm samples and part of water samples after nucleic acid extraction.

2.3. *Bacteroidales* and *Acinetobacter* qPCR

TaqMan qPCR assays for *Bacteroidales* and *Acinetobacter* targeting the 16S rRNA (Kildare et al. 2007; Schriewer et al. 2010; Silkie and Nelson 2009) were performed using a StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA). Assays for universal *Bacteroidales* derived from all warm-blooded animals as well as human-, bovine-, dog-, and horse-associated

Bacteroidales were applied to identify host-specific contributions to the fecal contamination of monitored sites. The *Acinetobacter* assay was used to calculate filtration recoveries by measuring concentrations of *Acinetobacter* in feed and retentate samples. Each 25-µL qPCR reaction volume contained 12.5 µL of TaqMan Environmental Master Mix 2.0 (Applied Biosystems, Foster City, CA), 10 µL of nucleic acid extract, and optimized concentrations of forward and reverse primers and probe. Thermal cycling conditions were 2 min at 50ºC and 10 min at 95ºC, followed by 40 cycles of 15 s at 95ºC and 1 min at 60ºC. For the horse assay, the 40 cycles were modified to 15 s at 92ºC and 1 min at 60ºC. A serial dilution approach was employed for each sample to mitigate potential inhibitors during qPCR reaction. The recoveries and sample limits of detection (S_{LOD}) of *Acinetobacter* and *Bacteroidales* genetic markers were calculated (Schriewer et al. 2010).

2.4 Statistical analysis

Data were analyzed using Excel 2007 (Microsoft Corp.). The probabilistic model (Wang et al. 2010) developed at UCD was applied to estimate true concentrations of host-associated *Bacteroidales* using the statistical package R (http://www.r-project.org/). The model was validated only for BacUni, BacHum, BacCow and BacCan assays (Kildare et al. 2007) and not the horse assay. Hence the data for horse-associated *Bacteroidales* measured in this study were not adjusted using the model. Analysis of variance (ANOVA) was used to investigate variations in the data between sites and sampling phases using SPSS Statistics 20.0 (Chicago, Illinois, USA). Differences were considered significant when the *p*-value was less than 0.05.

3. Results

Seven environmental monitoring events at the five creeks draining into the Pacific Ocean in the vicinity of the Reserve were carried out during the period of March 2012 through October 2012 (Table 2). Some environmental samples could not be collected due to conditions such as no streamflow during the dry season or heavy streamflow during the wet season, which was unsafe for sampling. FIB results from wet and dry seasons will be included in the SFEI report as part of pilot BMP water quality monitoring. FIB monitoring results throughout the 7 sampling events were used for reference but not included in this report.

3.1. *Bacteroidales* in surface water

Universal *Bacteroidales* were detected at all sites in 7 sampling events at concentrations ranging from 1.1 x 10^2 to 2.5 x 10^5 gc/mL (Tables 3 and 4). In the wet season events performed in March 2012, concentrations of universal marker during rain were significantly increased at all five creeks compared to concentrations before and after rain (ANOVA, $p < 0.05$). Approximately 10 to 500 times elevated concentrations of universal marker were observed at every site during rain. In addition, in the first flush sampling events conducted in October 2012, universal marker concentrations were up to 10-fold higher during first flush rain compared to those in the pre- and post-rain events. However, the differences between the three event types (pre-, during-, and post-rain) were not significant (ANOVA, $p = 0.08$). There were approximately 2.5 – 4 cm (3/14/12) and 1.5 - 3 cm (10/22/12) of rainfall on the days of wet season rain and first flush rain , respectively, in the vicinity of the Reserve according to Community Collaborative Rain, Hail, and Snow Network (http://www.cocorahs.org). In the dry season, universal *Bacteroidales* concentrations at all sites were generally lower than those in the wet season and first flush sampling events. According to the spatial distribution of universal *Bacteroidales,* universal marker concentrations were usually highest at Dean Creek and lowest at Martini Creek throughout the wet season samplings. In the dry season, Montara Creek showed the highest universal marker concentrations compared to other sites. In the first flush events, highest universal *Bacteroidales* concentrations were found at Montara (before and during the first flush rain) and San Vicente Creek (after the first flush rain).

Human-associated *Bacteroidales* were detected at Dean (326 gc/mL, pre-rain) and Montara Creek (66 gc/mL, during-rain) in the wet season. The concentration of human marker at Dean Creek contributed approximately 10% of universal *Bacteroidales* at the creeks. Except for the two sites, human marker was hardly detected in the wet season sampling events. In the first flush events, human-associated *Bacteroidales* were detected at all sampling sites during and after first flush rain. The concentrations ranged from 3.6 gc/mL at Montara Creek to 561 gc/mL at Kanoff Creek.

Bovine-associated *Bacteroidales* were detected at Kanoff and San Vicente Creek during rain in the wet season. In San Vicente Creek, bovine-associated *Bacteroidales* contributed a significant amount (44%) of universal *Bacteroidales* during the rain. However, bovine marker was not found in the dry season and first flush events, and the S_{LOD}s were lower than 5 gc/mL for the events.

Dog-associated *Bacteroidales* were the most frequently detected host marker at all sites in the wet season samplings, and its concentrations were also higher than those of other host markers. When it rained in the wet season, dog marker concentrations ranged from 3.2×10^3 to 1.5×10^5 gc/mL. On the contrary, dog-associated *Bacteroidales* were rarely detected in dry and first flush samples. The only sites that were positive for the dog marker in dry and first flush events were Montara and Dean Creek, respectively.

Horse-associated *Bacteroidales* were detected at high concentrations at Dean (3080 gc/mL) and San Vicente Creek (1079 gc/mL) when it rained in the wet season. In the dry season sampling event, horse marker was found at all sites; however, the concentration range was between 3.9 and 51 gc/mL, which contributed less than 5% of universal *Bacteroidales* at each site.

The bird-associated *Catellicoccus marimammalium* assay (Lu et al. 2009) was applied to wet season samples to investigate the prevalence of bird marker in water samples; however, all samples tested were negative (data not shown). The S_{LOD}s ranged from 0.7 to 24 gc/mL. The bird assay was not used for samples collected in dry and first flush events.

Among the 7 field blanks, universal marker was detected once in a blank sample of the wet season during rain sampling event (Table 6). A carryover might have occurred during the

filtration of samples with highest concentrations of the universal *Bacteroidales* detected in the wet season during rain events. However, the concentration was orders of magnitude lower than concentrations in samples collected at all sampling sites in the event. None of the host-associated markers was detected in any field blanks. The variability of field duplicates was calculated by comparing the concentrations of paired environmental samples and associated field duplicate samples (Table 7). High variability over 100% of replicate percent difference (RPD) was found in the human marker concentrations of the field duplicate 2. Relatively low concentrations of human-associated *Bacteroidales* in the water sample and duplicate (less than 100 gc/ml) could result in the high RPD. The contribution of human source to universal marker was less than 1% at the site. In addition, horse marker was detected in a sample of the post-first flush event but not in its duplicate (duplicate 7); the measured concentration in the positive sample was very low at 11 gc/mL.

3.2. *Bacteroidales* in sediments and biofilm

Throughout the wet and dry season events, a total 19 of sediment and 5 of biofilm samples were collected and analyzed (Table 4). Three biofilm and one sediment sample could not be collected in the wet season due to elevated water depth and heavy water flow. Universal *Bacteroidales* were detected in all samples. Biofilm samples usually contained higher universal marker concentrations compared to sediment samples collected at the same sites (ANOVA, $p < 0.05$), ranging from 2.7×10^3 to 9.9×10^5 gc/g of dry weight of biofilm. At all sites but Martini Creek universal *Bacteroidales* concentrations in sediments increased when it rained in the wet season. When rain stopped, the levels of universal marker in sediments decreased compared to those during rain; however, Dean and Montara Creek showed dramatically elevated concentrations of universal *Bacteroidales* in sediments after rain. In the dry season, all sites but Montara were found to have relatively high universal marker in sediments compared to surface water (ANOVA, $p < 0.05$).

Human-associated *Bacteroidales* were only detected in sediments at Dean Creek before and after rain during the wet season, which was consistent with results for surface water samples. However, the contribution of the human marker in sediments to the universal signals at the creek was less than 10%. In the dry season, the human marker was not found in any sediment or

biofilm samples. (Note that sediment and biofilm samples were not collected during first flush events).

Bovine-associated *Bacteroidales* were frequently found at all sites throughout the wet season ranging from 16 to 18660 gc/g of dry weight of sediment or biofilm. Moreover, the bovine marker was a dominant source of fecal pollution in sediments at Kanoff (during rain), Montara (after rain) and San Vicente Creek (during and after rain) in the wet season. Interestingly, bovine-associated *Bacteroidales* in water samples were only detected during rain but not before and after rain. During the dry season, bovine-associated *Bacteroidales* were not detected in any sediment or biofilm samples.

Dog-associated *Bacteroidales* were also commonly detected in sediments and biofilm at all creeks except at Dean Creek in the wet and dry seasons. The dog marker contributed a significant amount of universal *Bacteroidales* in sediments and biofilm at many sites. The highest concentrations were detected in biofilm at Kanoff (24337 gc/g of dry weight, pre-rain) and Montara Creek (16475 gc/g of dry weight, post-rain). The levels of dog marker in the overlying water, which was sampled concurrently with sediment and biofilm samples at Kanoff or Montara Creek, were not significant (less than 500 gc/mL). This result shows that previously introduced dog-associated *Bacteroidales* could be accumulated and could persist longer when associated with sediments and biofilm.

Horse-associated *Bacteroidales* were consistently detected at Martini, Montara, and San Vicente Creek in both the wet and dry seasons. Horse marker was more frequently detected in sediment and biofilm samples than in water samples. The range of detected horse-associated *Bacteroidales* concentrations at all sites was from 13 to 1861 gc/g of dry weight of sediments or biofilm.

Table 2. MST sampling schedule and water quality parameters

Event	Date	Site	Matrix[A]	Temp. (ºC)	pH	Conduc.[C] (µS)	Salinity (ppt)	DO[D] (mg/L)	TSS[E] (mg/L)
1	03/12/12	Dean	W, S	11.8	8.08	979	0.7	10.25	n.d.[F]
Wet season		Kanoff	W, S, B	14.4	7.89	549	0.3	10.03	17.6
(pre-rain)		Martini	W, S	10.3	8.59	177	0.1	11.20	n.d.
		Montara	W, S, B	11.0	8.58	512	0.3	12.03	16.0
		Vicente	W, S	11.6	8.34	242	0.2	10.72	n.d.
2	03/14/12	Dean	W, (S)[B]	13.5	8.26	247	0.2	10.37	527.5
Wet season		Kanoff	W, S, (B)	12.6	7.70	224	0.1	10.58	112.5
(during-rain)		Martini	W, S	12.7	7.94	192	0.1	10.92	290.0
		Montara	W, S, (B)	12.1	8.14	146	0.1	10.80	1170.0
		Vicente	W, S	12.5	7.81	187	0.1	10.51	1028.0
3	03/20/12	Dean	W, S	10.3	7.89	675	0.5	10.97	7.1
Wet season		Kanoff	W, S, (B)	10.4	7.94	513	0.3	10.91	n.d.
(post-rain)		Martini	W, S	10.4	7.91	175	0.1	11.70	9.0
		Montara	W, S, B	9.9	8.09	309	0.2	11.84	13.1
		Vicente	W, S	10.5	8.23	200	0.1	11.33	87.6
4	07/09/12	Dean	W, S	13.8	7.88	645	0.3	9.94	26.2
Dry season		Kanoff	W, S, B	15.5	7.52	441	0.3	10.72	7.9
		Martini	W, S	13.8	7.49	219	0.1	11.35	n.d.
		Montara	W, S, B	13.8	7.67	580	0.2	10.89	26.3
		Vicente	W, S	13.8	7.31	446	0.2	10.51	n.d.
5	10/21/12	Dean	(W)						
First flush		Kanoff	W	16.3	7.95	358	0.2	9.77	25.6
(pre-rain)		Martini	W	14.2	7.91	194	0.1	10.65	n.d.
		Montara	W	14.2	7.98	745	0.5	10.18	62.1
		Vicente	W	13.7	7.70	275	0.2	10.19	17.0
6	10/22/12	Dean	W	13.2	8.80	137	0.1	10.52	715.8
First flush		Kanoff	W	12.7	7.64	376	0.2	9.94	69.2
(during-rain)		Martini	W	12.9	7.79	211	0.1	10.61	12.7
		Montara	W	13.1	7.57	334	0.2	10.48	379.0
		Vicente	W	12.2	7.45	315	0.2	10.01	132.0
7	10/29/12	Dean	W	14.5	7.86	1313	0.8	9.17	10.8
First flush		Kanoff	W	16.6	7.65	421	0.2	9.74	8.8
(post-rain)		Martini	W	14.8	7.90	200	0.1	10.51	n.d.
		Montara	W	14.9	7.78	709	0.4	10.20	n.d.
		Vicente	W	14.4	7.66	263	0.2	9.95	13.0

[A] Environmental samples collected at the sites (W: water; S: sediments; B: biofilm).

[B] Samples in parenthesis were initially planned but not collected due to severe weather conditions

[C] Conductivity

[D] Dissolved oxygen

[E] Total suspended solids

[F] n.d. not detected. Laboratory limit of detection was 5 mg/L.

Table 3. Concentrations and sample limits of detection for host-associated *Bacteroidales* in water at creeks in wet and dry season

Sampling date	Site	Universal *Bacteroidales*		Human *Bacteroidales*		Bovine *Bacteroidales*		Dog *Bacteroidales*		Horse *Bacteroidales*	
		Concn[A]	S_{LOD}[B]	Concn	S_{LOD}	Concn	S_{LOD}	Concn	S_{LOD}	Concn	S_{LOD}
		(gene copies per mL)									
03/12/12	Dean	3110.7	1.2	325.8	0.7	n.d.	0.7	n.d.	1.3	n.d.	0.7
	Kanoff	107.7	16.6	n.d.[C]	10.4	n.d.	10.4	72.6	17.8	n.d.	10.4
	Martini	335.3	4.3	n.d.	2.7	n.d.	2.7	n.d.	4.6	n.d.	2.7
	Montara	1658.9	15.5	n.d.	9.7	n.d.	9.7	482.3	16.6	n.d.	9.7
	Vicente	882.4	8.6	n.d.	5.4	n.d.	5.4	674.9	9.3	n.d.	5.4
03/14/12	Dean	250950.3	39.1	n.d.	24.4	n.d.	24.4	154194.0	41.9	3079.6	24.4
	Kanoff	50939.5	20.5	n.d.	12.8	3936.9	12.8	6688.5	22.0	n.d.	12.8
	Martini	3235.8	31.5	n.d.	19.7	3.8	19.7	3235.8	33.8	n.d.	19.7
	Montara	78745.9	23.9	65.8	14.9	87.7	14.9	13106.1	25.7	n.d.	14.9
	Vicente	102544.5	27.4	n.d.	17.1	45257.0	17.1	30495.3	29.4	1078.9	17.1
03/20/12	Dean	3733.3	8.7	n.d.	5.5	n.d.	5.5	1178.4	9.4	n.d.	5.5
	Kanoff	1256.2	11.4	n.d.	7.1	n.d.	7.1	n.d.	12.2	n.d.	7.1
	Martini	133.8	12.0	n.d.	7.5	n.d.	7.5	n.d.	12.9	n.d.	7.5
	Montara	673.3	9.6	n.d.	6.0	n.d.	6.0	116.6	10.3	n.d.	6.0
	Vicente	1445.9	5.9	n.d.	3.7	n.d.	3.7	n.d.	6.3	n.d.	3.7
07/09/12	Dean	591.3	5.9	n.d.	3.7	n.d.	3.7	n.d.	6.3	4.7	5.2
	Kanoff*	815.1	3.6	n.d.	2.2	n.d.	2.2	n.d.	3.8	3.9	3.1
	Martini*	377.3	12.5	n.d.	7.8	n.d.	7.8	n.d.	13.4	16.0	11.0
	Montara*	2470.7	5.2	n.d.	3.3	n.d.	3.3	501.0	5.6	50.8	4.6
	Vicente*	235.5	4.0	n.d.	2.5	n.d.	2.5	n.d.	4.3	6.6	3.6

[A] Concentration

[B] Sample limit of detection

[C] n.d. not detected.

* DNA purification step was applied to the samples

Table 4. Concentrations and sample limits of detection of host-associated *Bacteroidales* in water in first flush events

Sampling date	Site name	Universal *Bacteroidales*		Human *Bacteroidales*		Bovine *Bacteroidales*		Dog *Bacteroidales*		Horse *Bacteroidales*	
		Concn[A]	S_{LOD}[B]	Concn	S_{LOD}	Concn	S_{LOD}	Concn	S_{LOD}	Concn	S_{LOD}
		(gene copies per mL)									
10/21/12	Dean	NA[C]									
	Kanoff*	2087.8	2.0	91.3	0.4	n.d.[D]	0.4	n.d.	2.1	n.d.	1.9
	Martini	1408.5	3.9	0.0	0.9	n.d.	0.9	n.d.	4.2	n.d.	3.9
	Montara	4415.9	3.2	0.0	0.7	n.d.	0.7	n.d.	3.4	n.d.	3.1
	Vicente*	3907.0	2.1	99.5	0.5	n.d.	0.5	n.d.	2.2	24.5	2.0
10/22/12	Dean	10315.6	5.1	168.9	0.6	n.d.	0.8	3188.3	3.9	n.d.	3.6
	Kanoff*	2446.0	2.3	560.6	0.3	n.d.	0.3	n.d.	1.7	n.d.	1.6
	Martini*	1666.1	13.5	78.4	1.6	n.d.	2.0	n.d.	10.3	n.d.	9.4
	Montara	21954.7	1.7	3.6	0.2	n.d.	0.3	n.d.	1.3	21.2	1.2
	Vicente*	8113.1	1.6	94.7	0.2	n.d.	0.2	n.d.	1.2	n.d.	1.1
10/29/12	Dean*	3775.7	4.6	99.0	0.7	n.d.	0.7	n.d.	2.6	10.5	2.3
	Kanoff*	4927.6	2.5	85.1	0.4	n.d.	0.4	n.d.	1.4	n.d.	1.2
	Martini*	2408.0	3.1	264.7	0.5	n.d.	0.5	n.d.	1.7	n.d.	1.5
	Montara*	2290.1	1.8	242.4	0.3	n.d.	0.3	n.d.	1.0	n.d.	0.8
	Vicente*	2209.9	1.8	89.9	0.3	n.d.	0.3	n.d.	1.0	10.6	1.1

[A] Concentration

[B] Sample limit of detection

[C] NA not available to collect

[D] n.d. not detected.

* DNA purification step was applied to the samples

Table 5. Concentrations and sample limits of detection of host-associated *Bacteroidales* in sediments and biofilm in wet and dry season

Sampling date	Site	Universal *Bacteroidales*		Human *Bacteroidales*		Bovine *Bacteroidales*		Dog *Bacteroidales*		Horse *Bacteroidales*	
		Concn[B]	S_{LOD}[C]	Concn	S_{LOD}	Concn	S_{LOD}	Concn	S_{LOD}	Concn	S_{LOD}
		(gene copies per g of dry weight of sediments or biofilm)									
03/12/12	Dean	396.3	30.7	26.2	19.2	100.9	19.2	n.d.	33.0	n.d.	19.2
	Kanoff	168.5	31.0	n.d.[D]	19.4	n.d.	19.4	n.d.	33.3	n.d.	19.4
	Martini	1655.4	22.6	n.d.	14.1	n.d.	14.1	892.2	24.2	22.7	14.1
	Montara	596.3	30.5	n.d.	19.0	n.d.	19.0	n.d.	32.7	46.4	19.0
	Vicente	3711.7	30.3	n.d.	18.9	89.6	18.9	286.8	32.5	253.3	18.9
	Kanoff[A]	79840.7	146.0	n.d.	91.0	18659.8	91.0	24337.2	156.5	1860.7	91.0
	Montara[A]	2691.9	360.7	n.d.	224.9	n.d.	224.9	515.3	386.8	210.5	224.9
03/14/12	Kanoff	1375.0	29.0	n.d.	18.1	347.7	18.1	383.7	31.1	n.d.	18.1
	Martini	349.2	30.3	n.d.	18.9	28.5	18.9	101.6	32.5	36.9	18.9
	Montara	1738.2	29.8	n.d.	18.6	16.0	18.6	196.7	32.0	12.8	18.6
	Vicente	5014.0	29.8	n.d.	18.6	2649.8	18.6	n.d.	31.9	76.0	18.6
03/20/12	Dean	10229.3	30.2	181.4	18.9	603.9	18.9	n.d.	32.4	90.3	18.9
	Kanoff	262.5	29.1	n.d.	18.2	77.4	18.2	n.d.	31.2	n.d.	18.2
	Martini	1520.6	31.6	n.d.	19.7	n.d.	19.7	n.d.	33.9	28.5	19.7
	Montara	18370.8	30.9	n.d.	19.3	6983.7	19.3	3898.0	33.2	48.1	19.3
	Vicente	636.1	30.9	n.d.	19.3	201.2	19.3	439.4	33.1	17.5	19.3
	Montara[A]	71535.4	130.8	n.d.	81.6	n.d.	81.6	16474.5	140.2	973.4	81.6
07/09/12	Dean	2551.1	15.3	n.d.	9.6	n.d.	9.6	n.d.	16.5	61.9	9.6
	Kanoff	3585.9	32.1	n.d.	20.0	n.d.	20.0	n.d.	34.4	271.8	20.0
	Martini	551.3	15.2	n.d.	9.5	n.d.	9.5	n.d.	16.3	30.6	9.5
	Montara	640.5	15.3	n.d.	9.5	n.d.	9.5	625.5	16.4	73.5	9.5
	Vicente	3655.6	15.4	n.d.	9.6	n.d.	9.6	1968.1	16.5	168.3	9.6
	Kanoff[A]	986013.4	113.0	n.d.	70.5	n.d.	70.5	n.d.	121.2	1255.2	70.5
	Montara[A]	582339.0	172.0	n.d.	107.2	n.d.	107.2	347711.1	184.4	1601.5	107.2

[A] Biofilm samples
[B] Concentration
[C] Sample limit of detection
[D] n.d. not detected

Table 6. Concentrations and sample limits of detection of host-associated *Bacteroidales* in field blanks

Sampling date	Sample	Universal *Bacteroidales*		Human *Bacteroidales*		Bovine *Bacteroidales*		Dog *Bacteroidales*		Horse *Bacteroidales*	
		Concn[A]	S_{LOD}[B]	Concn	S_{LOD}	Concn	S_{LOD}	Concn	S_{LOD}	Concn	S_{LOD}
		(gene copies per mL)									
03/12/12	Field blank 1	n.d.[C]	1.7	n.d.	1.1	n.d.	1.1	n.d.	1.8	n.d.	1.1
03/14/12	Field blank 2	14.8	3.7	n.d.	2.3	n.d.	2.3	n.d.	4.0	n.d.	2.3
03/20/12	Field blank 3	n.d.	1.4	n.d.	0.9	n.d.	0.9	n.d.	1.5	n.d.	0.9
07/09/12	Field blank 4	n.d.	8.2	n.d.	5.1	n.d.	5.1	n.d.	8.8	n.d.	7.2
10/21/12	Field blank 5	n.d.	0.5	n.d.	0.1	n.d.	0.1	n.d.	0.5	n.d.	0.5
10/22/12	Field blank 6	n.d.	0.6	n.d.	0.1	n.d.	0.1	n.d.	0.5	n.d.	0.5
10/29/12	Field blank 7	n.d.	0.5	n.d.	0.1	n.d.	0.1	n.d.	0.3	n.d.	0.2

[A] Concentration

[B] Sample limit of detection

[C] n.d. not detected

Table 7. Replicate percent differences in environmental samples and field duplicates (for water samples)

Sampling date	Sample	Universal *Bacteroidales*	Human *Bacteroidales*	Bovine *Bacteroidales*	Dog *Bacteroidales*	Horse *Bacteroidales*
				RPD[A]		
03/12/12	Field duplicate 1	28%	ND[B]	ND	23%	ND
03/14/12	Field duplicate 2	55%	118%	1%	57%	ND
03/20/12	Field duplicate 3	73%	ND	ND	ND	ND
07/09/12	Field duplicate 4	48%	ND	ND	ND	86%
10/21/12	Field duplicate 5	49%	32%	ND	ND	15%
10/22/12	Field duplicate 6	20%	4%	ND	ND	ND
10/29/12	Field duplicate 7	32%	72%	ND	ND	ND1[C]

[A] Replicate percent difference (RPD) = ($\left|\text{Concentration}_{sample}-\text{Concentration}_{duplicate}\right|$ / mean ($\text{concentration}_{sample}$, $\text{concentration}_{duplicate}$)) x 100%.
For reference, factors of 3- and 10-fold differences in concentrations equate to RPDs of 100% and 163.6%, respectively.

[B] ND = both the environmental and field duplicate were non-detect

[C] ND1 = measurement was positive in one sample and negative in the other

4. Discussion

The main goal of the MST study was to investigate the sources of fecal pollution in the five watersheds draining to the Pacific Ocean in the vicinity of the Reserve. Analysis of 34 water samples during 7 events in 2012 revealed that there is a temporal distribution of host-associated *Bacteroidales* in water in the Reserve. Dog-associated *Bacteroidales* were a predominant source of fecal contamination at several sites during the wet season, while the marker was hardly detected in water in first flush events. Interestingly, human-associated *Bacteroidales* were frequently found in the first flush sampling events but not often during the wet season. Bovine-associated *Bacteroidales* were only observed during rain in the wet season throughout all 7 events in 2012. Horse-associated *Bacteroidales* were detected at all creeks in dry season and at Dean, Montara, and San Vicente Creeks during wet season and first flush even though its contributions to universal *Bacteroidales* were still minor (less than 5% of universal *Bacteroidales* concentrations). In dry season event, at all sites except Montara Creek, less than 5% of universal *Bacteroidales* concentrations were made up of host markers tested in this project. This result indicates that uncharacterized fecal sources such as wildlife or other domestic animals contributed a large amount of fecal pollution during the dry season. The watersheds monitored in this study contain unincorporated communities, agricultural fields, equestrian facilities, a small commercial area, public beaches, and open space/recreational areas within coastal bluff, coastal scrub, and riparian habitat (California Coastal Commission, 2008). All creeks except Martini Creek flow through medium to high density residential areas. Overflows or leaking septic and/or sewer system pipes and runoff from residential and public areas could contribute to the elevated levels of human- and dog-associated *Bacteroidales*. The presence of bovine and horse markers could be derived from the use of manure compost and equestrian operations. In addition, it is plausible, given the large area of wildlife habitat, that various species of animals that reside along the waterways also made a significant contribution to fecal loading to the Reserve.

Regarding universal *Bacteroidales,* the concentrations generally increased when it rained in the wet season and first flush events. However, it was evident that the increased amounts during rainfall in the wet and first flush events were quite different. Even though there were similar amounts of rainfall during the rainy sampling days in March and October (2.5 – 4 versus 1.5- 3

cm), the increased levels of *Bacteroidales* were significantly higher in the wet season event compared to the first flush event. The fact that universal marker was detected at higher concentrations during rain in the wet season event could be due to unequal survival of *Bacteroidales* in the given environments or due to release of microorganisms from sediments and biofilm. It is plausible that the relatively cool water temperature in March (8ºC) compared to October (13ºC) enabled *Bacteroidales* in water to persist longer during the wet season (Okabe and Shimazu 2007). Dissolved oxygen concentrations in water were not very different in March and October (10.6 versus 10.3 mg/L), which indicates that dissolved oxygen concentrations in water were not the main effect of the different levels of *Bacteroidales*. It is also possible that the higher flow rate in creeks in the wet season could resuspend microorganisms in sediments and biofilm, which would then result in increased bacteria levels in the water. According to daily discharge data (http://waterdata.usgs.gov) of Pilarcitos Creek which located nearby the Reserve, considerably higher discharge was observed at the sampling time of wet season rain (approximately 40 cubic feet per second) than first flush rain (less than 10 cubic feet per second). Even though the rainfall totals were similar in the two events, the streamflow could be different due to the degree of ground saturation and groundwater levels. Flow rates at each creek were not directly measured during the events; however, the total suspended solids (TSS) levels during rain in the wet season were significantly higher in March compared to those in October (ANOVA, $p < 0.05$), indicating elevated flow rates during rain in the wet season compared to first flush. While survival of *Bacteroidales* is limited in surface water due to its obligate anaerobic metabolism, it may be possible for cells to grow or persist longer in sediments with anaerobic conditions. In addition, sediments can provide favorable environments for microorganisms (Anderson et al. 2005; Craig et al. 2004; Lee et al. 2006). Microorganisms in surface water could settle to the bottom and attach to sediments. Release of those cells in sediments and biofilm into the overlying water can occur during times of turbulence. Therefore, the accumulated microorganisms in sediments and biofilm along the waterway could be resuspended and transported with the elevated flow to the Reserve when it rained.

Sediment and biofilm samples evaluated in this study revealed the presence of high amounts of host-associated *Bacteroidales*. Universal *Bacteroidales* concentrations in sediments generally increased during rain. A probable reason is that the introduced amounts of *Bacteroidales* from

the water during the rain event were much higher than the levels of *Bacteroidales* released from sediments and biofilm at the creek confluence. Elevated levels of bovine-associated *Bacteroidales* at Kanoff and San Vicente Creek during rainfall were consistent with the increased presence and concentrations of the bovine marker in water in the same events. The fact that bovine-associated *Bacteroidales* were detected during, as well as, after rain even though the marker was only detected during rain in the overlying water suggests that *Bacteroidales* could persist longer when they were associated with sediments. It is important to remember that MST genetic analysis is based on the detection of DNA that can persist long after cell death. Recently, the application of propidium monoazide (PMA) prior to qPCR has been shown to be a useful technique for the discrimination of DNA from intact and impaired cells by inhibiting DNA amplification from damaged cells (Bae and Wuertz 2009). Since PMA reacts with DNA in impaired cell and extracellular DNA upon light exposure, high levels of solid particles (> 1000 mg/L) in a concentrated water sample after filtration can hinder the photolysis of PMA by blocking light exposure of impaired cells. Therefore, the PMA application was planned for sediment and biofilm samples rather than water samples because sediment eluants should have fewer solids than concentrated water after filtration. However, relatively high sample limits of detection of the eluants resulted in needs of higher eluant volumes to be concentrated on the sterile membrane filters during DNA extraction, which might result in the failure of the PMA application for sediment and biofilm samples in this study (data not shown). It is not known what mechanisms between PMA and eluants from sediment/biofilm samples produced the impractically high DNA concentrations in the PMA-treated samples compared to untreated samples. We applied nucleic acid purification with phenol-chloroform to minimize the unknown inhibitions but did not include the results of PMA-treated samples in this report. As part of resolving the PMA issue, samples without PMA treatments were also analyzed with/without DNA purification. Even though it is inevitable to lose a certain fraction of DNA during purification, every sample showed higher genetic marker concentrations after DNA purification compared with before purification. Host-associated *Bacteroidales* were detected at high concentrations compared to unpurified sample (unpurified sample data not shown). This result indicates that unknown inhibitors existed in the sediment and biofilm samples and controlled by DNA purification when PMA was not applied to the samples. Overcoming the problem in analyzing PMA treated environmental samples will be helpful for further monitoring studies.

5. Conclusions

- Universal *Bacteroidales* concentrations increased when it rained. The increased amounts should vary with the flow rate of the creek since a high flow rate could resuspend sediments, which results in an increase in host-associated genetic markers in the overlying water column.
- Human-associated *Bacteroidales* were detected once at Dean (pre-rain) and Montara (during rain) in the wet season but were not often detected in either wet or dry seasons. The human marker was prevalent in water throughout the first flush events.
- Bovine feces affected water quality at sampling sites during rain during the wet season but not during the dry season or first flush event. The marker considerably contributed to the fecal loading at Kanoff and San Vicente Creek.
- Dog-associated *Bacteroidales* contributed a significant amount of universal *Bacteroidales* detected at all creeks in the wet season. The dog marker was not found during the dry season and first flush events except at Montara Creek (dry season) and at Dean Creek (during rain in the first flush events).
- The fact that the high levels of bovine or dog marker were detected in sediments and biofilm when their levels in water were not high showed that previously introduced host markers in the creek could persist longer when associated with sediments and biofilm.
- Horse-associated *Bacteroidales* were found at high concentrations in water at Dean and San Vicente Creek during rain in the wet season. Horse feces were also present in all creeks in the dry season, but it is not a predominant fecal source. Higher concentrations of the horse genetic marker in sediments compared to those in water during the dry season suggest the accumulation or re-growth of horse-associated *Bacteroidales* in sediments in the dry season.
- The monitoring study provides good insights into the prevalence of host-associated *Bacteroidales* at five creeks draining into the Reserve.
- Future monitoring studies that can distinguish host-associated *Bacteroidales* DNA from intact and impaired cells will allow determination of the age of fecal pollution and may provide more information about potential health risks.

6. References

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