Microbial Water Quality at Minimally Human-Impacted Reference Beaches in Northern California

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Acronyms and Abbreviations

AB	Assembly Bill
ASTM	ASTM International, an international standards developing organization
C-CAP	Coastal Change Assessment Program
cfs	Cubic feet per second
DFW	Department of Fish and Wildlife
DNQ	Detected but not quantifiable
E. coli	*Escherichia coli*
FC	Fecal coliform
FIB	Fecal indicator bacteria
gc/ml	Gene copies per milliliter
HF183	Microbial source tracking (MST) marker targeting the HF183 16S sRNA gene cluster of members of *Bacteroides*, a genus of human-associated fecal microbes
IDEXX	IDEXX Laboratories, Inc.
in	Inches
LARWQCB	Los Angeles Regional Water Quality Control Board
LOQ	Level of quantification
MBAS	Monterey Bay Analytical Services
MTF	Multi-tube fermentation
NOAA	National Oceanic and Atmospheric Administration
PRISM	Parameter-elevation Relationships on Independent Slopes Model (calculates spatial climate data)
SFEI	San Francisco Estuary Institute
SC	Specific conductivity
SCCWRP	Southern California Coastal Water Research Project
SCPHRL	Sonoma County Public Health Regional Laboratory
SP	State Park
SWRCB	State Water Resources Control Board
TC	Total coliform
TMDL	Total maximum daily load
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey

Executive Summary

All of the coastal reference beaches that have been used by the State of California for quantifying "natural loads" of fecal indicator bacteria have been in Southern California. None has been in the northern and central coastal regions of the State. To fill this information gap, this study assessed bacteria concentrations at coastal reference beaches in Northern California, investigated possible factors influencing the bacteria concentrations, and evaluated how these concentrations compare to results from the Southern California reference beaches.

Reference beaches were defined as open beaches with breaking waves that receive runoff from undeveloped watersheds. The study beaches were selected to represent a variety of geographical conditions and watershed sizes in Northern California. Five reference beaches were sampled between January 2016 and September 2017 for a total of 25-30 sampling events, including 10 wet weather events, 10 winter dry events, and 10 summer dry events. The number of samples collected was admittedly small for characterizing variable bacteria concentrations but was deemed sufficient for providing initial information relevant to management questions. At each beach, samples were collected from an ocean site in the surf zone and from a freshwater site in the watershed draining to the beach. Samples were analyzed for total coliform, fecal coliform, *Escherichia coli* (*E. coli*), enterococci (ocean sites only), as well as a human-specific genetic marker (human *Bacteroides* HF183).

The study documented background concentrations of bacteria at Northern California reference beaches during two years that spanned extreme drought and high rainfall conditions. Bacteria concentrations were significantly higher during wet weather than during dry weather periods indicating that watershed loading is an important explanatory factor.

The study results suggest that the rates at which water quality objectives were exceeded at Northern California reference beaches were similar to those observed in Southern California. At Northern California reference beaches, 35% of ocean samples and 31% of freshwater samples collected during the first 24 hours of rain exceeded State of California water quality thresholds, compared to 27% of samples collected at Southern California beaches (Griffith et al. 2006). Therefore, the data collected for this study indicate that reference concentrations for Southern California can be used for Northern California beaches as a conservative (protective) assumption. The sample size for this study was not large enough to derive statistically valid reference concentrations for Northern California beaches. However, the study generated a database of results that can be built upon by future studies to achieve this goal.

The results from the *Bacteroides* genetic marker analyses suggest that the examined watersheds are minimally impacted compared to urban watersheds in Southern California (Cao et al. 2017). However, the genetic marker results were not well enough correlated with measured bacteria indicator concentrations to be a useful explanatory variable.

Introduction

California coastal beaches attract over 150 million day visits annually, for recreational activities such as swimming, wading, and surfing. Most of these visits are to Southern California beaches, but a number of Northern California beaches also experience significant recreational use (Dorfman and Rosselot 2008, SWRCB 2017). Northern California beaches include those in Monterey, Santa Cruz, San Mateo, Alameda, San Francisco, Contra Costa, Marin, Sonoma, Mendocino, Humboldt, and Del Norte counties. The beaches are located within the jurisdictions of the North Coast Regional Water Quality Control Board (Region 1), San Francisco Bay Regional Water Quality Control Board (Region 2), and the Central Coast Regional Water Quality Control Board (Region 3).

To protect beach users from exposure to fecal contamination, public health agencies and pollution control agencies monitor fecal indicator bacteria (FIB) concentrations at beaches where water contact recreation is common. FIB are used because they correlate well with the incidence of human illness in epidemiology studies at recreational beaches (e.g., Cabelli 1982, Haile et al. 1999, Colford et al. 2012). FIB are generally not pathogenic themselves and more quickly and cost-effectively enumerated than pathogens. The FIB monitored for this study were total coliform, *E. coli*, fecal coliform, and enterococci.

A number of coastal beaches in Northern California (Central Coast, San Francisco Bay, and North Coast regions) are on the State's Clean Water Act Section 303(d) list due to exceedances of water quality thresholds for FIB, and there are several Total Maximum Daily Load (TMDL) reports in development, which are plans to achieve water quality standards. An important step in the development of TMDLs and other water quality management plans is to determine what portion of water quality objective exceedances are caused by controllable (anthropogenic) sources in the watershed, so that management measures can focus on them. Bacterial water quality at beaches located at the mouth of undeveloped watersheds can serve as a reference condition for this determination. For example, regulators in in the Los Angeles region are using the level of contributions from minimally developed watersheds as a benchmark for quantifying "natural loads" of bacteria (LARWQCB 2002).

Currently, all coastal reference beaches used by the State for quantifying "natural loads" of FIB (i.e. the contribution of non-anthropogenic sources) are located in Southern California (Los Angeles and San Diego regions). There have been no previous studies characterizing the contributions of natural variability of FIB at Northern California beaches. It is therefore unclear whether conditions at the existing southern California reference beaches are appropriate

benchmarks for all of coastal California or whether there is a need for region-specific reference systems.

The goal of this study was to assess bacterial water quality conditions at coastal reference beaches in Northern California. Reference beaches were defined as beaches that are located at the mouth of watersheds that are minimally developed and minimally impacted by human activities (Griffith et al. 2006). The intended use of the data is for establishing a "reference level" to support decisions about appropriate water quality targets in Northern California's coastal regions. To that end, the following management questions were answered by this study:

1. What are the ranges of concentrations of fecal indicator bacteria (FIB) from non-anthropogenic sources at Northern California beaches and freshwater streams located at/within minimally developed watersheds?
    A. What are the FIB concentrations in the mixing zone and immediately upstream in the freshwater stream?
    B. What percent of FIB samples exceed water quality thresholds for recreation beneficial uses?
    C. Are bacteria water quality objectives exceedance rates at Northern California reference beaches and streams different from those in Southern California? Is it justifiable to use data from existing reference systems in Southern California for setting an allowable level of microbial water quality objective exceedances for impaired coastal beaches and streams throughout Regions 1, 2, and 3?

To better understand processes and factors influencing reference beach water quality, we also evaluated relationships between bacterial levels and

a) other contributing factors such as region, watershed size, presence or absence of a lagoon, and amount of annual rainfall/size of storm; and
b) the detection of a human-specific genetic marker (*Bacteroides* HF183).

Methods

Sites

Five coastal reference beaches were selected for the study (Table 1; Figure 1). The number of sites and the number of samples collected at each site was constrained by the available budget. The selection of reference beaches was based on the following four criteria (Jabusch *et al*. 2014) that were adapted from Schiff *et al.* (2005) and Griffith *et al.* (2006):

1) Each reference beach must be an open beach with breaking waves;
2) Each reference beach must have a freshwater input;
3) Freshwater input must come from a watershed of similar size to an impaired watershed; and
4) The watershed discharging to the reference beach must be >95% undeveloped.

All five reference beaches for this study met the selection criteria (Table 1). All five reference beaches are open with breaking waves and have freshwater inputs. The five watersheds that discharge to these reference beaches range from 5 to 152 km^2, representing the full range of watershed size categories for all the watersheds that drain to impaired beaches in Northern California. The five watersheds draining to the reference beaches were between 97% and 99% undeveloped based on the NOAA Coastal Change Assessment Program land cover layer.

The reference beach selection also considered the average annual precipitation (30-year normal mean) in the watershed (Table 1). The average annual precipitation for each watershed was based on the 30-year average, which was estimated using PRISM data[1] and zonal statistics. Selected reference beaches represent two of the three precipitation bins that were used to categorize impaired beaches in the scoping study (Jabusch *et al.* 2014). Garrapata State Beach and Andrew Molera State Park Beach represent watersheds receiving moderate amounts of rain. Stump Beach, Stillwater Cove Beach, and Big Creek represent watersheds in the "heavy precipitation" category. None of the selected watersheds represented the "dry watersheds" (<24.4 in) category. Based on the analyses conducted for the scoping study, the working assumption was that Southern California reference watersheds are potentially representative of watersheds within the dry category.

Sampling

There were two sampling locations at each reference beach. The primary sampling location at each site was in the ocean immediately in front of the freshwater input at the so-called ''wave wash", where the watershed discharge initially mixes with the ocean waves (ocean sampling site). The goal was to collect samples between ankle and knee depth on an incoming wave (Griffith *et al.* 2006). However, high tides and surf sometimes necessitated collecting samples outside this range of depths in order to protect the safety of the sampling team. The second sampling location was in the watershed discharge upstream of the beach (freshwater sampling site).

[1] Parameter-elevation Relationships on Independent Slopes Model (calculates spatial climate data).

All samples were analyzed for FIB and *Bacteroides* genetic markers. Samples were collected in sterilized 250 mL Nalgene wide mouth bottles (for FIB analysis) and sterile 120 mL Bacti Bottles (for *Bacteroides* analysis, 125 ml sterile plastic bottles without preservatives supplied by Environmental sampling supplies, San Leandro CA).

Sampling began in January 2016 and ended in September 2017 and covered three different types of events: wet weather, winter dry[2], and summer dry. There were a total of 30 sampling events, including 10 wet weather events, 10 winter dry events, and 10 summer dry events. Wet weather sampling consisted of the collection of one sample from each sampling site for each sampled storm. The winter dry events were sampled in two rounds. Each sample round included one sample per week for five consecutive weeks (i.e., five weekly samples in the 2015-2016 wet season and five weekly samples in the 2016-2017 wet season). Similarly, summer dry events were sampled in two rounds (one sample per week for 5 weeks) in the 2016 dry season and 2017 dry season. Wet season sampling (wet weather and winter dry) was initiated after first flush and when winter base flow conditions were established. Indicators for winter base flow conditions were season-to-date rainfall at reference weather stations and baseflow at reference gauges.[3] The wet weather sampling trigger was a predicted minimum rainfall estimate of 1 inch in 24 hours.

Laboratory Analysis

Concentrations of *E. coli*/total coliform, enterococci, and fecal coliform were measured using standard methods. Concentrations of total coliforms and *E. coli* were measured using Colilert-18™ (IDEXX/SM9223B) and enterococci were measured using Enterolert™ (IDEXX/ ASTM D6503-99). Concentrations of fecal coliforms were measured using multi-tube fermentation (MTF, SM 9221). Enterococci were only measured in seawater samples (ocean sampling site). Sonoma coast samples were received and analyzed by the Sonoma County Public Health Regional Laboratory (SCPHRL, Santa Rosa, CA) and Big Sur coast samples were received and analyzed by Monterey Bay Analytical Services (MBAS, Monterey, CA).

All samples were analyzed for human *Bacteroides* genetic markers. The purpose of this analysis was to evaluate the presence of human sources of indicator bacteria at each site. Following drop-off at the receiving laboratories (SCPHRL and MBAS), samples for bacterial source marker analysis were passed through membrane filters to concentrate *Bacteroides*. MBAS used 0.45-µm

[2] On dry weather days, after an antecedent dry period of 96 hours with less than 1 inch of rainfall.

[3] Season-to date rainfall criteria were >7 inches at the Venado rain gauge for Sonoma coast watersheds and >5 inches at the Big Sur Station rain gauge for Big Sur coast watersheds. Winter baseflow criteria were >10 cfs at the Gualala River flow gauge (USGS11467553) for Sonoma coast watersheds and >30 cfs at the Big Sur River flow gauge (USGS11143000) for Big Sur coast watersheds.

pore size polycarbonate (PC) membranes (Millipore, New Bedford, MA) and SCPHRL used 0.22-μm pore size polyethersulfone (PES) membranes (Pall Laboratory, Port Washington, NY). The filtered volume was 100 mL. Filters were immediately stored at -20°C and subsequently shipped overnight to Cel Analytical, Inc. (San Francisco, CA) for bacterial source marker analysis. The analysis was performed according to USEPA Method B using the human-specific HF183 marker and a non-specific total *Bacteroides* marker (Griffith *et al*. 2013, USEPA 2010a).

To test for any comparability between the PC membranes and the 0.22-μm pore size PES membranes, we spiked paired sets of both membranes with the same concentration of *B. thetaiotamicron* cells. Using method B, similar recovery was observed from PC and PES membranes.

Quality Assurance

Microbiological quality control data submitted by the laboratories were evaluated using the Indicator Bacteria In Freshwater Measurement Quality Objectives (MQOs) of the State of California Surface Water Ambient Monitoring Program (SWAMP). All laboratories reported field duplicates, laboratory duplicates, laboratory control samples, field blanks, and laboratory blanks. For genetic marker analyses, matrix spikes and negative controls were also reported (see Appendix 2).

Data Analysis

Data analysis focused on five elements. The first compared FIB concentrations and the frequency of water quality threshold exceedances during wet weather, winter dry weather, and summer dry weather. The comparisons were based on the California State Assembly Bill AB411 public health standards for marine bathing beaches for total coliform, fecal coliform, and enterococci; and on the USEPA criteria for fresh water bathing recreational waters for *E. coli* (USEPA 1986). Table 3 summarizes the threshold values used in the analyses.

The second analysis element compared the frequency of exceedance of water quality objectives at Northern California reference beaches to those in Southern California, as reported by Griffith *et al.* (2006) and Schiff *et al.* (2005). The margin of error from the binomial distribution was used as an estimate of uncertainty in the frequencies:

$$\text{Margin of Error in a proportion} = z * \sqrt{p * \frac{(1-P)}{n}}$$

where z is 1.96 (for α=0.05), p is the frequency of exceedance (as a proportion), and n is the sample size. Given the number of sampling events for the study (25-30), the margin of error will be approximately +/-15% but the exact amount depends on the proportion.

The third data analysis element focused on comparisons among the five reference beaches. The first comparison examined the relative frequency of exceedance for each sampling event type. Additional analyses evaluated relationships between bacterial levels and other contributing factors such as amount of annual rainfall/size of storm, region, watershed size, and the presence or absence of a lagoon. The effects of changes in annual rainfall were assessed by comparing the frequency of exceedance of FIB thresholds between years. Statistical significance was tested using estimated error bars based on the binomial distribution (see previous paragraph for details). Effects of storm size were further evaluated by simple linear regression between the 24-hour rainfall and the frequency of exceedances of FIB thresholds and FIB concentrations at individual beaches (log-transformed). The effects of watershed size, region, and lagoonal systems on FIB concentrates were evaluated using box and whisker plots and Kruskal-Wallis non-parametric tests for statistical significance.

The fourth analysis element compared FIB and *Bacteroides* concentrations to evaluate whether water quality exceedances correspond to the presence of human sources. The relationship between the human *Bacteroides* genetic marker and standard fecal indicators was evaluated using regression analysis.

The fifth element of the data analysis compared human *Bacteroides* levels across the five reference beach systems. This analysis element evaluated relationships between human *Bacteroides* levels and factors such as sampling event type and sampling year/water year[4] type. It also examined the relationship between human *Bacteroides* concentrations at freshwater sampling sites and ocean sampling sites. The analyses considered human *Bacteroides* concentrations and the ratios of human *Bacteroides* to total *Bacteroides* spp.

Human *Bacteroides* concentrations and the ratios of human *Bacteroides* to total *Bacteroides* spp. were categorized based on thresholds that have been published in the literature and used in assessments by the Wisconsin Department of Natural Resources (Koski et al. 2014, Sauer et al. 2011). These thresholds are summarized in Table 4.

[4] A water year describes a time period of 12 months for which precipitation totals are measured. A water year in California is defined as the period between October 1st of one year and September 30th of the next.

Results and Discussion

Sampling Events

The measured 24-hr rainfall on wet weather sampling days ranged from 1.9 to 7.2 inches on the Sonoma Coast, and from 0.5 to 7.2 inches on the Big Sur Coast (Table 2). However, rainfall data in each sampling region were not available for 2 out of the 10 sampling events. Therefore, the available data may not represent the full range of precipitation amounts for sampled wet weather events. Sampling Year 1 or Water Year 2016 (October 1, 2015 - September 30, 2016) was officially listed as "dry" statewide, whereas Sampling Year 2 or Water Year 2017 was the second wettest year on record in California.

The plan was to collect 10 samples of each type at each site, but the actual collection fell short of what was planned (Table 2). The Sonoma Coast and Big Sur sampling teams each sampled four wet weather events in the 2015-16 wet season and six in the 2016-17 wet season. The sampling sites at Andrew Molera State Park Beach, Big Creek, and Big Creek Cove became temporarily inaccessible due to road and trail closures from mid-January to mid-August 2017. For this reason, each of these sites was visited only once in the 2016-17 wet season, for a wet weather sampling event. Therefore, only 5 of 10 planned wet weather sampling events and 5 of 10 planned winter dry sampling events were completed at these sites.

Quality Assurance

Results were reported for a total of 270 of 300 planned samples (sample completeness 90%). All reported FIB results were considered useable. The human *Bacteroides* and total *Bacteroides* results from samples that were collected on January 12, 2016, during a winter dry event at the Sonoma Coast sites were rejected by the laboratory and excluded. See Appendix 2 for a summary of the quality assurance results.

The salinity measurements were not done as called for in the Sampling and Analysis Plan, which would have been to take specific conductance measurements at freshwater and ocean sites. The sampling teams for North Coast beaches did not monitor salinity at the freshwater sites, because they are too far upstream to be tidally influenced. The sampling teams for the Central Coast beaches did not monitor salinity at the ocean sites, because their sensor did not

work at ocean strength salinity. Without paired results for salinity at any of the beaches, analysis of dilution and mixing at the beaches cannot be completed.

FIB Concentrations Among Reference Beaches in Northern California

FIB Concentrations at Ocean and Freshwater Sampling Sites at Northern California Reference Beaches

The FIB concentrations measured at the ocean beach sites and freshwater sites covered a wide range and varied with site and weather conditions. Due to the large range in actual concentrations, the data were mostly analyzed using the frequency of exceedance of water quality objectives. However, the following general observations were apparent from the concentration data. Median concentrations of the different FIB's were 2-5 times higher during wet weather events than during dry weather events (Table 5). Big Sur reference sites (Garrapata, Andrew Molera, and Big Creek) had higher concentrations of total coliforms than Sonoma Coast sites, but concentrations of other FIBs were comparable across all sites (Figures 2-5). Finally, the ranges of concentrations for total coliforms, fecal coliforms, and *E. coli* are similar for ocean sites and their corresponding freshwater sites in each of the reference systems (Figures 2-5).

Frequency of FIB Threshold Exceedances at Northern California Reference Beaches

There were more water quality exceedances in total for each of the indicators in wet weather than in winter dry weather or summer dry weather (Table 6 and Figure 6), at both freshwater and ocean sampling sites. About 35% of ocean samples and 31% of freshwater samples collected during wet weather sampling exceeded water quality thresholds for at least one indicator. In winter dry weather, 15% of ocean samples and 4% of freshwater samples exceeded at least one water quality threshold. The fewest single-sample exceedances were observed in summer dry weather conditions, with only 4% of ocean samples and 0% of freshwater samples exceeding water quality thresholds.

Total coliform was the FIB that most frequently exceeded the water quality threshold in wet weather conditions but not always in dry weather conditions. All wet weather exceedances at freshwater sites and most wet weather exceedances at ocean sites were associated with total coliform threshold exceedances. That is, the frequency of wet weather samples with any exceedance closely matched the frequency of total coliform exceedances at ocean sites (35% vs 30%) and was identical with it at freshwater sites (both 31%). In contrast, during winter dry

weather, enterococci was the FIB that most frequently exceeded a water quality threshold at ocean sites and fecal coliform was the only FIB that exceeded a water quality threshold at freshwater sites in winter dry conditions (4%). All summer dry weather exceedances at ocean sites were associated with total coliform threshold exceedances (4%). The frequency of exceedance of the enterococci threshold at ocean sites in summer dry weather was 2%. There were no single-sample water quality exceedances at freshwater sites in dry weather.

The cumulative frequency of water quality exceedances across all weather conditions at individual sampling sites included in this study ranged from 0% to 30% (Figure 7). One-third of samples collected at the Garrapata Beach ocean sampling site exceeded water quality thresholds for at least one indicator. No single-sample water quality threshold exceedances were observed at the Big Creek Cove reference system sampling sites.

Some of the reference systems exhibited large differences in the frequency of water quality threshold exceedances between wet and dry weather conditions (Table 6). The Garrapata Beach reference system had the highest frequency of wet weather exceedances. Seven of 10 wet weather samples (70%) from the Garrapata State Beach ocean site and 5 of 10 samples (50%) from the Garrapata Creek freshwater site exceeded water quality thresholds. In contrast, only 1 of 10 (10%) summer dry weather samples from the Garrapata State Beach ocean site and none of the summer dry samples from the Garrapata Creek freshwater site exceeded any single-sample water quality thresholds. Similarly, 3 of 5 (60%) wet weather samples collected at the Andrew Molera SP Beach ocean site and 3 of 10 (30%) wet weather samples collected at the Big Sur River freshwater site exceeded at least one water quality threshold. None of the summer dry samples collected at the Andrew Molera SP sites exceeded any thresholds and there was only one winter dry sample from the ocean site exceeding a threshold. Single-sample water quality exceedances at the Miller Creek, Stillwater Cove Beach, and Stockhoff Creek sampling sites were observed in wet weather and winter dry conditions but not in summer dry conditions.

Overall, total coliforms were responsible for most water quality threshold exceedances at most of the sites. For example, all 7 of the 10 wet weather samples from Garrapata Beach with water quality exceedances exceeded the total coliform threshold. Enterococci exhibited the second-greatest rate of exceedance at ocean sites. For example, enterococci concentrations were responsible for the two water quality threshold exceedances in winter dry samples collected at Stump Beach. The only winter dry sample with an exceedance collected at Andrew Molera SP Beach was also due to enterococci. The indicator with the fewest exceedances was fecal coliform. The fecal coliform water quality threshold was exceeded at most in 1 of 10 samples at any given site in wet weather and winter dry conditions and was never exceeded in summer dry conditions.

Trends in FIB exceedances between Year 1 (January – July 2016) and Year 2 (December 2016 – September 2017) were different for ocean sites and freshwater sites (Figure 8). At ocean sites, there were more exceedances overall in Year 1, the drought year. However, the observed difference between Year 1 and Year 2 at the ocean sites is within the range of error of the results (decrease from 20% in Year 1 to 13% in Year 2, with an estimated 95% confidence interval of $\pm$ 14%). In contrast, the frequency of water quality threshold exceedances at freshwater sites increased by 5-fold from 4% in Year 1 (drought year) to 20% in Year 2 (wet year) (Figure 8). This change is potentially significant (estimated margin of error of $\pm$ 11%) and driven by an increased frequency of exceedances of the total coliform threshold from 3% in Year 1 to 17% in Year 2 and of the fecal coliform threshold from 0% in Year 1 to 9% in Year 2. In wet weather conditions, the frequency of total coliform and fecal coliform threshold exceedances increased from 10% in Year 1 to 48% in Year 2 and from 0% in Year 1 to 16% in Year 2, respectively. There were no exceedances of water quality thresholds in winter dry conditions in Year 1, whereas 10% of winter dry samples collected in Year 2 exceeded the fecal coliform threshold.

Results for 5-week geometric mean exceedances in dry conditions suggest potential regional differences between sites at the Sonoma Coast and at the Big Sur Coast (Table 7). While there were no exceedances of 5-week geometric means for FIB at Sonoma Coast sites, all Big Sur coast sites exceeded the total coliform 5-week geometric mean threshold in one or several dry sampling periods. All three Big Sur Coast freshwater sites exceeded the total coliform geometric mean threshold in both summer dry sampling periods. The Garrapata Creek freshwater site exceeded the total coliform geometric mean in all four dry sampling periods. The result may represent elevated loads of coliforms at the Big Sur Coast from sources that are not present or not as significant at the Sonoma Coast. The reference watersheds at the Big Sur Coast are larger than those at the Sonoma Coast and include areas used for cattle grazing and horseback riding. However, they are almost entirely undeveloped and the Big Creek watershed is located in a nature reserve. A more detailed investigation would be needed to determine the reason for the regional differences in total coliform levels.

In summary, this study demonstrated exceedances of State of California water quality thresholds for FIB at the selected beach reference systems during wet and dry conditions. The exceedance rate was considerably higher in wet weather conditions. At the ocean sampling sites, 30% of samples exceeded one or several water quality thresholds in wet weather, 15% in winter dry weather, and 4% in summer dry weather. At the freshwater sites, 31% of freshwater samples exceeded one or several water quality thresholds in wet weather, 4% in winter dry weather, and 0% in summer dry weather. Total coliform concentrations led to exceedances most frequently (29 times) and were associated with all wet weather and summer dry exceedances.

The finding that Big Sur Coast beaches and their freshwater sources frequently exceeded the geomean threshold for total coliforms in dry conditions and that Sonoma Coast sites never did suggests that there are potentially systematic differences between beaches of these two regions. A main difference is that the three monitored watersheds at the Big Sur Coast are much larger than the two Sonoma Coast watershed (Table 1). Therefore, the higher concentrations in the larger watersheds may simply represent increased natural loadings of total coliforms associated with sediment or decaying organic material from the watershed. Fecal coliforms and *E. coli* are better indicators of fecal material than are total coliforms. Higher concentrations of these indicators would also be expected if there were different sources of bacteria in the Big Sur watersheds. Additional study is needed to test this hypothesis and to determine the source of elevated total coliform concentrations at Big Sur Coast sites.

FIB Concentrations Between Northern and Southern California Beaches

Comparison to Southern California Reference Beaches

Griffith et al. (2006) reported the frequency of exceedance of state water quality objectives for certain FIBs at reference beaches in Southern California. Table 8 shows how the frequency of exceedance measured at Northern California reference beaches compare for the same FIBs. This comparison was made using frequency of exceedances rather than actual concentrations of FIBs because exceedances of water quality objectives is most important from a management perspective.

The results of this study suggest that the overall frequency of exceedance of FIB water quality objectives at Northern California reference beaches are similar to those observed at Southern California reference beaches (Table 8). This study found that 35% of ocean samples and 31% of freshwater samples collected during the first 24 hours of rain exceeded State of California water quality thresholds during wet weather, compared to 27% of samples collected at Southern California beaches (Griffith *et al.* 2006). Factoring in an estimated confidence interval for the Northern California data of $\pm$ 16%, the frequency of exceedance for the two regions were essentially the same. The storm events sampled in Northern California were much larger in magnitude than those sampled in Southern California, which may have contributed to the slightly higher exceedance rate. Northern California beaches had an average of 4 inches of total rainfall compared to 1 inch of total rainfall per storm event in Southern California (Griffith *et al.* 2006).

However, despite the similarity of the overall rate, the results suggest that there may be important differences between reference beaches in Northern California and Southern California. First, the water quality objective exceedance rates during winter dry weather could be higher at Northern California reference beaches than at Southern California reference beaches. A total of 15% of winter dry samples and 4% of summer dry samples from Northern California reference beaches exceeded water quality thresholds, compared to 1% and less than 1% of samples, respectively, from Southern California beaches. A direct comparison of the results from Northern and Southern California is difficult because of the different sampling criteria and triggers for dry period sampling. In this study, dry weather samples were collected after an antecedent dry period of 96 hours with less than 1 inches of rainfall. In Southern California, some of these sampling events may have met the wet weather sampling trigger of a predicted minimum rainfall estimate of 0.10 inch. Aside from these potential biases from the different study designs, there is a marked difference between Northern and Southern California in the magnitude of wave energy impacting beaches. Point Conception in southwestern Santa Barbara County marks the geographical divide between the Northern California and Southern California coastal regions. South of Point Conception the wave energy arriving from North Pacific storms is blocked by a significant change in California coast orientation and wave energy is significantly reduced compared to the California coast north of Pont Conception (Wilson and Beyene 2007). It is conceivable that increased wave energy at Northern California beaches from winter storms could keep more bacteria suspended in the nearshore water column during the winter months. High volumes of runoff could also explain this observation, if the water at the ocean beaches was predominantly freshwater during wet weather sampling. Unfortunately, this hypothesis cannot be tested because salinity measurements were not made at the ocean beaches with large watersheds in Big Sur (where high runoff volume would be most likely to occur). Future study designs should be sure to collect paired salinity measurements to be able to test this hypothesis.

Another apparent difference between the Northern California reference beaches and their Southern California counterparts is that different FIBs account for the majority of the water quality objective exceedances during wet weather. In Northern California, total coliform was the FIB with the most water quality objective exceedances. Whereas, in Southern California, enterococci was the FIB that accounted for the most exceedances. The difference in the exceedance rates between the two regions is greater than the margin of error (approximately +/- 15%) for total coliforms but not for enterococci (Table 8). These results suggest that there could be potentially differences in the composition of bacteria contributing to loadings from undeveloped watersheds in Northern California versus Southern California. However, total coliforms represent a broad group of bacteria which are routinely found in soil and decaying organic material, such as leaf litter, in the absence of fecal material. It is not clear whether the

higher concentrations represent controllable sources or bacteria associated with sediment loads or naturally decay processes.

The preceding analysis aimed to answer the management questions: "*Are bacteria water quality objectives exceedance rates at Northern California reference beaches and streams different from those in Southern California? Is it justifiable to use data from existing reference systems in Southern California applicable for setting an allowable level of microbial water quality objective exceedances for impaired coastal beaches and streams throughout Regions 1, 2, and 3?*" The study results suggest that the rates at which water quality objectives are exceeded at Northern California reference beaches are similar to those observed in Southern California. Therefore, the data collected for this study indicate that reference concentrations for Southern California can be used for Northern California beaches as a conservative (protective) assumption.

The sample size for this study was not large enough to derive statistically valid reference concentrations for Northern California beaches. However, the study generated a database of results that can be built upon by future studies to achieve this goal. Between 5 and 10 samples were collected at each of the five beaches, during each climatic period (e.g., wet weather, summer dry, winter dry). Approximately 30 samples from each beach in each climatic period are needed to derive statistically valid results. Future studies, using compatible protocols, can continue to build the database if deriving stand-alone reference concentrations for Northern California beaches is deemed a priority. Recommendations for future studies are listed in the last section of this report.

Processes and Factors Influencing Beach Water Quality

Annual Rainfall and Storm Size

As discussed previously, the data indicate differences in bacteria concentrations between Water Year 2016 (drought year) and Water Year 2 (extremely wet year). Freshwater sites experienced a 5-fold increase in water quality exceedances from Year 1 to Year 2. The increase in exceedances in the wet year at the freshwater sites may be explained by increased flows that mobilized and transported more bacteria from within the watershed.

To follow-up on this observation, a regression analysis was attempted to evaluate the potential relationship of the frequency of exceedances across all samples combined, all ocean sites, and all freshwater samples with storm size (amount of rainfall) for each region. No relationship was

detected between storm size and the frequency of water quality threshold exceedances or FIB concentrations for either region (Table 9). However, we cannot be confident of this result because a number of factors may have worked to obscure a potential relationship. For example, the exact timing of sample collection was driven by logistical considerations. Therefore, sampling events occurred at different time points relative to the onset of storm events and the hydrograph. Second, rainfall data are missing for two storms in each region. And, third, the small sample size (both the number of beaches and the samples collected at each beach) means that the test has low statistical power to detect the hypothesized relationship. A different monitoring design would be needed to quantitatively evaluate the effect of storm size on the frequency of water quality exceedances.

Region, Watershed Size, and Presence or Absence of Lagoons

The five beaches included in this study were in two regions (Sonoma and Big Sur) and ranged in size from 5 to 152 square kilometers. Two of the five beaches were lagoon systems. These factors are not independent because all of the beaches with medium-to-large watersheds and lagoons are in the Big Sur region. Similarly, all of the beaches with lagoons have medium-to-large watersheds. Plots of FIB data show that higher concentrations of total coliforms (statistically significant) were found in beaches with medium-to-large watersheds and lagoon systems (Figure 9). The were also statistically significant differences between the watershed size categories for fecal coliforms (Figure 10) and *E. coli* (Figure 11) but the pattern was less clear. The lowest concentrations were found in the medium size watersheds for these FIB. Enterococcus concentrations did not vary with any of these factors.

Flux of Bacteria from Watershed

Three findings suggest a strong linkage between flux of bacteria from watersheds and bacterial levels in the wave wash zone: a) the similarity of ranges of concentrations at ocean and freshwater sites observed in this study (Figures 2 to 5); b) strong correlation coefficients (typically above 0.5) between freshwater and ocean concentrations at each beach (Table 12); and c) water quality threshold exceedance rates were considerably higher in wet weather conditions in both Northern California and Southern California. Therefore, there is strong qualitative evidence for linkage between the watershed fluxes and beach water quality. However, if a quantitative linkage is desired, then a different study design would be needed to quantify this relationship. For example, samples for the Southern California study during each wet weather event were collected on four consecutive days per site (within 24 hours of recorded rainfall and the three days following recorded rainfall), salinity was measured in the surf zone, and flow was measured in the freshwater discharge (Griffith et al. 2006). A similar design or potentially

even higher sampling frequency along the hydrograph (combined with careful timing of the onset of sampling relative to the hydrograph) would be needed to calculate flux for one or several Northern California reference systems. At a minimum, paired salinity measurements should be made a fresh water sites and ocean beach sites to evaluate dilution of watershed loads at the beaches.

Human Sources of Bacteria from Human-Specific Bacteroides Marker HF183

HF183 *Bacteroides* genetic marker results served as an indicator to validate the key working assumption that the selected reference beach systems are minimally impacted by human activity. The results from the *Bacteroides* genetic marker analyses suggest that the examined watersheds are minimally impacted compared to urban watersheds in Southern California (Cao et al. 2017). In our study, the percentage of samples that tested positively above the LOQ ranged from 0 -13% across all sites, and concentrations ranged from 0 to 89 gc/ml. In comparison, HF183 was detected in 11 – 97% of samples in a recent study of 18 drainages in Southern California, with a concentration range of non-detect to 1.5×10^7 gc/ml (Cao et al, 2017). In summary, human-associated *Bacteroides* detection frequencies and concentration ranges from our study are at or below the low end of ranges reported from impacted California watersheds.

There was no correlation of human *Bacteroides* detections or human:total *Bacteroides* ratios and exceedances of FIB thresholds (Table 10, Table 11). A total of 3 samples exceeded a threshold of 50 gc/ml considered indicative of potential sewage pollution in some studies (Koski et al. 2014, Sauer et al. 2011), but none of these samples exceeded any FIB thresholds. Human *Bacteroides* was detected above the limit of quantification threshold (12 gc/ml) in 16 of 270 collected samples (6%), and the ratio of human to total *Bacteroides* concentrations exceeded the "moderate" threshold in 17 samples (6%). An additional 78 samples (29%) tested positive for the human *Bacteroide*s marker below the LOQ. There is currently no established baseline for detecting fecal pollution in environmental waters and no consensus on how to treat detected but not quantifiable (DNQ) results (Ahmed *et al*. 2016, Layton et al. 2013). In environmental samples, DNQ measurements may result from dilution or degradation of a human fecal source and represent true positives, thus increasing the sensitivity of testing. However, there is also an increased probability for false positive results at low target concentrations and a higher likelihood that DNQ results originate from cross-reactivity, for example, with deer, dog, or bird fecal sources (Stewart et al. 2013). Cross-reactivity was not tested for this study. Therefore, it is unclear in which of these categories DNQ results may actually belong, and they are thus reported and identified in a separate category (DNQ positive), as recommended by Stewart *et al*. (2013).

There was no statistically significant relationship of FIB threshold exceedances and *Bacteroides* detections; however, there were some apparent similarities in patterns of FIB exceedances and those in *Bacteroides* detections.

- There were more human *Bacteroides* detections and higher human to total *Bacteroides* ratios overall in wet weather than in winter dry weather or summer dry weather (Figure 13). Especially at freshwater sites, there were considerably less human *Bacteroides* detections in dry weather than in wet weather (Figure 14). Across all freshwater sites, about twice as many samples tested negative for human *Bacteroides* in winter dry weather (73%) and in summer dry weather (74%) than in wet weather (36%). At the ocean sites, the detection frequency for human *Bacteroides* was more similar for wet weather and dry weather for most sites. The notable difference is the Andrew Molera SP Beach ocean site, where 4 of 5 winter dry samples tested positive for human *Bacteroides*, with one detection above the threshold for potential sewage contamination. Summer dry samples had the fewest human *Bacteroides* detections at both freshwater and ocean sites (Figures 13-16).

- Sampling sites with the most frequent FIB exceedances were not the sites with the most human *Bacteroides* detections. For example, the Garrapata Beach ocean site had the highest FIB water quality threshold exceedance rate at 30% and human *Bacteroides* were detected in 27% of samples (of which 8% were detections above the LOQ). However, only 15% of samples from the Andrew Molera SP Beach ocean site exceeded FIB water quality thresholds but human *Bacteroides* were detected in 50% of samples from this site (of which 10% were above the LOQ).

- A Year 1 to Year 2 comparison reveals mixed trends for different human *Bacteroides* metrics (Figures 17-19). In Year 2, there were fewer human *Bacteroides* detections below the LOQ and more detections above the LOQ.

- Human *Bacteroides* concentrations at ocean sites generally have lower correlations with those at freshwater sites than for the FIB (Table 12). Thus, factors other than freshwater inputs may be responsible for water quality exceedances and the presence of human *Bacteroides* at the ocean sampling sites.

The data on human *Bacteroides* should be interpreted cautiously because there is risk of both false negatives and false positives. The potential for false negatives is illustrated by the following example. Human *Bacteroides* were detected more frequently in wet weather than in dry weather conditions, which is consistent with other studies in California (Cao et al. 2017).

They were also detected more frequently above the LOQ in Year 2 (wet year) than in Year 1 (dry year). These observations are consistent with increased mobilization of fecal contamination by increased flows. However, there were also fewer detections below the LOQ in Year 2. One potential explanation is increased sample matrix interference in Year 2 samples, which were generally more turbid than Year 1 samples. Even though the sample preparation included a column purification step to counter matrix interference, it is conceivable that inhibitory residues may have suppressed detections below the LOQ (Shanks et al. 2016). If this were the case, the number of samples testing positive for the marker would be lower than expected.

The potential for false positives is illustrated by the following example. Both the Northern California and the Southern California studies used human-specific markers to assess human contributions to concentrations of FIB at reference beaches[5]. The marker results from both studies indicate human source contributions in each of the watersheds. However, previous studies reported the occasional presence of the HF183 marker in non-human fecal samples, such as deer, dog, or bird, and an increased probability for false positive results at low target concentrations (Ahmed et al 2012, Ahmed et al. 2016, Stewart et al. 2013). Specificity testing using scat collected from regional animal populations to check for cross-reactivity with the HF183 human-associated marker, as well as the addition of an additional human-associated microbial source identification marker that does not target *Bacteroidales*, such as that recently published by Feng et al. (2018) would be useful to verify the presence of human contamination and rule out false positive results.

Recommendations for Future Work and Next Steps

The recommendations from this study fall into two categories.

Recommendations for studies to derive reference concentrations that are specific to Northern California beaches

- The data collected for this study indicate that reference concentrations for Southern California can be used for Northern California beaches as a conservative (protective) assumption. However, the data also suggest some potential differences in the type and seasonality of bacterial concentrations at Northern California beaches versus Southern California beaches. More data would be needed from these beaches to derive statistically valid reference concentrations for Northern California beaches. Between 5 and 10

[5] Griffith *et al.* (2006) used human enterovirus markers.

samples were collected at each of the five beaches, during each climatic period (e.g., wet weather, summer dry, winter dry). Approximately 30 samples from each beach in each climatic period would be needed to derive statistically valid results. Future studies, using compatible protocols, can continue to build the database if deriving stand-alone reference concentrations for Northern California beaches is deemed a priority.

Recommendations for source identification studies

- Evaluate the extent to which non-human sources contribute to human Bacteroides detections in targeted reference systems. Additional region-specific studies that include testing for cross-reactivity of human-associated markers with region specific fecal sources and the addition of an additional human-associated microbial source tracking (MST) marker are needed to evaluate the extent to reduce or rule-out false-positive results that may contribute to human *Bacteroides* detections in reference systems, especially for detections below the LOQ.
- Consider alternative sampling designs. Alternative sampling strategies should be evaluated to reduce data variability in future FIB and MST studies. For example, composite sampling could be a cost-effective approach to improve precision for assessing fecal indicator densities (USEPA 2010b).
- Quantify flux of natural bacteria from watersheds at representative reference beaches in Northern California. A different monitoring design, similar to the one used for Southern California by Griffith et al. (2006), is needed to quantify bacteria flux from watersheds and to evaluate how storm size and other factors affect natural bacterial loadings and the frequency of water quality exceedances. Griffith et al. (2006) demonstrated a quantitative linkage of flux from the watershed and FIB concentrations in the wave wash zone. They collected samples during each wet weather event at four consecutive days per site (within 24 hours of recorded rainfall and the three days following recorded rainfall). The study design would also require direct measurement of flow in the watershed discharge and salinity in the wave wash zone. Precipitation in sampled watersheds should be scaled from nearby gauges using the PRSM raster.

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Tables

Table 1. Reference beach and watershed characteristics.

Reference Beach	Watershed	County	Watershed Size		Annual Precipitation		Open Space	Lagoonal System	CEDEN Stn Code[2]
			km^2	bin[1]	in	bin	%		
Stump Beach	Miller Creek	Sonoma	5.1	small	49.2	heavy	97	No	113MR1171 (FW), 111MLB001 (Ocean)
Stillwater Cove Beach	Stockhoff Creek	Sonoma	5.2	small	48.6	heavy	97	No	113ST0986 (FW), 107PRB001 (Ocean)
Garrapata State Beach	Garrapata Creek	Monterey	27.4	med	38.1	moderate	99	Yes	308BGC008 (FW), 308BGCB01 (Ocean)
Andrew Molera State Park Beach	Big Sur River	Monterey	152.4	large	42.4	moderate	97	Yes	308BSR024 (FW), 308BSRB01 (Ocean)
Big Creek Cove Beach	Big Creek	Monterey	52.5	med	47.9	heavy	98	No	308GAR015 (FW), 308GARB01 (Ocean)

[1]The initial scoping study (Jabusch et al. 2015) employed a ranking method that prioritized candidate reference beaches based on proximity and similarity to impaired beaches. The two features used to assess similarity between reference beaches and impaired beaches were watershed size and average annual precipitation. The similarity analysis was based on binning both impaired and candidate reference beaches into three empirically determined categories for each feature:
Watershed size: small (<17 km^2) – medium (17 – 64 km^2) – large (> 64 km^2)
Precipitation: dry (> 28.8 in) – moderate (28.8 – 44.4 in) – heavy (> 44.4 in)

[2]Site Details

Stump Beach

- 113MR1171, Freshwater Site, Miller Creek at Highway 1 Crossing , Lat 38.5778 N, Lon -123.3317 W
- 111MLB001, Ocean Site, Stump Beach - at mouth of Miller Creek in Salt Point State Park , Lat 38.581404 N, Lon -123.336045 W

Stillwater Cove

- 113ST0986, Freshwater Site, Stockoff Creek at trail bridge upstream of HWY 1 , Lat 38.5484 N, Lon -123.2948 W
- 107PRB001, Ocean Site, Stillwater Cove Beach - at mouth of Stockhoff Creek , Lat 38.546861 N, Lon -123.297541 W

Garrapata Creek

- 308GAR015, Freshwater Site, Garrapata Creek - upstream of the lagoon, 200m from mouth , Lat 36.417122 N, Lon -121.914453 W
- 308GARB01, Ocean Site, Garrapata State Beach - at mouth of Garrapata Creek , Lat 36.417656 N, Lon -121.916113 W

Andrew Molera SP

- 308BSR024, Freshwater Site, Big Sur River - just upstream of the lagoon, 600m from the mouth , Lat 36.282793 N, Lon -121.855492 W

- 308BSRB01, Ocean Site, Big Sur River Beach - at mouth of Big Sur River in Andrew Molera State Park , Lat 36.280985 N, Lon -121.860095 W

Big Creek Cove

- 308BGC008, Freshwater Site, Big Creek- at HWY 1, 80m from mouth , Lat 36.070039 N, Lon -121.599758 W
- 308BGCB01, Ocean Site, Big Creek Cove Beach - at mouth of Big Creek , Lat 36.069918 N, Lon -121.600381 W

Table 2. Summary of sampling events. Numbers are precipitation in inches at the day of sampling and total amount of rainfall during storms events, as recorded at the reference rain gauges at Venado (for the Sonoma Coast) and Big Sur Station (for the Big Sur Coast).

Sampling Date	Region			
	Sonoma Coast		Big Sur Coast	
	24-hr	Total	24-hr	Total
Wet Weather				
01/13/16	2.4	2.64	-	
01/20/16	2.92	2.94	3.26	3.28
02/18/16	-		1.08	1.08
03/07/16	2.8	9.12	1.91	2.07
03/14/16	3.44	>3.44	0.95	1.75
12/16/16	No data	No data	-	
01/04/17	No data	>0.56	-	
01/05/17	-		7.24	7.5
01/19/17	-		1.32	3.65
02/03/17	3.0	6.88	-	
02/07/17	7.18	9.94	No data	>1.78
02/10/17	3.5	3.5	No data	>0.8
02/16/17	1.9	1.9	-	
03/25/17	-		0.45	0.63
04/07/17	-		3.12	3.4
Winter Dry				
01/12/16	0.12		-	
01/26/16	0.2		-	
01/27/16	-		0	
02/03/16	0.08		0	
02/09/16	0		-	
02/10/16	-		0	
02/16/16	0		-	
02/17/16	-		0	
02/24/16	-		0	
03/01/17	0		-	
03/10/17	0		-	
03/15/17	0		-	
03/30/17	0		0	
04/05/17	0		0	
04/11/17	-		0.02	
04/21/17	-		0	
04/28/17	-		0	
Summer Dry				

Sampling Date	Region			
	Sonoma Coast		Big Sur Coast	
	24-hr	Total	24-hr	Total
06/28/16	-		0	
06/29/16	0		-	
07/05/16	-		0	
07/06/16	0		0	
07/12/16	-		0	
07/13/16	0		-	
07/19/16	-		0	
07/20/16	0		-	
07/26/16	-		0	
07/27/16	0		-	
06/28/17	0		-	
07/06/17	0		-	
07/12/17	0		-	
07/19/17	0		-	
07/26/17	0		-	
08/29/17	-		0	
09/05/17	-		0	
09/12/17	-		0	
09/19/17	-		0	
09/26/17	-		0	

Table 3. Bacterial water quality standards for recreational waters in California.

Indicator	Single Sample Criteria Cells per 100 mL	Geomean of at least 5 weekly samples during any 30-day period Cells per 100 mL
Total coliform (TC)	10,000 1,000, if TC/FC ratio <10	1,000
Fecal coliform (FC)	400	200
Enterococcus	104	35
E. coli[1]	235	126

[1]Draft Guidance for Salt and Freshwater Beaches - Appendices Appendix B. US EPA Guidance for Recreational Waters and Beaches (DHS 2001).

Table 4. Genetic marker thresholds derived from studies conducted in Wisconsin (Koski et al. 2014, Sauer et al. 2011).

Threshold Category	**Human *Bacteroides* concentration** gc/ml	**Ratio human *Bacteroides*/ total *Bacteroides*** Percentage
Negative	Not detected/ no amplification	0%
Positive	> 0 *Detected below Limit of Quantification (LOQ)*	NA
Moderate	12 *Detected above LOQ*	0.1%
High	50 *Potential human waste pollution*	2.2%

Table 5. Summary statistics for FIB (TC, FC, *E. coli, enterococci*) at reference beaches and watersheds targeted in this study.

Reference Beach/ Watershed	Sampling site	Event type	n	Maximum	Median	Minimum
		Total Coliform (cells per 100 ml)				
All sites (cumulative)	Ocean	Wet	40	241,960	1,926	8
		Winter Dry	40	22,030	493	2
		Summer Dry	50	18,581	1047	5
	Freshwater	Wet	45	483,923	2,420	8
		Winter Dry	45	3,873	548	2
		Summer Dry	50	8,458	1.316	18
Stump Beach/ Miller Creek	Ocean	Wet	10	3,804	1,469	314
		Winter Dry	10	493	168	36
		Summer Dry	10	18,581	211	5
	Freshwater	Wet	10	2,131	1,321	403
		Winter Dry	10	411	271	115
		Summer Dry	10	1,918	719	415
Stillwater Cove Beach/ Stockhoff Creek	Ocean	Wet	10	2,420	965	282
		Winter Dry	10	8,753	196	16
		Summer Dry	10	154	414	5
	Freshwater	Wet	10	3,012	1,093	375
		Winter Dry	10	505	180	118
		Summer Dry	10	850	414	18
Garrapata State Beach/ Garrapata Creek	Ocean	Wet	10	241,960	22,382	2,400
		Winter Dry	10	22,030	1,714	496
		Summer Dry	10	11,867	2,498	20
	Freshwater	Wet	10	483,923	15,347	984
		Winter Dry	10	3,873	1,860	1,184
		Summer Dry	10	8,458	2,372	624
Andrew Molera SP Beach/ Big Sur River	Ocean	Wet	5	15,531	4,175	2,481
		Winter Dry	5	2,612	749	199
		Summer Dry	10	5,172	1,640	771
	Freshwater	Wet	10	51,721	3,966	14
		Winter Dry	10	2,612	969	13

Reference Beach/ Watershed	Sampling site	Event type	n	Maximum	Median	Minimum
		Summer Dry	10	4,106	1,873	727
Big Creek Cove Beach/ Big Creek	Ocean	Wet	5	1,698	269	8
		Winter Dry	5	798	624	2
		Summer Dry	10	3,068	1,636	7
	Freshwater	Wet	5	1,427	332	8
		Winter Dry	5	688	613	2
		Summer Dry	10	3,076	1,637	738
		Fecal Coliform (cells per 100 ml)				
All sites (cumulative)	Ocean	Wet	40	640	57	6
		Winter Dry	40	445	10	1
		Summer Dry	50	140	7	1
	Freshwater	Wet	45	800	30	8
		Winter Dry	45	470	11	1
		Summer Dry	50	72	9	1
Stump Beach/ Miller Creek	Ocean	Wet	10	540	86	24
		Winter Dry	10	255	14	9
		Summer Dry	10	9	2	1
	Freshwater	Wet	10	720	77	15
		Winter Dry	10	470	20	9
		Summer Dry	10	72	4	1
Stillwater Cove Beach/ Stockhoff Creek	Ocean	Wet	10	207	113	23
		Winter Dry	10	445	10	4
		Summer Dry	10	16	1	1
	Freshwater	Wet	10	250	131	41
		Winter Dry	10	400	22	10
		Summer Dry	10	33	6	1
Garrapata State Beach/ Garrapata Creek	Ocean	Wet	10	640	83	19
		Winter Dry	10	10	6	2
		Summer Dry	10	140	16	1
	Freshwater	Wet	10	800	45	8
		Winter Dry	10	22	6	1
		Summer Dry	10	50	17	1

Reference Beach/ Watershed	Sampling site	Event type	n	Maximum	Median	Minimum
Andrew Molera SP Beach/ Big Sur River	Ocean	Wet	5	110	50	22
		Winter Dry	5	33	17	1
		Summer Dry	10	79	32	5
	Freshwater	Wet	10	90	29	14
		Winter Dry	10	33	10	2
		Summer Dry	10	50	32	2
Big Creek Cove Beach/ Big Creek	Ocean	Wet	5	36	17	8
		Winter Dry	5	50	2	1
		Summer Dry	10	23	11	1
	Freshwater	Wet	5	50	17	8
		Winter Dry	5	9	2	1
		Summer Dry	10	13	8	1
		***E. coli* (cells per 100 ml)**				
All sites (cumulative)	Ocean	Wet	40	631	57	5
		Winter Dry	40	8,753	10	1
		Summer Dry	50	231	7	5
	Freshwater	Wet	45	413	20	5
		Winter Dry	45	56	10	1
		Summer Dry	50	41	10	5
Stump Beach/ Miller Creek	Ocean	Wet	10	232	113	5
		Winter Dry	10	64	15	5
		Summer Dry	10	20	10	8
	Freshwater	Wet	10	311	107	15
		Winter Dry	10	36	10	10
		Summer Dry	10	38	10	10
Stillwater Cove Beach/ Stockhoff Creek	Ocean	Wet	10	238	60	26
		Winter Dry	10	8,753	15	1
		Summer Dry	10	17	10	10
	Freshwater	Wet	10	187	69	36
		Winter Dry	10	31	13	10
		Summer Dry	10	21	10	10

Reference Beach/ Watershed	Sampling site	Event type	n	Maximum	Median	Minimum
Garrapata State Beach/ Garrapata Creek	Ocean	Wet	10	631	52	5
		Winter Dry	10	21	8	5
		Summer Dry	10	231	10	5
	Freshwater	Wet	10	202	38	5
		Winter Dry	10	20	7	1
		Summer Dry	10	31	9	5
Andrew Molera SP Beach/ Big Sur River	Ocean	Wet	5	58	50	20
		Winter Dry	5	85	10	5
		Summer Dry	10	41	15	5
	Freshwater	Wet	10	310	25	10
		Winter Dry	10	56	12	5
		Summer Dry	10	41	10	5
Big Creek Cove Beach/ Big Creek	Ocean	Wet	5	42	10	5
		Winter Dry	5	74	5	2
		Summer Dry	10	31	10	5
	Freshwater	Wet	5	19	8	5
		Winter Dry	5	16	3	2
		Summer Dry	10	21	10	5
		***Enterococci* (cells per 100 ml)**				
All sites (cumulative)	Ocean	Wet	40	623	31	5
		Winter Dry	40	1,223	5	1
		Summer Dry	50	192	6	5
Stump Beach	Ocean	Wet	10	452	52	8
		Winter Dry	10	172	5	5
		Summer Dry	10	56	5	5
Stillwater Cove Beach	Ocean	Wet	10	353	30	8
		Winter Dry	10	159	5	5
		Summer Dry	10	13	5	5

Reference Beach/ Watershed	Sampling site	Event type	n	Maximum	Median	Minimum
Garrapata State Beach	Ocean	Wet	10	623	33	5
		Winter Dry	10	8	5	5
		Summer Dry	10	192	18	5
Andrew Molera SP Beach	Ocean	Wet	5	123	51	5
		Winter Dry	5	1,223	5	5
		Summer Dry	10	25	5	5
Big Creek Cove Beach	Ocean	Wet	5	26	8	5
		Winter Dry	5	33	2	1
		Summer Dry	10	41	21	5

Table 6. Frequency of single-sample water quality threshold exceedances for FIB (TC, FC, *E. coli, enterococci*, and any indicator) expressed as a percent of samples during wet weather, winter dry weather, and summer dry weather, and across all samples at reference beaches and watersheds targeted during this study.

Reference Beach/ Watershed (Watershed size)	Sampling Site	Event Type	TC	FC	E. coli	Entero	Any Indicator
All sites (cumulative)	Ocean	Wet	30	8		13	35
		Winter Dry	2	3		10	15
		Summer Dry	4	0		4	4
		All Samples	12	3		8	17
	Freshwater	Wet	31	9	9		31
		Winter Dry	0	4	0		4
		Summer Dry	0	0	0		0
		All Samples	10	4	3		11
Stump Beach/ Miller Creek ($5.1\ km^2$)	Ocean	Wet	30	10		10	30
		Winter Dry	0	0		20	20
		Summer Dry	10	0		0	10
		All Samples	13	3		10	20
	Freshwater	Wet	40	10	20		40
		Winter Dry	0	10	0		10
		Summer Dry	0	0	0		0
		All Samples	13	7	7		17
Stillwater Cove Beach/ Stockhoff Creek ($5.2\ km^2$)	Ocean	Wet	0	0		10	10
		Winter Dry	0	10		10	20
		Summer Dry	0	0		0	0
		All samples	0	3		7	10
	Freshwater	Wet	20	0	0		20
		Winter Dry	0	10	0		10
		Summer Dry	0	0	0		0
		All samples	7	10	0		10
Garrapata State Beach/ Garrapata Creek ($27.4\ km^2$)	Ocean	Wet	70	20		20	70
		Winter Dry	10	0		0	10
		Summer Dry	10	0		10	10
		All samples	30	7		10	30
	Freshwater	Wet	50	20	0		50
		Winter Dry	0	0	0		0

		Summer Dry	0	0	0		0
		All samples	17	7	0		17
Andrew Molera SP Beach/ Big Sur River ($152.4\ km^2$)	Ocean	Wet	40	0		20	60
		Winter Dry	0	0		20	20
		Summer Dry	0	0		0	0
		All samples	10	0		10	20
	Freshwater	Wet	30	10	20		30
		Winter Dry	0	0	0		0
		Summer Dry	0	0	0		0
		All samples	10	3	7		10
Big Creek Cove Beach/ Big Creek ($52.5\ km^2$)	Ocean	Wet	0	0		0	0
		Winter Dry	0	0		0	0
		Summer Dry	0	0		0	0
		All samples	0	0		0	0
	Freshwater	Wet	0	0	0		0
		Winter Dry	0	0	0		0
		Summer Dry	0	0	0		0
		All samples	0	0	0		0

Table 7. Summary of 5-week geometric mean exceedances. The table lists observed 5-week geomean exceedances for each winter dry and summer dry sampling period by site and sampling year. TC = total coliform. For the "all beaches/watersheds" section, the total number of exceedances for each indicator is shown in parentheses. TC was the only FIB that exceeded thresholds for geometric means.

Reference Beach/ Watershed	Sampling Site	Sampling period	Year 1	Year 2
All beaches/watersheds	Ocean	Winter Dry	TC (1)	TC (1)
		Summer Dry	TC (3)	TC (1)
	Freshwater	Winter Dry	TC (1)	TC (1)
		Summer Dry	TC (3)	TC (3)
Stump Beach/ Miller Creek			No exceedances	
Stillwater Cove Beach/ Stockhoff Creek			No exceedances	
Garrapata State Beach/ Garrapata Creek	Ocean	Winter Dry	TC	TC
		Summer Dry	TC	-
	Freshwater	Winter Dry	TC	TC
		Summer Dry	TC	TC
Andrew Molera S.P. Beach/ Big Sur River	Ocean	Winter Dry	-	No samples
		Summer Dry	TC	TC
	Freshwater	Winter Dry	-	-
		Summer Dry	TC	TC
Big Creek Cove Beach/ Big Creek	Ocean	Winter Dry	-	No samples
		Summer Dry	TC	-
	Freshwater	Winter Dry	-	-
		Summer Dry	TC	TC

Table 8. Comparison of the frequency of single-sample water quality threshold exceedances for FIB (TC, FC, *enterococci*, and any indicator) at Northern and Southern California ocean sampling sites. The frequency of exceedances is expressed as a percent of samples during wet weather, winter dry weather, and summer dry weather.

Regions	Event type	TC[1]	FC	TC:FC[1]	E. coli[2]	TC:E.coli[2]	Entero	Any Indicator
Northern	Wet	30	8	0	3	0	13	35
California	Winter Dry	2	3	0	3	0	10	15
	Summer Dry	4	0	0	0	0	4	4
Southern	Wet[3]	12			9	5	21	27
California	Winter Dry	0			1	0	1	1
	Summer Dry	<1			<1	0	<1	<1

[1]For this comparison, the TC single-sample water quality threshold does not include TC exceedances of the 1,000 cells/mL threshold, if TC/FC <10, which are listed separately as the "TC:FC ratio".
[2]Southern California reference beaches studies substituted *E. coli* for FC (i.e. comparison of *E. coli* concentrations to FC water quality threshold of 400 cells/100 mL)(Griffith *et al.* 2006, Schiff *et al.* 2005).
[3]Within 24 h of recorded rainfall.

Table 9. Results from a regression analysis to evaluate the relationship of the frequency of water quality threshold exceedances and FIB concentrations during wet weather sampling events with storm size. The table provides the R^2 values ($p \leq 0.05$). The analyses for frequency of exceedance were performed with simple linear regression. The analyses for FIB concentrations were performed with log-linear regression. NS = not significant.

Reference Beach/ Watershed	TC	FC	E. coli	Enterococcus
Frequency of exceedance				
Sonoma Coast				
- All sites combined	NS	NS	NS	NS
- Ocean sites	NS	NS	NS	NS
- Freshwater sites	NS	NS	NS	-
Big Sur Coast				
- All sites combined	NS	NS	NS	NS
- Ocean sites	NS	NS	NS	NS
- Freshwater sites	NS	NS	NS	-
FIB concentrations				
Stump Beach/	NS	NS	-	NS
Miller Creek	NS	NS	NS	-
Stillwater Cove Beach/	NS	NS	-	NS
Stockhoff Creek	NS	NS	NS	-
Garrapata State Beach/	NS	NS	-	NS
Garrapata Creek	NS	NS	NS	-
Andrew Molera S.P. Beach/	NS	NS	-	NS
Big Sur River	NS	NS	NS	-
Big Creek Cove Beach/	NS	NS	-	NS
Big Creek	NS	NS	NS	-

Table 10. Summary of single-sample threshold exceedances and human *Bacteroides* detection results. ND = not detected. *=detected below reporting limit. The table lists the results for all samples exceeding any single-sample FIB standard and any additional samples with human *Bacteroides* detections. Samples that did not exceed FIB standards and had no human *Bacteroide*s detections are not listed. ND = not detected, * = detected below detection limit, **detected below reporting limit.

Reference Beach/ Watershed	Sampling site	Event type	Date	FIB Exceedances	Human *Bacteroides* (gc/ml)	Percentage Human/Total
Stump Beach/ Miller Creek	Ocean	Wet	01/13/16	TC, Entero	3*	0.01%
			03/07/16	TC, FC	ND	0.00%
			03/14/16	None	2*	0.06%
			01/04/17	None	54	0.04%
			02/03/17	TC	ND	0.00%
			02/16/17	None	13	0.07%
		Winter Dry	01/12/16	Entero	ND	0.00%
			01/26/16	Entero	1*	0.00%
			02/09/16	None	1*	0.00%
			03/01/17	None	3*	0.03%
			04/05/17	None	3*	0.29%
		Summer Dry	06/29/16	None	0.1*	0.00%
			07/06/16	None	2*	0.04%
			06/28/17	None	41	0.13%
			07/06/17	TC	ND	0.00%
	Creek	Wet	01/13/16	TC, E. coli	7**	0.02%
			03/07/16	TC	3*	0.00%
			03/14/16	None	2*	0.02%
			12/16/16	None	2*	0.02%
			01/04/17	None	88	0.16%
			02/03/17	TC	4*	0.00%
			02/07/17	None	5*	0.03%
			02/10/17	None	6*	0.12%
			02/16/17	FC, TC, E. coli	18	0.04%
		Winter Dry	03/10/17	FC	ND	0.00%
		Summer Dry	07/13/16	None	2*	0.01%
			07/20/16	None	2*	0.03%
			07/27/16	None	2*	0.03%
Stillwater Cove Beach/ Stockhoff Creek	Ocean	Wet	01/13/16	Entero	ND	0.00%
			03/14/16	None	4*	0.18%
			02/16/17	None	7**	0.01%
		Winter Dry	01/12/16	Entero	ND	0.00%
			01/26/16	FC, TC	1*	0.00%
			02/03/16	None	17	0.06%

Reference Beach/ Watershed	Sampling site	Event type	Date	FIB Exceedances	Human *Bacteroides* (gc/ml)	Percentage Human/Total
			02/09/16	ND	1*	0.00%
		Summer Dry	06/29/16	None	2*	0.02%
			07/27/16	None	2*	0.07%
	Creek	Wet	03/07/16	None	4*	0.03%
			01/04/17	None	2*	0.00%
			02/07/17	TC	8*	0.07%
			02/16/17	TC	4*	0.00%
		Winter Dry	02/03/16	None	1*	0.00%
			03/10/17	FC	ND	0.00%
		Summer Dry	06/29/16	None	17	0.16%
			07/06/16	None	3*	0.03%
Garrapata State Beach/ Garrapata Creek	Ocean	Wet	01/20/16	None	8**	0.68%
			02/18/16	TC	ND	0.00%
			03/07/16	TC	ND	0.00%
			03/14/16	None	1*	0.00%
			01/05/17	TC	ND	0.00%
			01/19/17	TC, FC, Entero	ND	0.00%
			02/07/17	TC, FC	ND	0.00%
			02/10/17	TC	12	0.02%
			03/25/17	None	4*	0.01%
			04/07/17	TC, Entero	ND	0.00%
		Winter Dry	01/27/16	None	20	0.03%
			02/17/16	TC	ND	0.00%
			03/30/17	None	11**	0.01%
		Summer Dry	07/26/16	TC, Entero	5*	0.01%
	Creek	Wet	01/20/16	None	25	3.78%
			02/18/16	None	1*	0.00%
			03/07/16	None	1*	0.00%
			01/05/17	TC	ND	0.00%
			01/19/17	TC, FC	ND	0.00%
			02/07/17	TC, FC	19	0.02%
			02/10/17	TC	6**	0.01%
			04/07/17	TC	14	0.06%
		Winter Dry	01/27/16	None	2*	0.04%
			03/02/16	None	5*	0.09%
			03/30/17	None	2*	0.00%
			04/05/17	None	2*	0.00%
			04/21/17	None	2*	0.01%
		Summer Dry	06/28/16	None	1*	0.01%
			07/19/16	None	3*	0.03%

Reference Beach/ Watershed	Sampling site	Event type	Date	FIB Exceedances	Human *Bacteroides* (gc/ml)	Percentage Human/Total
			07/26/16	None	15	0.10%
Andrew Molera S.P. Beach/ Big Sur River	Ocean	Wet	02/18/16	Entero	1*	0.00%
			03/07/16	TC	2*	0.01%
			01/05/17	TC	2*	0.00%
		Winter Dry	01/27/16	None	77	1.51%
			02/03/16	None	10**	0.18%
			02/17/16	Entero	2*	0.01%
			02/24/16	None	1*	0.00%
		Summer Dry	08/29/17	None	27	0.03%
	Creek	Wet	01/20/16	E. coli	0.3*	0.03%
			02/18/16	None	1*	0.00%
			03/14/16	None	1*	0.01%
			01/05/17	TC	0.2*	0.00%
			02/07/17	TC	7**	0.02%
			03/25/17	None	1*	0.01%
			04/07/17	TC. FC, E. coli	ND	0.00%
		Winter Dry	02/10/16	None	2*	0.02%
			02/17/16	None	4*	0.05%
			03/30/17	None	8**	0.04%
			04/05/17	None	4*	0.02%
			04/28/17	None	1*	0.01%
		Summer Dry	06/28/16	None	4*	0.01%
			07/19/16	None	8**	0.12%
			08/29/17	None	11**	0.05%
			09/12/17	None	4*	0.04%
Big Creek Cove Beach/ Big Creek	Ocean	Wet	01/20/16	None	1*	0.15%
			03/14/16	None	1*	0.01%
		Winter Dry	01/27/16	None	6*	0.31%
		Summer Dry	06/28/16	None	3*	0.08%
			07/19/16	None	2*	0.07%
	Creek	Wet	01/20/16	None	0.1*	0.01%
			02/18/16	None	1*	0.02%
			01/19/17	None	42	3.40%
		Winter Dry	01/27/16	None	10**	1.43%
		Summer Dry	07/26/16	None	5*	0.36%

Table 11. Results from a regression analysis to evaluate the relationship of the frequency of water quality threshold exceedances with human *Bacteroides* concentrations. The table provides the R^2 values ($p \leq 0.05$). The analyses were performed with simple linear regression. NS = not significant.

Sites	R^2	*P*
All sites combined	0.003	NS (p=0.77)
Ocean sites	-0.004	NS (p=0.49)
Freshwater sites	0.005	NS (p=0.19)

Table 12. Results from a correlation analysis to evaluate the relationship of ocean sample concentrations with freshwater sample concentrations. The table provides the correlation coefficients from a Spearman rank correlation analysis using untransformed values from all seasons at each beach.

Reference Beach/ Watershed	Event type	TC	FC	E. coli	Human *Bacteroides* (gc/ml)	Percentage Human/Total
Stump Beach/ Miller Creek	All samples	0.52	0.76	0.64	0.13	0.05
Stillwater Cove Beach/ Stockhoff Creek	All samples	0.54	0.70	0.73	0.23	0.19
Garrapata State Beach/ Garrapata Creek	All samples	0.66	0.62	0.51	0.38	0.39
Andrew Molera S.P. Beach/ Big Sur River	All samples	0.48	0.35	0.64	0.26	0.18
Big Creek Cove Beach/ Big Creek	All samples	0.87	0.42	0.43	0.21	0.25

Figures

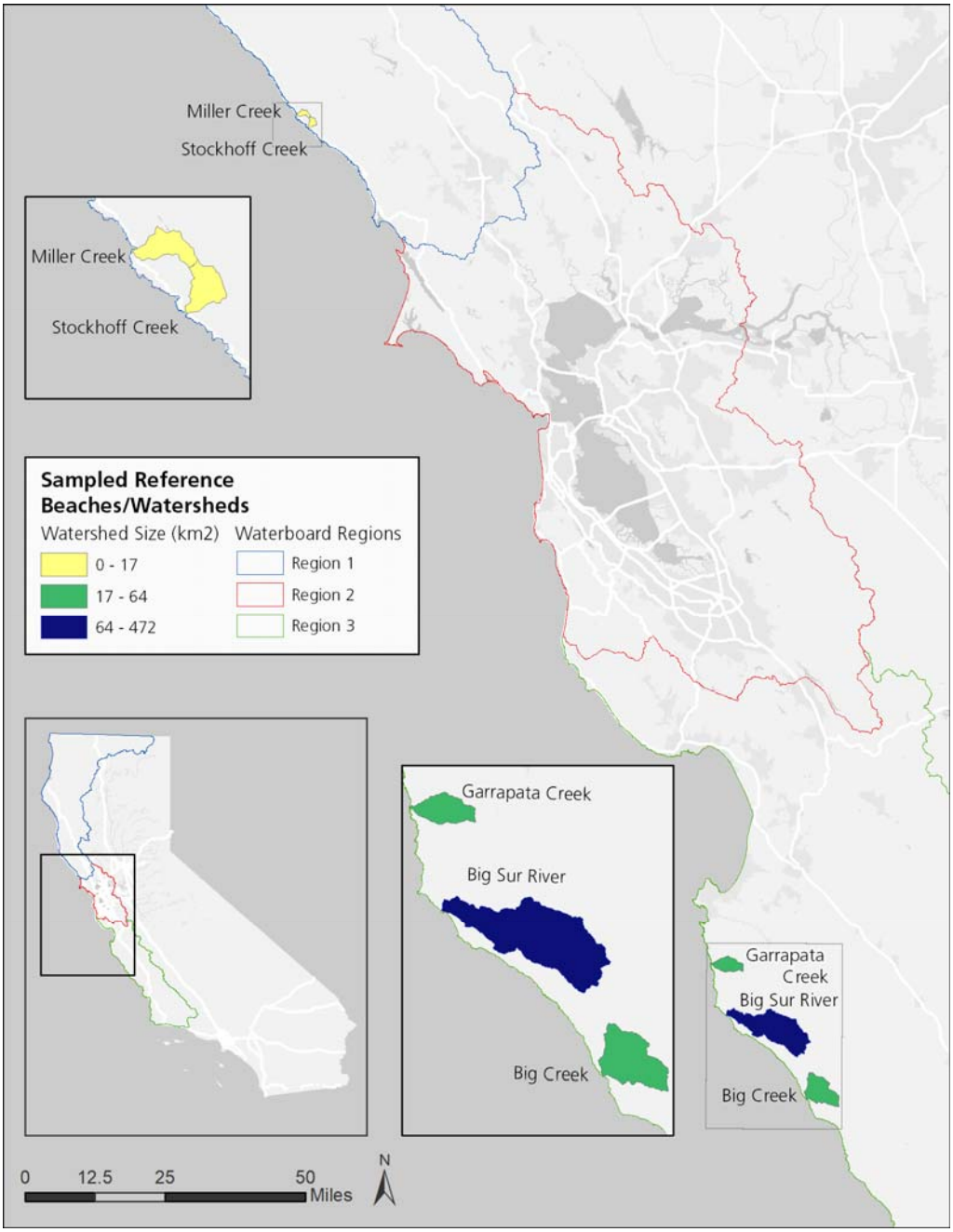

Figure 1. Map of reference beaches and watersheds.

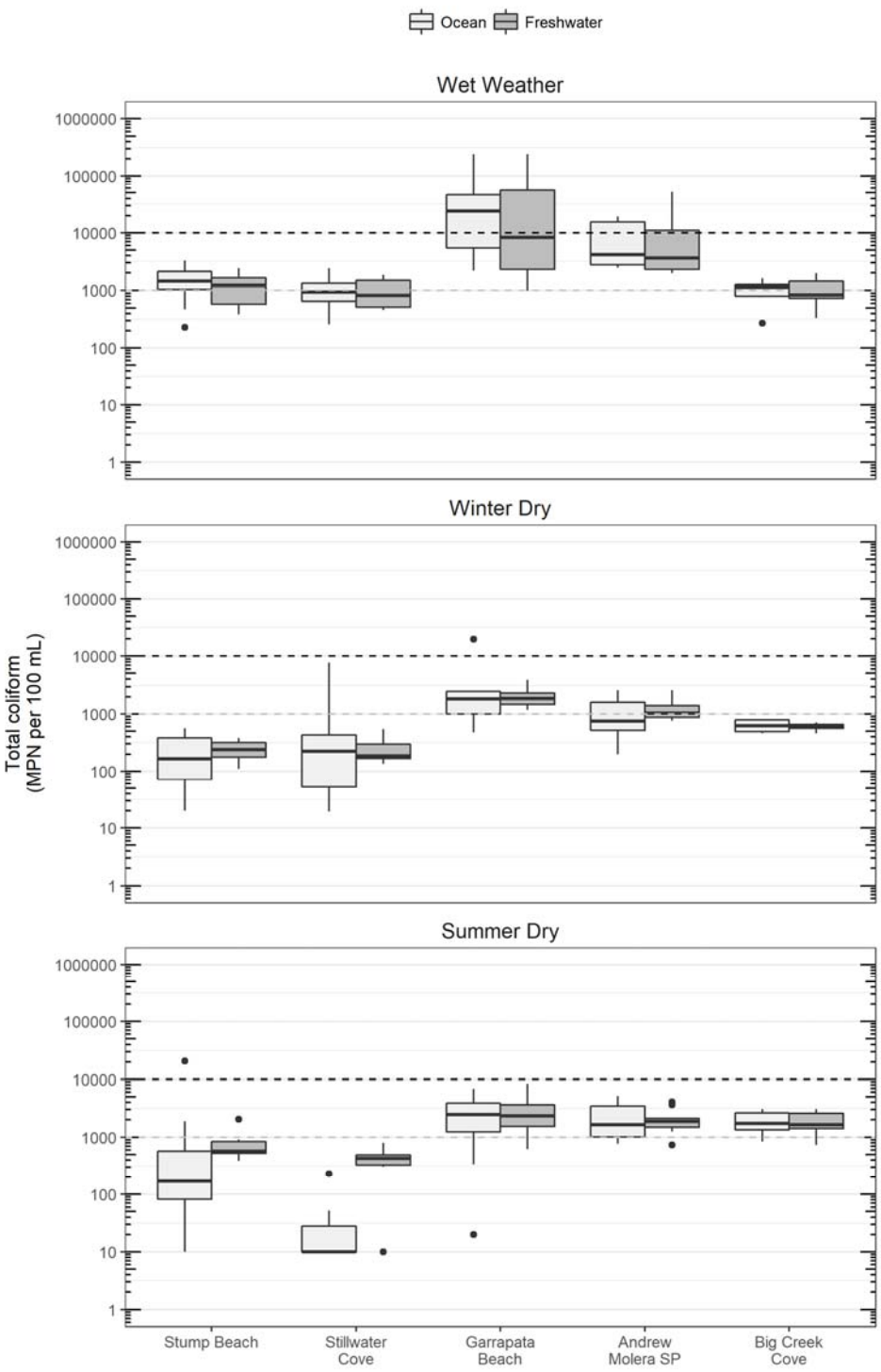

Figure 2. Ranges of total coliform concentrations in grab samples collected during wet-, winter dry- and summer dry events at reference beach ocean sampling sites and freshwater sampling sites. The dotted lines represent California State Assembly Bill AB411 public health standards for marine bathing beaches (see Table 3).

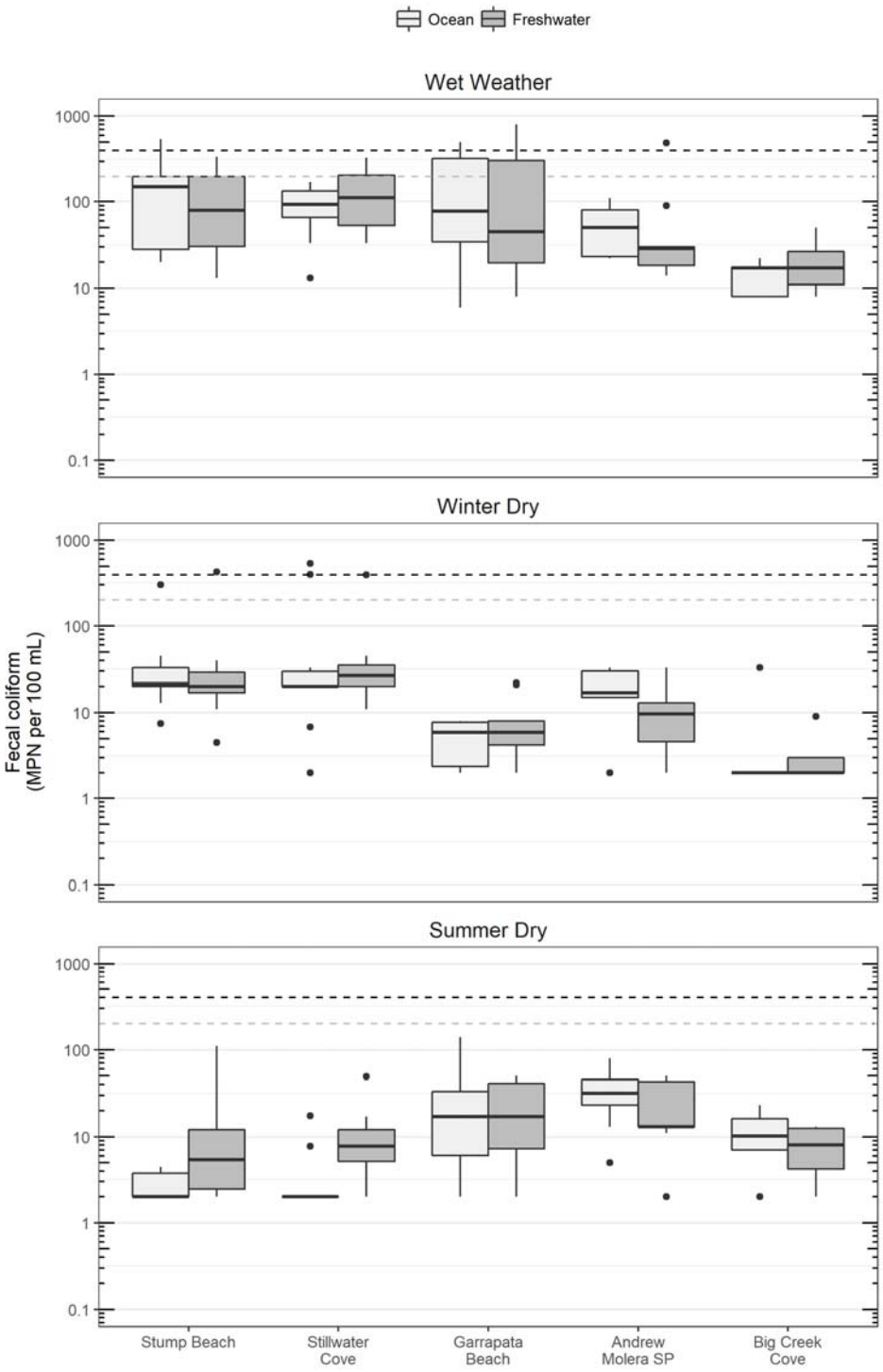

Figure 3. Ranges of fecal coliform concentrations in grab samples collected during wet-, winter dry- and summer dry events at reference beach ocean sampling sites and freshwater sampling sites. The dotted lines represents the California State Assembly Bill AB411 public health standard for marine bathing beaches (see Table 3).

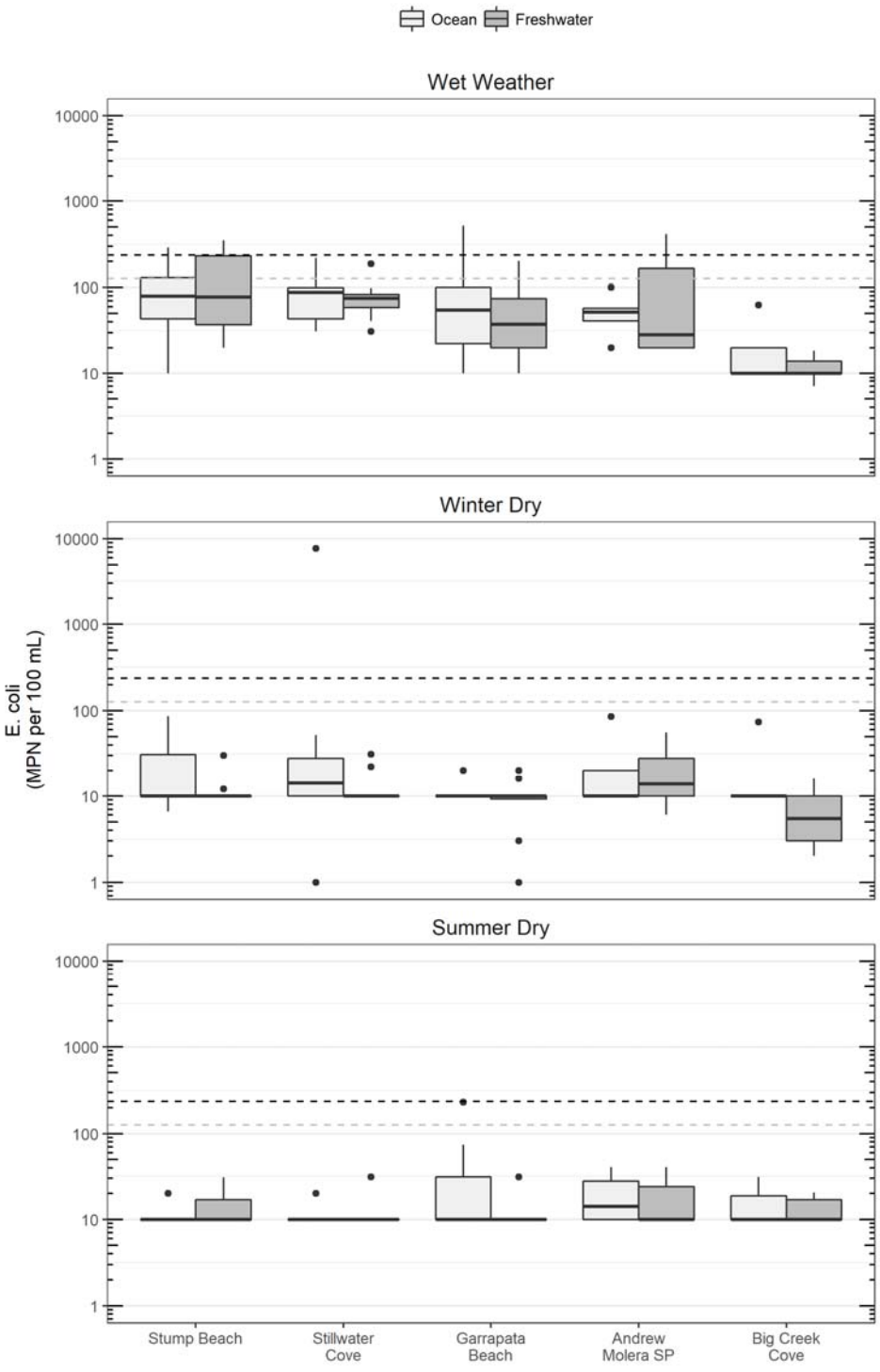

Figure 4. Ranges of *E. coli* concentrations in grab samples collected during wet-, winter dry- and summer dry events at reference beach ocean sampling sites and freshwater sampling sites. The dotted line represents the EPA recreational water quality criterion for freshwater (see Table 3).

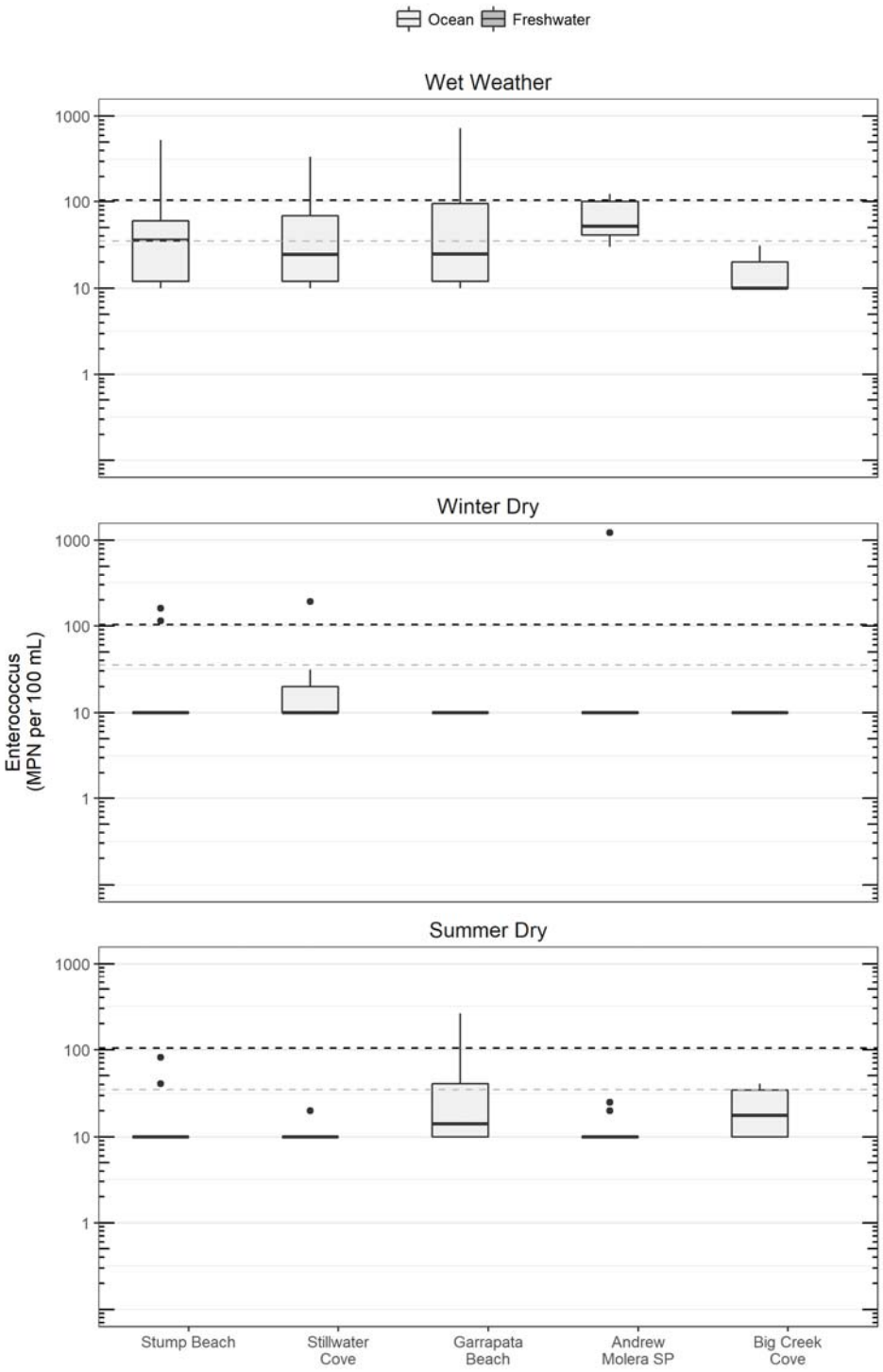

Figure 5. Ranges of *Enterococcus* concentrations in grab samples collected during wet-, winter dry- and summer dry events at reference beach ocean sampling sites. The dotted line represents the California State Assembly Bill AB411 public health standard for marine bathing beaches (see Table 3). Enterococcus was not measured at freshwater sites.

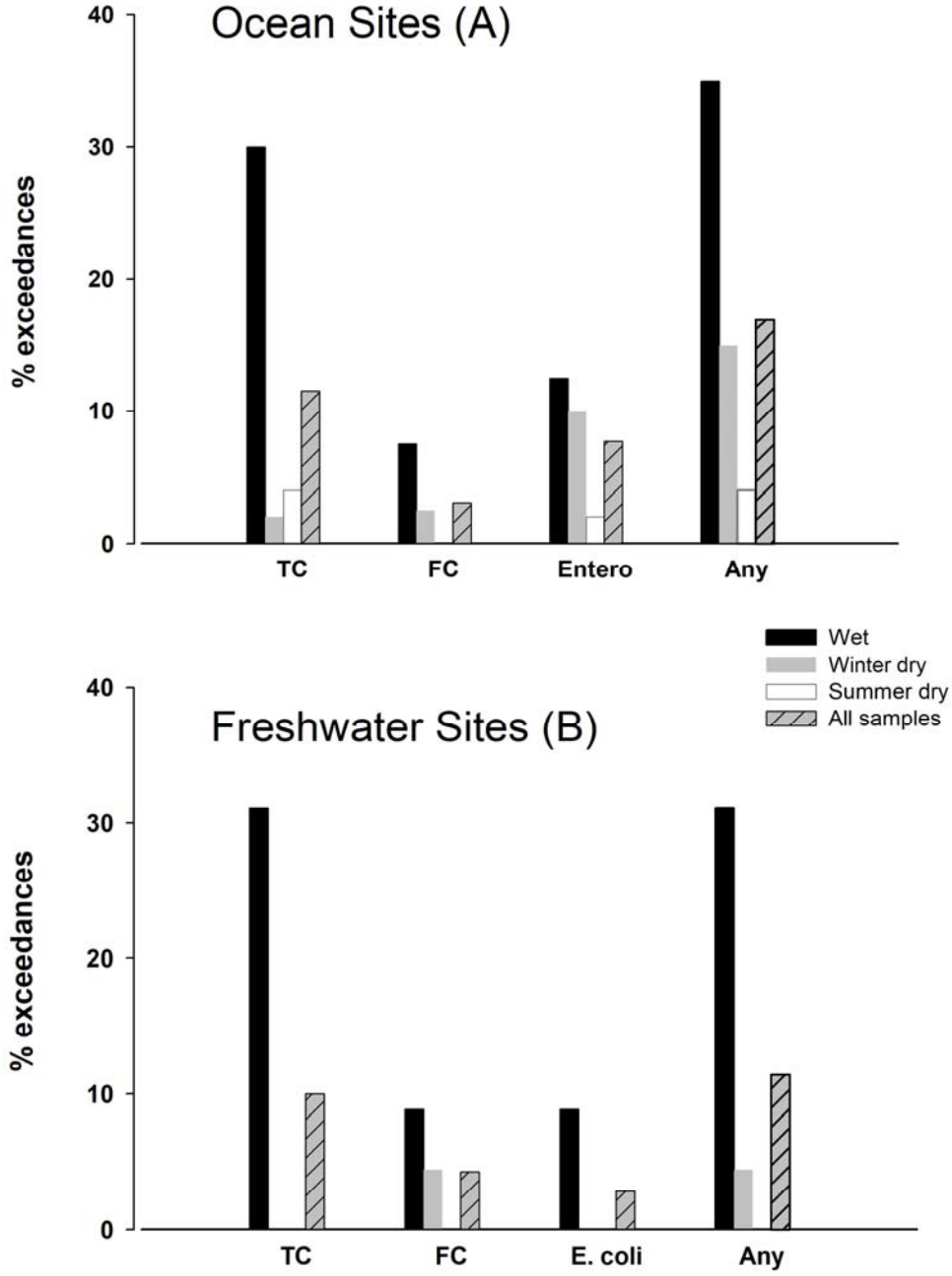

Figure 6. Frequency of water quality threshold exceedances of total coliform, fecal coliform, enterococci, and any threshold across all ocean sites (A) and freshwater sites (B). The graphs display percent exceedances by event for single sample exceedances of water quality criteria for recreational waters in California.

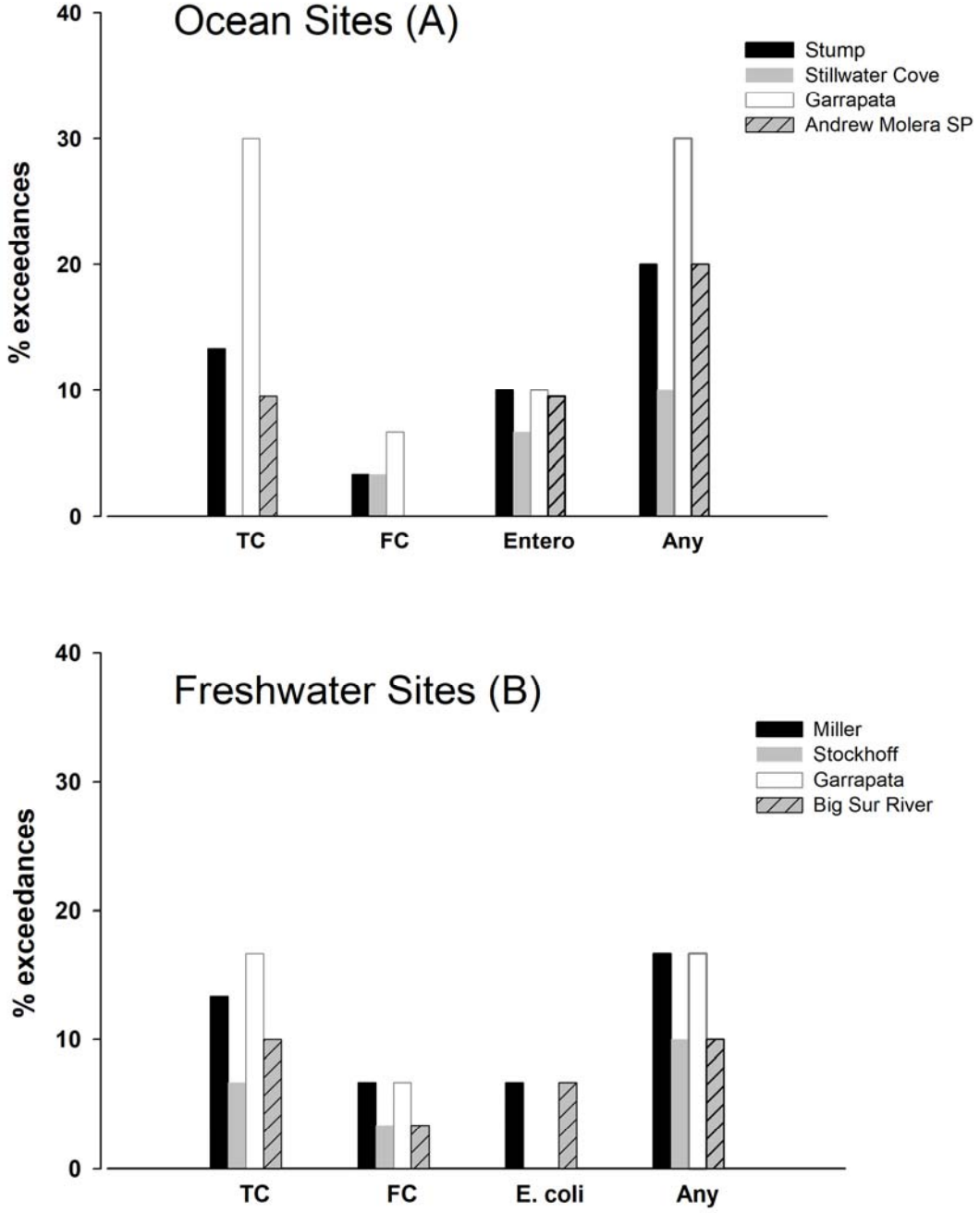

Figure 7. Frequency of water quality threshold exceedances of total coliform, fecal coliform, enterococci, and any threshold at individual ocean sites (A) and freshwater sites (B). The graphs display percent exceedances by event for single sample exceedances of water quality criteria for recreational waters in California. No exceedances were observed at Big Creek Cove Beach and Big Creek. These sites are therefore not displayed.

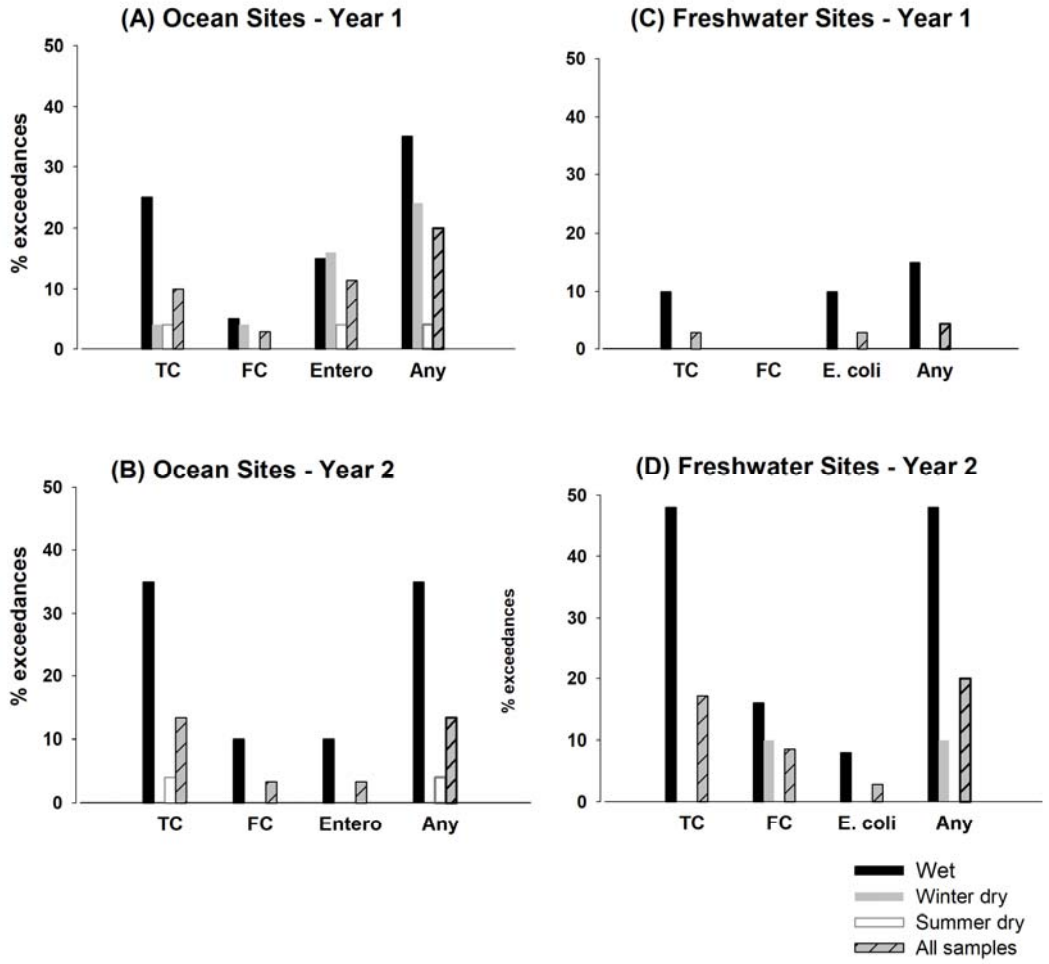

Figure 8. Comparison of the frequency of water quality threshold exceedances of total coliform, fecal coliform, enterococci, and any threshold in Year 1 (January – July 2016) vs. Year 2 (December 2016 – September 2017) across all ocean sites (A and B) and all freshwater sites (C and D). The graphs display percent exceedances by event for single sample exceedances of water quality criteria for recreational waters in California.

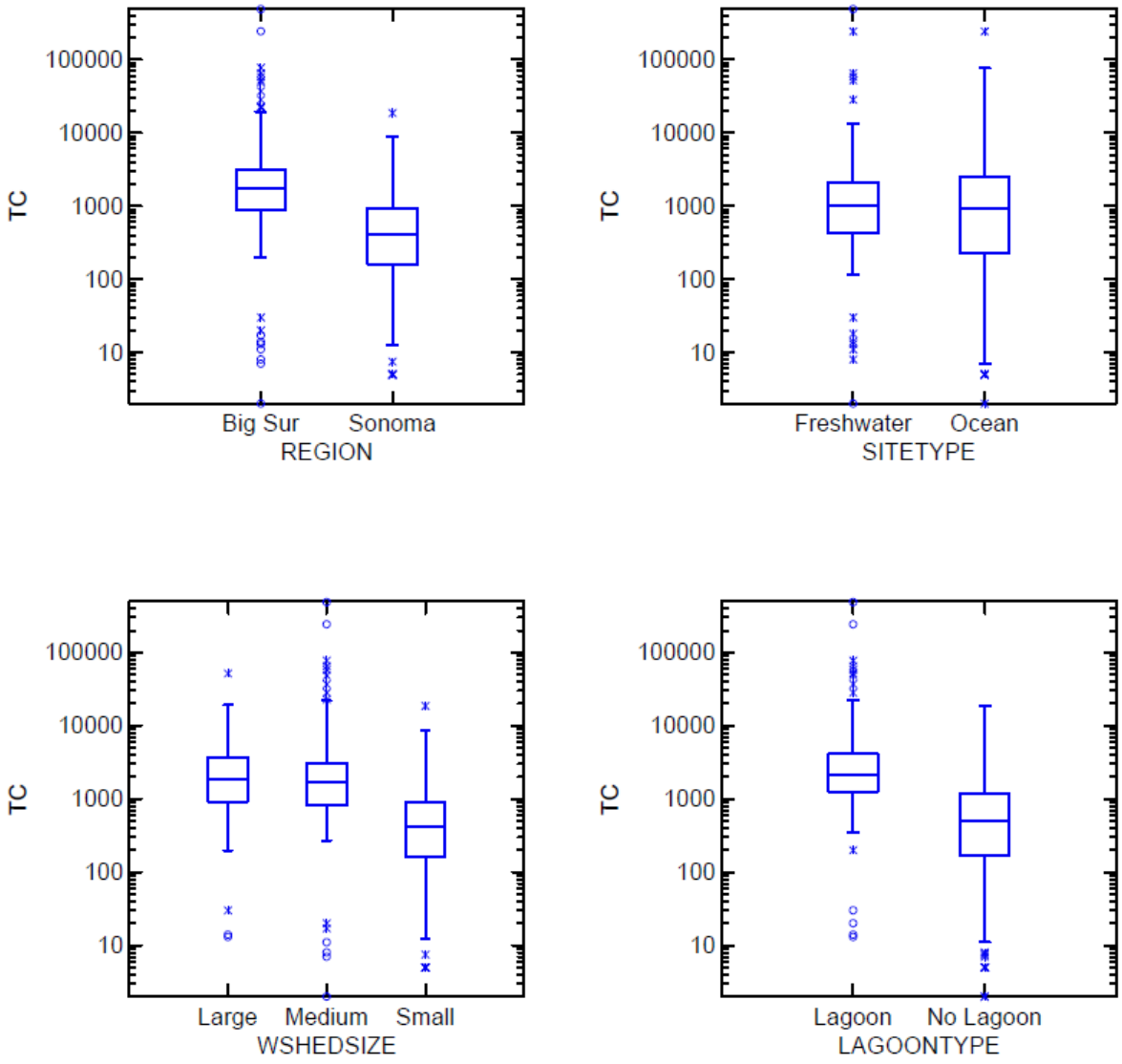

Figure 9: Comparison of total coliform concentrations (in MPN units) for different watershed characteristic factors. There are significant differences ($p<0.05$) among factors for region (top left), watershed size (bottom left), and lagoon type (bottom right) based on Kruskal-Wallis significance tests.

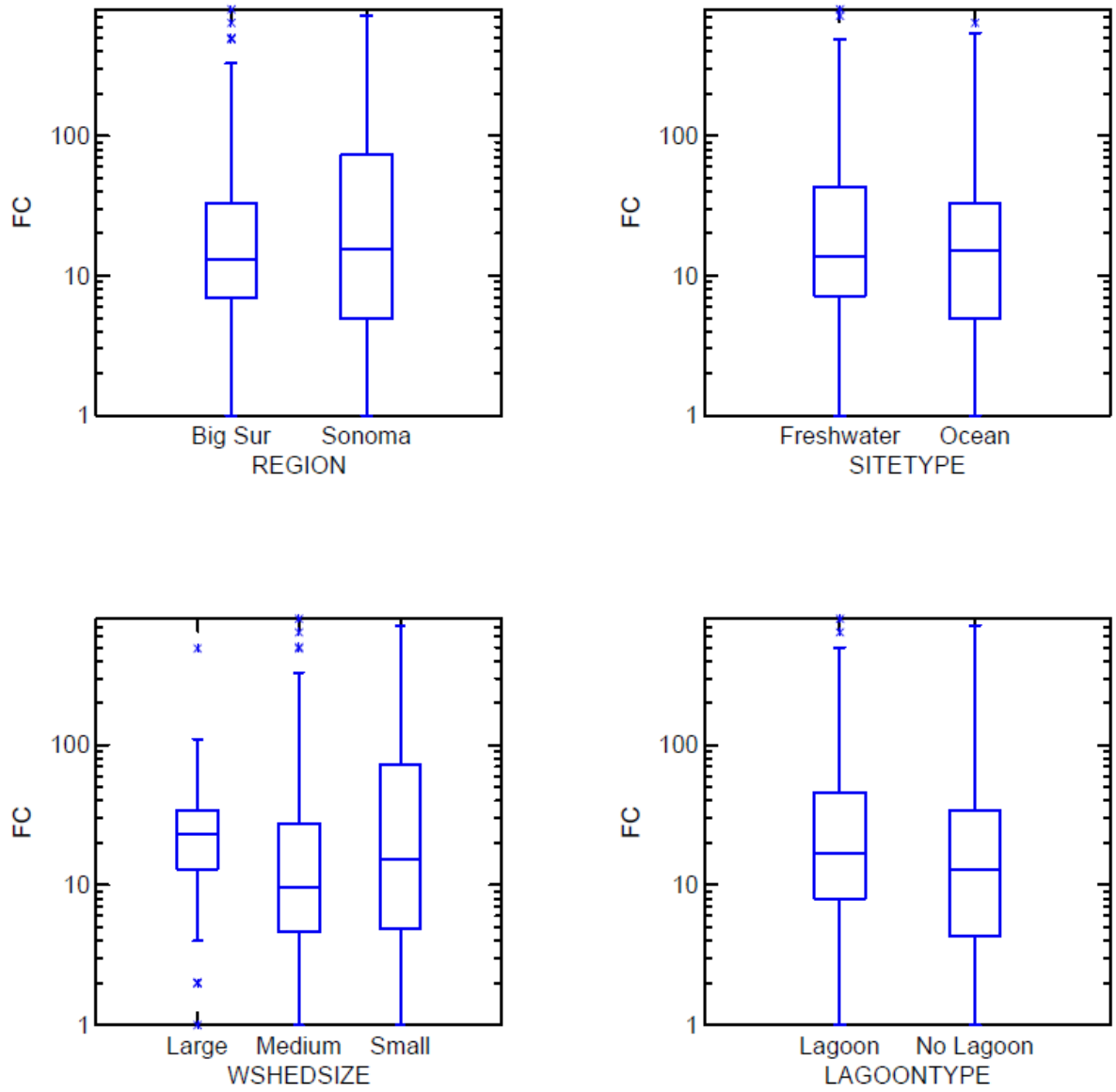

Figure 10: Comparison of fecal coliform concentrations (in MPN units) for different watershed characteristic factors. There are significant differences ($p<0.05$) among factors for watershed size (bottom left) based on Kruskal-Wallis significance tests.

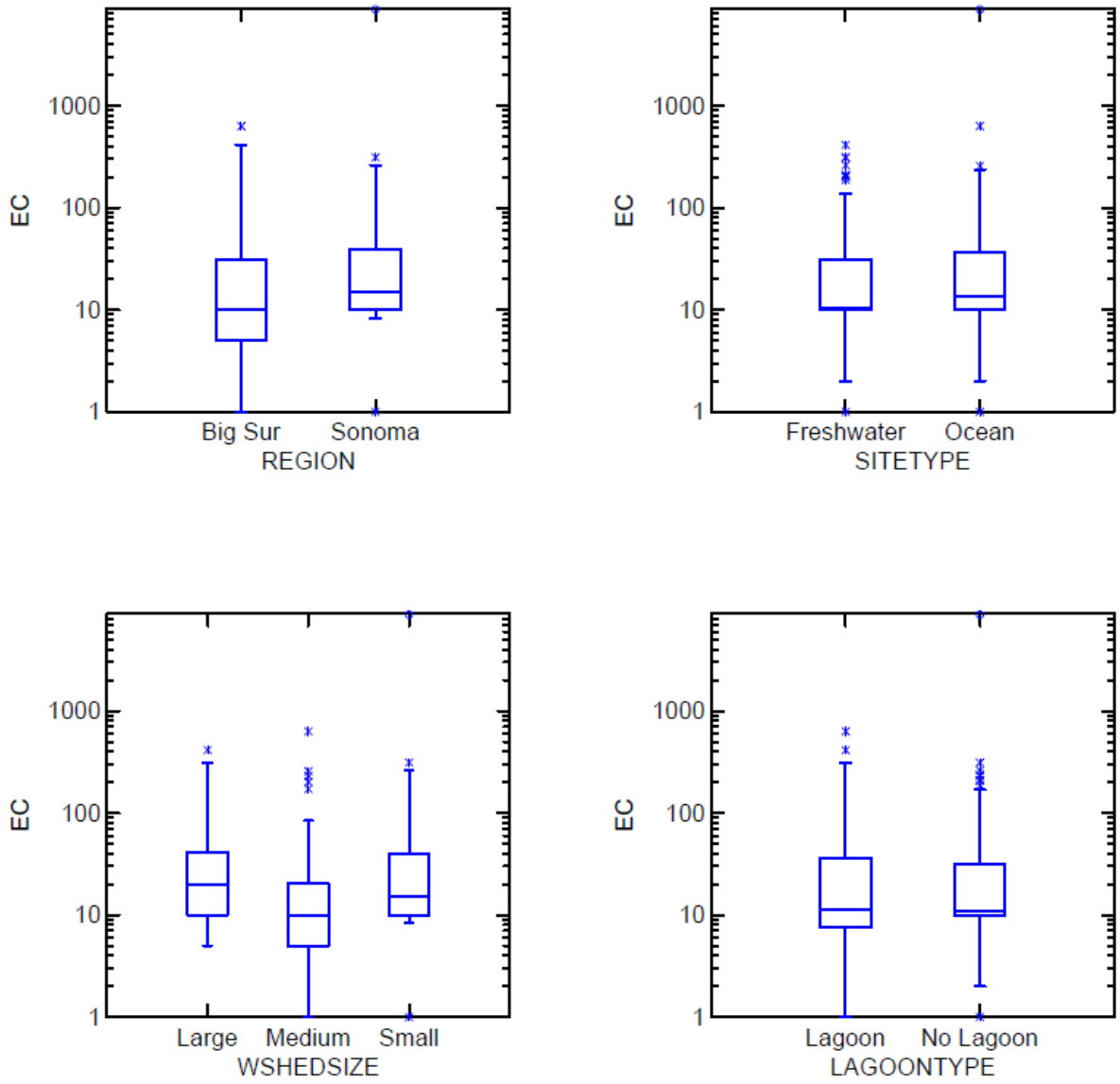

Figure 11: Comparison of *E. coli* concentrations (in MPN units) for different watershed characteristic factors. There are significant differences (p<0.05) among factors for region (top left), watershed size (bottom left), and lagoon type (bottom right) based on Kruskal-Wallis significance tests.

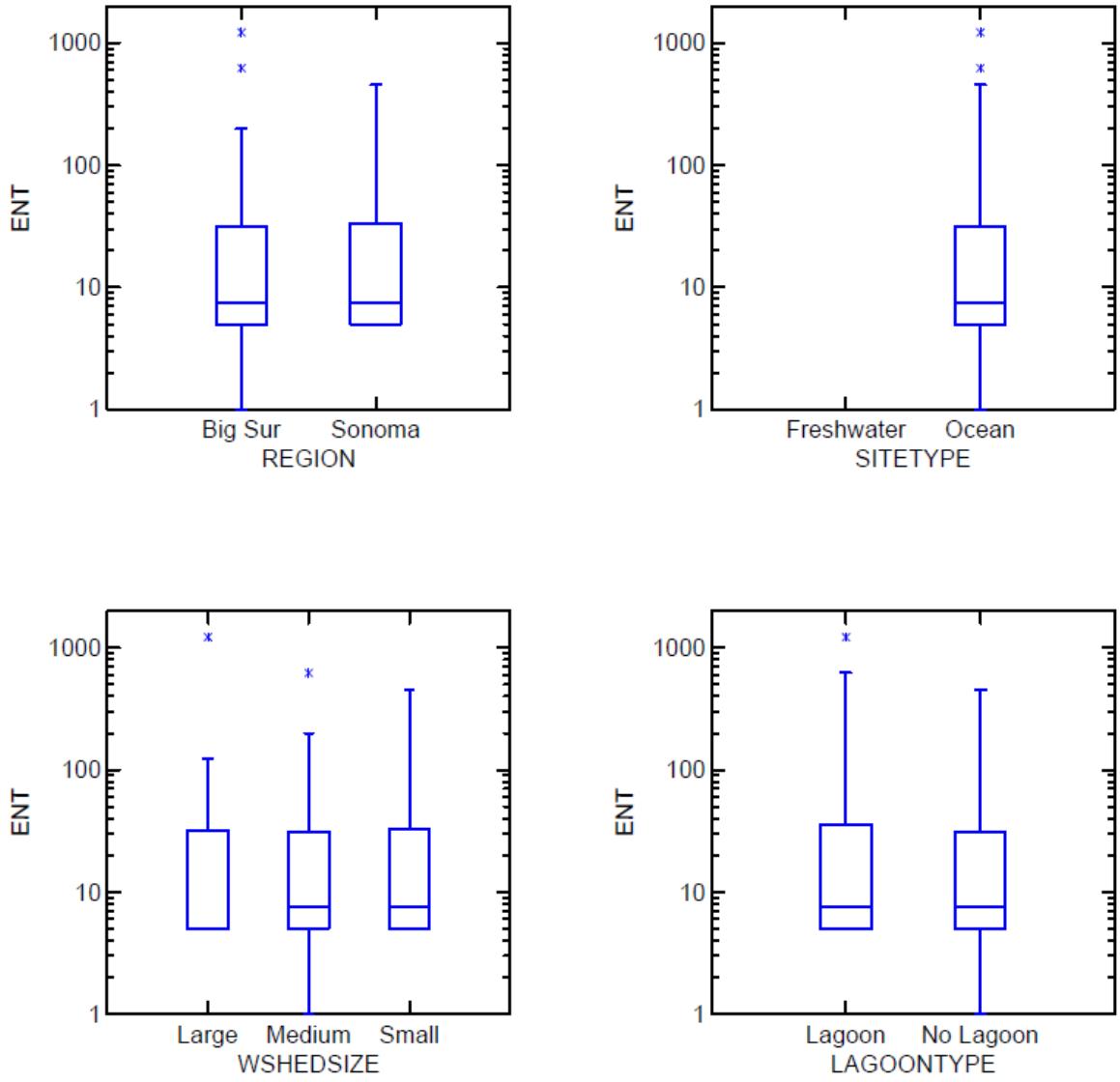

Figure 12: Comparison of enterococcus concentrations (in MPN units) for different watershed characteristic factors. There are no significant differences ($p<0.05$) among factors based on Kruskal-Wallis significance tests. Enterococcus was not measured at freshwater sites.

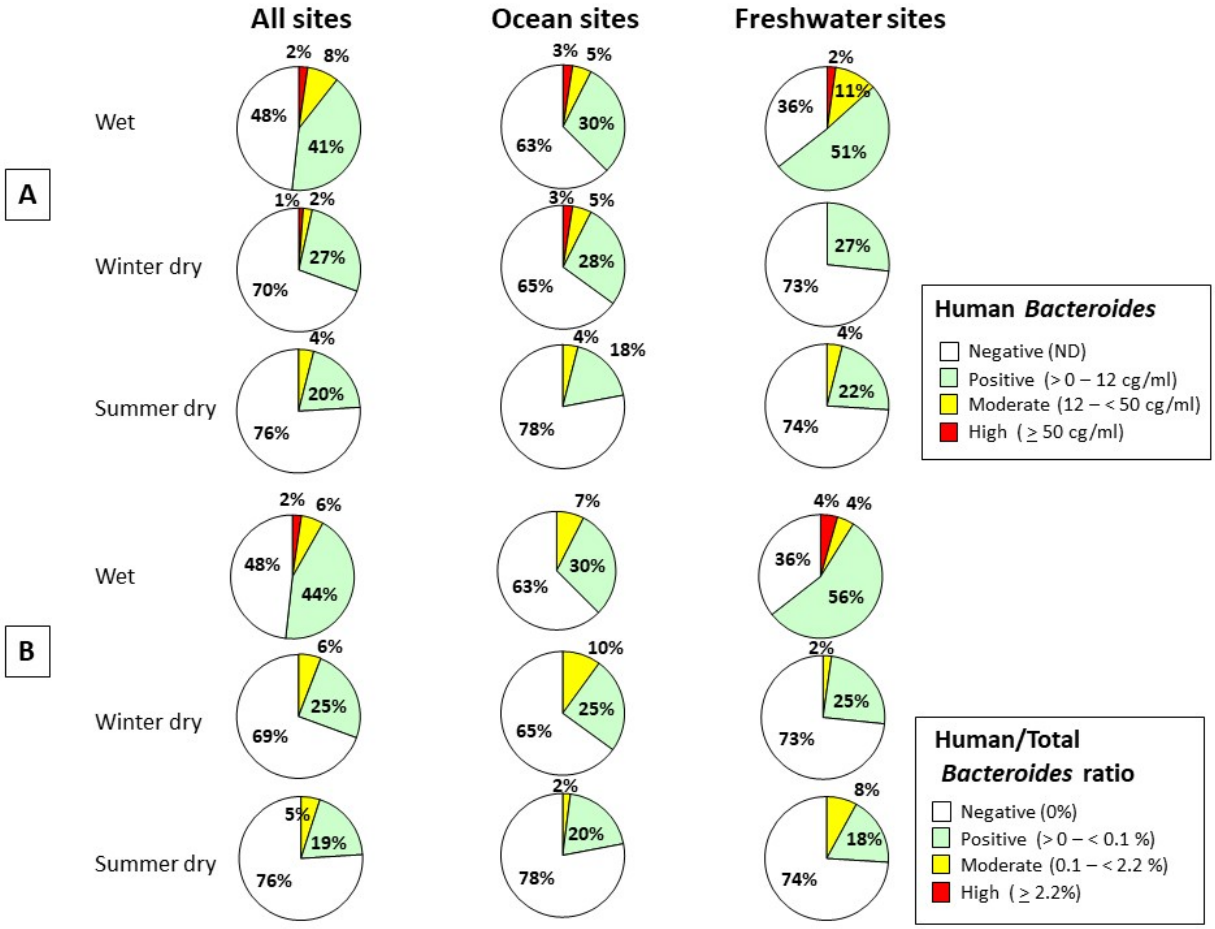

Figure 13. Comparison of genetic marker analysis results for wet, winter dry, and summer dry sampling events. The pie charts compare the distribution of human *Bacteroides* detections (Panel A) and the ratios of human *Bacteroides* to total *Bacteroides* spp (Panel B).

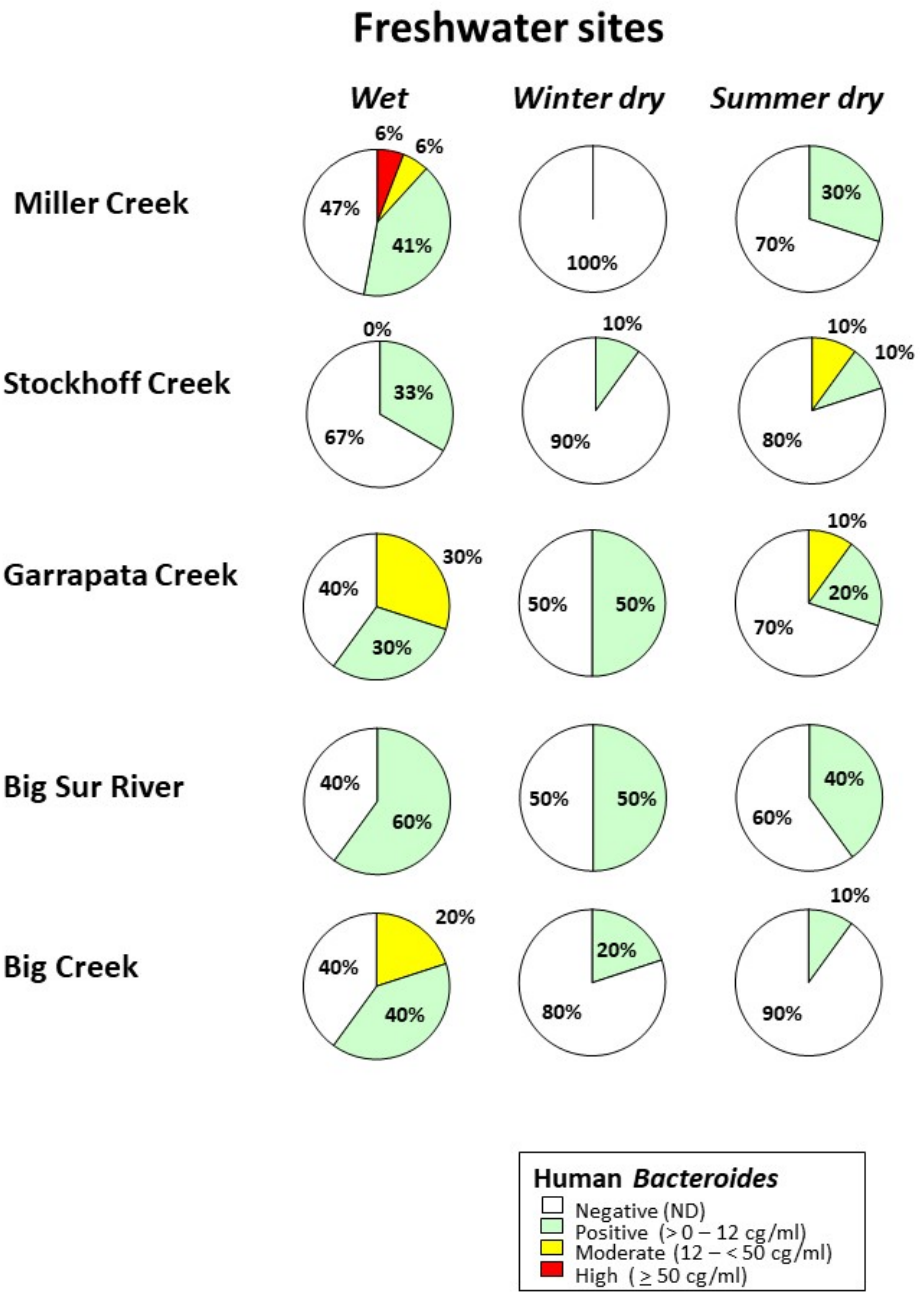

Figure 14. Comparison of human *Bacteroides* detections during wet, winter dry, and summer dry sampling events at freshwater sites.

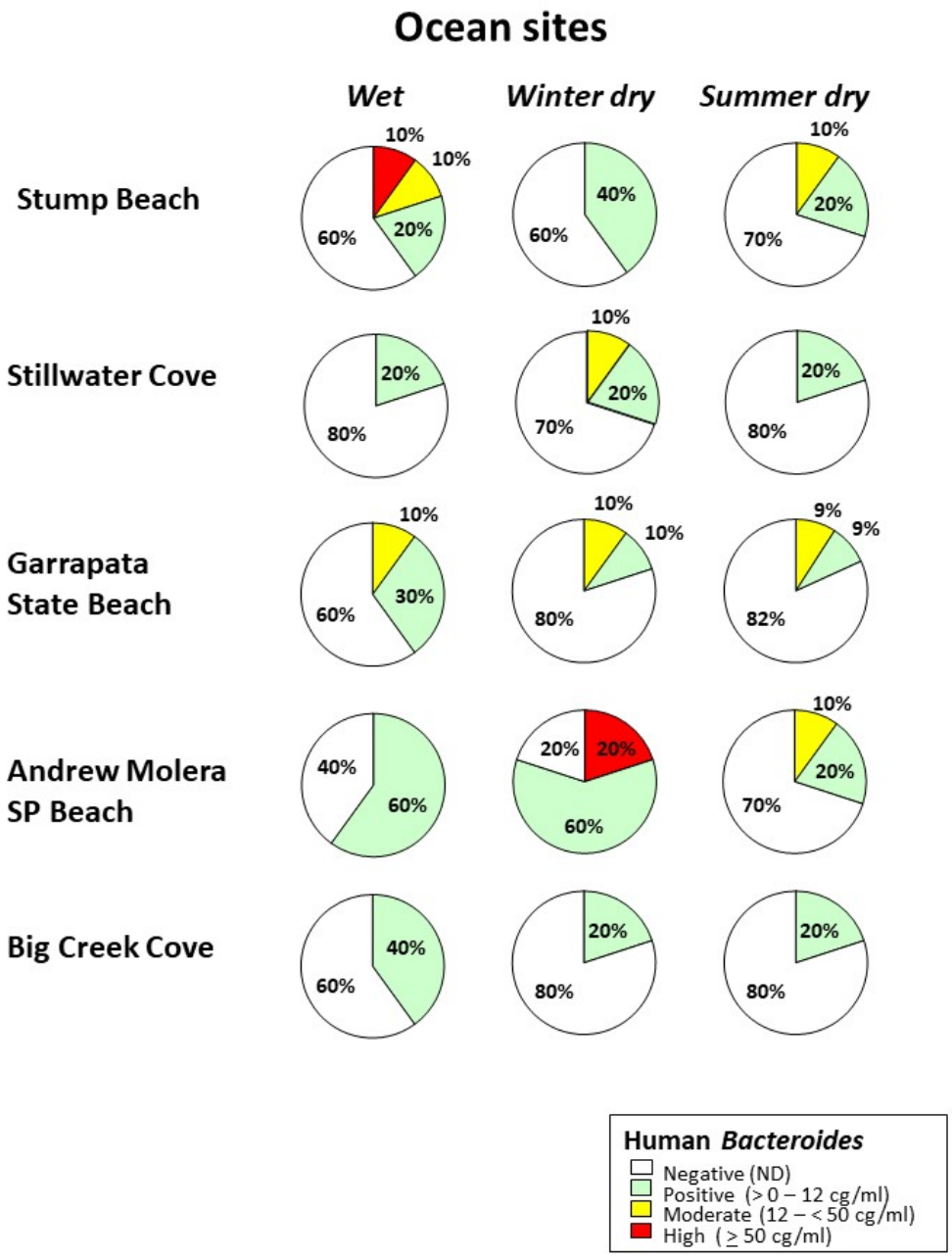

Figure 15. Comparison of human *Bacteroides* detections during wet, winter dry, and summer dry sampling events at ocean sampling.

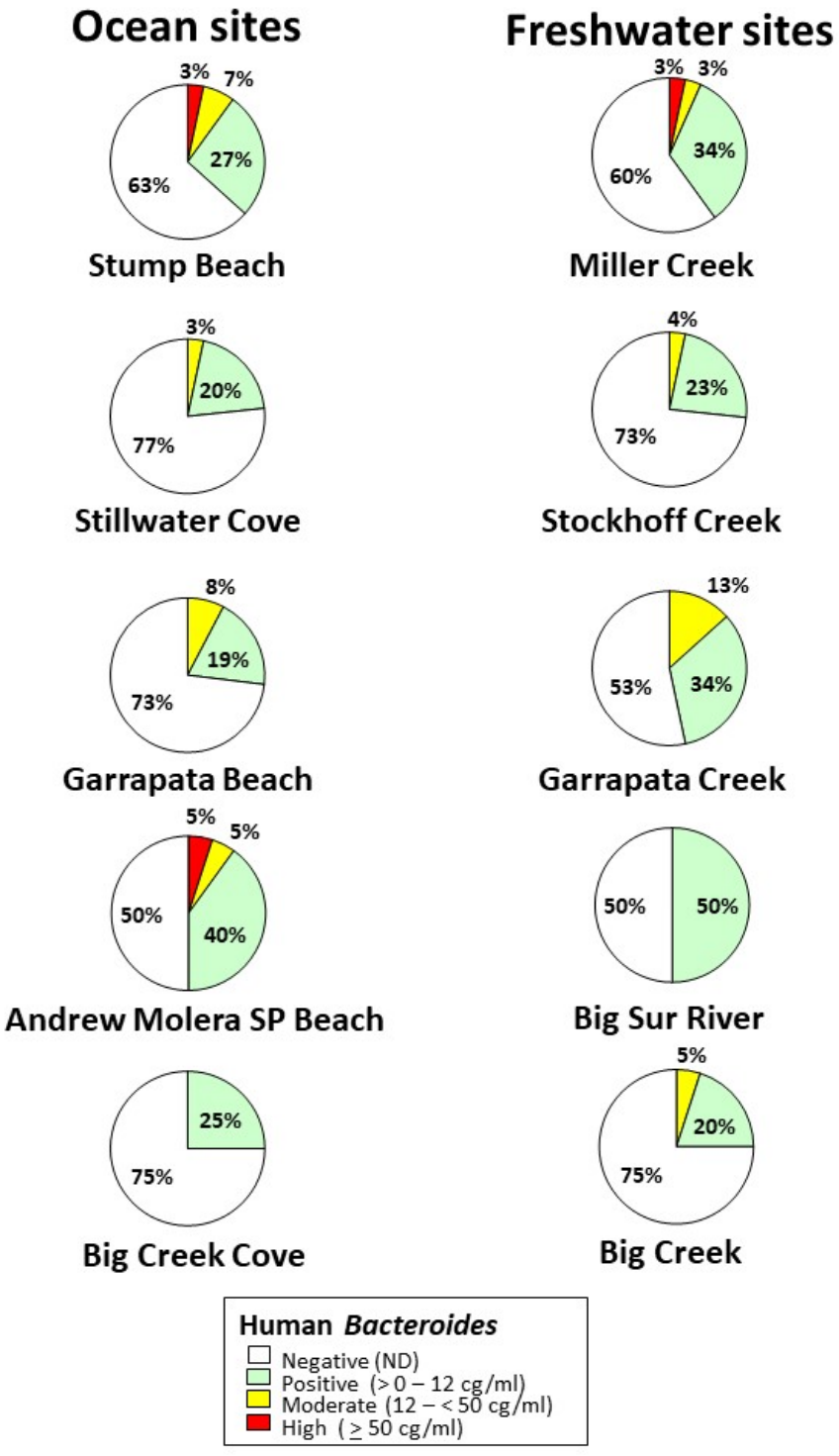

Figure 16. Distribution of human *Bacteroides* detections by site.

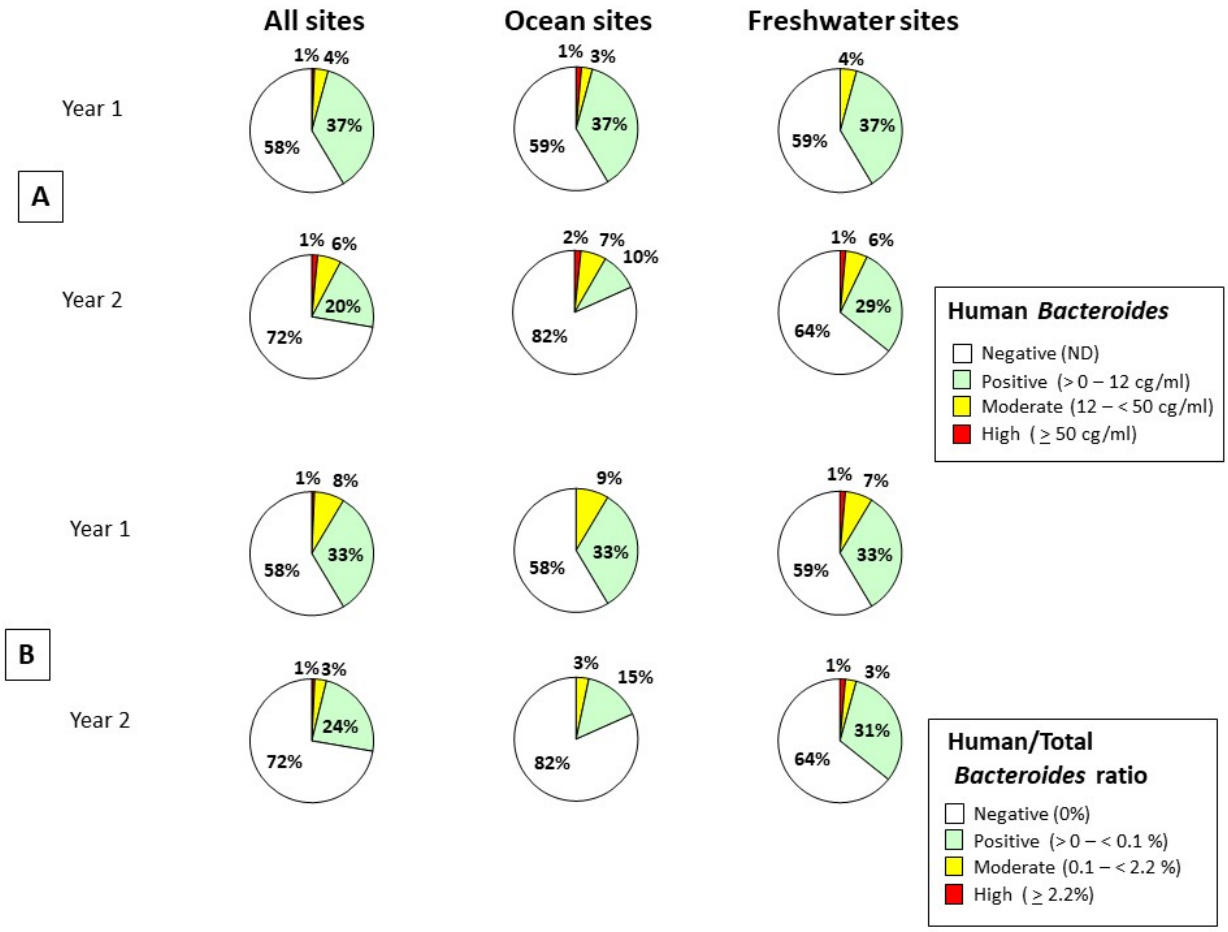

Figure 17. Comparison of genetic marker analysis results in Year 1 (January – July 2016) vs. Year 2 (December 2016 – September 2017). The pie charts compare the distribution of human *Bacteroides* detections (Panel A) and the ratios of human *Bacteroides* to total *Bacteroides* spp (Panel B).

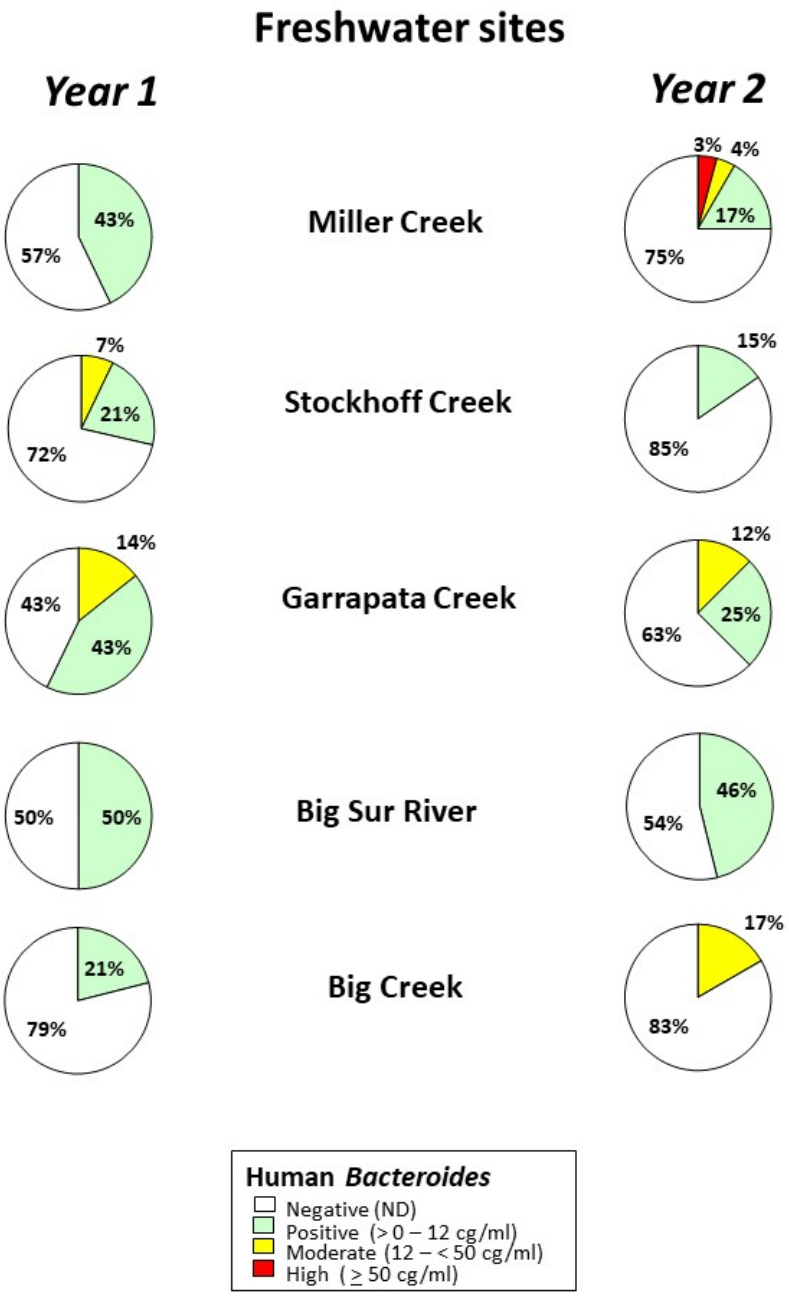

Figure 18. Comparison of human *Bacteroides* detections at freshwater sampling sites in Year 1 (January – July 2016) vs. Year 2 (December 2016 – September 2017).

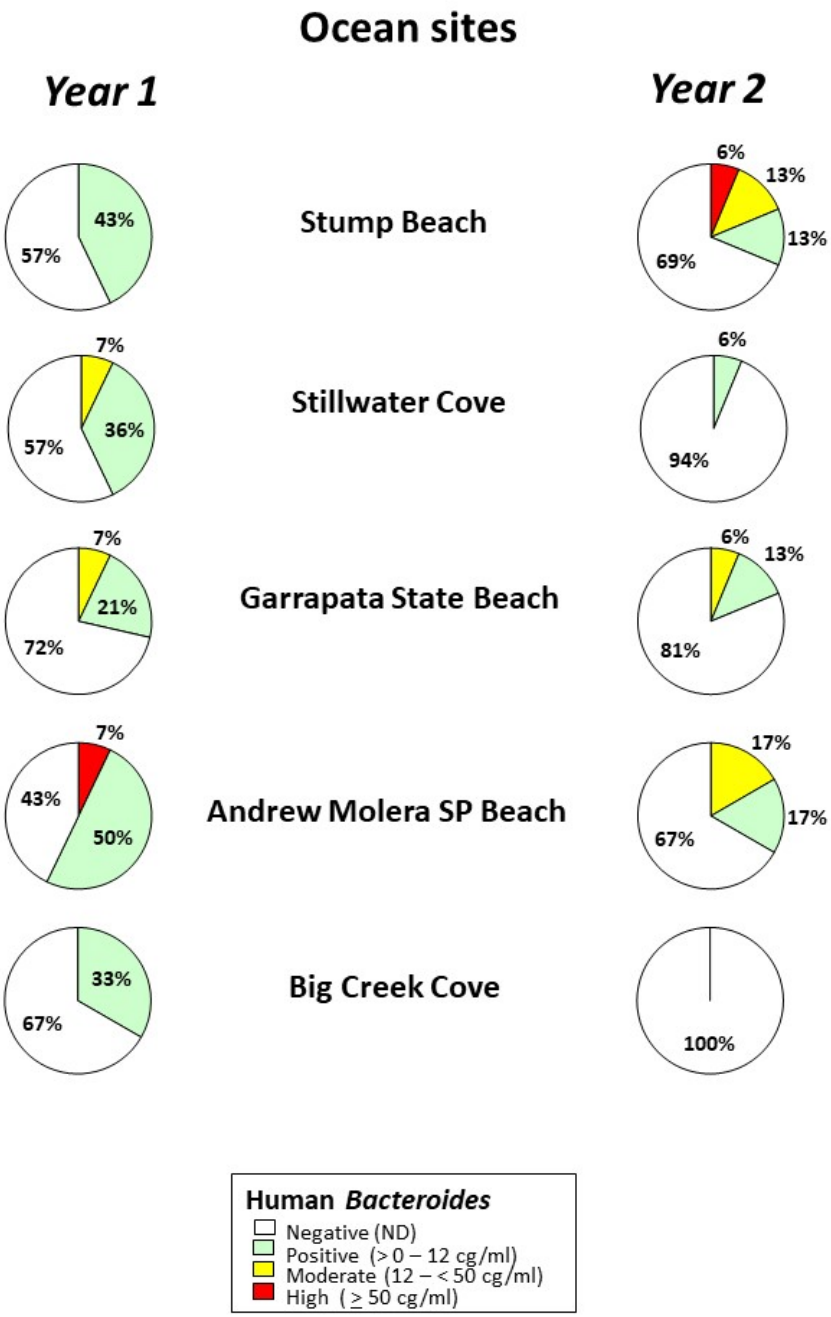

Figure 19. Comparison of human *Bacteroides* detections at ocean sampling sites in Year 1 (January – July 2016) vs. Year 2 (December 2016 – September 2017).

Appendices

Appendix 1. Sampling Plan

Appendix 2. Quality Assurance Summary Reports

Sampling Plan – Reference Systems Microbial Water Quality Sampling for Fecal Indicator Bacteria (*E. coli*/total coliforms, *Enterococcus*, fecal coliforms, *Bacteroides* HF183)

Samples will be collected for the Reference Systems Microbial Water Quality Study following the general field procedures described in the Surface Water Ambient Monitoring Program (SWAMP) Standard Operating Procedures (SOPs) for Conducting Field Measurements and the SWAMP Quality Control and Sample Handling Tables for Indicator Bacteria in Freshwater (revised 8/3/15, released 9/4/15). Specific sample handling procedures for the Northern California Coastal Reference Beaches Study are described in **Table 1**. The study will be performed as a joint collaboration between SFEI-ASC, the San Francisco Bay Regional Water Quality Control Board, and the North Coast Regional Water Quality Control Board. Laboratory or field services will be provided by Cel Analytical Inc., Marine Pollution Studies Lab (MPSL), Monterey Bay Analytical Services, Inc. (MBAS), and Sonoma County Public Health Laboratory. The Central Coast Regional Water Quality Control Board is participating in an advisory role. The San Francisco Bay Regional Water Quality Control Board is providing funding for the Reference Beaches Microbial Water Quality Study.

1.1 Sample Collection

The Reference Beaches Microbial Water Quality Study Design Summary specifies sampling of 10 wet events, 10 winter dry events, and 10 summer dry events over the course of the study (i.e., more than two years).

The sampling locations are described in the Study Design Summary. Field sample collection teams will collect samples at each sampling location based on the event triggers, criteria, and frequencies guidelines shown in **Table 2**. Safety and logistical considerations will be factored into the scheduling of sampling events. Over the course of the study, field sample collection teams will collect one field duplicate sample per event type (i.e., summer dry events, winter dry events, and wet events) for each indicator at each sampling location, which equates to 10% of field samples. Field teams will collect one field blank per event type for all FIB indicators, and at least one sample at each location that will be provided to Cel Analytical to perform a matrix spike. Field sample collection teams will fill one sterilized 250 mL glass jar for each FIB sample (one for E.coli/TC, one for enterococcus, one for FC) and one sterile Bacti Bottle (120 mL wide-mouth polypropylene container) for each *Bacteroides* HF183 sample and transport all samples to the laboratory on wet ice for analysis by standardized EPA methods. Specifications for sample handling are shown in **Table 1**. Field quality control (QC) measurements, QC sample information, and Method Quality Objectives (MQOs) are summarized in **Table 3**.

1.1.1 Equipment

The following equipment is necessary in addition to any standard SWAMP field equipment requirements:

- Sterile 120 mL Bacti Bottles for *Bacteroides* samples and sterilized 250 mL Nalgene wide mouth bottles for FIB
- Ziploc bags for separately containing samples during transportation
- Sterile gloves
- Cooler with wet ice

- YSI 6920 Water Quality Meter (for measuring SC), refractometer, or handheld probe (calibrated within 24 hours of a field deployment), if specific conductance is measured in the field
- Sample bottle for specific conductance measurement, if measured in the lab

1.1.2 Sample Collection Procedure

The field sample collection teams will collect samples by directly filling the sample container. Sample Collection Teams will fill out SWAMP field data sheets immediately after sample collection. All sample containers will be labeled with the date, SWAMP Station ID, parameters to be measured, preservation method, and sample matrix ("seawater" or "freshwater").

Prior to the field trip:

1. Using "Sharpie" waterproof felt-tip marker, fill out CryoLabel with sample location, station ID (the freshwater and saltwater locations will need different station IDs), sample ID number, collection date and time, and label as "seawater" or "freshwater".
2. Attach label to proper sample bottle. Dry surface before attaching. Tape over label with packing tape to ensure adhesion and waterproofness.

Sample collection will be performed as follows:

3. Wear gloves prior to start of sampling and change between each sampling stations.
4. Gloves and sampling poles should be treated using a mist bottle of ethanol or isopropyl alcohol and rinsed with a mist bottle containing sterile water prior to sampling and between sites.

Beach Sample Steps

5. The primary sampling location at each site is in the ocean immediately in front of the freshwater input at the so-called ''wave wash", where the watershed discharge initially mixes with the ocean waves.
6. Record SC measurement (if measured in field) as described in the SWAMP SOPs for Conducting Field Measurements (page 10).
7. Collect "wave wash" water samples (primary location) 6-12 inches below the surface between ankle and knee depth on an incoming wave.[1]
8. A sampling pole may be used, if necessary.
9. If a sampling device is used, secure the sample bottle onto the device. (Be sure to rinse with sterile water between each sampling location).
10. Collect FIB water samples following the collection procedure described in the SWAMP SOP for Bacteria and Pathogens in water samples (SWAMP SOPs for Conducting Field Measurements, pp. 49-51).
11. Install sample bottle cap.
12. Using "Sharpie" waterproof ink pen, label cap with sample location.
13. Collect HF183 samples as follows:
    a. Remove the seal completely from the bottle.
    b. Remove the lid carefully. Do not touch the inside of the bottle or lid.
    c. Collect sample by submerging the bottle and pushing forward with a slow, even motion. (Containers should be positioned such that the mouth of the container is pointed away from the sampler or sample point)

[1] Safety considerations may require a modification during storm events.

d. The sample should be collected with a single stroke
e. The sample bottle should be filled to just above the 100ml mark.
f. Replace cap and tighten.
g. Using “Sharpie” waterproof ink pen, label cap with sample location.

14. Place samples in individual Ziploc bags and place on ice immediately, and store on wet ice maintaining a sample temperature less than 10°C.
15. Enter data on appropriate field sheet including sample collection time.

Creek Sample Steps

16. The freshwater sampling location will be in the watershed discharge before entering the beach. specific conductance measurements will be used to confirm that there is no mixing of fresh/sea water at the creek sampling location (specific conductance <1,013 µS/cm).
17. Collect freshwater samples by submersing the sampling bottle 6-12 inches below the surface facing into the flow. Confirm that the specific conductance at the freshwater location is <1,013 µS/cm, prior to sampling or post-sampling in lab.
18. A sampling pole may be used, if necessary.
19. If a sampling device is used, secure the sample bottle onto the device. (Be sure to rinse with sterile water between each sampling location).
20. Collect FIB water samples following the collection procedure described in the SWAMP SOP for Bacteria and Pathogens in water samples (SWAMP SOPs for Conducting Field Measurements, pp. 49-51).
21. Install sample bottle cap.
22. Using “Sharpie” waterproof ink pen, label cap with sample location.
23. Collect HF183 samples as follows:
    a. Remove the seal completely from the bottle.
    b. Remove the lid carefully. Do not touch the inside of the bottle or lid.
    c. Collect sample by submerging the bottle and pushing forward with a slow, even motion. (Containers should be positioned such that the mouth of the container is pointed away from the sampler or sample point)
    d. The sample should be collected with a single stroke
    e. The sample bottle should be filled to just above the 100ml mark.
    f. Replace cap and tighten.
    g. Using “Sharpie” waterproof ink pen, label cap with sample location.
24. Install sample bottle cap.
25. Using “Sharpie” waterproof ink pen, label cap with sample location.
26. Place samples in individual Ziploc bags and place on ice immediately, and store on wet ice maintaining a sample temperature less than 10°C.
27. Enter data on appropriate field sheet including sample collection time.

After run completion:

28. Complete Chain of Custody (COC) and prepare samples for lab drop-off.

Table 1. Reference Beaches Microbial Water Quality Study Sample Handling Specifications

Parameter	Method	Sample Prep	Sample Size	Dilution	Container	Preservative	Hold Time
E. coli/ Total Coliform	Colilert-18 (IDEXX/SM9223B)	Unfiltered	250 mL		250mL sterilized Nalgene wide mouth bottle	1˚ - 10˚ C	8 hours
Enterococcus	Enterolert (IDEXX/ASTM D6503-99)	Unfiltered	250 mL		250mL sterilized Nalgene wide mouth bottle	1˚ - 10˚ C	8 hours
Fecal Coliform	SM 9221 (MTF)	Unfiltered	250 mL		250mL sterilized Nalgene wide mouth bottle	1˚ - 10˚ C	8 hours
HF183	EPA Method B	Unfiltered	100 mL		125 ml sterile polypropylene bottle	1˚ - 10˚ C	6 hours to filter, then $\leq$ 2 months to run sample after freezing

Table 2. Scope of Reference Beaches Microbial Water Quality Monitoring.

Event type	Sampling Triggers	Criteria	Event Frequency
Wet			
Significant winter storms	• 1" of rainfall in 24 hours • (If it would be projected that not enough samples would be collected, the trigger may be modified to ½" in 24 hours with approval by Principal Investigator) Suggested reference website for quantitative precipitation forecast: WFO San Francisco Bay / Monterey Precipitation Forecast	• During the rainy season (Nov. 1 through Apr. 30) • After 1st flush/1st significant winter storm. The following indicators will be considered to determine that these conditions have been achieved: – R1 watersheds- At least 7 inches of rainfall season to-date and peak flows exceeding 2,000 cfs for Austin Creek reference gauge – R3 watersheds- At least 6 inches of rainfall season to-date and reference gauge peak flows exceeding 400 cfs for Big Sur (Reference flow gauge: USGS11143000 Big Sur R.) • After stream reaches winter base flow conditions based on reference stream gauge. The following indicators will be considered to determine that winter base flow conditions have been established: – R1 watersheds- Baseflow exceeding 10 cfs	• 10 samples – sample collection at each selected event happens within the first 24 hours from the beginning of the storm event

Event type	Sampling Triggers	Criteria	Event Frequency
		for Gualala River reference gauge – R3 watersheds- Baseflow exceeding 30 cfs for Big Sur River reference gauge • Within the first 24 hours from the beginning of the storm event	
Dry			
Winter baseflow	• ASC staff will advise field crews based on: – Local flow monitoring gauge – 3-7 inches of preceding total rainfall that season – Peak flow in a local gauged watershed exceeding an annual 1" in 1-year return storm frequency.	• Requires a clear indication of a truly wet season flow signal, which may be based on either one of the above indicators or, as much as possible, a combination	• Two sample rounds • Each sample round contains 1 sample per week for five consecutive weeks (i.e., five samples in fall/winter 2015/2016; five samples in Fall/Winter 2016/2017). If it rains, skip a week.
Summer baseflow	• No triggers, can sample June 1 – August 31. The sampling is planned for June/July of 2016 and 2017. The exact dates will be determined based on the availability of the sampling team, as long as criteria are met.	On dry weather days, after an antecedent dry period of 96 hours with less than 1 inches of rainfall	Two sample rounds Each round contains 1 sample per week for five consecutive weeks (e.g., five samples in 2016 dry season; five samples in 2017 dry season).

Table 3. QC samples and MQOs.

Parameter	Method	QC measurement/ sample	Frequency	MQO
QC Samples				
E. coli/Total Coliform *Enterococcus*	Colilert-18 (IDEXX/SM9223B) Enterolert (IDEXX/ASTM D6503-99)	Field duplicate	One per event type (wet, winter dry, summer dry) at each sampling location	Not applicable (NA) – used to estimate sampling and laboratory analysis precision.
E. coli/Total Coliform *Enterococcus*	Colilert-18 (IDEXX/SM9223B) Enterolert (IDEXX/ASTM D6503-99)	Field blank	One per event type (wet, winter dry, summer dry)	No response
Fecal Coliform	SM 9221 (MTF)	Field duplicate	One per event type (wet, winter dry, summer dry) at each sampling location	NA – used to estimate sampling and laboratory analysis precision.
Fecal Coliform	SM 9221 (MTF)	Field blank	One per event type (wet, winter dry, summer dry)	No response
HF183	EPA Method B	Field duplicate	One per event type (wet, winter dry, summer dry) at each sampling location	NA – used to estimate sampling and laboratory analysis precision.
HF183	EPA Method B	Field blank	One per event type (wet, winter dry, summer dry)	No response
HF183	EPA Method B	Matrix Spike	One per sampling location	NA – percent recovery will be used to adjust detected marker concentrations

[1]RPD = relative percent difference; [2]RL = Reporting Limit.

1.2 Sample Shipment

Analytical laboratories and contact information are shown in **Table 3.** The Central Coast Sample Collection Team (MPSL) will deliver samples to Monterey Bay Analytical Services (Inc.). The North Coast Sample Collection Team (NQRWQCB) will deliver samples to the Sonoma County Public Health Laboratory. Samples must be kept on wet ice. The laboratories must filter the HF 183 samples within 6 hours of sample collection and start sample incubation for Quantitray and multi-tube fermentation (MTF) analyses no later than 8 hours from time of collection.

Table 4. **Analytical Laboratories and Contact Information.**

Analytical Lab	Address	Contact	Service
Cel Analytical Inc.	82 Mary Street Suite #2 San Francisco, CA 94103	Katherine Chandler katherine@celanalytical.com 415 882-1690	*Bacteroides* – HF183 qPCR assay
Monterey Bay Analytical Services, Inc.	4 Justin Ct, Suite D Monterey, CA 93940	David Holland Montereybayanalytical@usa.net 831 375-6227	*E. coli* plus Total Coliform (Colilert-18) *Enterococcus* (Enterolert) Fecal coliform (MTF) HF183 filtration
Sonoma County Public Health Regional Laboratory	3313 Chanate Road Santa Rosa, CA 95404	Michael Ferris Michael.Ferris@sonoma-county.org 707 565-4711	*E. coli* plus Total Coliform (Colilert-18) *Enterococcus* (Enterolert) Fecal coliform (MTF) HF183 filtration

Table 5. Roles and Responsibilities.

Role and Contacts	Responsibilities
San Francisco Bay Regional Water Board Contract Manager Farhad Ghodrati office: 510.622.2331 farhad.ghodrati@waterboards.ca.gov	In support of the Principal Investigator: ● Track Reference Beaches Microbial Water Quality Study sample collection schedule and activities ● Track, review, and approve project deliverables ● Communicate input and direction from San Francisco Regional Water Board and North Coast and Central Coast Regional Water Board staff to SFEI Principal Investigator ● Ensure timely completion of work according to the contract
SFEI Principal Investigator Thomas Jabusch, SFEI-ASC thomas@sfei.org office: 510.746.7340 mobile: 530.220.4185	Prepare and implement sampling plan, coordinate and direct project staff, and liaise with Water Board and State Board staff: ● Procure necessary sample collection equipment, including sample containers ● Prepare sample collection logistics ● Oversee sample collection by Central Coast and North Coast Sample Collection Team Leads ● Arrange for FedEx to deliver HF183 samples to Cel Analytical, and coordinate with Cel Analytical for sample receipt. ● Notify Regional Water Board liaison of sample collection schedule ● Respond to technical questions from laboratory, field sample collection teams, or San Francisco Regional Water Board ● Communicate to field crews when basic sampling criteria (winter baseflows, winter wet etc.) are met. ● Communicate input and direction from Regional Water Boards to SFEI-ASC staff, collaborators, and subcontractors ● Evaluate data against water quality thresholds, starting June 2016. ● Prepare summary statistics, graphical displays, and an evaluation of relationships of bacterial levels with potentially contributing factors ● Develop data summary products
SFEI Project Manager Amy Franz Aquatic Science Center amy@sfei.org 510.746.7394	In coordination with the principle investigator: ● Payment of laboratory, sample collection, and courier costs ● Review chain of custody forms and field data sheets for completeness and conformity to study plan ● Review of monitoring activities and consistency check with sample collection and CEDEN data reporting protocols ● Compile data, perform QA/QC, and upload to CEDEN through regional data center ● Submit SWAMP Excel data forms to labs with columns B-P completed within 3 weeks of sample date
Sample Collection Team Leads Autumn Bonnema bonnema@mlml.calstate.edu, office ph. 831.771.4175 Billy Jakl bjakl@mlml.calstate.edu, office	Under the direction of the principle investigator: ● Lead in-field sample collection efforts according to the work plan, SWAMP SOPs for Conducting Field Measurements, and applicable health and safety plans ● Note all variances to the work plan and SOPs ● Notify Field Collection Coordinator of significant problems, safety issues, or delays

ph. 831.771.4171 Steve Butkus Steve.Butkus@waterboards.ca.gov, office ph. 707.576.2834	● Complete chain of custody requirements ● MPSL complete standard SWAMP field data sheets for R3 sites, SFEI complete field sheets for R1 sites. ● Inform laboratories of sampling plans ● Deliver samples to appropriate regional laboratory for hand-off to laboratory staff

Attachment A

SWAMP SOP for Conducting Field Measurements:

Specific Conductance (µS/cm), p. 10

BACTERIA AND PATHOGENS IN WATER SAMPLES , pp. 49-51

Specific Conductance (µS/cm)

Specific conductance should be recorded for each SWAMP visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff.
See http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa for detailed information on data reporting.

Specific Conductance Sampling Equipment

The conductivity meter should be calibrated according to the recommended procedures for calibration and maintenance of SWAMP field equipment. Calibration directions are listed in the manufactures field equipment operations manual.

Specific Conductance Sampling Procedure

Preferably, conductivity is measured directly in-stream at the depth(s) specified earlier in this document. Allow the conductivity probe to equilibrate for at least one minute before specific conductance is recorded to three significant figures (if the value exceeds 100). The primary physical problem in using a specific conductance meter is entrapment of air in the conductivity probe chambers. The presence of air in the probe is indicated by unstable specific conductance values fluctuating up to +/-100 µS/cm. The entrainment of air can be minimized by slowly, carefully placing the probe into the water; and when the probe is completely submerged, quickly move it through the water to release any air bubbles.

If specific conductance cannot be measured in-stream, it should be measured in the container it can be measured in a bucket-Nalgene or plastic container. The following precautions are outlined above; “Temperature Measurement from a Bucket”.

Salinity (parts per thousand--ppt, or ‰)

The value for salinity is computed from chloride concentration or specific conductance. The calculation assumes a nearly constant ratio for major ions in an estuary when seawater is diluted by river water. This assumption does not hold for cases where salinity is less than about three parts per thousand. Salinity determinations at such low values are only approximate. In estuarine waters, salinity is a relevant and meaningful parameter. Often the salinity may be low, approaching that of freshwater. Nevertheless, this is useful information. Determine if a station is estuarine from historical records (i.e., experiences cases where salinity is >2.0 ppt) and always report salinity at this station, regardless of the salinity during periods of high flow.

Salinity is measured directly in-stream at the depth(s) specified earlier in this document. Salinity data should be recorded for each SWAMP visit in final form on a Field Data Sheet and submitted to the SWAMP data management staff. See http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#qa for detailed information on data reporting.

Values between 2.0 ppt and 1.0 ppt should be reported as <2.0 ppt rather than the actual value and values <1.0 ppt should be reported as <1.0 ppt. The field instruments compute salinity from specific conductance and temperature, and display the value in parts per thousand. Report salinity values above 2.0 ppt to the nearest 0.1 ppt.

BACTERIA AND PATHOGENS IN WATER SAMPLES

Summary of Collection Procedure (Based on EPA water quality monitoring procedures)

Make sure the containers are sterilized; either factory-sealed or labeled.

Whirl-pak® bags

- Label the bottle as previously described for SWAMP.
- Tear off the top of the bag along the perforation above the wire tab just prior to sampling. Avoid touching the inside of the bag. If you accidentally touch the inside of the bag, use another one.
- If wading into the stream, try to disturb as little bottom sediment as possible. Be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream. Collect the water sample on your upstream side, in front of you.
- If taking sample from a boat, carefully reach over the side and collect the water sample on the upstream side of the boat.
- Hold the two white pull-tabs in each hand and lower the bag into the water on your upstream side with the opening facing upstream. Open the bag midway between the surface and the bottom by pulling the white pull-tabs. The bag should begin to fill with water. You may need to "scoop" water into the bag by drawing it through the water upstream and away from you. Fill the bag no more than 3/4 full.
- Lift the bag out of the water. Pour out excess water. Pull on the wire tabs to close the bag. Continue holding the wire tabs and flip the bag over at least 4-5 times quickly to seal the bag. Don't try to squeeze the air out of the top of the bag. Fold the ends of the wire tabs together at the top of the bag, being careful not to puncture the bag. Twist them together, forming a loop.
- If the samples are to be analyzed in the lab, place them in a cooler with ice or cold packs for transport to the lab.

Screw cap containers

- Label the bottle as previously described for SWAMP.
- Remove the plastic seal from the bottle’s cap just before sampling. Avoid touching the inside of the bottle or cap. If you accidentally touch the inside, use another bottle.

- If wading into the stream, try to disturb as little bottom sediment as possible. Be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream. Collect the water sample on your upstream side, in front of you.
- If taking sample from a boat, carefully reach over the side and collect the water sample on the upstream side of the boat.
- Hold the bottle near its base with polyethylene gloves and submerge the bottle in the water with the cap on. Open the bottle collecting the water sample 0.1m beneath the surface. When the bottle is filled to the desired level recap the bottle and remove from water. You can only use this method if the sample bottles do not contain sodium thiosulfate.
- Turn the bottle underwater into the current and away from you. In slow moving stream reaches, push the bottle underneath the surface and away from you in an upstream direction.
- Alternative sampling method: In case the sample bottle contains preservatives/chlorine removers (i.e. Sodium-Thiosulfate), it cannot be plunged opening down. In this case hold the bottle upright under the surface while it is still capped. Open the lid carefully just a little to let water run in. Fill the bottle to the fill mark and screw the lid tight while the bottle is still underneath the surface.
- Leave a 1-in. air space so that the sample can be shaken just before analysis. Recap the bottle carefully, remembering not to touch the inside.
- If the samples are to be analyzed in the lab, place them in a cooler with ice or cold packs for transport to the lab. Samples should be placed immediately on ice to maintain temperature at 6 ^{o}C

Pouring from another clean bottle

- Due to different sampling conditions (high turbidity, rough water etc.) it is sometimes easy to pour water from another clean bottle into the bacteria bottle. This helps to make sure that the sample water is only being filled to the desired line and no overfilling occurs.

TOXICITY IN WATER

Sample Collection

Using the standard grab sample collection method described previously for water samples, fill (for typical suite of water toxicity tests conducted) the required amount of 2.25-L amber glass bottles with sub surface water. Since the size of the 2.25-L amber bottle is bigger than your average sample bottle, find a spot in the centroid of the stream to completely submerge the toxicity bottle if possible. A clean water organics(1-L glass amber) bottle can be used if there is no sampling point deep enough to submerge a large toxicity bottle. If the stream is not deep enough to submerge any bottle, then comments should be made on the field data sheets that surface water was collected. Depth should also equal 0 for the sampling depth. All toxicity samples should be. put on ice, and cooled to 4 °C. Label the containers as described above and notify the laboratory of the impending sample delivery, since there is a 48-hr maximum sample hold time. Sample collection must be coordinated with the laboratory to guarantee appropriate scheduling.

Attachment B

SWAMP Quality Control and Sample Handling Tables for Indicator Bacteria in Fresh Water

Indicator Bacteria in Fresh Water

The following tables are not applicable to marine water samples.

A list of species included in this category may be found in the associated QAPrPTableReference.

Terms appearing in the tables are defined in the *Surface Water Ambient Monitoring Program Quality Assurance Program Plan*, which contains a glossary (Appendix E), as well as a list of abbreviations and acronyms (Appendix F).

Table 1: Quality Control[1]: Indicator Bacteria in Fresh Water

Laboratory Quality Control	Frequency of Analysis	Measurement Quality Objective
Sterility Checks[3]	Per new lot of dehydrated culture media as instructed in SM 9020B.4.i.5[2] and SM 9222D.1.a	No growth
	For non-sterile filters and pads per lot as instructed in SM 9020B.4.h.1.1	No growth
	Membrane Filter Media, filters, buffered dilution water, rinse water, and all equipment per series of samples as instructed in SM 9020B.8.a.5[2]	No growth
	Multiple Tube Media, dilution water, and glassware as instructed in SM 9020B.8.a.5[2]	No growth
Laboratory Positive Control	Per new lot of dehydrated culture media for the following methods: Colilert, Colilert -18, Colisure, Enterolert, or other chromogenic/fluorogenic methods. Per new lot of commercially-prepared culture media ampules for USEPA-approved fecal coliform and E. coli membrane filter methods (e.g. SM 9222, m-ColiBlue24, EPA 1603) Per batch for laboratory-prepared culture media for USEPA-approved fecal coliform and E. coli membrane filter methods (e.g., SM 9222)	Positive response
Laboratory Negative Control	Per new lot of dehydrated culture media for the following methods: Colilert, Colilert -18, Colisure, Enterolert, or other chromogenic/fluorogenic methods. Per new lot of commercially-prepared culture media ampules for USEPA-approved fecal coliform and E. coli membrane filter methods (e.g. SM 9222, m-ColiBlue24, EPA 1603) Per batch for laboratory-prepared culture media for USEPA-approved fecal coliform and E. coli membrane filter methods (e.g., SM 9222)	Negative response
Laboratory Duplicate	Per 10 samples or per analytical batch, whichever is more frequent	$R_{log} \leq 3.27 \times \overline{R}^{4}$ Computation of R from duplicate laboratory sample analyses
Laboratory Blank[5]	Per 10 samples or per analytical batch, whichever is more frequent	No growth

Field Quality Control[6]	Frequency of Analysis	Measurement Quality Objective
Field Blank, Equipment Blank	Per method or SOP	Negative response

[1] Unless method specifies more stringent requirements

[2] Citations from *Standard Methods for the Examination of Water and Wastewater*, 20th edition(4)

[3] Sterility Checks

The specific type and number of sterility checks are method-dependent. For example, membrane filter tests require the testing of filters for sterility, while multiple-tube or pour plate procedures do not.

[4] Method for Determining Precision

In order to determine precision for bacterial analysis, the following procedure (adapted from Standard Methods 9020 Section 8.b) will be used. Note: When determining the precision of bacterial analyses, it is important to distinguish between different matrices (drinking water, wastewater, ambient water). Duplicate results from different matrices must be kept separate when calculating precision.

In order to calculate the laboratory precision for bacterial analyses, the results from the preceding 15 positive samples of a specific type (matrix) are used to calculate a running mean. The results used to calculate the running mean must all correspond to the same quality control parameter, in this instance laboratory duplicates (as opposed to field duplicates). The results of different quality control parameters such as laboratory and field duplicates must not both be used to calculate a single running mean. Note: Field duplicates are not a current SWAMP requirement (see footnote 6).

Step 1: Record the results from duplicate analyses (these results are here designated as D_1 and D_2).

Step 2: Calculate the logarithm (here designated as L_1 and L_2) of each duplicate result. Note: If either of the values D_1 or D_2 are less than 1, add 1 to both values before calculating the logarithms.

$$L_1 = \log D_1$$
$$L_2 = \log D_2$$

Step 3: Calculate the range of logarithms (R_{log}) for each pair of duplicates. R_{log} is equal to the absolute value of the difference between the two numbers.

$$R_{log} = |L_1 - L_2|$$

Step 4: Calculate the mean of R_{log} ($\overline{R}$) for the duplicates analyzed

$$\overline{R} = \sum \frac{R_{log}}{n}$$

Where

$\sum R_{log}$ = the sum of the ranges of logarithms calculated for each pair of duplicates
n = the number of pairs of duplicates (in this case, n = 15)

Step 5: Assess the precision of the duplicate analyses. In order for the laboratory to demonstrate an acceptable level of precision, the range of logarithms for a particular duplicate must be less than the mean of the range of logarithms multiplied by 3.27.

$$R_{log} \leq 3.27 \times \overline{R}$$

[5] Laboratory Blanks

Analysis and reporting of laboratory blanks is required only when samples are diluted prior to analysis. If samples are not diluted in the sample batch, no laboratory blanks are required for that specific sample batch.

[6] Field Duplicates

While SWAMP recommends that field duplicates be collected and analyzed, they are not a current SWAMP requirement. Projects are encouraged to require field duplicates in their QA project plan (QAPP) if it supports their specific quality objectives.

Table 2: Sample Handling: Indicator Bacteria in Fresh Water

Recommended Container	Recommended Preservation	Required Holding Time[1,2]
Factory-sealed, pre-sterilized, disposable whirlpak bags or 125-mL sterile plastic (high density polyethylene, polystyrene, or polypropylene) or glass container	Cool to ≤10 °C; for samples containing chlorine, sodium thiosulfate is pre-added to the containers in the laboratory	8 hours *for compliance monitoring*
		24 hours *for routine ambient monitoring*

[1] Each "Required Holding Time" is based on the assumption that the "Recommended Preservation" (or a method-mandated alternative) has been employed. All samples analyzed past the 8 hour compliance holding time will be flagged for user notification, however, will still be considered SWAMP compliant for routine ambient use. If the 24 hour holding time for analysis is not met, the project manager and SWAMP Quality Assurance Officer must be notified and the data must be flagged accordingly.

[2] Sample analysis should begin as soon as possible after receipt, a holding time of no more than 8 hours is highly recommended. For purposes of compliance monitoring, sample incubation must be started no later than 8 hours from time of collection and no later than 24 hours for routine ambient monitoring. (1, 2, 3, 4)

Table 3: Corrective Action: Indicator Bacteria in Fresh Water

Laboratory Quality Control	Corrective Action
Sterility Checks	Identify contamination source and take appropriate action; discard membrane filter/pad or prepared media lot; discard sample results if checks made during analysis
Laboratory Positive Control	Identify cause and take appropriate action; discard prepared media and remake from start or purchase new lot
Laboratory Negative Control	Identify cause and take appropriate action; discard prepared media and remake from start or purchase new lot
Diluent Control	Identify contamination source and take appropriate action; qualify data as needed
Laboratory Duplicate	Verify results; qualify data as appropriate
Laboratory Blank	Identify contamination source and take appropriate action; qualify data as needed
Field Quality Control	**Corrective Action**
Field Blank, Equipment Blank	Examine field log; identify potential contamination source; qualify data as needed

References:

(1) Meyers, D.N., et. al. 2014. U.S. Geological Survey TWRI Book 9. Fecal Indicator Bacteria. Ch. 7, V. 2.

(2) Pope, M.L., et. al. 2003. Assessment of the Effects of Holding Time and Temperature on *Escherichia coli* Densities in Surface Water Samples. Applied and Environmental Microbiology, Vol. 69, No. 10, p. 6201-6207.

(3) Standard Methods Committee. SM Section 9060. Standard Methods for the Examination of Water and Wastewater. Version 2006.

(4) Standard Methods Committee. Standard Methods for the Examination of Water and Wastewater, 20th Edition.

Attachment C

Field Data Sheet

SWAMP R2 Field Data Sheet (Water Chemistry & Discrete Probe) - EventType=WQ				Entered in d-base (initial/date)	Pg of Pgs
***StationID:**	***Date (mm/dd/yyyy):** / /			***Site Type:** Targeted	**Agency:** RWQCB2
***Funding:**	ArrivalTime:	DepartureTime:		***SampleTime (1st sample):**	Protocol: MPSL-DFG_Field_v1.1
*Personnel:	**Project Code: RWB2_FIB_REF_SYS_15_16**		**Purpose:** WaterChem Habitat FieldMeas		Purpose Failure:

*Location: Bank Midchannel OpenWater Intertidal Beach	*GPS	**Lat (dd.ddddd)**	**Long (ddd.ddddd)**	OCCUPATION METHOD: Walk-in Bridge R/V _____ Other
GPS Device:	**Target:**	in database	in database	STARTING BANK (facing downstream): **LB** / RB / NA
Datum: **NAD83** Accuracy (ft / m):	***Actual:**		-	Point of Sample (if Integrated, then -88 in dbase)

Habitat Observations (CollectionMethod = Not Applicable)			DISTANCE FROM BANK (m):	STREAM WIDTH (m):
SITE ODOR:	None, Sulfides, Sewage, Petroleum, Smoke, Other_____			WATER DEPTH (m):
SKY CODE:	Clear, PartlyCloudy, Overcast, Fog, Smoky, Hazey		**HYDROMODIFICATION:** None, Bridge, Pipes, ConcreteChannel, GradeControl, Culvert, AerialZipline, Other	
WATERCOLOR:	Colorless, Green, Yellow, Brown		**PHOTOS** (RB & LB assigned when facing downstream):	**LOCATION (to sample):** US / DS / WI / NA
DOMINANTSUBSTRATE:	Bedrock, Concrete, Cobble, Gravel, Sand, Mud, Unknown, Other_____			1: (RB / LB / BB / US / DS / ##)
WATERCLARITY:	Clear (see bottom), Cloudy (>4" vis), Murky (<4" vis)	**PRECIPITATION:**	None, Fog, Drizzle, Rain, Snow	
WATERODOR:	None, Sulfides, Sewage, Petroleum, Mixed, Other_____	**PRECIPITATION (last 24 hrs):**	Unk, <1", >1", None	2: (RB / LB / BB / US / DS / ##)
OTHERPRESENCE:	Vascular, Nonvascular, OilySheen, Foam, Trash, None, Other_____	**EVIDENCE OF FIRES:**	No, <1 year, <5 years	
OVERLAND RUNOFF (last 24 hrs):	Light Precip (no overland runoff), Moderate-Heavy Precip (probable/definite overland runoff), None, Unk			3: (RB / LB / BB / US / DS / ##)
OBSERVED FLOW:	NA, DryWaterbodyBed, NoObsFlow, IsolatedPool, Trickle(<0.1cfs), 0.1-1cfs, 1-5cfs, 5-20cfs, 20-50cfs, 50-200cfs, >200cfs			

Field Measurements (SampleType = FieldMeasure; Method = Field)

	DepthCollec (m)	Sp Cond (uS/cm)	Salinity (ppt)					
SUBSURF/MID/ BOTTOM/REP	0.1							

Instrument(s): _____ Calibration Date(s): _____

Notes:

Samples Taken (# of containers filled) - Method=Water_Grab

Field Dup YES / NO: (SampleType = **Grab** / Integrated; LABEL_ID = FieldQA; create collection record upon data entry

SAMPLE TYPE: **Grab** / Integrated | COLLECTION DEVICE: **Indiv bottle (by hand**, by pole, by bucket); Teflon tubing; Kemmer; Pole & Beaker; Other _____

	DepthCollec (m)	E. Coli & Total Coliforms (NoPrepPres)	Fecal Coliforms (NoPrepPres)	Enterococcus (NoPrepPres)	HF183 & Universal Bacteroides (LabFiltered)						
Sub/Surface	0.1										

COMMENTS:

MBAS Pathogens QA Review

Pathogens (E. Coli, Total Coliform, Enterococcus, Fecal Coliform)

QA Issues for Project Manager to Review

Data submission was evaluated using the SWAMP Indicator Bacteria In Freshwater MQOs.
https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/mqo/updated_ind_bact_water.pdf

Reporting Issues for Lab to Review

Rlog comments Rlog XX < YY oddly seem to have a nearly constant ratio of YY/XX ~ 1.65 (roughly 3.27/2). It is likely that YY target is calculated incorrectly, one would expect that since the target YY is the mean of 15 past XXs times 3.27, YY/XX should average around 3, but should vary around that (sometimes 4, sometimes 2, but never <1 since that would be an MQO deviation)
Most Rlogs recalculated by SFEI agreed with those from MBAS, although about ⅓ were either lower or higher than those provided by MBAS, so these should be double checked.

Formatting Issues for Data Manager to Review

Hold time review (especially desired by stormwater programs)

Analysis dates or sample dates were originally scrambled for some samples, but later fixed, with hold times <0.3 days (~8hrs) except for 2 samples needing dilution and reanalysis to report (1 day hold).

QA Review

Dataset completeness

Fecal Coliform, Total Coliform, and E. coli, and results were reported for all sites.
3 sites were reported for 20 events, and 3 sites for 30 events. Enterococcus was reported for 4 stations (3 stations for all their events, and 1 station only twice), Field replicates, laboratory replicates, laboratory control samples (LCS's), field blanks, and lab blanks were also reported. All data was reported not blank corrected.

Overview

Overall, the data are acceptable, although Rlogs may need to be recalculated and their target values updated. All analytes were quantified in about half or more of the samples. Recoveries on control samples were within limits, and precision was within SWAMP QAPP requirements for Rlogs.The only issue requiring flagging was 2 samples reanalyzed beyond hold time.

Sensitivity

Results were reported "<" the method detection limits (effectively non-detects; NDs) for Fecal Coliform (7%), and E. coli (32%), and 51% of samples for Enterococcus.

Blank contamination

None of the pathogens were found in the 30 method blanks above the method detection limits (all results flagged as "<" the MDLs; effectively NDs). Likewise, none of the pathogens were detected in the 3 field blanks.

Recovery

Accuracy was examined using the laboratory control samples. The unspiked laboratory control samples (LCS's) for Total Coliform and E. coli were negative (non-detects).

The spiked LCS samples for Enterococcus had average recovery of 65% (error 35%), E. coli recovery averaged 116% (43% average error), and Total coliform averaged 130% recovery (54% error). Only one Fecal Coliform LCS was reported and had 100% recovery. The SWAMP QAPP only specifies "positive response", and the recoveries on average were within 50% of expected results, so no recovery flags were added.

Precision

Precision was evaluated through calculation of Rlogs (where possible) as well as RSDs. Rlog is the log of the ratio of duplicate concentrations, and on any given pair should be equal to or less than the 15 pair running average Rlog * 3.27. Once pairs with one or more ND were eliminated, all individual Rlogs were <3.27 * the running average of calculable Rlogs within the previous 15 duplicate pairs.

Rlogs recalculated by SFEI often agreed with those from MBAS, although ⅓ were either lower or higher than those provided by MBAS. Additionally, the upper bound permissible Rlog in the MBAS comments appears to be calculated incorrectly, as always 1.635* the individual pair Rlog, rather than 3.27* the 15 pair running average. By our calculations, all the Rlogs should be within the SWAMP target criteria, so none are flagged, but the data comments will need to be updated to reconcile where the Rlogs do not match, and for all comments to provide the proper Rlog target.

The average lab replicate RSDs for Fecal Coliform, Total Coliform, E. coli, and Enterococcus were 38%, 17%, 45%, and 42% respectively. The average RSDs combining by site and event all lab and field replicates were 39%, 17%, 39%, and 42% for those same analytes respectively.

Comparison of dissolved and total phases

Not applicable.

Comparison to previous years

This is the first data submission for this study so there were no previous results for comparison.

<u>Ratio Checking Summary</u>

As would be expected, the Fecal Coliform results were <10% of Total Coliform on all samples.

<u>Sums Summary</u>

Not Applicable

Sonoma County Public Health Lab Pathogens QA Review

QA/QC Summary:

QA Issues for Project Manager to Review

Data submission was evaluated using the SWAMP Indicator Bacteria In Freshwater MQOs.

https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/mqo/updated_ind_bact_water.pdf.

Dataset completeness

Fecal Coliform, Total Coliform, and E. coli, results were reported for 120 water samples, and Enterococcus results for 60 water samples analyzed in 132 lab batches. Field replicates, laboratory replicates, laboratory control samples (LCS's), field blanks, and lab blanks were also reported. All data was reported not blank corrected.

Analysis dates for some samples were reported without times so it could not be determined whether samples were analyzed within the 8 hour hold time. However, all samples were analyzed the same day of collection.

Overall acceptability

Results flagged as being "<" the method detection limits (effectively non-detects; NDs) were report for Total Coliform (6.35%), Fecal Coliform (22.22%), and E. coli (38.89%). Extensive non-detects (<'s greater than 50%) were reported for Enterococcus (53.17%).

None of the pathogens were found in the seven method blanks at numbers greater than the method detection limits (all results were flagged as being "<" the MDLs; effectively NDs).

Likewise, none of the pathogens were reported in the 28 field blanks (12 FB + 8 FB lab replicates + 8 FB field replicates).

Accuracy is not required, but was examined using the laboratory control samples. The unspiked laboratory control samples (LCS's) for Total Coliform and E. coli were negative (non-detects). The spiked LCS samples for Enterococcus and Fecal Coliform were positive (detected) with an average %error for Enterococcus of 17.97% and for Fecal Coliform of 50.87%.

Precision was evaluated through calculation of Rlogs (where possible) as well as RSDs. Rlog is the log of the ratio of duplicate concentrations, and on any given pair should be equal to or less than the 15 pair running

average Rlog * 3.27. I conservatively flagged thee batches with the flag “VIL” for poor precision based on the Rlog criteria; one for Fecal Coliform (~3% of results), two for Total Coliform (~6% of results), and one for E. coli (~3% of results).

The average lab replicate RSDs for Fecal Coliform, Total Coliform, E. coli, and Enterococcus were 48%, 22%, 45%, and 45% respectively. The average RSDs for lab replicates and field replicates combined were 50%, 24%, 47%, and 43%.

This is the first data submission for this study so there were no previous results for comparison.

CEL Bacteroides QA Review

QA/QC Summary:

QA Issues for Project Manager to Review

Data submission was evaluated using the SWAMP Indicator Bacteria In Freshwater MQOs.

https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/mqo/updated_ind_bact_water.pdf

This is a research method, no certification or proficiency testing reports are available.

Dataset completeness

Human Bacteroidales and Universal Bacteroidales results were reported for 270 water samples analyzed in 66 lab batches. Field replicates, laboratory replicates, matrix spikes (MS), laboratory control samples (LCS's), field blanks, lab blanks, and negative controls (NEC) were also reported. All data was reported not blank corrected.

The results for samples collected on 1/12/2016 were rejected by the laboratory (LRQ -Data rejected - Based on professional judgement QA/QC protocols were not met, flagged by lab) and were excluded from the QA review.

Samples were analyzed between 7 and 199 days after collection. Although this research method may not exactly mirror those in the SWAMP QAPP for bacteria, similar issues with viability of analyses after extended holding may apply, so those hold time targets were applied. The processing hold time (filtering and freezing to occur within 6 hours of collection) was met for ~96% of samples. The analytical hold time was <= 2 months to run sample after filtering, with 58.7% of samples analyzed more than 60 days after collection and flagged with the flag "VH" for hold time violations.

Overall acceptability

Extensive non-detects (>50% ND's) were reported for Human Bacteroidales (~89%). No NDs were reported for Universal Bacteroidales.

Human Bacteroidales and Universal Bacteroidales were not found in the method blanks at numbers greater than the method detection limits (all NDs).

Human Bacteroidales were not found in the field blanks, but Universal Bacteroidales were detected in one field blank at a concentration level of 179.42 gc/ml (0.8% of the mean field sample concentration).

Human Bacteroidales negative controls were non-detects; Universal Bacteroidales negative controls were non-detects (NDs) except for one control which had a concentration of 48.42 gc/ml (0.2% of the mean field sample concentration).

Accuracy was examined for Universal Bacteroidales using the matrix spike samples (no matrix spike samples were analyzed for Human Bacteroidales). The average %error for Universal Bacteroidales was ~53%, with average recovery of ~153%. Percent recoveries for the individual matrix spikes ranged from -2814% to 738%; the acceptance range based on EPA determinations for these tests is detect to 3473%. Universal Bacteroidales results were flagged with the qualifiers VIU,VJ to alert users to the high variability of the results.

Precision was evaluated through calculation of Rlogs (where possible) as well as RSDs. Rlog is the log of the ratio of duplicate concentrations, and on any given pair should be equal to or less than the 15 pair running average Rlog * 3.27. There were not 15 calculable duplicate pairs (at least one of the pair concentrations being non-detect) for Human Bacteroidales so a quantitative target for Rlogs using the SWAMP method could not be applied, but all results were within 3.27 * the mean Rlog of all two reportable pairs. Universal Bacteroidales duplicate pair Rlogs were less than the 15 pair running average Rlog * 3.27 so no qualifiers were added.

The average lab replicate RSDs for Human Bacteroidales and Universal Bacteroidales were ~4%, and ~15% respectively (for all detected pairs). The average RSDs for lab replicates and field replicates combined were respectively ~63%, and ~19%. Laboratory control sample (LCS) average RSDs were ~20% and ~19%.

There were no previous results readily available for comparison.