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Alternative Indicators of Human Fecal Contamination in Beaches and Estuaries: A Reference System Study in Southern California

Final Report

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EXECUTIVE SUMMARY

With the overall goal of beginning to establish a microbial water quality reference system dataset in Southern California, concentrations of general, alternative, and human-associated fecal indicators and enteric viruses were measured in water samples collected from beaches and estuaries with different levels and types of anthropogenic impact (Figure 1). A total of 371 grab samples were collected between November 1, 2021 and January 17, 2024, distributed across rainy season (wet weather), rainy season (dry weather), and dry season (dry weather) conditions. Samples were analyzed for traditional fecal indicator bacteria (*E. coli* and enterococci), alternative viral fecal indicators (somatic coliphages), human-associated fecal biomarkers (HF183 and PMMoV), and human pathogens (adenovirus and norovirus).

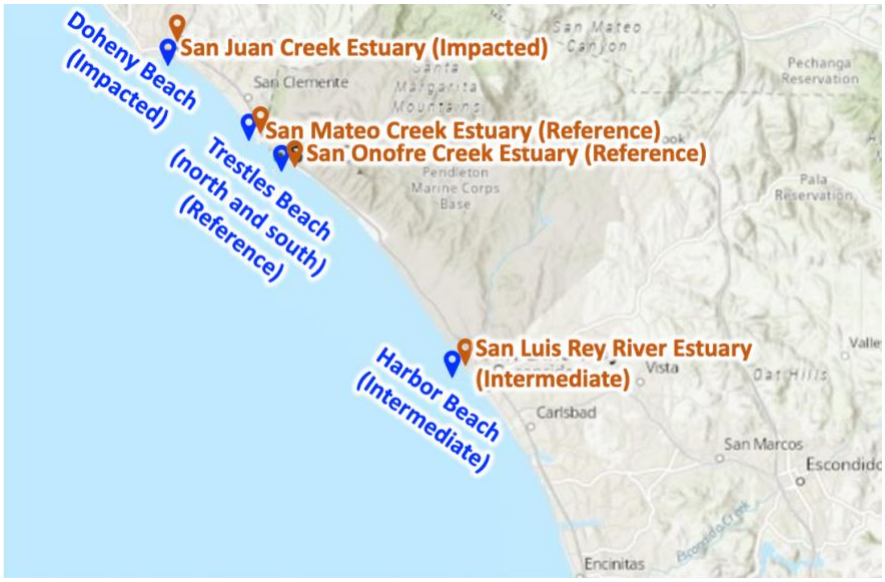

Figure 1. Map of sampling locations

Fecal indicator concentrations were significantly greater at impacted sites than they were at reference sites, and the magnitude of these differences were affected by weather and season. In samples from beaches, *E. coli* concentrations were significantly higher during the dry season than they were during the rainy season, but there were no seasonal differences in the *E. coli* concentrations in the estuary samples. Enterococci concentrations were significantly greater during wet weather conditions than they were during rainy season (dry weather) conditions, and this was true for both beach and estuary samples. There were no significant differences between *E. coli* concentrations at the different beach sites, but the enterococci concentrations at the impacted beach site (Doheny Beach) were significantly greater than they were at the reference beach sites (Trestles Beach). The *E. coli* and enterococci concentrations in the impacted estuary site (San Juan Creek) were significantly greater than they were in the reference estuary sites (San Mateo Creek and San Onofre Creek). REC-1 bacterial water quality objectives for *E. coli* were consistently met for San Mateo Creek, but not for any of the other estuary sites, and REC-1 bacterial water quality objectives for enterococci were not met for any of the beach sites, except for Harbor Beach under dry weather conditions during the rainy season.

San Luis Rey River and Harbor Beach, sites considered to have intermediate anthropogenic impact, had enterococci concentrations that were significantly lower than the impacted sites, but not significantly different than the reference sites. However, *E. coli* concentrations in San Luis Rey River were significantly greater than the reference estuaries but not significantly different than the impacted estuary. San Luis Rey River and Harbor Beach samples also had sporadic detection of HF183 and PMMoV, but no more frequently than the reference sites (San Mateo Creek, San Onofre Creek, and Trestles Beach). Enteric virus detection was also sporadic and occurred at almost all sites at similar frequencies and concentrations.

San Juan Creek and Doheny Beach had more frequent detection and higher concentrations of human-associated fecal biomarkers (HF183 and PMMoV) than the reference sites (San Mateo Creek, San Onofre Creek, and Trestles Beach), but there was still sporadic detection of HF183, PMMoV, and enteric viruses at the reference sites. At estuary sites, *E. coli* concentrations significantly coincided with the detection of HF183, but *E. coli* did not coincide well with the presence of PMMoV or enteric viruses. At estuaries and beaches, enterococci concentrations coincided very well with the presence of HF183, but not with the presence of PMMoV or enteric viruses, except for in the dry season, when enterococci concentrations were greater by 0.55-$\log_{10}$ units when enteric viruses were present. Somatic coliphage concentrations significantly coincided with the presence of HF183, but only in estuary sites. Somatic coliphage concentrations did not coincide well with the presence of the human-associated fecal biomarkers.

We assessed the potential for using the ratio of HF183:PMMoV to predict the presence and concentration of pathogens, given that PMMoV is known to have greater persistence in the environment relative to HF183. Past studies done by our group have shown that the $\log_{10}$-transformed HF183:PMMoV ratio is generally positive in fresh, untreated sewage, but decreases with respect to time and exposure to environmental conditions. In the present study, we found that the HF183:PMMoV ratio was greater at almost all sites during rainy season wet weather conditions than it was during dry weather conditions, which may indicate seasonal differences in the source, freshness, and/or trajectory of fecal pollution in these watersheds. Furthermore, we found that the HF183:PMMoV ratio was significantly greater when enteric viruses were detected compared to when they were not detected, and the HF183:PMMoV ratio also correlated stronger with enteric virus concentrations than HF183 or PMMoV concentrations by themselves.

The results of this study confirmed that, based on this reference system, culturable *E. coli* and somatic coliphages were good indicators of fecal pollution in estuaries, but not in beaches, and that culturable enterococci was a good indicator in both estuaries and beaches. However, none of these general fecal indicators reliably coincided with human-associated biomarkers or pathogenic enteric viruses. The $\log_{10}$ ratio of HF183:PMMoV, however, provided a better indication of the presence and concentration of enteric viruses, relative to any single indicator by itself, although the number of samples where enteric viruses were detected was relatively small (N = 12). Because of this, more research would be needed to further evaluate the use of the HF183:PMMoV ratio for this purpose.

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INTRODUCTION

Problem Definition and Background

Contact recreation in contaminated surface waters in the United States is responsible for 90 million illnesses, costing between $2.2 and $3.7 billion annually (DeFlorio-Barker et al., 2018). These illnesses can be caused by a wide range of different microbial pathogens, including viruses, bacteria, protists, and helminths. Compared to bacteria, viruses are more persistent in the environment, more infectious (higher potency), and are excreted in higher numbers (Aw, 2019). There are many different types of enteric viruses in wastewater that can cause a wide variety of illnesses, including gastroenteritis, hepatitis, myocarditis, and meningitis (Aw, 2019). Overall, there may be more than 200 different types of pathogenic viruses in wastewater, with the most common ones including enteroviruses, adenoviruses, noroviruses, hepatitis viruses, sapoviruses, rotaviruses, polyomaviruses, and astroviruses (Ibrahim et al., 2021). With approximately 685 million annual cases and 200,000 annual deaths worldwide, norovirus (NoV) is one of the leading causes of viral gastroenteritis, accounting for 18% of all diarrheal diseases globally (Farkas et al., 2020; Katayama and Vinjé, 2019). Human adenovirus (HAdV) is another group of enteric viruses that can cause both gastrointestinal and respiratory illnesses in humans, accounting for 5 to 10% of all febrile illnesses in infants and young children—it has been proposed as an indicator for viral pathogens due to their high concentrations in wastewater, and their high resistance to many chemical and physical agents, including UV light (Allard and Vantarakis, 2019).

In California, the Regional Water Quality Control Boards are charged with protecting beneficial uses of water bodies, such as the water contact recreation (REC-1) beneficial use category. This beneficial use involves recreational activities where the body contacts the water and where the unintentional ingestion of water is possible. Some examples are swimming, wading, water-skiing, skin and scuba diving, surfing, and fishing. The primary concerns for supporting REC-1 in oceans and beaches are microbial pollutants that pose a public health risk to people participating in water contact recreation activities. The REC-1 bacteria water quality objectives (WQOs), published in the Water Quality Control Plan for the San Diego Basin (i.e., the "Basin Plan"), are based on two thresholds—a geometric mean and a statistical threshold value (STV) (SD RWQCB, 2021). The geometric mean is defined as a six-week rolling geometric mean and is calculated based on the five most recent samples for a particular site. The STV is defined as the threshold concentration that is not to be exceeded by more than 10 percent of the samples collected in any calendar month, calculated in a static manner. For ocean waters (defined in the Basin Plan as waters where the salinity is greater than 1 ppt more than 5 percent of the time), enterococci is the recommended fecal indicator. The six-week rolling geometric mean concentration of enterococci cannot exceed 30 CFU (or MPN) per 100 mL and the STV cannot exceed 110 CFU (or MPN) per 100 mL. For other waters where the salinity is less than or equal to 1 ppt at least 95 percent of the time, *E. coli* is the recommended fecal indicator. The six-week rolling geometric mean *E. coli* concentration cannot exceed 100 CFU (or MPN) per 100 mL and the STV cannot exceed 320 CFU (or MPN) per 100 mL (SD RWQCB, 2021).

Traditionally, fecal indicator bacteria (FIBs) like *Escherichia coli* and enterococci have been utilized as indicators for assessing the microbial quality of recreational waters. The idea is that if FIB are present in high concentrations, there is a greater likelihood of fecal contamination and the presence of human

pathogens. However, FIB are not specific to human feces, and they are generally less persistent in the environment than enteric viruses and other non-bacterial pathogens (Murphy, 2019). The relationship between fecal indicator bacteria and waterborne viruses has been described as tenuous at best (Korajkic et al., 2018), and monitoring recreational surface waters with fecal indicator bacteria and viruses can lead to contradictory conclusions (Li et al., 2021). There is also evidence that some species of FIB can regrow in natural environments (Pote et al., 2009), making them potentially unsuitable for indicating the presence of human pathogens in water. Viral fecal indicators, such as coliphages (viruses that infect *E. coli*), have been suggested as alternative fecal indicators (US EPA, 2017, 2015). Relative to traditional FIB, somatic coliphages have been found to provide better predictions of the presence of enteric viruses in coastal bathing waters (Mocé-Llivina et al., 2005). In one study, coliphage concentrations in recreational coastal waters were more strongly associated with illnesses for beachgoers than enterococci concentrations (Benjamin-Chung et al., 2017). Nonetheless, neither FIB nor coliphages are specific to humans.

The main source of microbial pollutants that pose risks associated with water contact recreation is human fecal pollution (e.g., from sanitary sewer overflows, treated wastewater discharges, seepage from leaking sewers and septic leach fields, stormwater runoff from contaminated soils, etc.). Domesticated animal or wildlife sources of fecal pollution can also pose a risk, specifically from zoonotic pathogens such as *Cryptosporidium*, *Giardia*, and *Salmonella*. However, the risk of illness from animal fecal pollution is considerably lower than the risk from human fecal contamination, with some exceptions. For example, one study reported that the gastrointestinal illness risks from exposure to recreational waters contaminated by fresh cattle feces may be comparable to the risks from human fecal pollution, but that the risks from contamination by fresh gull, chicken, or pig feces were considerably lower (Soller et al., 2010).

To address this gap, the field of microbial source tracking (MST) was developed around the turn of the 21st century. MST relies on the detection of genetic biomarkers associated with host-associated fecal microorganisms—those that are excreted by specific groups of wild or domesticated animals (e.g., dogs, gulls, deer, cow, etc.), or those that are associated primarily with humans. There is still some research needed to standardize MST methods and implement this approach into the regulatory setting, but recent progress has been made, such as the standardization of US EPA Method 1696, to quantify HF183 in surface waters, a genetic MST biomarker from *Bacteroides dorei* that is associated with human fecal pollution (US EPA, 2019). Still, there is more work needed to transition MST from a research tool to a potential regulatory and water quality management approach. The current precedent for regulation of ambient microbial water quality is still the use of FIB, specifically *E. coli* and enterococci. Historically, FIBs such as *E. coli* and enterococci have also been used in regulatory settings in part due to the wide availability of laboratory methods to provide consistent results across different waterbody types and conditions. However, more recent advances in molecular biology and the development of new genetic methods to detect microorganisms, including PCR, qPCR, and digital PCR, has allowed for the measurement of human-associated fecal MST markers and even to directly measure human pathogens in ambient waters with better sensitivity and specificity than ever before. The use of these new approaches has revolutionized the research field of health-related water microbiology over the past two decades, and regulatory agencies including the US EPA are now starting to capitalize on the potential for integrating these new approaches into water quality monitoring (Demeter et al., 2023).

Several studies have investigated the correlation between traditional FIB, alternative indicators (e.g., coliphage), and human-associated (e.g., MST) fecal indicators with pathogens, yet results vary across different locations (Goh et al., 2019). Environmental factors can significantly influence these outcomes. For example, rainfall and salinity significantly affected microbial concentrations in contaminated rivers, and indicator concentrations clustered with pathogen concentrations to a greater extent when there was more rainfall, higher turbidity, and lower temperatures (González-Fernández et al., 2021). Only about half of previous studies in the scientific literature have found significant relationships between fecal indicators and pathogens, but it is more common to see significant relationships between FIB and bacterial or protist pathogens than pathogenic viruses (Korajkic et al., 2018), although small sample sizes may have prevented the discovery of significant correlations (Wu et al., 2011).

Overall Goal and Specific Objectives

The goal of this project is to investigate the suitability of alternative indicators of fecal contamination, including somatic coliphages, as well as human-associated markers from *Bacteroides* (HF183), pepper mild mottle virus (PMMoV), human adenovirus (HAdV), and norovirus (NoV). These alternative indicators have the potential to detect human fecal contamination with certainty and improve the Water Board's REC-1 assessments. The overall strategy will be to measure these alternative indicators – along with the conventional indicators for comparison – during dry and wet weather at beach sites that are: (1) minimally impacted by human activities ("reference" condition), and (2) impacted by human activities. The expected outcomes include data that will assist the Water Boards in the evaluation and development of water-quality thresholds or numeric targets for protecting water quality using a reference-system approach, and information about the potential use of viral source tracking markers and HF183 as a regulatory measure to assess water-quality conditions for protection of the REC-1 beneficial use.

The overall goal of this project was to begin the establishment of a reference system dataset in Southern California that includes general (traditional and alternative) and human-associated indicators of fecal contamination at beaches and nearby estuaries. We investigated the suitability of traditional alternative indicators in recreational surface waters, to determine if they have better correlation with pathogens (which would mean a better correlation to potential public health risks). To our knowledge, this is the first such microbial reference beach study in southern California with both dry season and wet season sampling. To achieve our overall goal, we collected samples from four beaches (Doheny Beach, Harbor Beach, Trestles Beach north, and Trestles Beach south) and four adjacent estuaries (San Juan Creek estuary, San Luis Rey River estuary, San Mateo Creek estuary, and San Onofre Creek estuary) approximately every two weeks over the course of two years, during rainy and dry weather conditions, and analyzed them for the concentrations of the following microbial groups:

1. Fecal indicator bacteria (FIB), *E. coli* and enterococci
2. Viral fecal indicators, somatic coliphages
3. Human-associated MST bacterial fecal biomarker, HF183
4. Human-associated MST viral fecal biomarker, pepper mild mottle virus (PMMoV)
5. Enteric virus pathogens, human adenovirus (HAdV), and norovirus genogroup II (NoVGII)

These microbial groups were chosen for their importance from a public health perspective and their potential to serve as indicators of human pathogens, based on findings from the literature. Specifically, coliphages have been found to be better predictors of the presence of enteric viruses and associated illnesses for beachgoers than bacterial indicators (Benjamin-Chung et al., 2017; Griffith et al., 2016; Mocé-Llivina et al., 2005). The combined use of coliphages and PMMoV was found to be the best indication of the presence of pathogens such as *Giardia*, *Cryptosporidium*, *Salmonella*, and norovirus genogroup I (NoVGI) in rivers near ocean outfalls, and PMMoV (in addition to enterococci and coliphage) was found to best indicate the presence of pathogens in ocean waters (González-Fernández et al., 2021). Likewise, HF183 has been used to predict risks of gastrointestinal illness at beaches (Boehm et al., 2015).

The specific objectives and associated research questions are as follows:

1. **Objective 1. Compare the concentrations and WQO exceedance frequencies of general and alternative fecal indicators in samples collected from reference sites and impacted sites during different seasons and weather conditions.**

   Associated Research Question: Are the concentrations and exceedance frequencies of fecal indicators at impacted sites significantly greater than they are at reference sites and are these differences affected by weather and season?

2. **Objective 2. Compare the concentrations of general and alternative indicators of fecal pollution in samples where human-associated fecal pollution is detected compared with samples where human-associated fecal pollution is not detected.**

   Associated Research Question: Is there a difference in the concentrations of general and alternative indicators of fecal pollution when human fecal pollution is detected vs. when it is not detected?

3. **Objective 3. Assess the use of the ratio of HF183:PMMoV to predict the presence of human pathogens.**

   Associated Research Question: Do trends in the HF183:PMMoV ratio correlate with the levels of general and alternative fecal indicators or the detection of human pathogens at impacted and reference sites, in different seasons and during different weather conditions?

METHODOLOGY

Sample Locations

The following beaches located within the boundaries of the SD RWQCB's jurisdiction were selected, as well as the estuaries of four rivers adjacent to these beaches that empty into the Pacific Ocean. Samples were collected during the dry season (from April to October) and during the wet season (from October to April), after either dry or rainy weather conditions.

1. Doheny Beach and nearby San Juan Creek estuary
2. Harbor Beach and nearby San Luis Rey River estuary
3. Trestles Beach (north) and nearby San Mateo Creek estuary
4. Trestles Beach (south) and nearby San Onofre Creek estuary

Two additional sites (Laguna Beach and the nearby estuary of Aliso Creek) were also sampled for the first four sampling events but were later excluded due to logistical challenges with sample holding times. The sample locations were selected based on the extent of development in their watersheds, and due to their historical concentrations of fecal indicator bacteria based on data retrieved from the California Environmental Data Exchange Network (CEDEN) database (https://ceden.org/). For example, Aliso County Beach (near Aliso Creek estuary) and Doheny State Beach (near San Juan Creek estuary) both experienced a high number of exceedances for the enterococci statistical threshold value (STV), which means that the 90th percentile of the enterococci concentrations exceeded 110 MPN per 100 mL, which is the STV threshold specified in the Basin Plan (SD RWQCB, 2021) (Table 1). Harbor Beach (near San Luis Rey River estuary) has had a much lower number of enterococci exceedances compared to Aliso State Beach and Doheny State Beach—its historical exceedance rate is more similar to the two sites on Trestles Beach (near San Mateo Creek and San Onofre Creek estuaries). Harbor Beach was selected because the watershed of the San Luis Rey River has a much higher level of land development than the watersheds of San Mateo and San Onofre Creeks (see Table 2). Thus, Aliso Beach and Doheny Beach could be considered a potentially impacted beach (i.e., more likely to have human-associated fecal pollution) due to the high extent of development in the watersheds of Aliso Creek and San Juan Creek and the high historical rates of enterococci STV exceedances.

For this study, Trestles Beach was considered a reference beach (i.e., minimally impacted by anthropogenic activities) due to the lower rates of enterococci STV exceedances and the low level of urbanization in the San Mateo Creek and San Onofre Creek watersheds (Table 2). Harbor Beach has a lower historical level of enterococci STV exceedances, but the watershed of the San Luis Rey River is much larger and is a mix of urban and agriculture development (Table 2). Therefore, Harbor Beach and the San Luis Rey are referred to as "Intermediate" throughout this report. The Upper San Luis Rey River is disconnected from the lower watershed by Lake Henshaw, which captures and diverts water from the upper watershed for drinking water use.

Table 1. Summary of enterococci statistical threshold value (STV) exceedances for the study sites.

Beach Name	Name of Adjacent Water Body	FID	Station Code and Name	Enterococci STV Exceedances (>110 MPN / 100 mL)
Aliso Beach	Aliso Creek	236	C1 – Aliso County Beach, Orange	686 of 1065 (64.4%)
Doheny Beach	San Juan Creek	430	S-0 – Doheny State Beach, Orange	595 of 1728 (34.4%)
Harbor Beach	San Luis Rey River	672	OC-100 – Harbor Beach, San Diego	168 of 2119 (7.9%)
Trestles Beach N	San Mateo Creek	588	EH-520 – San Onofre State Beach	66 of 578 (11.4%)
Trestles Beach S	San Onofre Creek	590	EH-510 – USMC Camp Pendleton	30 of 419 (7.2%)

Table 2. Summary of land development in the basins of the water bodies selected for the study (NLCD 2016).

Station	Latitude (degrees)	Longitude (degrees)	USGS Gage	Watershed Size (km^2)	Percent Agriculture	Percent Urban	Paved Intersections	Road Density (km/km^2)
Aliso Creek	33.510682	-117.75275	No	90.4	0.2%	54.8%	350	10.9
San Juan Creek	33.462387	-117.68381	Yes	455.9	0.5%	19.4%	647	4.3
San Luis Rey River*	33.206254	-117.38579	Yes	912.5	5.1%	8.9%	621	3.0
San Mateo Creek	33.385861	-117.59358	Yes	345.2	0.7%	1.5%	123	1.3
San Onofre Creek	33.380989	-117.57865	No	110.5	0.2%	3.2%	67	1.4

*Below Henshaw Reservoir, which captures upper watershed flow for diversion

Sample Collection

A total of 371 grab samples were collected for this project between November 1, 2021 and January 17, 2024 (Table 3). Approximately half of those samples were collected in the dry season (from May 1 until September 30), and approximately one-third were collected in the rainy season (from October 1 until April 30) but during dry weather conditions. The remaining 25% were collected in the rainy season during or shortly following wet weather events[1]. Samples were collected following the US EPA's guidelines for sampling recreational waters (US EPA, 2010) and were also consistent with sampling conducted under AB411. Sampling took place in targeted areas of beaches close to river mouths. The collection of samples took place in the morning to offer a balance between practicality and the generation of scientifically rigorous and actionable data. At estuary sites, conductivity measurements were taken in the field. These samples were not always consistent with freshwater, depending on upstream access. Grab samples were typically collected between the hours of 9:00 AM and 1:00 PM and were typically delivered to the laboratory by 2:00 PM on the same day of sample collection, where they were generally processed immediately.

[1] Defined as within 72 hours of a rainfall event of 0.1 inches or greater

Table 3. Summary of sampling plan, showing the breakdown of the number of samples collected at reference sites, impacted sites, and intermediate sites, at beaches and estuaries, during the dry season (dry weather) and during the rainy season (dry weather and wet weather).

Sample Site	Sample Location*	Number of Samples			
		Dry Season, Dry Weather	Rainy Season, Dry Weather	Rainy Season, Wet Weather	Subtotal
Reference Sites (Trestles Beach, San Mateo Creek, and San Onofre Creek)	Beach	40	30	22	**92**
	Estuary	40	28	22	**90**
Impacted Sites (Doheny Beach, San Juan Creek)	Beach	20	17	13	**50**
	Estuary	20	15	13	**48**
Intermediate Sites (Harbor Beach, San Luis Rey River)	Beach	20	15	11	**46**
	Estuary	20	14	11	**45**
TOTAL NUMBER OF SAMPLES		**160**	**119**	**92**	**371**

* Beach water samples were collected at the locations used by counties for routine monitoring; estuary samples were collected from ebb tide flows at the point where the creek/river enters the ocean, above the tidal prism, and far enough upstream to consist of freshwater.

Beach samples were collected at a depth of approximately 10 cm from the water surface, at a location within the shore zone but ideally past the breakpoint. If it was not practical to wade past the breakpoint, then the samples were collected at a depth of 10 cm from the water surface within the surf zone on an incoming wave, by wading to a safe depth, typically ankle to knee deep. If the sample was collected within the surf zone, it was collected in a way to minimize the amount of sediment in the sample due to wave action. Previous research has shown mixed results about the influence of sample depth on the density of fecal indicators (coliforms, *E. coli*, and enterococci) in beaches. In one study, when samples were collected in the surf zone, the fecal indicator concentrations at 10 cm from the surface differed from the concentrations at 10 cm from the bottom, but when samples were collected at offshore sites, no difference was noted (Le Fevre and Lewis, 2003). In another study, concentrations of fecal indicators in offshore samples collected at the surface and at 1.0 m depth did not vary, nor did concentrations of fecal indicators in shoreline samples collected at ankle depth and at waist depth (Boehm et al., 2003). The US EPA's EMPACT studies similarly found a lack of significant differences in the geometric mean concentrations of fecal indicators from samples collected from beaches at different depths.

Figure 2. Photos of the four sites chosen to be included in the study: a) San Mateo Creek at Trestles Beach North (reference); b) San Onofre Creek at Trestles Beach South (reference); c) San Juan Creek near Doheny Beach (impacted); and d) San Luis Rey River near Harbor Beach (intermediate).

Estuary samples were collected from the surface while standing on the riverbank using a telescoping dipstick sampler. If possible, these samples were collected at a location where the water was not completely stagnant, but displayed slow, steady flow. Care was taken to not disturb sediments from the river bottom before or during sample collection. Table 4 shows a summary of field analytical methods used at each sample site during each collection date to confirm basic water quality characteristics. In the field, samples were stored in a cooler with ice.

Table 4. Summary of field analytical methods used

Analyte/Parameter	Method Description	Reference/Standard
Water temperature	Commercial portable water quality meter with a temperature probe	SM 2550
Acidity/alkalinity (pH)	Commercial portable water quality meter with a pH probe (with a glass electrode)	SM 2310 B.2
Dissolved oxygen	Commercial portable water quality meter with a dissolved oxygen probe (electrochemical sensor)	SM 4500-O G
Conductivity	Commercial portable water quality meter with a conductivity probe	SM 2510

Laboratory Analysis

Once in the lab, samples were stored at 4°C until they were processed. Samples were split into five aliquots for analysis: two were used for the analysis of FIB (enterococci and *E. coli*), one was used for somatic coliphage analysis, and the last two were used for nucleic acid extraction (for qPCR analysis). Extracted nucleic acids were analyzed for the HF183/BacR287 gene target of *Bacteroides dorei* (HF183), pepper mild mottle virus (PMMoV), human adenovirus (HAdV), and norovirus genogroup II (NoVGII). The first seven batches of samples were also analyzed for norovirus genogroup I (NoVGI), but then later we switched to NoVGII, given its higher prevalence in California (Chen et al., 2022). All methods are based on protocols that have either been standardized or have been published in the peer-reviewed scientific literature (Table 5).

Table 5. Summary of laboratory analytical methods used

Analyte	Method Description	Standardized or Based on Literature	Reference/Standard
Enterococci	IDEXX Enterolert	Standardized	ASTM D6503-19
E. coli	IDEXX Colilert-18	Standardized	SM 9223 B-2004
Somatic coliphages	Single agar layer method	Standardized	EPA Method 1602
HF183	qPCR	Standardized	EPA Method 1696
PMMoV	RT-qPCR	Based on literature	Haramoto et al. (2013)
HAdV	qPCR	Based on literature	Jothikumar et al. (2005)
NoVGI	RT-qPCR	Based on literature	Svraka et al. (2006)
NoVGII	RT-qPCR	Standardized	Fout et al. (2014)

E. coli and enterococci

Standard method SM 9223 B-2004 (Rice et al., 2012) was used with the IDEXX Colilert-18 kit and the Quanti-Tray 2000 system to analyze samples for *E. coli*. The ASTM D6503-19 method was used with the IDEXX Enterolert kit and the Quanti-Tray 2000 system to analyze samples for enterococci. Both methods are based on the most probable number (MPN) concept. Briefly, two aliquots of 100 mL each were mixed with Enterolert and Colilert-18 media packs, respectively. Samples with historically high concentrations were diluted by a factor of 1:5 or 1:10 prior to mixing with the media packs (and MPN concentrations

were corrected for any dilutions made). Samples were analyzed within the maximum holding time of 8 hours from sample collection to sample incubation (per SWAMP MQO).

Somatic coliphages

One aliquot of 100 mL was used for somatic coliphage analysis using EPA Method 1602. Briefly, this single agar layer method allows a culture of *E. coli* to grow on a petri dish with tryptic soy agar, and then 100 mL aliquots of samples are added. After an incubation period, any coliphages present create plaque forming units (PFUs) in the lawn of bacterial growth. Samples were analyzed within the maximum holding time of 48 hours from sample collection to sample incubation (per US EPA Method 1602).

Human-associated biomarkers and pathogens

Bacteria and viruses were concentrated from an aliquot of at least 500 mL (and often up to 1,000 mL) using membrane filtration and the adsorption-extraction method, respectively. For membrane filtration, magnesium chloride ($MgCl_2$) was added to each sample at a final concentration of 0.25 mM, then the samples were filtered through a 0.45-µm pore size electronegative HAWP-type membrane, which was pretreated with 2.5 mM $MgCl_2$. These samples were extracted to analyze HF183 and PMMoV. For HAdV and NoV, the adsorption-extraction method was used (Symonds et al. 2014; 2017; Ahmed et al. 2015; 2021). Briefly, the pH was adjusted to 3.5 – 4.0 and magnesium chloride ($MgCl_2$) was added to samples at a final concentration of 0.25 mM to enhance virus adsorption. After this pretreatment, samples were filtered through a 0.45-µm pore size electronegative HAWP-type membrane, which had been pretreated with 2.5 mM $MgCl_2$. Prior to sample concentration, a selection of samples were spiked with approximately 500,000 copies of bovine rotavirus (Calf-Guard) as a viral process recovery control. All membranes were subsequently transferred aseptically to bead-beating tubes with lysis buffer and 10% beta-mercaptoethanol (βME). Then, as described in EPA Method 1696, a volume of 600 µL of a working stock of salmon sperm DNA with a concentration of 0.2 µg/mL was added to each bead-beating tube as an extraction control. After concentration, filters were placed immediately in lysis buffer with 10% beta-mercaptoethanol (βME) for nucleic acid extraction. Concentrated viruses and bacteria were lysed using bead beating, and their nucleic acids were extracted and purified within 24 hours using the Qiagen RNeasy PowerWater kit or the Thermofisher MagMAX Ultra Environmental kit, following the manufacturer's recommended protocol for the simultaneous extraction and purification of DNA and RNA into a final volume of 70 to 100 µL. The purified nucleic acids were stored at -80°C to protect the integrity of RNA.

The purified nucleic acids were then analyzed for HF183, PMMoV, HAdV, and NoVGII using RT-qPCR with the primer/probe concentrations and cycling conditions shown in Table 6. For HF183 and HAdV, qPCR was used with Taqman Environmental Master Mix 2.0 (Applied Biosystems) on the QuantStudio 3 system, with the primer/probe sequences, concentrations, volumes, and thermocycling conditions reported in EPA 1696 or in the literature (Jothikumar et al. 2005). Each sample was analyzed in duplicate (HAdV) or triplicate (HF183) reactions, each with a volume of 25 µL, with 2 to 5 µL of template DNA per reaction. For PMMoV and NoVGII, RT-qPCR was used with Taqman Fast Virus One-Step Master Mix (Applied Biosystems) on QuantStudio 3 with primer/probes, concentrations, volumes, and thermocycling conditions reported in the literature (Haramoto et al. 2013; Svraka et al. 2006). Samples were analyzed in duplicate reactions, each with a volume of 20 µL, with 2 to 5 µL of template RNA per reaction.

Table 6. Assay cycling conditions, primers, probes, and concentrations used for (RT-)qPCR

Assay	Primers and Probes (5' to 3')[2]	Concentrations for (RT-)qPCR[1]	Amplicon Length	Cycling Conditions[1]	References
HF183/ BacR287	F: ATCATGAGTTCACATGTCCG R: CTTCCTCTCAGAACCCCTATCC P: [FAM]-CTAATGGAACGCATCCC-[MGB]	Primers: 1,000 nM Probe: 80 nM	191 bp	95°C 10 min; 40x: (95°C 30 s, 60°C 1 min)	(US EPA, 2019)
	IAC P: [VIC]-AACACGCCGTTGCTACA-[MGB]	Probe: 80 nM			
PMMoV	F: GAGTGGTTTGACCTTAACGTTTGA R: TTGTCGGTTGCAATGCAAGT P: [FAM]-CCTACCGAAGCAAATG-[MGB]	Primers: 400 nM Probe: 200 nM	125 bp	50°C 5 min; 95° 20 s; 40x: (95° 3 s, 60° 30 s)	(Haramoto et al., 2013)
HAdV	F: GGACGCCTCGGAGTACCTGAG R: ACIGTGGGGTTTCTGAACTTGTT P: [FAM]-CTGGTGCAGTTCGCCCGTGCCA-[IABkFQ]	Primers: 250 nM Probe: 150 nM	96 bp	95°C 10 min; 40x: (95°C 30 s, 60°C 1 min)	(Jothikumar et al., 2005)
NoVGII	F: ATGTTCAGRTGGATGAGRTTCTCWGA R: TCGACGCCATCTTCATTCACA [FAM]-AGCACGTGGGAGGGCGATCG-[IABkFQ]	Primers: 500 nM (F) 900 nM (R) Probe: 250 nM	89 bp	50°C 5 min; 95° 20 s; 40x: (95° 3 s, 60° 30 s)	(Fout et al., 2014)
Sketa22	F: GGTTTCCGCAGCTGGG R: CCGAGCCGTCCTGGTC P: [FAM]-AGTCGCAGGCGGCCACCGT-[TAMRA]	Primers: 1,000 nM Probe: 80 nM	77 bp	95°C 10 min; 40x: (95°C 30 s, 60°C 1 min)	(US EPA, 2019)
BRV	F: GATATTGGACCATCTGATTCTGCTTCAAA R: GAAATCCACTTGATCGCACCCAA P: [CFO560]-TCGAATGCAGTTAAGACAAATGCAGACGCT-[BHQ1]	Primers: 500 nM Probe: 150 nM	155 bp	50°C 5 min; 95° 20 s; 40x: (95° 3 s, 60° 30 s)	(Schroeder et al., 2012)

[1] Concentrations of primers and probes and cycling conditions for (RT-)ddPCR will be consistent for all assays at 900 nM (primers) and 250 nM (probes), with the following cycling conditions for ddPCR: 95° for 10 min, followed by 40 cycles of 94° for 30 s and 60° for 1 min. RT-ddPCR have the following cycling conditions: 50°C for 60 min for reverse transcription, followed by 95° for 10 min and 40 cycles of 94° for 30 s and 60° for 1 min.

[2] FAM = 6-Carboxyfluorescein (on 5' end); IABkFQ = Iowa Black FQ quencher (on 3' end); IAbRQSp = 3' Iowa Black® RQ; MGB = minor groove binder

For both qPCR and RT-qPCR analyses, each plate contained a standard curve with at least five points at tenfold dilutions, run in duplicate (HAdV, PMMoV, NoVGII) or triplicate (HF183), and at least one no template control (NTC). Standards consisted of serially diluted stocks of gBlocks with the amplicon sequences and 10 base pairs on either end. Other quality control considerations were adhered to in accordance with the guidelines proposed in the scientific literature (Bustin et al. 2009). To correct for the one-cycle offset in the number of copies produced between the double-stranded DNA gBlock standards and the single-stranded RNA genomes from template RNA, standard concentrations were divided by two prior to developing the equation for the standard curve that was used to quantify the concentration of copies in unknown samples. The Sketa22 assay was used to detect Salmon sperm DNA as an extraction recovery control. The method described by Schroeder et al. (2012) was used to detect attenuated strains of bovine rotavirus (BRV) (Lincoln isolate, serotype G6; B223 isolate, serotype G10) as a virus concentration and RNA process recovery control, as described in the QAPP. The maximum holding times were 12 hours from sample collection to the initiation of filtration, 24 hours from sample collection to nucleic acid extraction and purification, 12 months from nucleic acid extraction to RT-qPCR analysis (RNA targets), and 24 months from nucleic acid extraction to qPCR analysis (DNA targets).

Statistical Methods

Objective 1. Fecal Indicator Concentrations and WQO Exceedances at Reference and Impacted Sites

The first objective was to compare the concentrations and WQO exceedance frequencies of general and alternative fecal indicators in samples collected from reference sites and impacted sites during different seasons and weather conditions. To do this, we used a two-way analysis of variance (ANOVA) with post-hoc Tukey test to assess the concentrations of *E. coli*, enterococci, and somatic coliphages with respect to sample site and season/weather conditions. For samples where there were non-detects, we used the parametric two factor fixed effects ANOVA method for censored data in the NADA2 package (Julian and Helsel, 2021) for R statistical software, which uses likelihood ratio tests to produce similar results to the usual method of moments ANOVA tests. In addition to comparing concentrations between sample sites and weather conditions, concentrations of enterococci and *E. coli* were also compared to the REC-1 criteria for geometric mean and STV. The exceedance rate for the latter was reported as the number of samples exceeding the STV divided by the total number of samples. Exceedance rates were calculated using the overall data sets, and the data subsets for each season / weather condition.

Objective 2. Concentrations of Human-Associated Fecal Indicators and Pathogens

The second objective was to compare the detection rates and concentrations of general and alternative indicators of fecal pollution in samples where human-associated fecal pollution is detected compared with samples where human-associated fecal pollution is not detected. To do this, we calculated the detection rates for each of the human-associated biomarkers and pathogens (HF183, PMMoV, HAdV, and NoVGII), then used a chi squared test to determine if the rates of detection at any of the sites or during any of the season/weather conditions were significantly greater than the overall average. For samples where those biomarkers were detected, we used ANOVA to determine if the concentrations were significantly greater at any of the sites or under any of the season/weather conditions. Then, we calculated the $\log_{10}$ differences between the average concentrations of general fecal indicators (*E. coli*, enterococci, and somatic

coliphages) when the human-associated biomarkers (e.g., HF183 and PMMoV) or enteric viruses (HAdV and NoVGII) were and were not detected. Then, we used a two-tailed t-test to determine if the general fecal indicator concentrations were significantly greater when the human-associated biomarkers were detected.

Objective 3. The HF183:PMMoV Ratio by Sample Site, Season, and Weather Conditions

The third objective was to compare the ratio of HF183:PMMoV in samples collected from reference sites and impacted sites, in the dry season and rainy season, during dry weather and wet weather conditions, and in samples where pathogens are detected compared to samples where pathogens are not detected. To do this, we used two-way ANOVA to determine if there were any significant differences in the HF183:PMMoV ratios with respect to sample site and season/weather conditions. Then, we used a two-tailed t-test to determine if the human-associated biomarkers or if the $\log_{10}$ HF183:PMMoV ratio were significantly greater when the human enteric viruses were detected, compared to when they were not detected.

Quality Assurance and Quality Control (QA/QC)

Quality assurance and quality control (QA/QC) activities consisted of the calculation of data quality indicators (DQIs), which are specific calculations of statistics that measure performance as discussed in the Environmental Protection Agency (EPA) report QA/G-5i (EPA, 2001) and in the State Water Resources Control Board (SWRCB) Surface Water Ambient Monitoring Plan (SWAMP) Measurement Quality Objectives (MQOs). DQIs specify tolerable levels of error in the data and ensure that the data generated meet the standards for publication in the peer-reviewed literature. Each DQI category used for this project is described in more detail below. In general, the DQIs described in the standardized methods used in this study were followed. For non-standardized methods, the DQIs typically reported in the peer-reviewed scientific literature were followed. For this project, the DQIs generally included measurements of precision, accuracy, recovery, bias, and sensitivity. The completeness and representativeness of samples were also assessed. The full plan for QA/QC is described in the approved Quality Assurance Project Plan (QAPP) for this project.

Laboratory quality control (QC) checks

Laboratory QC checks included sterility checks, laboratory positive controls, laboratory negative controls (method blanks), laboratory duplicates, and laboratory blanks, which were used to evaluate the analytical process for contamination, accuracy, and reproducibility. Blanks were used to verify that the equipment, sample containers, and reagents were not a source of contamination, and that the sampling techniques used were non-contaminating. Both field and laboratory blanks were included in the program. Field blanks were collected by sampling contaminant-free deionized water in the field during a sampling event. The same equipment used for collection of the grab samples was used to transfer the blank water into the blank sample containers. Method blanks were run by the analytical laboratory to determine the level of contamination associated with laboratory reagents and equipment. Method blanks were clean samples in a known matrix that was subjected to the same complete analytical procedure as the submitted samples to determine if contamination has been introduced into the samples during processing. Results of method

blank analysis should be less than the method limit of quantification, or less than 5% of the native sample concentration. If results from field blanks, extraction blanks, or method blanks were more than 20% of the native sample concentration, then the native sample was labeled as a non-detect.

Matrix spikes (MS) and internal amplification controls (IAC) were used to assess the bias, precision, and accuracy of the laboratory methods. A MS is created by adding a known quantity of the analyte (i.e., the target microorganism or genome) to an aliquot of field sample. After accounting for native concentrations, the percent recovery is calculated as the proportion of the known compound in the sample. The acceptable recovery limits were determined in accordance with standard methods. Percent recovery is calculated as the difference between the spiked concentration and the native sample concentration divided by the original spiked amount. An internal amplification control (IAC) is analogous to a matrix spike for qPCR. The IAC used in this study is based on a composite primer technique where the target and the IAC are simultaneously amplified with a common set of primers, under the same conditions in the same reaction, with the only difference being the probe sequence and respective reporter molecules (which fluoresce at different wavelengths, allowing the assays to be run in duplex). Duplicates were used in this project to check for consistency in the results, either periodically or constantly, at the level of the field (field duplicates), the laboratory (process duplicates), or the equipment used for analysis (analytical duplicates). Process controls will also be used in this project to measure percent recovery, for example during the concentration of viruses from large-volume samples or for the recovery of nucleic acids during the extraction and purification process.

The precision of individual measurements, as defined in QA/G-4 (US EPA, 2000), is a measure of mutual agreement among individual measurements of the same property under similar conditions, and it describes how well repeated measurements agree with each other. Statistically, it is generally expressed in terms of the standard deviation (SD), the coefficient of variation (CoV), or the relative percent difference (RPD). The precision measurements used in this study consisted of field duplicates, laboratory duplicates, and instrument duplicates, and it was quantified using the RPD between duplicate analyses. Accuracy describes how close a measurement is to its true value. The accuracy of microbial measurements for this study was measured during sample processing and laboratory analysis. For somatic coliphage analysis, accuracy was measured as the percent recovery of PhiX174 (based on EPA method 1602). Acceptable recoveries of PhiX174 for initial and ongoing precision and recovery are specified in the EPA standard method (EPA method 1602).

For qPCR and qPCR with reverse transcription (RT-qPCR), accuracy during nucleic acid extraction was measured as the percent recovery of salmon sperm DNA (based on EPA method 1696). For the filtration of samples and the subsequent measurement of viruses PMMoV, NoV, and HAdV using (RT-)qPCR specifically, accuracy was also measured based on the percent recovery of bovine rotavirus (BRV) in a percentage of samples. Acceptable recoveries of salmon sperm DNA are described in EPA method 1696, which also allows for the correction of sample concentrations based on the measured recovery of salmon sperm DNA. The method of assessing virus recovery during filtration based on the use of BRV is not standardized, so the following guidelines were used, based on what is generally considered acceptable in the peer-reviewed scientific literature. Recoveries of at least 10% were considered good, between 1% and 10% were considered acceptable, and recoveries of less than 1% were considered unacceptable.

For all qPCR and RT-qPCR methods, accuracy was also measured using the calculated efficiency and the coefficient of determination (the R^2 value) of standard curves. Based on the guidelines proposed by Bustin et al. (2009), known as the Minimum Information for the publication of Quantitative real-time PCR Experiments (MIQE), and based on the more recent guidelines proposed by Borchardt et al. (2021), known as the The Environmental Microbiology Minimum Information (EMMI) Guidelines, the acceptable efficiency of standard curves is between 90% and 110%. While not specifically addressed by Bustin et al. (2009) or Borchardt et al. (2021), R^2 values for (RT-)qPCR standard curves are generally considered acceptable if they are greater than or equal to 0.98.

Bias, which is defined in QA/G-4 (US EPA, 2000) as the systematic or persistent distortion of a measurement process that causes errors in one direction (*i.e.*, an underprediction or overprediction of measured values relative to the true values), was assessed through the analysis of field blanks (one per sample batch, always collected at one of the impacted sites). For the analysis of samples for fecal indicator bacteria and somatic coliphages, bias was measured using media sterility checks, laboratory positive controls, laboratory negative controls, and laboratory blanks, as described in the SWAMP MQOs for Indicator Bacteria in Fresh Water. For (RT-)qPCR, bias was assessed at the level of sample processing by analyzing extraction blanks and extraction spikes with salmon sperm DNA. At the analytical level, bias will be assessed by analyzing no template controls (NTCs) and internal amplification controls (IACs). Detectable quantities in any of the field blanks, extraction blanks, or no template controls would indicate positive bias. Recovery of less than specified thresholds for extraction spikes and IACs would indicate negative bias potentially due to losses experienced during sample extraction or PCR inhibition, respectively.

For this study, sensitivity relates to the presence of HF183 and PMMoV in sewage samples. Since, these two biomarkers are proposed as human-associated indicators of fecal pollution in environmental waters, it is defined as the number of sewage samples with positive detection divided by the total number of samples analyzed. Sensitivity was assessed by analyzing a total of 98 residential sewage samples for HF183 and PMMoV. Composite samples (24-hour) were collected using Teledyne ISCO model 3710 autosamplers, approximately 3 times per week between August 2021 until May 2022, from two sites that collected sewage from residence halls at a university campus. The first site served two buildings housing a total of 1038 people. The second site catered to one wing of another residence hall that housed 438 students. Four of the 98 samples were not analyzed because the extracted nucleic acid volume was insufficient. Of the remaining 94 samples, 91 had detectable levels of HF183 (sensitivity = 96.8%), and 90 had detectable levels of PMMoV (sensitivity = 95.7%). The full results of the sensitivity study were published by Fani et al. (2023). The host specificity of HF183 and PMMoV is defined as the proportion of nontarget host fecal samples that produce negative results (Harwood et al., 2014), was not measured for this study, as it was outside of the scope of the contract. However, it should be noted that the specificity for these two human-associated markers has already been thoroughly assessed in the scientific literature, with several of the studies taking place in southern California (e.g., Ahmed et al., 2016; Hamza et al., 2011; Li et al., 2021; Rosario et al., 2009). In many previous studies, measured host specificity values have been close to 100% for both HF183 and PMMoV (Ahmed and Harwood, 2019; Harwood et al., 2019).

Completeness describes the success of sample collection and laboratory analysis and is measured as the fraction of samples sampled and analyzed relative to the quantity targeted in the study design. This accounts for adverse weather conditions, safety concerns, and equipment problems. The target completeness for this study was 90% for all analyses, since a loss of 10% of the samples would represent a minimal loss in statistical power to address the study objectives. The completeness results for the different analytes measured in this study are summarized in Table 7.

Table 7. Summary of the completeness of each analyte measured in this study.

Analyte	Target Number of Samples	Number of Samples Analyzed	Number of Controls Analyzed*	Completeness (%)
E. coli	360	371	>200	103%
Enterococci	360	371	>200	103%
Somatic coliphages	360	332	>200	92%
HF183	360	356	>200	99%
PMMoV	360	356	>200	99%
HAdV	360	356	>200	99%
NoVGII	360	356	>200	99%

* Controls analyzed included the following:

- For *E. coli* and enterococci: Field duplicates, field blanks, method blanks, and media sterility blanks
- For somatic coliphages: Initial precision and recovery (IPR) controls, field blanks, method blanks, media sterility blanks, matrix spikes, and ongoing precision and recovery (OPR) controls
- For HF183: Initial precision and recovery (IPR) controls, internal amplification controls (IAC), sample processing control (SPC), field blanks, method blanks, extraction blanks, and instrument blanks (no template controls)
- For PMMoV, HAdV, and NoVGII: Virus recovery controls, matrix spikes, field blanks, method blanks, extraction blanks, and instrument blanks

RESULTS AND DISCUSSION

Objective 1. Traditional and Alternative General Fecal Indicators

The first objective was to compare fecal indicator concentrations and WQO exceedance frequencies at reference sites and impacted sites during different seasons and weather conditions. We answered the following question: *Are the concentrations and exceedance frequencies at impacted sites significantly greater than they are at reference sites and are these differences affected by weather and season?*

E. coli

The concentrations of *E. coli* at the different sites are shown in Figure 3. At the beach sites, the one-way ANOVA by sample site for *E. coli* was not significant ($p = 0.999$), meaning that there were no significant differences between the *E. coli* concentrations. However, the two-way ANOVA revealed a significant effect from season/weather ($p < 0.001$), but not by site ($p = 0.977$), and the interaction effect was also not significant ($p = 0.903$). Concentrations were significantly higher during the dry season than they were during the rainy season (wet weather) conditions ($p = 0.036$) and during the rainy season (dry weather) conditions ($p < 0.001$). At the estuary sites, the one-way ANOVA by sample site was significant ($p < 0.001$), meaning that there were significant differences between the *E. coli* concentrations. Two-way ANOVA revealed significant differences by site ($p < 0.001$) and by season/weather ($p = 0.0432$), with no significant interaction ($p = 0.111$). The post-hoc Tukey test revealed that the concentrations in San Juan Creek were significantly greater than the concentrations in San Mateo Creek ($p < 0.001$) and San Onofre Creek ($p < 0.001$) but not San Luis Rey River ($p = 0.075$), but the concentrations in San Luis Rey River were significantly greater than the concentrations in San Mateo Creek ($p = 0.0045$) and San Onofre Creek ($p = 0.0021$). The difference between San Mateo Creek and San Onofre Creek was not significant.

Table 8 shows the exceedance frequencies relative to the REC-1 *E. coli* WQOs (SD RWQCB, 2021). San Juan Creek and San Luis Rey River were the only two estuary sites where geometric mean *E. coli* concentrations were above the REC-1 criteria of 100 MPN per 100 mL, using the overall data set and also when using only data from the dry season and rainy season (dry weather) samples. During rainy season wet weather conditions, in addition to San Juan Creek and San Luis Rey River, the geometric mean *E. coli* concentration at San Onofre Creek also exceeded the REC-1 threshold. Regarding the STV for *E. coli*, using all data, San Mateo Creek was the only estuary site that did not exceed the threshold of 320 MPN per 100 mL, neither when using the entire data set nor when using only data from rainy season wet weather conditions. During dry weather conditions (rainy season and dry season), San Juan Creek and San Luis Rey River were the only two estuary sites that exceeded the STV.

The concentrations of *E. coli* at impacted sites were significantly greater than they were at reference sites, but only for estuaries. This confirms that ***E. coli* is a good indicator of fecal pollution in estuaries, but not in beaches**[2]. The concentrations measured at the San Luis Rey River estuary site were significantly greater than the reference estuary sites, but not significantly different from the impacted estuary site.

[2] This is consistent with California's statewide bacteria water quality objectives, which species the use of *E. coli* for inland fresh surface waters.

Table 8. Summary of exceedances at estuary sites relative to the REC-1 *E. coli* water quality objectives (geomean of 100 CFU per 100 mL and STV of 320 CFU per 100 mL)

Season/Weather Conditions	Sample Site	N	Geomean (MPN/100 mL)	STV Exceedance Rate (percent of samples above 320 MPN/100 mL)
Rainy Season (Wet Weather)	San Juan Creek (impacted)	11	839	73%
	San Mateo Creek (reference)	11	52.2	9%
	San Onofre Creek (reference)	11	148	27%
	San Luis Rey (intermediate)	11	148	18%
Rainy Season (Dry Weather)	San Juan Creek (impacted)	14	287	50%
	San Mateo Creek (reference)	14	26.2	7%
	San Onofre Creek (reference)	14	21.6	7%
	San Luis Rey (intermediate)	14	58.6	14%
Dry Season (Dry Weather)	San Juan Creek (impacted)	20	681	65%
	San Mateo Creek (reference)	20	47.3	5%
	San Onofre Creek (reference)	20	23.0	5%
	San Luis Rey (intermediate)	20	680	65%
Overall (all samples)	San Juan Creek (impacted)	45	548	62%
	San Mateo Creek (reference)	45	40.3	7%
	San Onofre Creek (reference)	45	35.6	11%
	San Luis Rey (intermediate)	45	219	38%

Note: Red cells indicate sites with values that exceed established thresholds for the geomean or the STV. Note that the geomean and STV exceedance rates were developed using all data from this study. This differs slightly than the REC-1 criteria, where the geometric mean is defined as a six-week rolling geometric mean, calculated based on the five most recent samples for a particular site, and where the STV is defined as the threshold concentration that is not to be exceeded by more than 10 percent of the samples collected in any calendar month, calculated in a static manner.

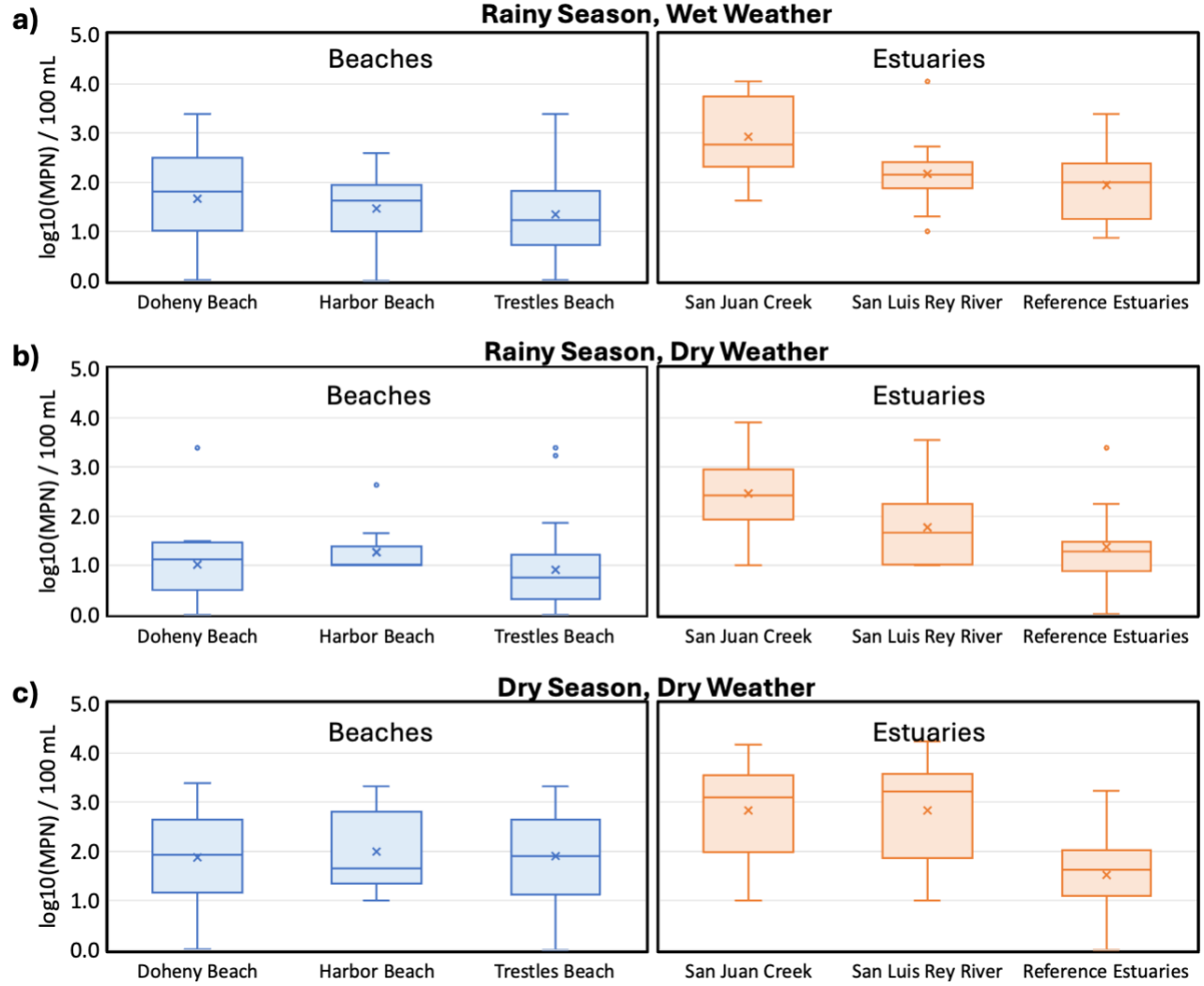

Figure 3. Box and whisker plots of the *E. coli* concentrations at the impacted sites (Doheny Beach and San Juan Creek), the reference sites (Trestles Beach, San Mateo Creek, and San Onofre Creek), and the intermediate test sites (Harbor Beach and San Luis Rey River).

Enterococci

The concentrations of enterococci at the different sites are shown in Figure 4. At the beach sites, the one-way ANOVA tests were significant based on site ($p < 0.001$) and season/weather ($p = 0.037$). The two-way ANOVA revealed significant effects from season/weather ($p = 0.0184$) and by site ($p < 0.001$), and the interaction effect was not significant ($p = 0.466$). Concentrations were significantly higher during wet weather conditions than they were during the rainy season under dry weather conditions ($p = 0.0282$). The post-hoc Tukey test revealed that the concentrations at Doheny Beach were significantly greater than the concentrations at Harbor Beach ($p < 0.001$), Trestles Beach North ($p = 0.0034$), and Trestles Beach South ($p = 0.0149$). There were no significant differences between the concentrations at Harbor Beach or either

of the two sites on Trestles Beach. At the estuary sites, the one-way ANOVA tests were significant ($p < 0.001$) and the two-way ANOVA revealed significant differences by site ($p < 0.001$) and by weather ($p = 0.002$), and the interaction effect was not significant ($p = 0.159$). Concentrations were significantly higher during wet weather conditions than they were during dry weather conditions, both in the dry season ($p = 0.0158$) and in the rainy season ($p = 0.0174$). The post-hoc Tukey test revealed that the concentrations in San Juan Creek were significantly greater than the concentrations in San Mateo Creek ($p < 0.001$), San Onofre Creek ($p < 0.001$), and San Luis Rey River ($p < 0.001$). The concentrations in San Luis Rey River were not significantly different than the concentrations in San Mateo Creek ($p = 0.0736$) or San Onofre Creek ($p = 0.825$), and there was no significant difference between the concentrations in San Mateo Creek and San Onofre Creek ($p = 0.394$).

Table 9 shows the results of the exceedance frequencies relative to the REC-1 bacteria WQOs (SD RWQCB, 2021). All beach sites had exceedances under all seasons and weather conditions, except for Trestles Beach North during rainy season (dry weather) conditions, which only exceeded the STV (but not the geomean), and Harbor Beach during rainy season (dry weather) conditions, which did not exceed either threshold.

Table 9. Summary of exceedances at beach sites relative to the REC-1 enterococcus water quality objectives (geomean of 30 CFU per 100 mL and STV of 110 CFU per 100 mL)

Season/Weather Conditions	Sample Site	N	Geomean (MPN/100 mL)	STV Exceedance Rate (percent of samples above 110 MPN/100 mL)
Rainy Season (Wet Weather)	Doheny Beach (impacted)	11	551	82%
	Trestles Beach North (reference)	11	53.5	18%
	Trestles Beach South (reference)	11	94.4	55%
	Harbor Beach (intermediate)	11	43.2	27%
Rainy Season (Dry Weather)	Doheny Beach (impacted)	15	115	47%
	Trestles Beach North (reference)	15	27.1	13%
	Trestles Beach South (reference)	15	39.6	20%
	Harbor Beach (intermediate)	15	20.9	7%
Dry Season (Dry Weather)	Doheny Beach (impacted)	20	149	55%
	Trestles Beach North (reference)	20	67.9	35%
	Trestles Beach South (reference)	20	71.8	40%
	Harbor Beach (intermediate)	20	46.3	20%
Overall (all samples)	Doheny Beach (impacted)	46	187	59%
	Trestles Beach North (reference)	46	47.5	24%
	Trestles Beach South (reference)	46	63.1	37%
	Harbor Beach (intermediate)	46	35.1	17%

Note: Red cells indicate sites with values that exceed established thresholds for the geomean or the STV. Note that the geomean and STV exceedance rates were developed using all data from this study. This differs slightly than the REC-1 criteria, where the geometric mean is defined as a six-week rolling geometric mean, calculated based on the five most recent samples for a particular site, and where the STV is defined as the threshold concentration that is not to be exceeded by more than 10 percent of the samples collected in any calendar month, calculated in a static manner.

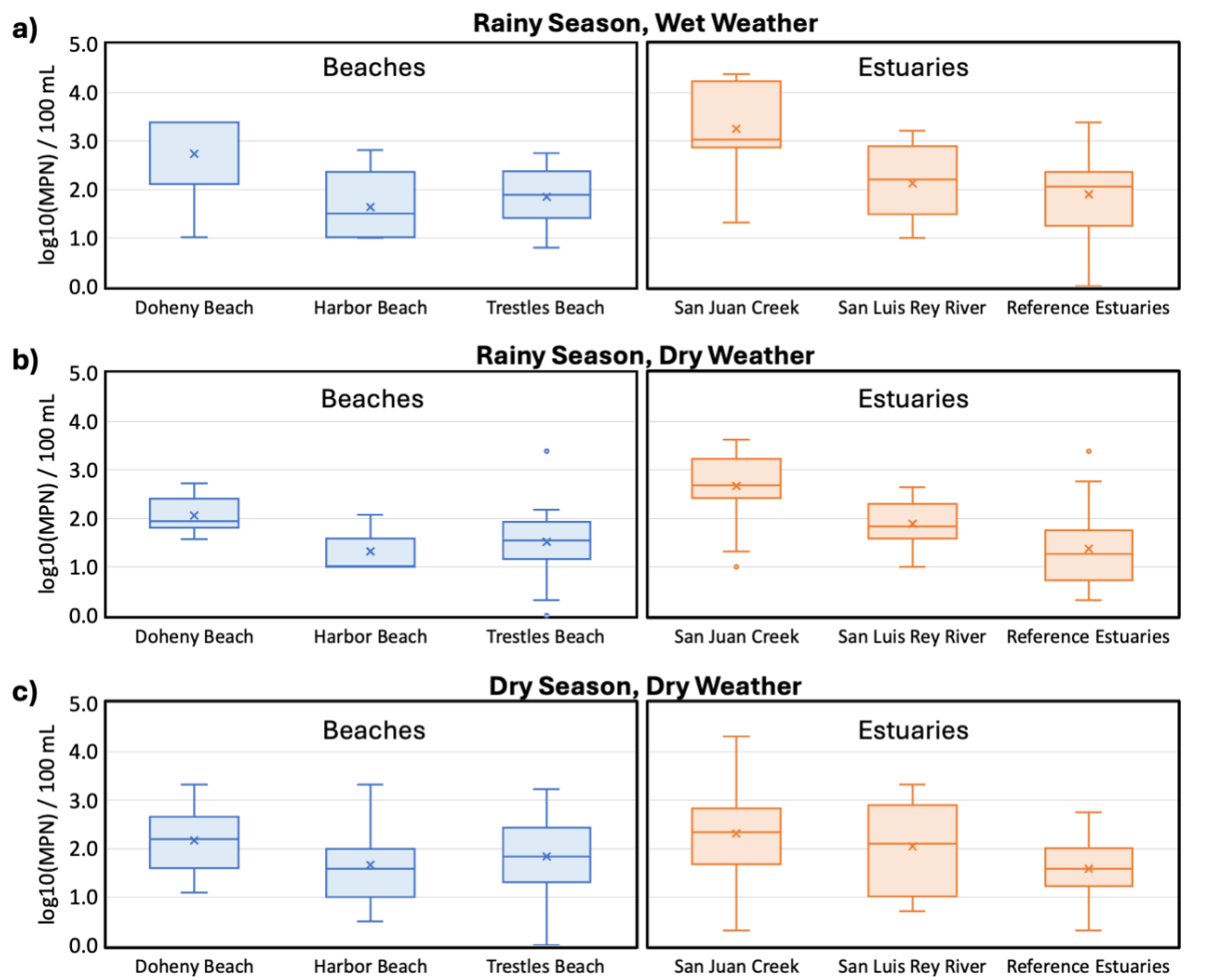

Figure 4. Box and whisker plots of the enterococci concentrations at the impacted sites (Doheny Beach and San Juan Creek), the reference sites (Trestles Beach, San Mateo Creek, and San Onofre Creek), and the intermediate test sites (Harbor Beach and San Luis Rey River).

The concentrations of enterococci at the impacted estuary and beach sites were significantly greater than they were for the reference sites, meaning that **enterococci appear to be good indicators of fecal pollution in estuaries and beaches**. The concentrations measured at San Luis Rey River and Harbor Beach were significantly lower than the concentrations measured at the impacted sites, but not significantly different from the concentrations measured at the reference sites.

Somatic Coliphages

The concentrations of somatic coliphages at the different sites are shown in Figure 5. At the beach sites, the one-way ANOVA tests were significant based on site ($p = 0.0145$) and season/weather ($p < 0.001$). The two-way ANOVA revealed significant effects from season/weather ($p < 0.001$) and by site ($p = 0.004$), but the interaction effect was not significant ($p = 0.728$). Concentrations were significantly lower during the dry season (dry weather) conditions than they were in the rainy season, both during rainy weather conditions ($p = 0.0016$) and during dry weather conditions ($p < 0.001$). The post-hoc Tukey test revealed that the concentrations at Doheny Beach were significantly greater than the concentrations at Trestles Beach South ($p = 0.0101$), but they were not significantly different than the concentrations at Trestles Beach North ($p = 0.0964$) or Harbor Beach ($p = 0.167$). There were no significant differences between the concentrations at Harbor Beach and the concentrations at Trestles Beach North ($p = 0.997$) or Trestles Beach South ($p = 0.715$), and there were no significant differences between the north and south sampling sites on Trestles Beach ($p = 0.826$).

At the estuary sites, the one-way ANOVA tests were significant by site ($p < 0.001$) and by weather ($p = 0.014$), the two-way ANOVA also revealed significantly differences by site ($p < 0.001$) and by weather ($p < 0.001$), but the interaction effect was not significant ($p = 0.0964$). Concentrations were significantly higher during wet weather conditions than they were during dry season (dry weather) conditions, but there were no significant differences between rainy season (wet weather) samples and rainy season (dry weather) conditions ($p = 0.598$), nor were there differences between rainy season (dry weather) and dry season (dry weather) conditions ($p = 0.132$). The post-hoc Tukey test revealed that the concentrations in San Juan Creek were significantly greater than the concentrations in San Mateo Creek ($p < 0.001$), San Onofre Creek ($p < 0.001$), and San Luis Rey River ($p < 0.001$). The concentrations in San Luis Rey River were not significantly different than the concentrations in San Mateo Creek ($p = 0.899$) or San Onofre Creek ($p = 0.527$), and there was no significant difference between the concentrations in San Mateo Creek and San Onofre Creek ($p = 0.911$).

In summary, the concentrations of somatic coliphages at the impacted estuary site (San Juan Creek) were significantly greater than they were at the reference estuary sites (San Mateo and San Onofre Creeks), meaning that **somatic coliphages appear to be good indicators of fecal pollution in these estuaries**. The concentrations of somatic coliphages at the impacted beach site (Doheny Beach) were significantly greater than one of the two reference beach sites (Trestles Beach South), meaning that these coliphages either may not be the best indicators in beach sites, or there may be a virus fecal pollution source at only one end of the reference beach (i.e., the north end of Trestles Beach). Concentrations of somatic coliphages in the San Luis Rey River were significantly lower than they were in the impacted estuary site (San Juan Creek), but they were not significantly different from the reference estuary sites (San Mateo and San Onofre Creeks). As for Harbor Beach, the concentrations of somatic coliphages were not significantly different from the impacted beach site nor from the reference beach sites.

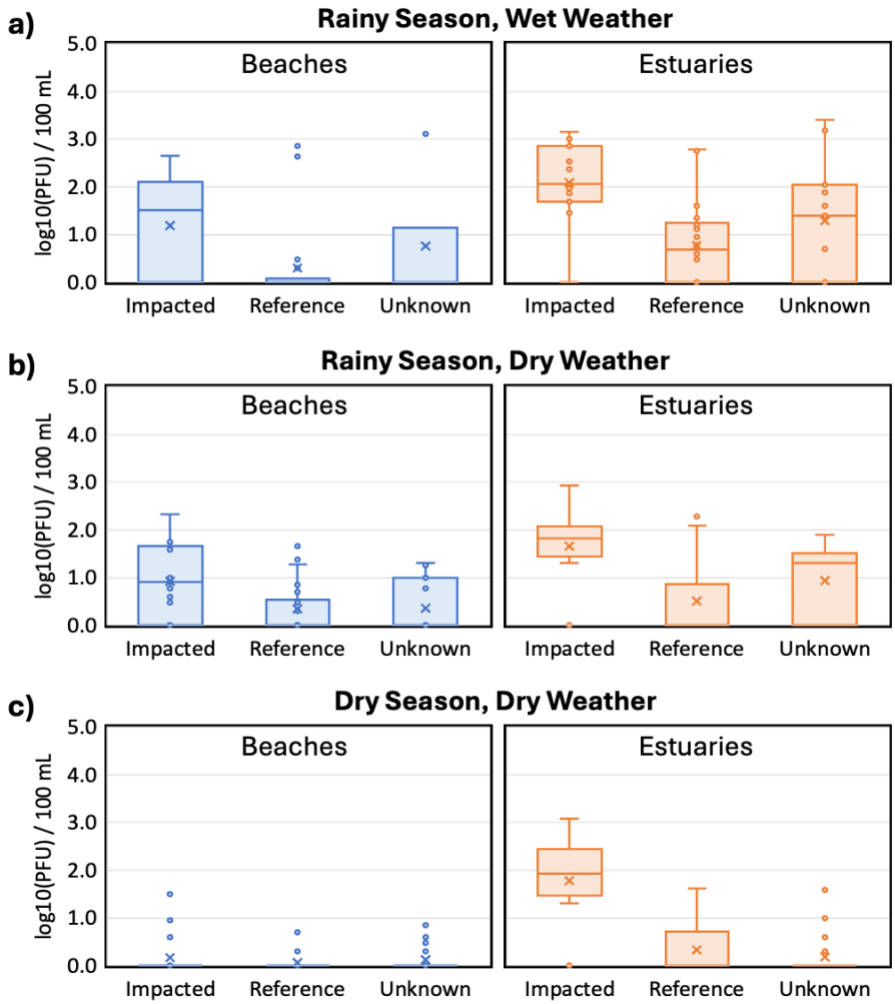

Figure 5. Box and whisker plots of the somatic coliphage concentrations at the impacted sites (Doheny Beach and San Juan Creek), the reference sites (Trestles Beach, San Mateo Creek, and San Onofre Creek), and the intermediate test sites (Harbor Beach and San Luis Rey River).

Objective 2. Human-Associated Fecal Indicators and Pathogens

The second objective was to compare the detection rates and concentrations of general and alternative indicators of fecal pollution in samples where human-associated fecal pollution is detected compared with samples where human-associated fecal pollution is not detected. For this objective, our task was to answer the following research question: *Is there a difference in the concentrations of general and alternative indicators of fecal pollution when human fecal biomarkers are detected vs. when they are not detected?*

HF183 detection rates and concentrations

The detection rates and average $\log_{10}$-transformed concentrations of HF183 are shown in Tables 10 and 11. Overall, HF183 was detected in 103 of 356 samples (28.9%). HF183 was detected at all sites, but the

detection rate in San Juan Creek estuary was significantly higher than it was in the other sites based on the chi squared test ($p = 0.015$). The differences between the concentrations at each of the sites were not statistically significant ($p = 0.0859$), but the geometric mean concentration in San Juan Creek estuary was the highest of all sites (2.40 copies/mL), and Doheny Beach (SJCO) had the highest geometric mean concentrations of all beach sites (1.36 copies/mL). At the reference sites, detection rates and geometric mean concentrations were lower in the estuaries than they were at the beaches. The opposite was true at the impacted sites, where the detection rate and concentrations were greater in the estuary than they were at the beach. For San Luis Rey River and Harbor Beach (the intermediate sites), HF183 was detected more frequently and at higher average concentrations in the estuary than at the beach. Based on this assessment, San Luis Rey River is likely impacted by HF183 in a way that is more consistent with San Juan Creek than with San Mateo or San Onofre Creeks.

HF183 detection rates were significantly greater during wet weather conditions than they were during dry weather conditions (both in the rainy season and in the dry season), according to the chi squared test ($p = 0.007$). A two-way ANOVA also revealed that concentrations were significantly different for different season/weather conditions ($p = 0.0019$). The concentrations were greatest during rainy season (wet weather) conditions, followed by rainy season (dry weather), then dry season (dry weather) conditions. HF183 was only detected twice at Trestles Beach South, but the concentration was quite high (50.5 copies/mL) in one of those samples, which is why the average shown in Table 11 appears much higher than the other sites. The highest concentration detected in a single sample was 85.6 copies/mL, in San Juan Creek during wet weather conditions on 12/12/2022.

Table 10. Detection rates of HF183 at each site and under each season and weather condition

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	9/11 (81.8%)	5/13 (38.5%)	8/20 (40%)	22/44 (50%)
SJCO	Impacted	Beach	8/11 (72.7%)	2/14 (14.3%)	8/20 (40%)	18/45 (40%)
SMAE	Reference	Estuary	0/11 (0%)	4/13 (30.8%)	5/20 (25%)	9/44 (20.5%)
SMAS	Reference	Beach	4/11 (36.4%)	3/14 (21.4%)	4/20 (20%)	11/45 (24.4%)
SONE	Reference	Estuary	3/11 (27.3%)	1/13 (7.7%)	2/20 (10%)	6/44 (13.6%)
SONS	Reference	Beach	6/11 (54.5%)	2/14 (14.3%)	3/20 (15%)	11/45 (24.4%)
SLRE	Intermediate	Estuary	8/11 (72.7%)	5/13 (38.5%)	1/20 (5%)	14/44 (31.8%)
SLRS	Intermediate	Beach	5/11 (45.5%)	3/14 (21.4%)	4/20 (20%)	12/45 (26.7%)
		Overall	**43/88 (48.9%)**	**25/108 (23.1%)**	**35/160 (21.9%)**	**103/356 (28.9%)**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach

Table 11. Average log_{10}-transformed concentrations of HF183 at each site and under each season and weather condition (in samples where HF183 was detected)

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	7.16	3.04	0.61	2.40
SJCO	Impacted	Beach	3.36	0.89	0.61	1.36
SMAE	Reference	Estuary	-	1.80	0.25	0.60
SMAS	Reference	Beach	0.42	4.92	0.91	1.09
SONE	Reference	Estuary	0.47	1.62	0.15	0.40
SONS	Reference	Beach	0.81	11.17	0.42	1.09
SLRE	Intermediate	Estuary	1.33	0.41	0.04	0.68
SLRS	Intermediate	Beach	1.09	0.17	0.33	0.46
		Overall	**1.71**	**1.38**	**0.44**	**1.02**

PMMoV detection rates and concentrations

The detection rates and concentrations of PMMoV are shown in Tables 12 and 13. Overall, PMMoV was detected in 142 of 356 samples (39.9%). PMMoV was detected at all sites, and while the detection rate in San Juan Creek was greater than other sites, the difference was not significant ($p = 0.390$). The geometric mean concentration in San Juan Creek (0.292 copies/mL) was higher than the concentrations at other sites (0.119 to 0.216 copies/mL). Differences between the concentrations at different sites were not significant ($p = 0.167$), but the detection frequencies and average concentrations of PMMoV in San Luis Rey River and Harbor Beach samples were more similar to San Juan Creek and Doheny Beach than they were to San Mateo Creek, San Onofre Creek, and Trestles Beach. The frequency of PMMoV detection was higher in the dry season than in the rainy season, but the difference was not significant ($p = 0.141$). Average concentrations in the dry season were lower than the rainy season (especially during wet weather).

Table 12. Detection rates of PMMoV at each site and under each season and weather condition

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	6/11 (54.5%)	5/13 (38.5%)	12/20 (60%)	23/44 (52.3%)
SJCO	Impacted	Beach	0/11 (0%)	4/14 (28.6%)	10/20 (50%)	14/45 (31.1%)
SMAE	Reference	Estuary	4/11 (36.4%)	4/13 (30.8%)	10/20 (50%)	18/44 (40.9%)
SMAS	Reference	Beach	3/11 (27.3%)	5/14 (35.7%)	5/20 (25%)	13/45 (28.9%)
SONE	Reference	Estuary	2/11 (18.2%)	5/13 (38.5%)	8/20 (40%)	15/44 (34.1%)
SONS	Reference	Beach	3/11 (27.3%)	5/14 (35.7%)	10/20 (50%)	18/45 (40%)
SLRE	Intermediate	Estuary	7/11 (63.6%)	6/13 (46.2%)	8/20 (40%)	21/44 (47.7%)
SLRS	Intermediate	Beach	5/11 (45.5%)	5/14 (35.7%)	10/20 (50%)	20/45 (44.4%)
		Overall	**30/88 (34.1%)**	**39/108 (36.1%)**	**73/160 (45.6%)**	**142/356 (39.9%)**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach

Table 13. Average log_{10}-transformed concentrations of PMMoV at each site and under each season and weather condition (in samples where PMMoV was detected)

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	0.33	0.41	0.24	0.29
SJCO	Impacted	Beach	-	0.15	0.11	0.12
SMAE	Reference	Estuary	0.16	0.21	0.13	0.15
SMAS	Reference	Beach	0.11	0.32	0.10	0.16
SONE	Reference	Estuary	0.41	0.12	0.11	0.13
SONS	Reference	Beach	0.14	0.14	0.12	0.13
SLRE	Intermediate	Estuary	0.44	0.24	0.11	0.22
SLRS	Intermediate	Beach	0.29	0.40	0.13	0.21
			0.26	0.23	0.13	0.18

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach

Enteric virus detection rates and concentrations

The detection rates and average log_{10}-transformed concentrations of enteric viruses are shown in Tables 14 and 15. Overall, enteric viruses were detected in only 12 of 356 samples (3.4%). Specifically, HAdV was detected in 5 samples and NoVGII was detected in 7 samples (no single sample amplified for both enteric virus biomarkers). The only sites where enteric viruses were not detected were Doheny Beach and Trestles Beach South. In San Mateo Creek and San Onofre Creek samples, HAdV was the only virus detected, and in Trestles Beach North samples, NoVGII was the only virus detected. Both viruses were detected at least once in San Juan Creek, San Luis Rey River, and Harbor Beach.

The sites with the highest rates of detection of either enteric virus were San Juan Creek (3 of 44 samples) and Harbor Beach (3 of 45 samples), but the differences in the detection frequencies were not significant, according to the chi squared test ($p = 0.245$). All three samples in San Juan Creek with positive enteric virus detection happened during rainy season wet weather conditions (3 of 11 samples, 27% detection rate), whereas the samples from Harbor Beach with positive enteric virus detection were spread out across different seasons and weather conditions. The highest enteric virus concentration in a single sample was at Trestles Beach South, where HAdV was detected at a concentration of 0.30 copies/mL. Other than that sample, the highest enteric virus concentrations detected were both at San Juan Creek, where NoVGII was detected at concentrations of 0.26 and 0.14 copies/mL.

Enteric viruses were detected most frequently during rainy season wet weather conditions, followed by rainy season dry weather conditions, and dry season (dry weather) conditions. The greater frequency of detection during wet weather conditions was significant, according to the chi squared test ($p = 0.008$). Only NoVGII was detected once during the dry season dry weather.

Table 14. Detection rates of viruses (HAdV or NoVGII) at each site under each season/weather condition

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	3/11 (27.3%)	0/13 (0%)	0/20 (0%)	3/44 (6.8%)
SJCO	Impacted	Beach	0/11 (0%)	0/14 (0%)	0/20 (0%)	0/45 (0%)
SMAE	Reference	Estuary	0/11 (0%)	1/13 (7.7%)	0/20 (0%)	1/44 (2.3%)
SMAS	Reference	Beach	1/11 (9.1%)	1/14 (7.1%)	0/20 (0%)	2/45 (4.4%)
SONE	Reference	Estuary	1/11 (9.1%)	0/13 (0%)	0/20 (0%)	1/44 (2.3%)
SONS	Reference	Beach	0/11 (0%)	0/14 (0%)	0/20 (0%)	0/45 (0%)
SLRE	Intermediate	Estuary	0/11 (0%)	2/13 (15.4%)	0/20 (0%)	2/44 (4.5%)
SLRS	Intermediate	Beach	1/11 (9.1%)	1/14 (7.1%)	1/20 (5%)	3/45 (6.7%)
		Overall	**6/88 (6.8%)**	**5/108 (4.6%)**	**1/160 (0.6%)**	**12/356 (3.4%)**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach

Table 15. Average $\log_{10}$-transformed concentrations of viruses (HAdV or NoVGII) at each site and under each season and weather condition in samples where enteric viruses were detected

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	0.14	-	-	0.14
SJCO	Impacted	Beach	-	-	-	-
SMAE	Reference	Estuary	-	0.11	-	0.11
SMAS	Reference	Beach	0.07	0.02	-	0.04
SONE	Reference	Estuary	0.30	-	-	0.30
SONS	Reference	Beach	-	-	-	-
SLRE	Intermediate	Estuary	-	0.03	-	0.03
SLRS	Intermediate	Beach	0.05	0.03	0.03	0.04
		Overall	**0.12**	**0.04**	**0.03**	**0.07**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach

The detection frequencies and concentrations of HF183 and PMMoV were significantly greater in impacted sites (San Juan Creek and Doheny Beach) than they were in reference sites (San Mateo Creek, San Onofre Creek, and Trestles Beach). However, San Mateo Creek, San Onofre Creek, and Trestles Beach have some limitations as reference sites, since human-associated fecal biomarkers or enteric viruses were detected at least once in each of these sites. Enteric virus detection was sporadic and occurred at almost all sites at similar frequencies and concentrations. Based on a comparison of the detection frequencies and concentrations of enteric viruses and other human-associated fecal biomarkers, **San Luis Rey River and Harbor Beach are likely impacted by human-associated fecal pollution, but not necessarily any more than the reference sites**.

E. coli concentrations in the presence and absence of human-associated biomarkers

The log_{10} differences between the average *E. coli* concentrations when HF183, PMMoV, and enteric viruses were vs. were not detected are shown in Tables 16, 17, and 18. Overall, *E. coli* concentrations were greater by 0.14 log_{10} units in samples were HF183 was detected, but the difference was not statistically significant ($p = 0.210$). *E. coli* concentrations were also greater overall by 0.15 log_{10} units in samples were PMMoV was detected, but the difference was not statistically significant ($p = 0.165$). When enteric viruses (HAdV or NoVGII) were present, *E. coli* concentrations were greater on average by 0.37 log_{10} units, but the difference was not statistically significant ($p = 0.074$). In estuary sites however, *E. coli* concentrations were significantly greater when HF183 was detected than when HF183 was not detected ($p = 0.045$), but there was no significant difference when PMMoV was vs. was not detected ($p = 0.182$). When considering only the beach sites, there were no significant differences in the *E. coli* concentrations, neither in samples where HF183 was vs. was not detected ($p = 0.819$), nor in samples where PMMoV was vs. was not detected ($p = 0.955$).

E. coli concentrations significantly coincided with the presence of human-associated biomarkers, but only in estuary sites. *E. coli* concentrations did not coincide well with the presence of enteric viruses.

Table 16. Log_{10} differences in the average concentrations of *E. coli* in samples where HF183 was detected, compared with samples where HF183 was not detected (positive values indicate *E. coli* concentrations were higher when HF183 was detected).

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	0.80	-0.29	0.58	0.38
SJCO	Impacted	Beach	0.10	-0.27	-0.58	-0.09
SMAE	Reference	Estuary	ND	0.32	-0.03	0.06
SMAS	Reference	Beach	0.04	-0.50	-0.14	-0.22
SONE	Reference	Estuary	0.41	-0.41	0.00	0.33
SONS	Reference	Beach	0.34	0.00	0.22	0.11
SLRE	Intermediate	Estuary	-0.13	-0.33	-1.44	-0.61
SLRS	Intermediate	Beach	0.30	0.04	0.04	0.06
		Overall	**0.40**	**0.07**	**-0.01**	**0.14**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach; ND = not enough data

Table 17. Log_{10} differences in the average concentrations of *E. coli* in samples where PMMoV was detected, compared with samples where PMMoV was not detected (positive values indicate *E. coli* concentrations were higher when PMMoV was detected).

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	-0.06	0.81	0.15	0.32
SJCO	Impacted	Beach	ND	0.09	-0.45	-0.15
SMAE	Reference	Estuary	0.30	0.14	0.04	0.16
SMAS	Reference	Beach	-0.34	0.05	0.16	-0.08
SONE	Reference	Estuary	1.26	0.46	-0.40	0.02
SONS	Reference	Beach	-0.50	-0.30	-0.38	-0.23
SLRE	Intermediate	Estuary	0.29	0.21	-0.60	-0.21
SLRS	Intermediate	Beach	0.67	0.18	0.32	0.42
		Overall	**0.36**	**0.25**	**-0.10**	**0.15**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach; ND = not enough data

Table 18. Log_{10} differences in the average concentrations of *E. coli* in samples where enteric viruses (HAdV or NoVGII) was detected, compared with samples where enteric viruses were not detected (positive values indicate *E. coli* concentrations were higher when enteric viruses were detected).

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	-0.27	ND	1.13	0.31
SJCO	Impacted	Beach	0.93	ND	0.30	0.71
SMAE	Reference	Estuary	ND	0.75	0.49	0.55
SMAS	Reference	Beach	-1.47	-1.06	0.48	-0.34
SONE	Reference	Estuary	0.35	ND	ND	0.95
SONS	Reference	Beach	ND	ND	0.79	1.24
SLRE	Intermediate	Estuary	-0.67	-0.64	1.63	-0.33
SLRS	Intermediate	Beach	0.79	-0.29	1.24	0.52
		Overall	**0.25**	**-0.27**	**0.73**	**0.37**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach; ND = not enough data

Enterococci concentrations in the presence and absence of human-associated biomarkers

The log_{10} differences between the average enterococci concentrations when HF183, PMMoV, and enteric viruses were and were not detected are shown in Tables 19, 20, and 21. Overall, enterococci concentrations were significantly greater by 0.40 log_{10} units in samples were HF183 was detected ($p < 0.001$). Enterococci concentrations were greater overall by only 0.02 log_{10} units on average in samples were PMMoV was detected, and this small difference was not statistically significant ($p = 0.858$). When enteric viruses (HAdV or NoVGII) were present, enterococci concentrations were greater on average by 0.31 log_{10} units, but the difference was not statistically significant ($p = 0.068$). In estuary sites, enterococci concentrations were significantly greater in samples where HF183 was detected compared to samples where HF183 was not detected ($p < 0.001$), but there was no significant difference when PMMoV was vs. was not detected ($p = 0.156$). When considering only the beach sites, enterococci concentrations were also significantly greater in samples where HF183 was detected compared to samples where HF183 was not detected ($p = 0.035$), but the same was not true for enterococci and PMMoV ($p = 0.105$).

Enterococci concentrations coincided well with the presence of HF183, both in estuaries and in beaches, but they did not coincide as well with the presence of PMMoV nor with enteric viruses, except for the samples collected during the dry season dry weather period, where enterococci concentrations were greater by 0.55-log_{10} units when enteric viruses were present, compared to when they were not present.

Table 19. Log_{10} differences in the average concentrations of enterococci in samples where HF183 was detected, compared with samples where HF183 was not detected (positive values indicate that enterococci concentrations were higher when HF183 was detected).

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	0.01	0.19	0.68	0.61
SJCO	Impacted	Beach	0.99	0.20	-0.38	0.29
SMAE	Reference	Estuary	ND	0.24	-0.23	-0.10
SMAS	Reference	Beach	0.23	0.39	-0.38	0.04
SONE	Reference	Estuary	0.41	-0.84	0.22	0.33
SONS	Reference	Beach	0.87	-0.02	0.07	0.37
SLRE	Intermediate	Estuary	0.08	0.26	0.20	0.22
SLRS	Intermediate	Beach	0.45	0.22	-0.55	0.03
		Overall	**0.73**	**0.27**	**0.05**	**0.40**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach; ND = not enough data

Table 20. Log_{10} differences in the average concentrations of enterococci in samples where PMMoV was detected, compared with samples where PMMoV was not detected (positive values indicate that enterococci concentrations were higher when PMMoV was detected).

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	-0.52	-0.20	-0.04	-0.23
SJCO	Impacted	Beach	ND	0.16	-0.49	-0.45
SMAE	Reference	Estuary	0.39	0.47	0.17	0.34
SMAS	Reference	Beach	0.53	-0.22	-0.27	-0.10
SONE	Reference	Estuary	1.15	0.17	0.09	0.15
SONS	Reference	Beach	-0.02	-0.43	-0.29	-0.25
SLRE	Intermediate	Estuary	0.16	0.29	-0.03	0.14
SLRS	Intermediate	Beach	0.27	-0.28	0.37	0.18
		Overall	**0.16**	**0.00**	**-0.02**	**0.02**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach; ND = not enough data

Table 21. Log_{10} differences in the average concentrations of enterococci in samples where enteric viruses (HAdV or NoVGII) were detected, compared with samples where enteric viruses were not detected (positive values indicate that enterococci concentrations were higher when enteric viruses were detected).

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	-0.38	ND	1.08	0.47
SJCO	Impacted	Beach	0.71	ND	0.00	0.29
SMAE	Reference	Estuary	ND	1.61	0.09	0.57
SMAS	Reference	Beach	-0.36	0.11	0.59	0.25
SONE	Reference	Estuary	-0.06	ND	ND	0.55
SONS	Reference	Beach	ND	ND	0.13	0.22
SLRE	Intermediate	Estuary	-1.30	0.18	1.53	-0.04
SLRS	Intermediate	Beach	-0.65	-0.31	1.20	0.09
		Overall	**-0.04**	**0.19**	**0.55**	**0.31**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach; ND = not enough data

Somatic coliphage concentrations in the presence and absence of human-associated biomarkers

The log_{10} differences between the average somatic coliphage concentrations when HF183, PMMoV, and enteric viruses were and were not detected are shown in Tables 22, 23, and 24. Overall, somatic coliphage concentrations were significantly greater by 0.40 log_{10} units in samples were HF183 was detected ($p = 0.001$). Somatic coliphage concentrations were also greater overall by 0.12 log_{10} units in samples were PMMoV was detected, but the difference was not significant ($p = 0.227$). There was no significant difference in the concentrations of somatic coliphages when enteric viruses (HAdV or NoVGII) were or were not present ($p = 0.894$).

In estuary sites alone, somatic coliphage concentrations were significantly greater when HF183 was present ($p < 0.001$), but the same was not true for beach sites ($p = 0.258$). Somatic coliphage concentrations were also greater when PMMoV was detected in estuary sites, but the difference was not significant ($p = 0.058$) and there was no significant difference between the coliphage concentrations at beach sites when PMMoV was vs. was not detected ($p = 0.138$).

Somatic coliphage concentrations significantly coincided with the presence of HF183, but only in estuary sites. They did not coincide as well with the presence of PMMoV or enteric viruses.

Table 22. Log_{10} differences in the average concentrations of somatic coliphages in samples where HF183 was detected, compared with samples where HF183 was not detected (positive values indicate coliphage concentrations were higher when HF183 was detected).

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	-0.26	0.40	0.04	0.17
SJCO	Impacted	Beach	0.85	-0.44	-0.21	0.17
SMAE	Reference	Estuary	ND	0.04	0.33	0.09
SMAS	Reference	Beach	-0.45	-0.02	0.10	-0.11
SONE	Reference	Estuary	0.49	-0.48	-0.35	0.09
SONS	Reference	Beach	0.39	-0.07	-0.02	0.22
SLRE	Intermediate	Estuary	0.08	0.39	ND	0.78
SLRS	Intermediate	Beach	-0.97	0.84	0.03	0.04
		Overall	**0.33**	**0.36**	**0.26**	**0.40**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach; ND = not enough data

Table 23. Log_{10} differences in the average concentrations of somatic coliphages in samples where PMMoV was detected, compared with samples where PMMoV was not detected (positive values indicate coliphage concentrations were higher when PMMoV was detected).

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	0.45	-0.31	0.35	0.19
SJCO	Impacted	Beach	ND	0.47	0.08	-0.24
SMAE	Reference	Estuary	0.59	0.04	-0.06	0.06
SMAS	Reference	Beach	-0.26	0.49	-0.02	0.12
SONE	Reference	Estuary	1.10	0.15	-0.15	0.11
SONS	Reference	Beach	-0.29	0.03	-0.05	-0.14
SLRE	Intermediate	Estuary	0.04	-0.06	-0.03	0.12
SLRS	Intermediate	Beach	-0.55	-0.03	-0.08	-0.22
		Overall	**0.29**	**0.11**	**0.18**	**0.12**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach; ND = not enough data

Table 24. Log_{10} differences in the average concentrations of somatic coliphages in samples where enteric viruses (HAdV or NoVGII) were detected, compared with samples where enteric viruses were not detected (positive values indicate coliphage concentrations were higher when enteric viruses were detected).

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	-1.24	ND	-0.29	-0.69
SJCO	Impacted	Beach	0.34	ND	-0.15	-0.18
SMAE	Reference	Estuary	ND	1.15	-0.39	0.00
SMAS	Reference	Beach	-0.32	0.08	0.08	-0.05
SONE	Reference	Estuary	0.68	ND	ND	0.90
SONS	Reference	Beach	ND	ND	-0.02	-0.16
SLRE	Intermediate	Estuary	-1.58	0.78	-0.22	-0.10
SLRS	Intermediate	Beach	-0.83	0.66	-0.14	-0.05
		Overall	**-0.25**	**0.61**	**-0.21**	**-0.02**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach; ND = not enough data

Objective 3. The HF183:PMMoV Ratio by Sample Site, Season, and Weather Condition

The third objective was to compare the ratio of HF183:PMMoV in samples collected from reference sites and impacted sites, in the dry season and rainy season, during dry weather and wet weather conditions, and in samples where pathogens are detected compared to samples where pathogens are not detected. This objective addressed the following research question: *Do trends in the HF183:PMMoV ratio correspond with the levels of general and alternative fecal indicators or with the detection of human pathogens at impacted and reference sites, in different seasons and during different weather conditions?*

HF183:PMMoV log_{10} ratio

Table 25 shows the average log_{10}-transformed HF183:PMMoV ratio at each site, under rainy season wet weather conditions, rainy season dry weather conditions, and dry season (dry weather) conditions. The ratios are calculated for all samples where at least one of the two biomarkers was detected. If one of the biomarkers was not detected, then its lowest detected value (*i.e.*, the highest Cq values in samples with true amplification and no QA/QC issues, which was often below the 95% probability of detection), was used to estimate the HF183:PMMoV ratio. Overall, the log_{10} HF183:PMMoV ratios were higher during wet weather conditions and lower during dry weather conditions. This was also true for each site, apart from San Mateo Creek (SMAE), where the ratio was lowest during rainy season (wet weather) conditions and highest during rainy season (dry weather) conditions.

Table 25. Average log_{10}-transformed HF183:PMMoV concentration ratio at each site, during each season and weather condition in samples where at least one biomarker was detected

Site*	Type	Location	Rainy Season (Wet Weather)	Rainy Season (Dry Weather)	Dry Season (Dry Weather)	Overall
SJCE	Impacted	Estuary	1.53	0.48	-0.14	0.54
SJCO	Impacted	Beach	2.16	-0.13	0.29	0.75
SMAE	Reference	Estuary	-1.02	0.57	-0.31	-0.19
SMAS	Reference	Beach	0.44	0.08	0.31	0.27
SONE	Reference	Estuary	0.48	-0.43	-0.55	-0.34
SONS	Reference	Beach	0.96	0.08	-0.41	0.09
SLRE	Intermediate	Estuary	0.42	-0.07	-0.78	-0.08
SLRS	Intermediate	Beach	0.45	-0.69	-0.37	-0.24
		Overall	**0.85**	**0.01**	**-0.23**	**0.14**

* SJCE = San Juan Creek; SJCO = Doheny Beach; SMAE = San Mateo Creek; SMAS = Trestles Beach North; SONE = San Onofre Creek; SONS = Trestles Beach South; SLRE = San Luis Rey River; SLRS = Harbor Beach

A boxplot of the log_{10} HF183:PMMoV ratios at the different sites is shown in Figure 6. At the beach sites, the two-way ANOVA was significant, with significant effects from season/weather ($p < 0.001$) and by site ($p = 0.035$), but the interaction effect was not significant ($p = 0.334$). The post-hoc Tukey test revealed that the ratios were significantly higher during wet weather conditions than they were during rainy season dry weather conditions ($p = 0.002$) and during dry season dry weather conditions ($p = 0.001$). The post-hoc Tukey test also revealed that the ratios at Doheny Beach were significantly greater than the ratios at

Harbor Beach (p = 0.023), but not Trestles Beach North (p = 0.534) or Trestles Beach South (p = 0.205). There were no significant differences between the ratios at Harbor Beach and Trestles Beach. Considering only the estuary samples, the two-way ANOVA was significant, with significant effects from season/weather (p = 0.0105) but not by site (p = 0.084), and the interaction effect was not significant (p = 0.213). The post-hoc Tukey test revealed that the ratios were significantly higher during rainy season wet weather conditions than they were during dry season dry weather conditions (p = 0.010), but not rainy season dry weather conditions (p = 0.497). There was no significant difference between the ratios during rainy season dry weather conditions and dry season (dry weather) conditions (p = 0.185). Differences between the ratios detected at the different estuary sites were not significant at the 0.05 level.

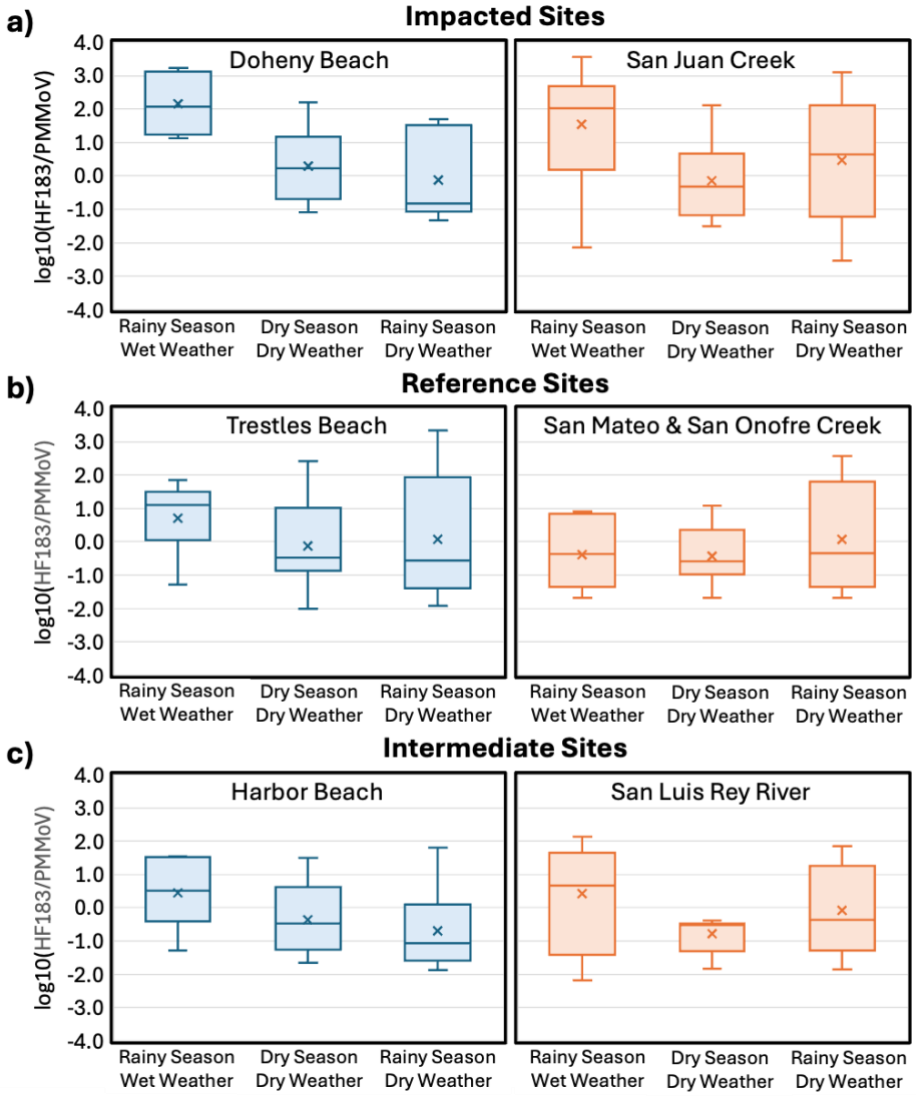

Figure 6. Box and whisker plots of the log_{10}-transformed HF183:PMMoV ratios under rainy weather, dry season (dry weather), and rainy season (dry weather) conditions at: a) the impacted sites (Doheny Beach and San Juan Creek); b) the reference sites (Trestles Beach, San Mateo Creek, and San Onofre Creek); and c) the intermediate test sites (Harbor Beach and San Luis Rey River).

The log_{10} HF183:PMMoV ratio was significantly greater in the impacted sites than in the other sites, but only during rainy weather conditions. In general, the log_{10} HF183:PMMoV ratios were greater after wet weather than they were during dry weather conditions, and they were lowest during the dry season months.

Figure 7 shows box and whisker plots of the log_{10}-transformed HF183 and PMMoV concentrations, as well as the log_{10} HF183:PMMoV ratio, in samples where enteric viruses (HAdV or NoVGII) were detected vs. not detected. For samples where enteric viruses were detected, Figure 7 also shows scatter plots of the concentrations of the enteric viruses vs. HF183, PMMoV, and the log_{10} ratio of HF183:PMMoV. Using a two-tailed t-test, HF183 concentrations were significantly greater when enteric viruses were detected than when enteric viruses were not detected ($p = 0.013$; homoscedasticity assumed, F-test $p = 0.093$). In contrast, PMMoV concentrations were not significantly different when enteric viruses were detected vs. not detected ($p = 0.982$; homoscedasticity assumed, F-test $p = 0.528$). The log_{10} HF183:PMMoV ratio was significantly greater when enteric viruses were present compared to when they were absent ($p = 0.013$; homoscedasticity assumed, F-test $p = 0.493$). Furthermore, there was a significant correlation between the log_{10} HF183:PMMoV ratio and the concentration of enteric viruses (Pearson's $r = 0.910$, $p = 0.004$, $n = 7$), but correlations between HF183 and enteric viruses (Pearson's $r = 0.034$, $p = 0.949$, $n = 6$) or between PMMoV and enteric viruses (Pearson's $r = 0.167$, $p = 0.788$, $n = 5$) were not significant.

There was no significant difference in the PMMoV concentrations when enteric viruses (HAdV, NoVGII) were detected vs. when they were not detected. The HF183 concentrations and the log_{10} HF183:PMMoV ratios were both significantly greater when enteric viruses were detected compared to enteric viruses were not detected, however the log_{10} HF183:PMMoV ratio showed a stronger correlation with enteric virus concentrations than the HF183 concentration alone.

CONCLUSIONS AND RECOMMENDATIONS

In summary, we compared the concentrations of general and alternative fecal indicators, human-associated microbial source tracking markers, and two enteric viruses, at four beaches and four nearby estuaries, using a reference system approach. The concentrations of fecal indicators at impacted sites were significantly greater than they were at reference sites, but these differences were affected by weather and season. REC-1 WQOs for *E. coli* were consistently met for one of the reference estuaries (San Mateo Creek), but not for any of the other estuary sites. REC-1 WQOs for enterococci were not met for any of the beach sites, except for Harbor Beach under dry weather conditions during the rainy season.

Relative to the impacted sites, the reference sites had lower concentrations of fecal indicators, but there was still periodic detection of fecal indicators, including human-associated fecal biomarkers and even enteric viruses. As such, the reference sites cannot be considered perfect reference sites. However, based on the frequency of detection and the concentrations of human-associated fecal biomarkers, the impacted sites do appear to be more impacted by human fecal pollution than the reference sites. The detection frequencies and concentrations of human-associated fecal biomarkers (HF183 and PMMoV) were significantly greater in San Juan Creek and Doheny Beach than they were in San Mateo Creek, San Onofre

Creek, and Trestles Beach. San Luis Rey River samples had *E. coli* concentrations that were significantly greater than the reference estuaries, but not significantly different than the impacted estuary site. Ironically, enterococci concentrations in San Luis Rey River and Harbor Beach samples were significantly lower than the concentrations at the impacted sites, but not significantly different from the reference sites. San Luis Rey River and Harbor Beach samples also had sporadic detection of HF183 and PMMoV, but no more than the reference sites (San Mateo Creek, San Onofre Creek, and Trestles Beach). Enteric virus detection was also sporadic and occurred at almost all sites at similar frequencies and concentrations.

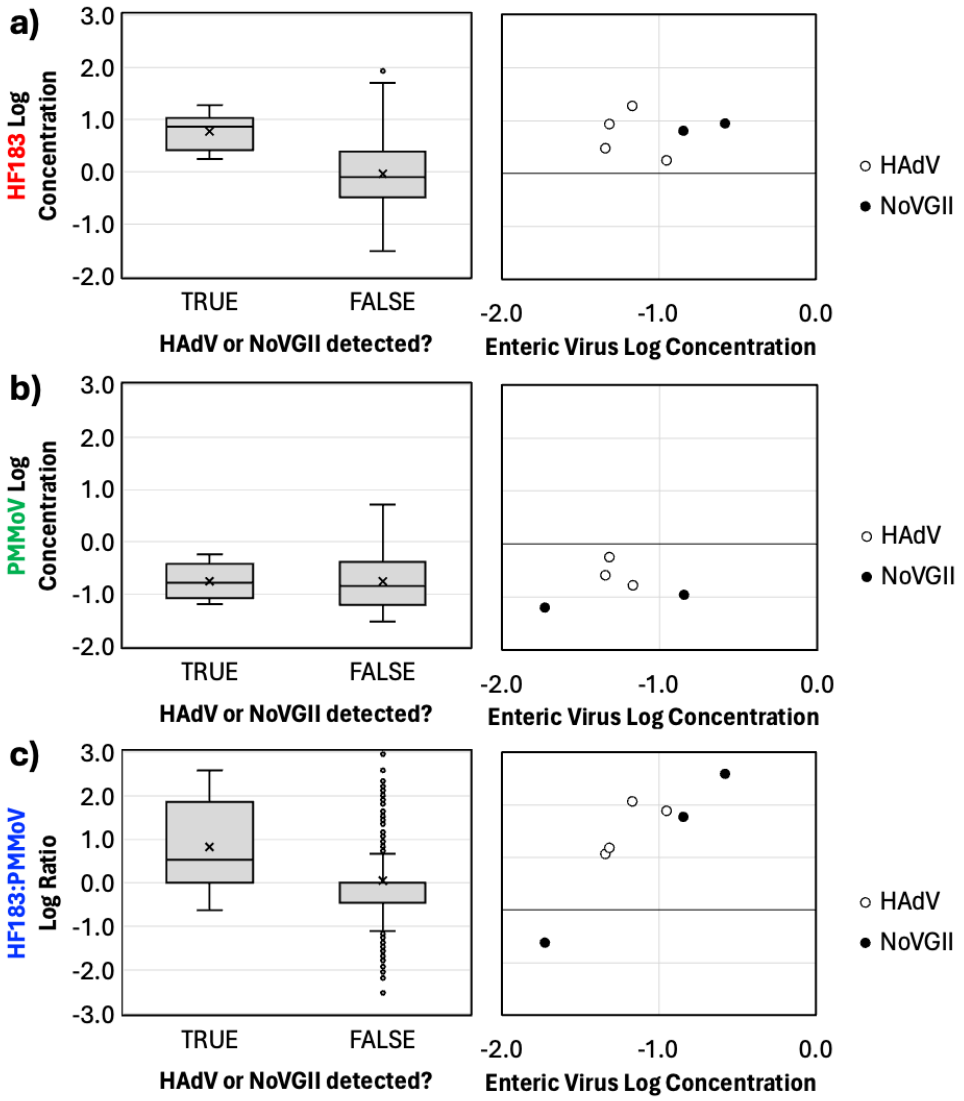

Figure 7. Plots of: a) the HF183 concentrations; b) the PMMoV concentrations; and c) the HF183:PMMoV ratios in samples where enteric viruses (HAdV or NoVGII) either were or were not detected (left panel), as well as the relationship between enteric virus concentrations and concentrations of HF183, PMMoV, or the HF183:PMMoV ratios, in samples where enteric viruses were detected (right panels).

A comparison of the concentrations of fecal indicators in samples where human-associated biomarkers were vs. were not detected revealed significant differences. For example, *E. coli* concentrations significantly coincided with the detection of HF183, but only in estuary sites. *E. coli* concentrations did not coincide well with the presence of PMMoV or enteric viruses. Enterococci concentrations coincided very well with the presence of HF183 in estuaries and beaches, but it did not coincide well with the presence of PMMoV or enteric viruses, except for the samples collected during the dry season, where enterococci concentrations were greater by 0.55-$\log_{10}$ units when enteric viruses were present, compared with when they were not present. Somatic coliphage concentrations significantly coincided with the presence of HF183, but only in estuary sites. Somatic coliphage concentrations did not coincide well with the presence of PMMoV or enteric viruses.

Finally, we assessed the potential for using the $\log_{10}$-transformed ratio of HF183:PMMoV to predict the presence and concentration of pathogens. We found that trends in the $\log_{10}$ HF183:PMMoV ratio had some correlation with the detection of human pathogens at both impacted and reference sites—it was found to be significantly greater for the impacted sites than it was for the other sites, but only during rainy season wet weather conditions. In general, the $\log_{10}$ HF183:PMMoV ratio was greater after rainy season wet weather than during dry weather conditions, and it was lowest during the dry season, which may indicate differences in the fecal pollution source(s), freshness, or trajectory. The $\log_{10}$ HF183:PMMoV ratio was significantly greater when enteric viruses were detected compared to enteric viruses were not detected, and it showed a stronger correlation with enteric virus concentrations than the HF183 or PMMoV concentrations alone.

Based on these results and using this reference system, *E. coli* and somatic coliphages were good indicators of fecal pollution in estuaries, but not in beaches, and enterococci was a good indicator in both estuaries and beaches. Still, none of these general fecal indicators reliably coincided with human-associated biomarkers or pathogens. The $\log_{10}$ ratio of HF183:PMMoV appeared to be a better indication of the presence and the concentration of enteric viruses, relative to either of the two biomarkers by themselves. More research would be needed to confirm the use of the HF183:PMMoV ratio for this purpose. More research is also needed to improve methods for monitoring ambient water quality for enteric viruses, especially given their lack of correlation with FIB concentrations and exceedances of current WQO thresholds. Recent advances in molecular biology and the proliferation of new genetic methods such as PCR, qPCR, and digital PCR, have revolutionized the research field of health-related water microbiology and microbial source tracking, enabling the detection and quantification of human-associated fecal biomarkers. However, these methods do not measure viability of the organisms, which may make it difficult to predict risks associated with contact water recreation.

References

Ahmed, W., Harwood, V., 2019. Human and animal enteric viral markers for tracking the sources of faecal pollution, in: Michigan State University, Rose, J.B., Jiménez Cisneros, B., UNESCO - International Hydrological Programme (Eds.), Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). Michigan State University. https://doi.org/10.14321/waterpathogens.8

Ahmed, W., Hughes, B., Harwood, V., 2016. Current Status of Marker Genes of Bacteroides and Related Taxa for Identifying Sewage Pollution in Environmental Waters. Water 8, 231. https://doi.org/10.3390/w8060231

Allard, A., Vantarakis, A., 2019. Adenoviruses, in: Michigan State University, Rose, J.B., Jiménez Cisneros, B., UNESCO - International Hydrological Programme (Eds.), Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). Michigan State University. https://doi.org/10.14321/waterpathogens.11

Aw, T., 2019. Environmental Aspects and Features of Critical Pathogen Groups, in: Michigan State University, Rose, J.B., Jiménez Cisneros, B., UNESCO - International Hydrological Programme (Eds.), Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). Michigan State University. https://doi.org/10.14321/waterpathogens.2

Benjamin-Chung, J., Arnold, B.F., Wade, T.J., Schiff, K., Griffith, J.F., Dufour, A.P., Weisberg, S.B., Colford, J.M., 2017. Coliphages and Gastrointestinal Illness in Recreational Waters: Pooled Analysis of Six Coastal Beach Cohorts. Epidemiology 28, 644–652. https://doi.org/10.1097/EDE.0000000000000681

Boehm, A.B., Soller, J.A., Shanks, O.C., 2015. Human-Associated Fecal Quantitative Polymerase Chain Reaction Measurements and Simulated Risk of Gastrointestinal Illness in Recreational Waters Contaminated with Raw Sewage. Environ. Sci. Technol. Lett. 2, 270–275. https://doi.org/10.1021/acs.estlett.5b00219

California SWRCB, 2022. Surface Water Ambient Monitoring Program Quality Assurance Program Plan (No. v. 2.0). California State Water Resources Control Board (SWRCB), Surface Water Ambient Monitoring Program (SWAMP), Sacramento, CA, USA.

Chen, A., Hatada, A., Chao-Yang, P., Padilla, T., Morales, C., Wadford, D.A., 2022. California Norovirus Laboratory Network (NLN) Report for September 2021 – June 2022 (No. September 2022), CDPH/VRDL NLN Annual Report. California Department of Public Health Viral and Rickettsial Disease Laboratory (VRDL) Respiratory and Gastroenteric Diseases Section, Sacramento, CA, USA.

DeFlorio-Barker, S., Wing, C., Jones, R.M., Dorevitch, S., 2018. Estimate of incidence and cost of recreational waterborne illness on United States surface waters. Environ Health 17, 3. https://doi.org/10.1186/s12940-017-0347-9

Demeter, K., Linke, R., Ballesté, E., Reischer, G., Mayer, R.E., Vierheilig, J., Kolm, C., Stevenson, M.E., Derx, J., Kirschner, A.K.T., Sommer, R., Shanks, O.C., Blanch, A.R., Rose, J.B., Ahmed, W., Farnleitner, A.H., 2023. Have genetic targets for faecal pollution diagnostics and source tracking revolutionized water quality analysis yet? FEMS Microbiology Reviews 47, fuad028. https://doi.org/10.1093/femsre/fuad028

Farkas, K., Walker, D.I., Adriaenssens, E.M., McDonald, J.E., Hillary, L.S., Malham, S.K., Jones, D.L., 2020. Viral indicators for tracking domestic wastewater contamination in the aquatic environment. Water Research 181, 115926. https://doi.org/10.1016/j.watres.2020.115926

Fout, S., Brinkman, N., Cashdollar, J., Griffin, S., McMinn, B., Rhodes, E., Varughese, E., Karim, M., Grimm, A., Spencer, S., Borchardt, M., 2014. Method 1615: Measurement of Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR (No. 600/R–10/181). US Environmental Protection Agency, Office of Research and Development, Cincinnati, OH, USA.

Goh, S.G., Saeidi, N., Gu, X., Vergara, G.G.R., Liang, L., Fang, H., Kitajima, M., Kushmaro, A., Gin, K.Y.-H., 2019. Occurrence of microbial indicators, pathogenic bacteria and viruses in tropical surface waters subject to contrasting land use. Water Research 150, 200–215. https://doi.org/10.1016/j.watres.2018.11.058

González-Fernández, A., Symonds, E.M., Gallard-Gongora, J.F., Mull, B., Lukasik, J.O., Rivera Navarro, P., Badilla Aguilar, A., Peraud, J., Brown, M.L., Mora Alvarado, D., Breitbart, M., Cairns, M.R., Harwood, V.J., 2021. Relationships among microbial indicators of fecal pollution, microbial source tracking markers, and pathogens in Costa Rican coastal waters. Water Research 188, 116507. https://doi.org/10.1016/j.watres.2020.116507

Griffith, J.F., Weisberg, S.B., Arnold, B.F., Cao, Y., Schiff, K.C., Colford, J.M., 2016. Epidemiologic evaluation of multiple alternate microbial water quality monitoring indicators at three California beaches. Water Research 94, 371–381. https://doi.org/10.1016/j.watres.2016.02.036

Hamza, I.A., Jurzik, L., Überla, K., Wilhelm, M., 2011. Evaluation of pepper mild mottle virus, human picobirnavirus and Torque teno virus as indicators of fecal contamination in river water. Water Research 45, 1358–1368. https://doi.org/10.1016/j.watres.2010.10.021

Haramoto, E., Kitajima, M., Kishida, N., Konno, Y., Katayama, H., Asami, M., Akiba, M., 2013. Occurrence of Pepper Mild Mottle Virus in Drinking Water Sources in Japan. Appl Environ Microbiol 79, 7413–7418. https://doi.org/10.1128/AEM.02354-13

Harwood, V., Shanks, O., Korajkic, A., Verbyla, M., Ahmed, W., Iriarte, M., 2019. General and host-associated bacterial indicators of faecal pollution, in: Michigan State University, Rose, J.B., Jiménez Cisneros, B., UNESCO - International Hydrological Programme (Eds.), Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). Michigan State University. https://doi.org/10.14321/waterpathogens.6

Harwood, V.J., Staley, C., Badgley, B.D., Borges, K., Korajkic, A., 2014. Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between

pathogens and human health outcomes. FEMS Microbiol Rev 38, 1–40. https://doi.org/10.1111/1574-6976.12031

He, L.-M., Lu, J., Shi, W., 2007. Variability of fecal indicator bacteria in flowing and ponded waters in southern California: Implications for bacterial TMDL development and implementation. Water Research 41, 3132–3140. https://doi.org/10.1016/j.watres.2007.04.014

Ibrahim, Y., Ouda, M., Kadadou, D., Banat, F., Naddeo, V., Alsafar, H., Yousef, A.F., Barceló, D., Hasan, S.W., 2021. Detection and removal of waterborne enteric viruses from wastewater: A comprehensive review. Journal of Environmental Chemical Engineering 9, 105613. https://doi.org/10.1016/j.jece.2021.105613

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D., Erdman, D.D., 2005. Quantitative Real-Time PCR Assays for Detection of Human Adenoviruses and Identification of Serotypes 40 and 41. Appl Environ Microbiol 71, 3131–3136. https://doi.org/10.1128/AEM.71.6.3131-3136.2005

Julian, P., Helsel, D., 2021. NADA2: Data Analysis for Censored Environmental Data. R package.

Katayama, H., Vinjé, J., 2019. Norovirus and other Caliciviruses, in: Michigan State University, Rose, J.B., Jiménez Cisneros, B., UNESCO - International Hydrological Programme (Eds.), Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). Michigan State University. https://doi.org/10.14321/waterpathogens.14

Korajkic, A., McMinn, B.R., Harwood, V.J., 2018. Relationships between Microbial Indicators and Pathogens in Recreational Water Settings. IJERPH 15, 2842. https://doi.org/10.3390/ijerph15122842

Li, D., Van De Werfhorst, L.C., Steets, B., Ervin, J., Murray, J.L.S., Blackwell, A., Devarajan, N., Holden, P.A., 2021. Sources of Low Level Human Fecal Markers in Recreational Waters of Two Santa Barbara, CA Beaches: Roles of WWTP Outfalls and Swimmers. Water Research 202, 117378. https://doi.org/10.1016/j.watres.2021.117378

Mocé-Llivina, L., Lucena, F., Jofre, J., 2005. Enteroviruses and Bacteriophages in Bathing Waters. Appl Environ Microbiol 71, 6838–6844. https://doi.org/10.1128/AEM.71.11.6838-6844.2005

Murphy, H., 2019. Persistence of Pathogens in Sewage and Other Water Types, in: Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). Michigan State University. https://doi.org/10.14321/waterpathogens.51

Pote, J., Haller, L., Kottelat, R., Sastre, V., Arpagaus, P., Wildi, W., 2009. Persistence and growth of faecal culturable bacterial indicators in water column and sediments of Vidy Bay, Lake Geneva, Switzerland. Journal of Environmental Sciences 21, 62–69. https://doi.org/10.1016/S1001-0742(09)60012-7

Rice, E.W., Baird, R.B., Eaton, A.D., APHA, AWWA, WEF (Eds.), 2012. Standard methods for the examination of water and wastewater, 23rd ed. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, USA.

Rosario, K., Symonds, E.M., Sinigalliano, C., Stewart, J., Breitbart, M., 2009. *Pepper Mild Mottle Virus* as an Indicator of Fecal Pollution. Appl Environ Microbiol 75, 7261–7267. https://doi.org/10.1128/AEM.00410-09

Schroeder, M.E., Bounpheng, M.A., Rodgers, S., Baker, R.J., Black, W., Naikare, H., Velayudhan, B., Sneed, L., Szonyi, B., Clavijo, A., 2012. Development and performance evaluation of calf diarrhea pathogen nucleic acid purification and detection workflow. J VET Diagn Invest 24, 945–953. https://doi.org/10.1177/1040638712456976

SD RWQCB, 2021. Water Quality Control Plan for the San Diego Basin. California Regional Water Quality Control Board - San Diego Region, San Diego, CA, USA.

Soller, J.A., Schoen, M.E., Bartrand, T., Ravenscroft, J.E., Ashbolt, N.J., 2010. Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. Water Research 44, 4674–4691. https://doi.org/10.1016/j.watres.2010.06.049

US EPA, 2019. Method 1696: Characterization of Human Fecal Pollution in Water by HF183/BacR287 TaqMan Quantitative Polymerase Chain Reaction (qPCR) Assay (No. EPA 821-R-19-002). US Environmental Protection Agency, Office of Water, Washington, DC, USA.

US EPA, 2017. Proceedings from the U.S. Environmental Protection Agency (EPA) Coliphage Experts Workshop, in: March 1-2, 2016. US Environmental Protection Agency: Office of Water, Office of Science and Technology, Health and Ecological Criteria Division, Washington, DC, USA.

US EPA, 2015. Review of coliphages as possible indicators of fecal contamination for ambient water quality (No. 820- R-15–098). US Environmental Protection Agency, Washington, DC, USA.

Wu, J., Long, S.C., Das, D., Dorner, S.M., 2011. Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. Journal of Water and Health 9, 265–278. https://doi.org/10.2166/wh.2011.117