

Inner Cabrillo Beach Microbial Source Tracking and Quantitative Microbial Risk Assessment (QMRA)

Final Report



Courtesy of the Port of Los Angeles

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Southern California Coastal Water Research Project

Technical Report 1068

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EXECUTIVE SUMMARY

Fecal indicator bacteria (FIB) such as *Enterococcus* are the foundation of current water quality objectives. However, FIB do not come exclusively from human sources and can be shed in the feces of any warm-blooded animal (e.g., birds). The risk of swimmer illness associated with FIB from human sources may be much greater than the FIB associated with non-human sources. To account for the inconsistent risk of FIB observed at beaches impacted by non-human sources, the USEPA has allowed for risk-based models, termed Quantitative Microbial Risk Assessments (QMRA), for setting site-specific water quality objectives. However, a dry weather QMRA has not previously been conducted at a beach in California for setting site-specific objectives. The objective of this project was to conduct a QMRA at a California marine beach to establish a case study precedent for technical QMRA implementation. A goal of this case study was to ensure a sufficient technical foundation was built to support policy discussions and decision making for creating FIB site-specific water quality objectives or total maximum daily load (TMDL) numeric targets based on quantified swimmer health risk.

This QMRA project was comprised of five basic steps:

- 1) Beach selection
- 2) Source identification
- 3) Pathogen loading
- 4) Risk assessment
- 5) Sensitivity analysis

Beach Selection

An *ad hoc* committee of the State Water Resources Control Board (SWRCB) and staff from five different Regional Water Quality Control Boards (RWQCBs) agreed upon nine beach selection factors for QMRA applicability. These nine factors all converge on the concept of seemingly unfixable beach bacteria exceedances that are not of human fecal origin. The nine factors included: persistent low level FIB exceedances; a high level of effort to eliminate human sources; willing partners; a high volume of beach use; a completed or adopted Total Maximum Daily Load (TMDL); septic tanks are minimal and not a prominent sewage treatment solution; implemented dry weather flow diversions, and; a well-defined, small watershed.

Inner Cabrillo Beach (ICB) is a beach in the Los Angeles RWQCB that met the nine beach selection criteria developed by the State. ICB is located in Los Angeles Harbor, adjacent to the outer breakwater. Recent FIB exceedances averaged between 10% and 15% per year during the summer swimming season. As this beach approaches its TMDL compliance deadline, an excess of \$20 million has been spent trying to identify and remove human and non-human sources of FIB. More than 1 million beach goers attend this enclosed beach annually and the lack of large surf attracts many children.

Beach Monitoring for Sources in 2016

A rigorous source identification plan was prepared and implemented in the summer of 2016 following the State of California's Source Identification Project Plan. Potential FIB sources at this beach include human (i.e., sewage infrastructure along the beach, an offshore Water Reclamation Plant outfall, etc.), animals (i.e., shore birds), and/or environmental regrowth (e.g., beach sand, nearshore eel grass beds).

The source identification sampling campaign during the summer of 2016 was designed to determine the spatial gradient of FIB across ICB, as well as characterize the frequency and magnitude of human source(s) and non-human source(s) of fecal pollution using the latest technology.

There were three primary conclusions from summer 2016 source identification sampling campaign:

- There was a spatial relationship of *Enterococcus* concentrations along ICB, with the highest concentration and *Enterococcus* water quality objective exceedance rates mid-beach, coincident with the TMDL compliance site.
- There was a spatial relationship of avian-specific marker along ICB, which mimicked and was highly correlated to, *Enterococcus* concentrations.
- There was a persistent, but low-level occurrence of the human-specific marker HF183. This persistent low-level occurrence did not have a spatial relationship across ICB and was not correlated to *Enterococcus*.

Thus, birds are likely driving the FIB concentrations at ICB, but there also exists an unknown human source. Pathogen loading from shore birds has been studied previously and, while some local pollutant loading confirmation would be required, existing data suggests a reduced risk level compared to loading from human fecal contamination.

Harbor Monitoring for Human Marker in 2017

One potential source of human-specific markers at ICB is the Terminal Island Water Reclamation Plant (WRP) outfall, approximately 4 km east of ICB in Los Angeles Harbor. To identify if the WRP outfall could be a source of human fecal pollution to ICB, a gradient-based sampling design between the outfall and beach was conducted in 2017. There were two primary conclusions from the harbor monitoring study:

- No consistently clear pattern of HF183 human-specific marker was observed; however, some localized patterns of HF183 were detected. The greatest concentration of HF183 human-specific marker occurred at station CB02 at ICB. The second greatest concentration of HF183 human-specific marker occurred at station HW29 north of ICB and south of Watchorn Basin. Concentrations of HF183 human-specific marker also occurred in the array of stations by the WRP outfall.
- Concentrations of the HF183 human-specific marker varied between two tidal conditions. During spring tide, concentrations of HF 183 were detectable at most stations. The greatest concentration of the HF183 human-specific marker occurred at stations CB02 at

ICB and HW29 south of Cabrillo Marina, decreasing moving towards the outer harbor. During neap tide, much fewer stations detected the HF183 human-specific marker with the greatest concentrations detected closest to the Terminal Island WRP outfall.

While some general patterns were observed during the harbor screening survey, caution is advised when interpreting the results. This was just a screening survey and more information would be required before making any confirmations about sources and transport. However, the results obtained from this screening survey were sufficient for the Advisory Committee to not recommend any more harbor water investigations, effectively moving the WRP low on the list of sources impacting ICB.

Confirming Human Source Removal at ICB in 2018

Following the 2016 and 2017 survey findings of human fecal markers at ICB, the beach owners inspected and implemented repairs of the sewage infrastructure at ICB. A repeat of the 2016 survey was conducted in 2018 to confirm that the infrastructure repairs successfully removed human sources. Focused specifically on the times and locations where HF183 human-specific marker occurred, a single conclusion was surmised:

- There was little change in HF183 concentrations or frequency indicating that the infrastructure repairs were insufficient to remove the human sources of fecal contamination.

Final QMRA Determination and Next Steps

Because there are still low but persistent levels of HF183 at ICB that has yet to be identified, implementing a comprehensive, precedent-setting QMRA is problematic. Without knowing the origin of the source(s) of HF183 human-specific genetic marker, scientists are unable to assess what pathogens might be present at ICB that could lead to illness. The final determination at ICB was that a comprehensive QMRA could not be completed with the existing information.

Among the potential next steps for moving towards a QMRA at ICB, there are two that the Advisory Committee discussed at length identifying the advantages and disadvantages:

- 1) *Quantify groundwater for human fecal contamination.*
One potential origin of human fecal contamination at ICB is conveyance through groundwater that surfaces at or just offshore ICB. Groundwater is targeted because there are no other surface water discharges at ICB and the harbor survey did not indicate a strong gradient of HF183 human-specific genetic marker impacting ICB from offshore. Specifically, the goal of measuring human fecal contamination in groundwater is to quantify pathogen loading, a critical step in the QMRA process.

One advantage of measuring groundwater for pathogen loading is the ability to measure inputs to ICB before mixing and diluting with beach receiving waters. If groundwater is the primary conveyance of human fecal contamination, then both HF183 human-genetic

marker and difficult-to-detect pathogens should be more concentrated than after mixing in receiving waters. This concentrated discharge enhances the opportunity to quantify pathogens for loading estimates. The approach of looking closer to sources for pathogen loading is consistent with the QMRA process identified by the USEPA.

Although the technology exists for sampling and measuring groundwater, there are also unique challenges to measuring groundwater for pathogen loading. For example, providing sufficient measurements that this conveyance is well-quantified for pathogen concentrations and groundwater flow will likely require substantial effort and will be fiscally burdensome.

- 1) *Measure the beach water for pathogens using low level detection limits.*
A second option to pursue is to forego pathogen loading altogether and measure pathogens in (REC-1) beach water. This greatly simplifies the QMRA process providing a direct measure of swimmer exposure.

The advantage of direct pathogen measurement means foregoing measuring all sources or conveyances, both human and non-human. This option also removes uncertainty associated with estimating fate and transport once sources or conveyances enter the receiving water, which is sometimes a challenge when considering swimmer exposure. If low detection limits could be achieved, then this would help not just ICB, but all other beaches considering QMRA.

The disadvantage of direct pathogen measurement in marine receiving waters is that current methodology is not capable of quantifying many pathogens at levels sufficiently low enough to estimate risk; pathogen concentrations below detection limits could still result in substantial swimmer illness. This is particularly true for highly infective viral pathogens such as norovirus, one of the most common etiological agents of swimming-related gastrointestinal illness.

New technology such as droplet digital PCR provides the opportunity to start testing new low level viral detection methods. However, selecting this option will require research to develop and validate the method before it could be used as a QMRA tool.

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INTRODUCTION

The current water quality objectives for marine water contact recreation are based on epidemiology studies from beaches impacted by known human sources (i.e., treated municipal wastewaters) of pollution (USEPA 1986, Haile et al. 1996). These water quality objectives focus on concentrations of fecal indicator bacteria (FIB) that do not cause illness, but are easier and cheaper to measure than the human pathogens that cause illness. While many epidemiology studies have shown that FIB correlate with illness at beaches impacted by human sources of FIB (see Wade et al. 2003 for a review), epidemiology studies at beaches impacted by non-human sources of FIB do not always demonstrate this same positive relationship (Colford et al. 2007, Calderon et al. 1991). Likely, the water quality-human health relationship is confounded because non-human sources of FIB do not carry the same human specific pathogenic load compared to point sources such as sewage (Schoen et al. 2011, Soller et al. 2010a, b, 2014).

The USEPA and State of California have recently promulgated new marine recreational water quality criteria (USEPA 2012, SWRCB 2018). To account for the confounding observed at beaches impacted by non-human sources of FIB, the USEPA has allowed for risk-based models, termed Quantitative Microbial Risk Assessments (QMRA), for setting site-specific water quality objectives. The goal of QMRAs are to provide a tool to local regulators and stakeholders for setting appropriate water quality objectives that protect public health. Not many QMRAs have been conducted in marine waters (Soller et al. 2015, Soller et al. 2017, Dickenson et al. 2013, Tseng and Jiang 2012) and none have been used for setting site-specific objectives.

The issue of setting protective water quality objectives is crucial in regulatory actions such as restoring impaired waterbodies through total maximum daily loads (TMDLs). At the start of this project, there were 169 impaired waterbody listings for FIB exceedances that required TMDLs in California. Virtually all of these impaired waterbodies name nonpoint, possibly non-human, sources as potential contributors to the FIB-impaired beaches. As a result, numeric targets based on current water quality objectives may be over-protective resulting in prohibitively expensive management measures.

In order to resolve the potential discrepancy between existing objectives and targets at marine beaches relative to swimmer risk, the goal of this study was to conduct a QMRA to quantify swimmer risk and test EPA's proposed QMRA approach at a marine beach in California. The test beach was Inner Cabrillo Beach, where, despite over \$20 million expended on remediation, there are persistent low-level water quality standard exceedances of FIB. The test aimed to evaluate study design, methodology, sensitivity, and utility of QMRA in realistic conditions and with local FIB sources. Ultimately, two goals were intended: 1) regulators may use this information to decide if water quality objectives and/or TMDL targets are appropriate at the test beach; and 2) the study would provide information to document the usefulness of QMRAs for other California beaches.

THE QMRA PROCESS

The generalized QMRA process to consider site-specific water criteria for recreational waters at ICB consists of four steps. The critical first step is to identify all sources of FIB at ICB. Ideally, no human sources will be identified because if human sources are prevalent, existing water quality objectives still apply. The second step is to determine the pathogen load associated with each source. Pathogen loading measures the priority viral and bacterial pathogens as well as FIB to support swimmer exposure estimates. The third step is risk modeling. Risk modeling combines swimmer exposure data with dose-response relationships to generate probabilities of illness. The risk modeling also includes sensitivity analyses to test assumptions and evaluate the confidence in the results. The last step in the process is to engage an Advisory Panel comprised of both technical experts as well as regulated, regulatory, and non-governmental agency decision makers. Ultimately, the communication among stakeholders is where the decision about acceptable levels of risk for a site-specific objective occurs.

Step 1. Identifying Sources of Fecal Indicator Bacteria

The purpose of this task is to identify the sources of FIB and potential pathogens to the selected beach. This task is critical for ensuring that the significant sources of bacteria are identified and their relative contribution to impacted water quality is quantified. The State of California has a standardized, tiered microbial source identification manual (Griffith et al. 2015). The concept of the tiered approach is to start with simple and easy methods first, slowly building towards more involved and complex strategies and methods as the potential list of sources narrow. This strategy has proven effective at other marine beaches and adapts well to a toolkit/multiple line of evidence approach (Boehm et al. 2004, Noble et al. 2006).

The first tier starts with observational data and a listing of all potential sources. The second tier includes analyzing existing data to identify the times and/or locations of greatest concern. The third tier includes sampling, typically at the times and/or locations of greatest concern. Sampling is often focused on standard methods initially when many samples are required, then moves onto more detailed or complicated methods in future tiers as the number of sources narrows. Standard methods may include FIB measurements plus the use of infrastructure maps, flow tracking, and dye or smoke testing. Once the times and locations of the contamination are more clearly defined, tier four utilizes more complicated laboratory methods to assess the extent and magnitude of human fecal sources. These laboratory methods potentially include a variety of human-specific genetic markers, as well as chemical tracers of sewage. If the spatial extent and magnitude of human fecal contamination is large, then the QMRA may be paused until this source is eliminated. If the spatial extent and magnitude of human fecal contamination is minimal, then non-human sources need to be quantified as part of tiers five and six. Advanced genetic methods for non-human host-specific markers may be used including advanced microbiological methods with host-specific DNA targets (tier five) or community-based analysis (tier six). Currently, there is no consensus or regulatory definition of what constitutes a “large” or “small” spatial extent or magnitude of human fecal contamination.

Step 2: Pathogen Loading

Based on the outcome from the source identification (step 1), the next goal is to determine the pathogen load from each source. First, source-specific fecal sampling is required. This is typically accomplished by securing fresh effluent (for point sources), fresh scat (for local animal contributions), and/or fresh scat from surrogates of native animals (animals in captivity such as marine bird and mammal care facilities). Replicates from multiple hosts should be sampled and analyzed for a variety of priority pathogens including norovirus, enterovirus, adenovirus, *Salmonella* and *Campylobacter*, at a minimum. The number of replicates is determined by confidence in loading, which is a function of host density, host pathogen concentration, and host population infection rate. For equivalent levels of confidence, low density, low concentration, low infection rate sources will require more samples than high density, high concentration, high infection rate sources. Multiple methods may be required to measure pathogens including culture-based techniques and/or DNA and RNA based methods of quantitative polymerase chain reaction (qPCR) or droplet digital polymerase chain reaction (ddPCR). The FIB will also be measured from these samples to determine if relationships between FIB and pathogens can be used for inferring exposure in the next step.

Step 3. Risk Modeling

The goal of this task is to conduct the risk modeling for assessing illness rates in swimmers. This task will require two sub-steps: (A) risk modeling and (B) sensitivity analysis.

Step 3A. Risk Modeling

Risk modeling predicts the illness rates in swimmers based on the number of pathogens ingested (dose). Dose is a function of the volume of water ingested, and the probability of infection given exposure to a particular dose (dose-response relationship). Exposure is estimated from the pathogen loading and perhaps receiving water processes such as dilution, advection, and die-off. Swimmer ingestion estimates are most frequently derived from the literature. Collection of local information to justify literature assumptions is recommended, when possible. Estimating the dose response relationship for each pathogen is perhaps the most difficult of the steps in this sub-task. Much of the data that currently exists is found in the published literature based on feeding studies done in the past or through outbreaks (USEPA 2012). Using standard, accepted peer-reviewed dose response relationships for this task is acceptable, and conducting pathogen dosing studies is inappropriate. Because of this limitation in dose-response, a focus on sensitivity analysis of dose-response for the risk modeling is appropriate (see step 3B).

Task 3B: Sensitivity analysis

Sensitivity analysis is conducted to quantify uncertainty and confidence in modeling results. Sensitivity analysis typically employs Monte Carlo based simulations (Soller et al. 2010). The sensitivity analysis helps assess if the selection of one dose-response relationship over another is an important component of the risk modeling. The sensitivity of illness rates for dose-response should be compared to the uncertainty from the other elements of the risk assessment model

including pathogen loading and swimmer exposure. This process helps to determine the most critical factors in the model to help refine assumptions for this (and other) QMRA interpretation.

Most importantly, the sensitivity analysis provides decision-makers with estimates of confidence in the risk analysis. This estimate of uncertainty is crucial for managers to determine if risks of swimming-related illnesses are greater or less than existing predictions of illness rates based on commonly used FIB water quality objectives, and if further consideration of regulatory options are warranted.

Step 4. Advisory Panel

The last step in the QMRA is communication and outreach. In this project, we are using an Advisory Panel to ensure technical integrity and address management issues. The role of the Panel is to ensure technical integrity and to integrate management issues as they arise. Because there are so many policy implications related to a QMRA, having management review and input from the beginning will be crucial to its success. In general, four types of organizations should comprise the Advisory Panel: 1) State regulators who make the decisions regarding compliance with regulatory requirements including TMDLs or NPDES permits; 2) Federal regulators that approve state water quality objectives; 3) Regulated agencies including the beach owner, local stormwater and/or wastewater dischargers who are responsible for implementing remediation measures and maintaining beach water quality; and 4) a Non-Governmental Organization (NGO) who ensure that the public interest is being protected in light of revised water quality objectives and/or TMDL numeric targets. Collectively, these experts will provide important feedback on how the application of QMRA could or should be used.

BEACH SELECTION

On May 14, 2013, the State Water Resources Control Board Division of Water Quality convened an *ad hoc* committee of five coastal Regional Water Boards, State Water Board Ocean Unit, and State Water Board Division of Financial Assistance to define which beaches might be appropriate for a QMRA. The outcome of this *ad hoc* committee was that an appropriate beach should have the following attributes:

- Persistent low level of FIB exceedances
- High level of effort to eliminate human sources
- Willing partners
- High volume of beach use
- Total Maximum Daily Load (TMDL) completed/adopted
- Septic tanks are minimal and not a prominent sewage treatment solution
- Implemented dry weather flow diversions
- Well defined, small watershed

One beach recommended by the *ad hoc* committee was ICB. The project Beach Confirmation Report (SCCWRP 2016a) documents how ICB meets these criteria. The beach confirmation focused exclusively on the ICB beach face during the summer season (Memorial Day to Labor Day). The rationale for the spatial and temporal focus is relatively simple; this is where and when the most human exposure occurs.

Description of Inner Cabrillo Beach (ICB)

The ICB is a relatively small beach, approximately 300 m north to south, in the very southwest corner of inner Los Angeles Harbor (Figure 1). Like most inner harbor beaches protected from surf, ICB has reduced wave action and long-shore circulation patterns, making it an ideal beach for families.

The ICB has a long history in Los Angeles, with shoreline activities dating back to the turn of the 20th century. Fort McArthur, located atop the bluffs in San Pedro, has trained and housed military personnel since the end of World War I. The Cabrillo Marine Aquarium, originally located in the bath house, and now located in its own facility, attracts millions of visitors each year.

ICB has a number of amenities to support beach visitors, including ample parking for more than 900 vehicles (for both the beach and aquarium), a boat launch ramp, three bathrooms, a playground, lifeguard services and a swim area (Figure 1).

General water circulation patterns at ICB favor a south to north current along the beach face, from the accretion beach towards the Cabrillo Marina. Superimposed on this current circulation pattern at ICB are wind driven vertical circulation patterns (Evans Hamilton 2004). During the quiescent morning, little water movement is measurable in the vertical direction. As westerly winds predictably increase mid-day during the summer, surface layer water is pushed offshore at ICB, entraining deeper (5 m) water to the beach.



Figure 1. Map of Study Site, Inner Cabrillo Beach, located in Los Angeles Harbor (inset), California.

Persistent low level of FIB exceedances

There are two historical monitoring locations at ICB (Figure 2), but only one location that is the focus of this study: CB02. CB02 is located on the beach face of ICB, is located within the summertime swim area, and is the location of the greatest recreational body contact. Moreover, CB02 is the TMDL compliance point. Location CB01, located at the foot of the launch ramp, is still undergoing a number of management actions and is not part of this QMRA investigation.

The historical monitoring at CB02 consists of sampling five times per week – Tuesday through Saturday – for three fecal indicator bacteria: *Enterococcus*, fecal coliforms (*E. coli*), and total coliforms. The City of Los Angeles typically collects these samples just below the surface near

the shore at approximately 8:00 AM. The laboratory uses chromogenic substrate as its laboratory methodology.

Of the three indicators, *Enterococcus* is by far the most sensitive; this water quality objective was exceeded up to 20 times more frequently than either the fecal or total coliform objective over the 12-year period 2004 to 2015. As a result, the remaining focus of this section will be on *Enterococcus*.

The City of Los Angeles Harbor Department (known and referred to in this document as the Port of Los Angeles) conducted a TMDL-based analysis on the six-year period from 2010-2015 (Table 1). The analysis compared the number of days that exceeded water quality objectives for *Enterococcus* to the number of allowable days of exceedance defined in the TMDL for different seasons of the year. Focusing specifically on summer dry weather, the time period of the QMRA in the current study, the allowable exceedance days is zero. The actual number of exceedance days has ranged from 79 to 16, generally decreasing over time.

Table 1. Number of *Enterococcus* single sample water quality standard exceedances days by year in different seasons (from Weston 2016). Single sample standard for this time period is 104 MPN per 100 ml

Compliance Season	Compliance Season Dates	Allowable Exceedance Days	2010	2011	2012	2013	2014	2015
Summer Dry	Apr 1 – Oct 31	0	74	79	63	52	34	16
Winter Dry	Nov 1 – Mar 31	0	47	39	31	28	31	12
Wet Weather	Year-Round	17	16	31	20	23	10	14
Geometric Mean	Year-Round		53	52	43	36	24	2



Figure 2. Map of historical monitoring locations at Inner Cabrillo Beach.

High Level of Effort to Eliminate Human Sources

A critically important element of initiating a QMRA that could lead to considering site-specific water quality criteria is that the beach manager(s) have made all attempts at finding and removing human sources. In the case of ICB, substantial effort has been expended prior to this project, partially through the TMDL. Additional details can be found in the Beach Confirmation Report (SCCWRP 2016a).

Description of Current Human Sources

There are a number of potential human sources/conveyances of fecal contamination to ICB (Figure 1). These include:

- Bathrooms adjacent to the beach, east side of beach parking lot
- Bath house in the south end of the beach parking lot
- Marine aquarium in the west end of beach parking lot
- Supporting collection system for these facilities, including the lift pump located roughly central of the beach parking lot
- Storm drain located in the southern corner of the beach
- Abandoned sewer outfall bisecting beach
- Boats discharging holding tanks located 1 km to the north in the Cabrillo Marina
- The Terminal Island Treatment Plant outfall located 4 km to the east discharging in 8 m depth

As-built maps are an important precursor to microbial source identification (Griffith et al. 2015) and ICB as-built maps can be found in the Beach Confirmation Report (SCCWRP 2016a) which includes water, wastewater, electrical, and storm drain.

Infrastructure Removal

Probably the single largest infrastructure improvement at ICB was the removal of sections, then capping in place the remainder of, an old abandoned sewer outfall directly offshore CB02 (KLI and DMJM Harris 2006). This outfall was originally built of vitrified clay pipe in 1917 and discharged into the intertidal zone. At this time, there was no breakwater and the rocky shoreline had breaking turbulent waves. In 1927, the outfall was replaced with cast iron when the discharge was extended into -7.2 ft depth MLLW. Finally, in 1932, the outfall was diverted at the base of the bluff along Shoshone Drive to the City's Terminal Island Wastewater Treatment Plant. After long investigations in 2006, using magnemometer diver surveys, and finally fencepost auger surveys, the old outfall was located at -12 ft MLLW running under the aquarium to the base of the bluff. The old outfall was removed where it transited under the sand, and capped at both the parking lot and at the water line.

Infrastructure Testing and Repairs.

In 2005-06, extensive testing of the sanitary sewer collection system occurred to assess if leaks existed (KLI and DMJM Harris 2006). The tests included visual inspections, dye testing, pressure testing, and microbiological sampling. Locations included all three beach bathrooms, south and north beach lifeguards, bath house, aquarium, lift station, and conveyances in between.

Also included are testing from the launch ramp and wetland areas, which do not directly impact ICB at CB02.



Figure 3. Map of active and abandoned sewer and storm drain infrastructure at ICB, including notes on test results, management activities, and implementation results. See Table 2 for a summary.

The investigation included both positive and negative results. On the positive, the beach restrooms and associated conveyances appeared intact and functioning properly based on visual inspections, no drop in pressure, and lack of dye in adjacent groundwater samples or beach receiving water samples. Similar results were observed for the aquarium and its conveyance system.

On the negative, the conveyance system for the bath house and the lifeguard facilities along the south beach were in disrepair. Visual inspections indicated poor condition and pressure testing indicated leaks, although little dye and only modest microbiological contamination was observed in beach receiving water or beach sand interstitial water. Some of the materials of construction for the bath house conveyances dated back to the old sewer outfall, circa 1920s, and were observed to be cast iron. These conveyances were near poor performing storm drain systems at

the south end of the parking lot and circle drive, leading to the possibility of leakage from the sanitary system into the storm drain system.

As a result, the Port of Los Angeles replaced the sewer conveyance along the south accretion beach to the lifeguard station with 4-inch HDPE and upgraded the sewer conveyances to the Bath House with 18-inch reinforced concrete pipe. The storm drain collection system was replaced with 30-inch HDPE and reinforced concrete junction boxes for manhole access and a concrete headwall at the outfall to dissipate energy at the beach. Finally, a dry weather diversion of the storm drain to the sanitary sewer collection system was installed, just upstream of the storm drain outfall. This diversion was designed to re-route all dry weather flow and initial portions of wet weather flow.

Updated Dye studies

In June 2014, the Port of Los Angeles conducted dye studies to reassess the potential for human fecal contamination from the beach-side bathrooms (Weston 2012, Appendix A). The Port's contractor poured two types of fluorescent dye (Rhodamine and Fluorescein) in each of the ICB bathrooms. Water samples from 10 groundwater wells between the bathrooms and the beach and 8 beach receiving water sites were measured for dye for up to one week. No dye was detected during the study, leading to the conclusion that the sewage infrastructure for the bathrooms remained intact.

Human-specific marker sampling and analysis

The Port of Los Angeles has been conducting human-specific marker analysis dating back to 2005. However, technology has evolved over this time period, and the most trustworthy analyses – those conducted for *Bacteroidales* HF183 by qPCR as described in the State Water Board's SIPP Manual - have only been conducted since 2012. Since 2012, a total of 53 seawater samples have been analyzed for HF183. Many, but not all, of these samples were collected at CB02. The additional samples were collected either up or down coast from CB02 along the beach face. Up to the start of the current project, not a single sample had detected quantifiable levels of HF183 human-specific marker using qPCR (Weston 2012).

High Level of Effort to Remove Non-Human Sources

While a high level of effort to remove non-human source of FIB was not on the list of criteria from the State Water Board, we have added it here as a potential option for future consideration by the State Water Resources Control Board for selecting QMRA beaches. In the case of ICB, there has been tremendous effort to mitigate non-human sources of FIB. These strategies include removing birds, removing other nuisance animals, increasing circulation, replacing beach sand, and removing the flow-through aquarium discharge.

Birds are assumed to be a common source of FIB to many beaches because avian sources of *Enterococcus* can be quite large. In the case of ICB, informal bird counts routinely exceed 100

(Julianne Passerelli, personal communication). Moreover, genetic testing by the Port of Los Angeles has routinely found gull genetic marker in seawater samples at ICB (Weston 2016). In an attempt to exclude birds, the Port of Los Angeles has constructed large exclusion devices 10 m tall for the entire width of the beach and 300 m in length (Figure 4). Based on personal observation, birds do not congregate under the exclusion device, but they do congregate to the north and south of the device. It appears that FIB levels may have decreased partially as a result of the bird exclusion device, but FIB exceedances of the water quality criterion continue (Table 1).



Figure 4. Photo of bird exclusion devices at ICB.

The Port of Los Angeles also attempts to discourage other nuisance animals including feral cats and raccoons by placing lids on trash cans, emptying trash frequently, and posting signs to not feed the animals.

Water circulation is always an issue in enclosed beaches, where dilution and advection are minimal. At ICB, there have been numerous attempts to increase circulation to improve water quality (Appendix A). In general, water circulation moves slowly from south to north along the beach face (Evans Hamilton 2004). Attempts to improve water circulation have included removing the breakwater groin at the north end of the beach (KLI 2010). While water movement may have increased, it did not appear to improve FIB water quality. An alternative approach to improving water quality was augmenting circulation through the use of submersible pumps at the south end of the beach, facing north (Appendix A). Once again, these methods did not appear to produce long lasting improvements in beach water quality (KLI and DMJM Harris 2008).

The Port of Los Angeles has also measured FIB in beach sand. Measurable concentrations of *Enterococcus* were consistently found and resuspension from sediment is one theory put forward to explain FIB concentrations measured in the water column (Weston 2013). In an attempt to reduce water column concentrations, the Port of Los Angeles replaced the beach sand at ICB (KLI 2010). Once again, this strategy did not provide a long-term solution to high FIB concentrations in seawater at ICB.

The Cabrillo Marine Aquarium historically had a flow-through seawater system to supplement its exhibits, which were then discharged to ICB. While the aquarium does not maintain marine bird or marine mammal exhibits, both of which could contribute FIB, the aquarium decided to recycle much of its aquaria water system. The recycling process created a closed loop system, cleaning up the aquaria flow-through water, then sending all reject water to the sanitary sewer system. No aquarium water is discharged to ICB.

Adopted TMDL

The Los Angeles Harbor Bacteria TMDL was promulgated in 2004 (RWQCB 2004), and focused on ICB. The numeric targets focused on “exceedance days”, or allowable days of water quality objective exceedances. At ICB, the water quality objective of greatest concern is for *Enterococcus*, hence the focus on *Enterococcus* in this document. The numeric target for summer dry weather was zero exceedance days.

Part of the TMDL implementation has been the establishment of a work plan towards corrective actions, many of which were described in the previous sections. Table 2 describes the TMDL Work Plan agreed to by the RWQCB and the TMDL Stakeholders. The inability of the implementation plan to achieve the desired numeric target is what has led, at least in part, to the current project on QMRA: assessing health risk in light of the observed bacteria levels.

Table 2. Work Plan milestones and schedule requirements by tier for the Los Angeles Harbor Bacteria TMDL

Implementation Actions	TMDL Date Required	Date Accomplished
Tier 1		
Work plan for Tier 1 BMP's	Sept. 2005	Sept. 2005
<ul style="list-style-type: none"> • Additional Trash Pick up • Educational Signage • Storm Drain Repairs • Gravity Sewer Repairs • Sand Cleaning • Repair Bird Exclusion Structure 	March 2006 March 2006 March 2006 March 2006 March 2006 March 2006	Dec. 2005 Sept. 2005 July 2004 July 2004 Sept. 2005 Nov. 2005
Report-Tier 1 Complete	March 2006	March 2006
Tier 2		
Work plan for Tier 2 BMP's	Sept. 2005	Sept. 2005
<ul style="list-style-type: none"> • Alteration of Bird Structure • Beach Management Plan-Cats, Sand • Remove Old Outfall Line • Recontour/Replace Beach Sand (Phase 1) • Replace Beach Face Sand • Remove Groin • Redirect Aquarium Discharge to Sanitary 	March 2007 March 2007 March 2007 March 2007 March 2007 March 2007 March 2007	June 2009 Nov. 2008 June 2007 June 2007 June 2007 June 2007 June 2007
Degree of Compliance Report after Tier 1 and 2 implementation	March 2007	Oct 2008
Tier 3		
Work plan for Tier 3 BMP's	March 2008	Jan. 2009
<ul style="list-style-type: none"> • Nearshore Circulation Field Tests • Provide Nearshore Circulation • Shallow Water Improvement • Eelgrass Management/Coordination 	NA March 2010 March 2010 March 2010 March 2010	Jul. 04, Jul. 05 & Sept.-Dec. 06 Feb. 2010 Feb. 2010 Ongoing March 2010
Report- Compliance with TMDL Bacterial Exceedance Criteria		

Willing Partners

This project has a complete set of willing partners that encompasses multiple sectors of beach management decision makers (Table 3). The TMDL responsible parties include the Port and the City. The beach regulators are the agencies making decisions regarding any revisions to numeric targets or site-specific objectives should there be a significant reduction in public health risk relative to FIB concentration. The Environmental Advocacy Group is critical to ensure that the public interest is represented in the decision-making process, as public health is clearly not just a scientific exercise, but a social decision. In this project, all of these agencies serve on an Advisory Committee to ensure that the applicability and completeness of the technical material is sufficient for use in their management decision making.

Table 3. Beach decision maker agencies serving on the Advisory Committee at ICB.

Agency Category	Agency Name
TMDL responsible parties	Port of Los Angeles
	City of Los Angeles Watershed Protection Division
Beach regulators	Regional Water Quality Control Board
	State Water Resources Control Board
Environmental Advocacy	Heal the Bay
Beach monitoring laboratory	City of Los Angeles Environmental Monitoring Division
Facilitator	Southern California Coastal Water Research Project

High volume of beach use

Beach usage criteria was developed as a metric to justify the need for a QMRA for site-specific criteria effort. If beaches are well used, then there is greater exposure and therefore an increased likelihood of illness among the population (attributable cases). At ICB, the Cabrillo Marine Aquarium estimates almost half a million visitors per year, mostly school-aged children and the lifeguards estimate nearly a million beachgoers per year, mostly families with children (RWQCB 2004). This estimate of beach usage exceeds the level established by AB411 (1998) for mandatory beach monitoring.

Septic tanks are minimal and not a prominent sewage treatment solution

There are no known septic systems in the ICB watershed. However, there is the Terminal Island Water Reclamation Plant (WRP) outfall located nearly 4 km east of ICB. The WRP discharges up to 30 MGD of tertiary treated wastewater to outer Los Angeles Harbor (Figure 5). As part of their NPDES monitoring requirements, the City of Los Angeles monitors a number of locations throughout outer Los Angeles Harbor for fecal indicator bacteria, including *Enterococcus*. This monitoring is conducted weekly and was compiled for the 16-year period 1996 to 2011. While the WRP is clearly a possible source of human fecal contamination to ICB, the historical monitoring data near the outfall during dry weather rarely detects *Enterococcus* (Table 4, City of Los Angeles 2013). Unlike the water samples at ICB, surface water samples between the WRP outfall and ICB are routinely low (Table 4). This is supported by special studies conducted by the Port of Los Angeles to assess onshore transport of bacteria from offshore sources (KLI and DMJ Harris 2006).

Cabrillo Marina is located < 1 km from ICB and contains 885 boats slips. While not a septic system, the marina has a pumpout system and most boats of berthing size have a waste holding tank for their on-board head which is capable of discharging raw sewage to inner Los Angeles Harbor. The coast guard and marina operators both have strict rules for holding tank discharges and maintain both a pump-out dock and a mobile pump-out service for emptying holding tanks safely. The historical monitoring location at Cabrillo marina has indicated very low FIB concentrations over the 16-year time period. During dry weather, only three samples have exceeded the water quality objective for *Enterococcus*. Moreover, the water circulation at ICB is from south to north (KLI and DMJ Harris 2006), and Cabrillo Marina is located north of ICB. From these data, boats at Cabrillo Marina do not appear to be a source of FIB and pathogens to ICB.



Figure 5. Outer Los Angeles Harbor with locations of possible distant human sources, Cabrillo Marina and Terminal Island Treatment Plant Outfall.

Table 4. Frequency of water quality standard exceedance for *Enterococcus* in Outer Los Angeles Harbor near Terminal Island Treatment Plant (TITP), Cabrillo Marina, and Offshore ICB during dry weather 1996 – 2011 (see Figure 5 for site locations) (City of Los Angeles 2013). Water Quality Standard is 104 MPN per 100 ml.

SITE	Percent of <i>Enterococcus</i> sample exceedances
Shoreline Inner Cabrillo Beach (CB02)	40% ^a
Offshore Inner Cabrillo Beach (HW49)	0.1% ^b
Cabrillo Marina (HW29)	0.3%
Halfway to TITP Outfall (HW56)	0.2%
TITP Outfall (HW33)	0.3%

^asummer dry weather only, ^b summer and winter dry weather

Well-defined, small watershed

The ICB has a well-defined, small watershed (Figure 2). Since the beach is surrounded by bluffs, it has a self-contained hydrology. Storm drains on top of the bluff are plumbed to the north, draining to the marsh north of CB02 and north of the boat launch ramp. What little drainage does reach the beach near CB02, is through a single outfall, as described above in the section on human sources of contamination. This storm drain was completely removed, then reconstructed on-grade of sealed, inert materials such as HDPE. Moreover, a dry weather diversion was installed just upstream of the storm drain outfall to the beach, which sends all dry weather flows and initial storm flows to the sanitary sewer system.

BEACH MONITORING FOR SOURCES IN 2016

General Approach

SCCWRP performed a source tracking study during the summer of 2016 following the general approach outlined in the State Water Board's Source Identification Protocol (Griffith et al. 2013). This approach follows six tiered steps to perform a hypothesis-driven, science-based microbial source identification study. Prior to the start of this study, the Port of Los Angeles had performed the first two steps in the tiered approach: 1) Watershed characterization, infrastructure inspection, and listing potential sources and 2) Examination of historical FIB monitoring data. The results from these steps are summarized in the Beach Confirmation section above and Beach Confirmation Report (SCCWRP 2016a).

SCCWRP undertook a source tracking study focused on steps three through five of the tiered approach: 3) Sampling to determine spatial or temporal patterns to the FIB using traditional methods, 4) Detection and quantification of human source markers, and 5) Detection and quantification of avian source markers. To accomplish this, SCCWRP sampled water, sand, and eelgrass wrack five days a week for 15 weeks. During this 15-week timeframe, SCCWRP staff also performed a high temporal resolution study during a Spring Tide, collecting water, sand, and eelgrass every 2 hours over 36 hours. The approach is summarized in the Sampling and Analysis Plan (SCCWRP 2016c).

Sampling Methods

Samples were collected once-per-day across a gradient of six sites along the beach and one site on Outer Cabrillo Beach (Figure 6) to determine the extent and source of FIB contamination. The 7 sites sampled incorporated historic monitoring sites: CB02 (ICB 3) and SDS7 (ICB 7). Between June 1 and September 4, 2016, grab samples for water, sand, and, when available, eelgrass wrack were collected Wednesday through Sunday between 6:00 and 8:00 AM and transported to SCCWRP for FIB analysis and filtered for bacterial DNA.

During the Spring Tide from June 3-4, 2016, a high-resolution temporal study was performed collecting water, wet sand, and eelgrass wrack every 2 hours from 8:00 PM June 3 until 8:00 AM on June 5, 2016. Temperature and salinity were measured using a YSI-Pro30 Conductivity/Temperature meter. Wind speed and direction was collected at each site using a hand-held wind sensor. Water was filtered onto 0.4 μm polycarbonate or 0.45 μm type HA mixed cellulose ester filters for DNA and the filters were flash frozen in liquid N_2 and stored at -80°C . Wet sand (40 g) and eelgrass wrack (10 g) were measured out and shaken with filtered, autoclaved phosphate buffered saline for a final concentration of wash from 10 g sand and 5 g eelgrass per 100 ml. This wash was filtered onto a 0.4 μm polycarbonate filter for DNA, flash frozen in liquid N_2 , and stored at -80°C .

Laboratory Methods

Laboratory methods can be found in Appendix A and in the Sampling and Analysis Plan (SCCWRP 2016c). In brief, to measure cultivable FIB in water, sand, and eelgrass, total coliform bacteria and *E. coli* were analyzed using IDEXX Colilert-18 kits, and Enterococci was analyzed

using IDEXX Enterolert kits at two dilutions in duplicate for each sample. DNA was extracted using GeneRite kits according the protocol outlined in the State Source Tracking Manual (Griffith et al. 2013). Genetic markers for *Enterococcus* (Entero1A: Cao et al. 2015), human-associated *Bacteroidetes* (HF183: Cao et al. 2015), and Gull-associated *Catelliboccus* (LeeSeaGull: Lee et al. 2013) were quantified using digital PCR. The limit of quantification for the digital PCR assays was approximately 40 gene copies/100 ml, or a minimum of 3 positive droplets above the baseline threshold. Results meeting this threshold were designated quantifiable. Assays with 1 or 2 positive droplets above baseline were designated below the limit of quantification (BLOQ). For data analysis, these samples were assigned a value of 23 copies per 100 ml. Assays with zero positive droplets above baseline were designated as zero. Any samples that did not pass QA/QC for cultivation or molecular-based analyses were excluded from the dataset.

Data Analysis

Data analysis followed a four-step process. First, a data inventory was compiled documenting sampling success. Second, Analysis of Variance (ANOVA) was conducted to assess if spatial patterns in fecal indicator bacteria concentrations were evident; significant differences among sites were calculated using a Tukey *post hoc* test. Spatial differences were also estimated by examining frequency of single sample water quality objectives. These objectives are defined by the SWRCB (2015): *Enterococcus* > 104 MPN/100 ml, fecal coliform > 400 MPN/100 ml, total coliform > 10,000 MPN/100 ml. In this case, we used *E. coli* instead of fecal coliform because *E. coli* are the major component of fecal coliforms in fresh human fecal sources and *E. coli* is the routine measurement by the historical monitoring program at this beach. Third, ANOVA/Tukey *post hoc* testing for spatial differences in bird- and human-specific genetic markers was conducted for spatial differences in these source tracking indicators. Fourth, evidence for source attribution utilized Pearson correlation tests to examine relationships between *Enterococcus* concentrations and bird- or human-associated genetic markers. Pearson correlation tests between *Enterococcus* concentrations, bird- and human-associated genetic markers were also calculated with potential confounding factors including tide, time of day, day of week, presence of humans or birds, and wind speed.



Figure 6. Map of the western end of Los Angeles Harbor showing Inner and Outer Cabrillo beach and sampling locations as red dots designated sites ICB 1-7. Also visible is the Cabrillo Marina, salt marsh, Cabrillo Marine Aquarium, and breakwater

Results and Discussion

A total of 602 water samples, 600 sand samples, and 270 eelgrass wrack samples were collected across the 7 study sites during weekdays, weekends, and over 36 hours during a spring tide. A total of 476 water samples were collected during the daily sampling (7 sites x 68 sampling days); 126 water samples were collected during the 36-hour event (7 sites x 18 bi-hourly sampling periods). The reduction in eelgrass sample size relative to water and sediment reflected a lack of eelgrass on the beach. More than 97% of the sand samples, 99-100% of the water samples, and 100% of the eelgrass samples were successfully processed and analyzed (Table 5). All samples were successfully collected, however there were 6 samples (1 water sample and 4 sand samples) which did not meet QA/QC objectives for holding times or incubation temperatures for the IDEXX kits. These samples were excluded from further analysis.

Enterococcus displayed a spatial pattern across the beach.

Enterococcus was routinely detected at all six sites at Inner Cabrillo Beach and at site ICB7 (SDS7) located at Outer Cabrillo Beach. The percent of daily samples in exceedance of the *Enterococcus* single sample water quality standard (104 MPN/100 ml) ranged from 0% at Outer Cabrillo Beach (site ICB7 or SDS7) to 28% at the historical compliance site (ICB3 or CB02) in the middle of Inner Cabrillo Beach (Figure 7A, Table 6). The overall percentage of single sample exceedance was 14.5% across all sites and 16.9% across the six Inner Cabrillo Beach sites. *E.coli* had a total of 1.9% of samples in exceedance across the entire study and total coliforms had only 0.2% of samples in exceedance across the entire study (Table 6).

Enterococcus concentrations showed a spatial pattern that peaked at sites 3, 4, and 5. Concentrations dropped as sites were further from the center of the beach (Figure 7A). Mean concentrations were not significantly different between sites 2, 3, 4, and 5 (all p-values > 0.2), but the mean concentrations at sites 1 and 6 were significantly different from sites 3, 4, and 5 (all p-values < 0.05). The same pattern was shown, with slightly higher concentrations, by the digital PCR *Enterococcus* assay. These higher concentrations may be due to the variable number of gene copies per cell (Cao et al. 2015), although it is possible that the digital PCR is measuring a number of inactive or dead *Enterococcus*. *E.coli* and total coliforms displayed a similar spatial pattern to *Enterococcus* with their highest values near the center of Inner Cabrillo Beach.

Table 5. Percent sampling and processing success from the Source Tracking Study. NA indicates no samples collected.

Site	Water IDEXX	Sand IDEXX	Eelgrass IDEXX	Water Human Marker	Water Gull Marker	Water <i>Enterococcus</i> Marker
1	99%	99%	100%	100%	100%	100%
2	99%	99%	100%	100%	100%	100%
3 (CB02)	99%	99%	100%	100%	100%	100%
4	99%	99%	100%	100%	100%	100%
1	99%	99%	100%	100%	100%	100%
6	99%	99%	100%	100%	100%	100%
7 (SDS7)	99%	97%	NA	100%	100%	100%

Table 6. Percent and number of days with water samples exceeding FIB water quality objectives at Cabrillo Beach. Each site contains 68 samples.

Site	<i>Enterococcus</i> ^a		<i>E. coli</i> ^b		Total Coliforms ^c	
	Percent of Days Exceeding	Number of Days Exceeding	Percent of Days Exceeding	Number of Days Exceeding	Percent of Days Exceeding	Number of Days Exceeding
1	7	5	0	0	0	0
2	15	10	4	3	0	0
3 (CB02)	28	19	4	3	0	0
4	22	15	1	1	1	1
5	25	17	1	1	0	0
6	6	4	1	1	0	0
7 (SDS7)	0	0	0	0	0	0

- a. 104 MPN/100 ml
- b. 400 MPN/100 ml
- c. 10,000 MPN/100 ml

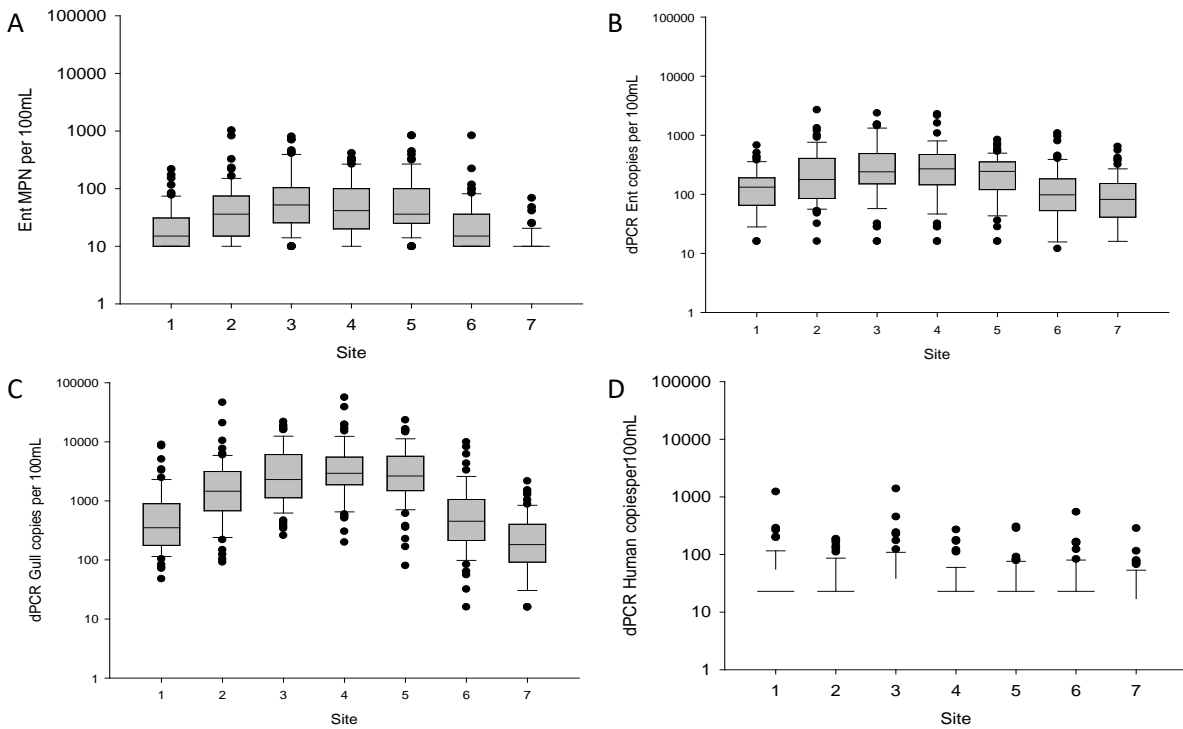


Figure 7. Box plots showing concentrations of A) cultivable *Enterococcus*, B) *Enterococcus* genetic copies by digital PCR, C) Gull-specific genetic marker by digital PCR, and D) Human-specific genetic marker by digital PCR in daily water samples at each site from Cabrillo Beach. Each site contains 68 samples. Boxes show 25th, 50th, 75th percentiles. Whiskers show the 5% and 95% range.

Birds are an influential source of *Enterococcus*

Avian fecal material, measured using digital PCR assays for gull-specific genetic markers, was detected in 98-100% of the water samples at the sites at Inner Cabrillo Beach, and 96% at the Outer Cabrillo Beach site (Table 3). Gull-specific genetic markers showed a similar pattern to *Enterococcus* with a peak in concentrations at sites 3, 4, and 5 at the center of the beach (Figure 7C). Concentrations of Gull-specific genetic markers averaged 5- to 10-fold higher than *Enterococcus* across the beach and detected in more samples. This similarity in spatial pattern was also shown in the Pearson correlation between gull-specific genetic markers and *Enterococcus* by digital PCR ($r=0.58$, $p<0.001$; Figure 8A). This correlation was the strongest relationship found in the source identification study.

Taken together, this provides multiple lines of evidence that shorebirds are a major source of *Enterococcus* to Inner Cabrillo Beach. The correlated and clearly similar spatial pattern leaves little doubt that bird feces are a major contributor to the *Enterococcus* concentrations found. This matches well with the historical source tracking data and responsiveness of *Enterococcus* concentrations to management actions focused on bird exclusion (Weston Solutions 2013).

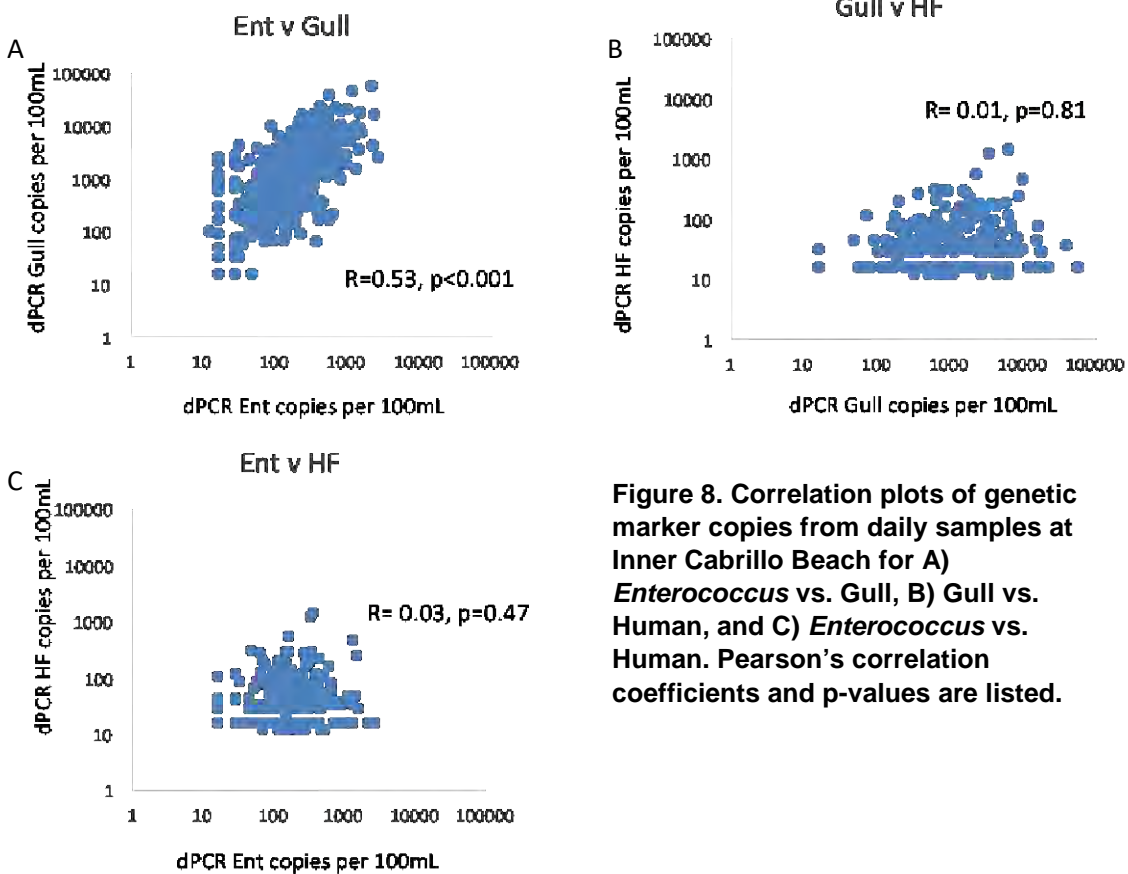


Figure 8. Correlation plots of genetic marker copies from daily samples at Inner Cabrillo Beach for A) *Enterococcus* vs. Gull, B) Gull vs. Human, and C) *Enterococcus* vs. Human. Pearson's correlation coefficients and p-values are listed.

Human sources frequently present at low levels

Human-specific genetic marker (HF183) was detected in 52% of the 476 daily water samples collected at Inner and Outer Cabrillo Beach sites (Figure 9, Table 7). The frequency of detectable human-specific genetic marker ranged from 25-67% among the seven Cabrillo Beach sites. However, concentrations of human-specific genetic marker were generally low, with 19% of the 476 samples detected above the limit of quantification (HF Quantifiable > 40 gene copies/100 ml). The detection frequency of quantifiable human-specific marker ranged from 12-28% among sites (Figure 9).

In contrast to the *Enterococcus* and gull-specific marker, quantifiable human-specific genetic marker (HF Quantifiable) did not display a spatial pattern (Figure 8D, 9). Human-specific genetic markers did not correlate with gull-specific genetic markers ($r=0.01$, $p=0.81$) or *Enterococcus* ($r=0.03$, $p=0.47$; Figure 8B, C). Instead, there appeared to be a relatively consistent detection rate and concentration among Inner Cabrillo Beach sites. None of the human-specific marker mean concentrations were statistically different between Inner Cabrillo Beach sites ($p > 0.05$). Modestly greater concentrations were observed at sites 1 and 3; this is matched by the greater percentage of samples with quantifiable detections at those sites. When examining any detectable sample (detections both above and below the quantitation limit or HF Quantifiable + HF BLOQ), sites 1 and 6 show the greatest detection frequency (Table 7, Figure 9).

Taken together, the data suggests that human sources are not a major contributor to *Enterococcus* or gull-specific genetic marker. This low level, diffuse human signal suggests a distributed source, rather than a concentrated source such as the avian source on the beach identified by the spatial pattern in the gull-specific marker and the *Enterococcus* concentrations.

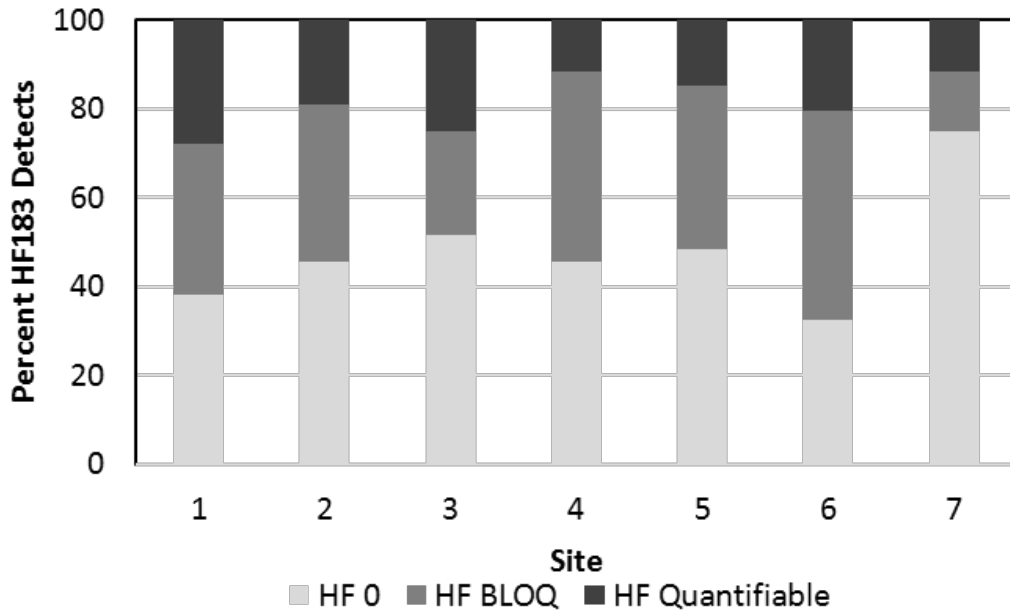


Figure 9. Percent human-specific genetic marker (HF183) in daily water samples at each site. Detection categories are the quantifiable range (HF Quantifiable), below the limit of quantification (HF BLOQ), and non-detects (HF 0). Each site contains 68 samples.

Table 7. Percent of water samples with genetic markers detected by digital PCR at Cabrillo Beach. Each site contains 68 samples.

Site	<i>Enterococcus</i>	Gull Marker	Human Marker
1	100%	98%	62%
2	100%	100%	54%
3 (CB02)	98%	100%	49%
4	88%	100%	54%
5	97%	100%	51%
6	93%	98%	67%
7 (SDS7)	94%	96%	25%

Potential explanatory and contextual variables

Enterococcus concentrations in sand samples were similar in spatial pattern to *Enterococcus* concentrations in water samples (Figure 7A, 10A). The *Enterococcus* sand concentrations observed at Inner Cabrillo Beach were within the range of sand concentrations observed at other Southern California beaches, but most closely resembled Doheny State Beach (Figure 10B). Doheny also had a large shorebird population and low magnitude concentrations of human-specific marker (Layton et al. 2015). Assuming the sand is a reservoir of *Enterococcus* from water samples, then shorebirds could also be a major contributor to the *Enterococcus* in the sand. Gull-specific marker was not measured in sand.

In contrast to sand, the *Enterococcus* concentrations found in eelgrass wrack collected on the beach did not show the same spatial pattern as the water (Figure 10C). Site 3 exhibited the highest wrack concentrations measured, followed by site 1, although site 4 had a higher median concentration than either site. The lack of spatial pattern may be due to the prevailing current, which deposited the eelgrass mainly at sites 1-3, while site 4 and particularly 5 and 6 had few samples (shown by the single bar instead of the box).

We found no relationship between tide, beach usage, or day of the week and the concentrations of *Enterococcus*, gull-, or human-specific genetic markers in the daily water samples. There was no appreciable difference in the concentrations of any analyte between the daily water samples collected at low tide, mid tide or high tide, as shown when broken down into four height categories (Figure 11). The daily samples could miss a pattern that occurred over the tidal cycle, however there was no pattern found in gull- or human-specific genetic markers (Figure 12A) during the intensive 36-hour Spring tide study which included some of the highest and lowest tides observed during the summer. No relationship to windspeed was found in human- or gull-specific markers (Figure 12B).

Beach usage showed a slightly negative, although not statistically significant, relationship with *Enterococcus* or the gull- or human-specific genetic marker concentrations. Daily water samples categorized by the number of people observed in the water showed that the concentrations of *Enterococcus*, human-specific, and gull-specific genetic markers decreased when the number of people increased (Figure 13). The decrease is greater for the gull-specific marker (Figure 13C) compared to the *Enterococcus* or human-specific marker (Figure 13A, B). We did observe the shorebirds move away from where people were swimming during the study. Although this apparent negative pattern was found when the daily samples were considered in aggregate, there was no relationship observed during the peak usage times (Saturday afternoon) during the 36-hour study (Figure 12).

In spite of the increased beach visitation on weekends during the summer, there was no pattern shown by the *Enterococcus*, gull-, or human-specific genetic marker concentrations in daily samples when binned by day of the week (Figure 14). In contrast to the relationship between beach use and gull-specific genetic markers (Figure 13C), gull-specific genetic markers did not change with day of the week (Figure 14B). This would suggest that even though gulls are impacted by people at the beach during the day, this impact is short-lived when birds re-populate on the beach to roost overnight.

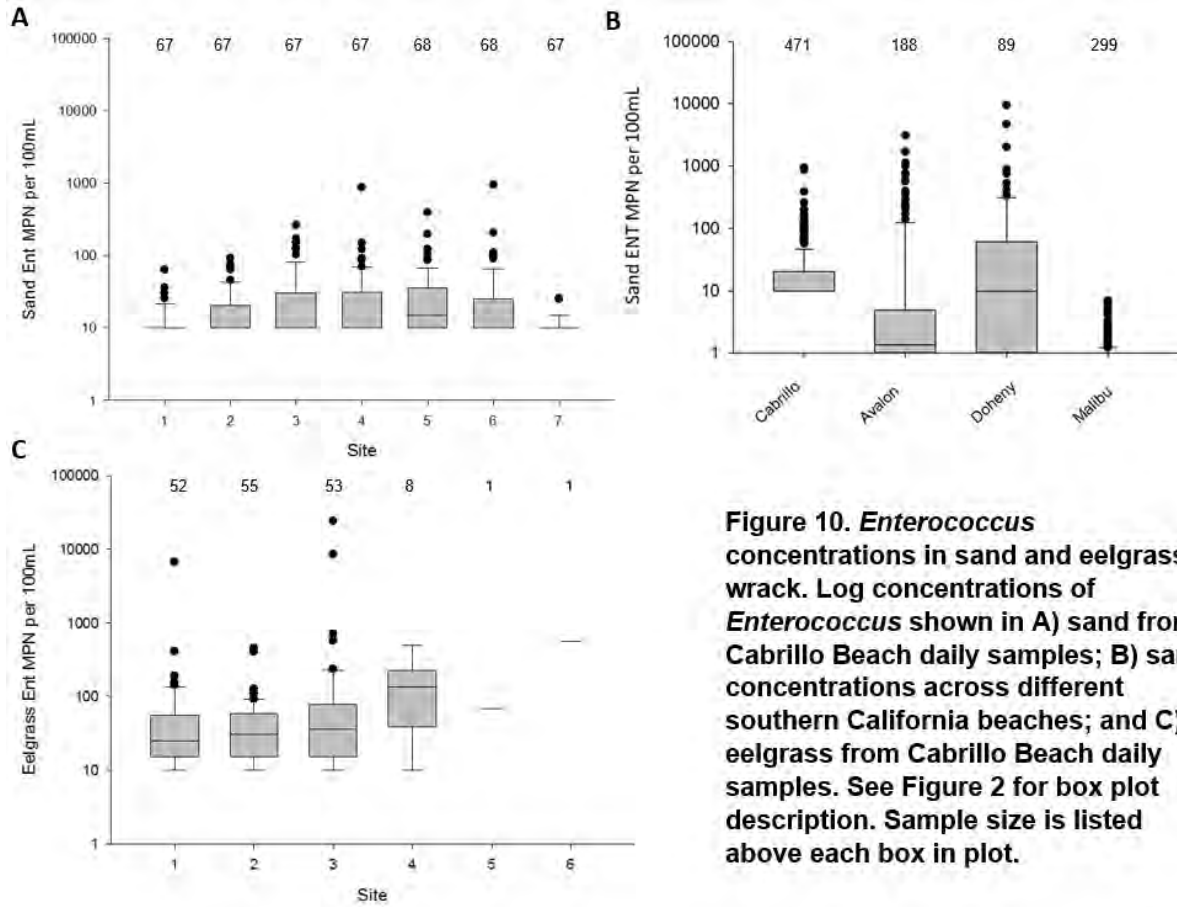


Figure 10. *Enterococcus* concentrations in sand and eelgrass wrack. Log concentrations of *Enterococcus* shown in A) sand from Cabrillo Beach daily samples; B) sand concentrations across different southern California beaches; and C) eelgrass from Cabrillo Beach daily samples. See Figure 2 for box plot description. Sample size is listed above each box in plot.

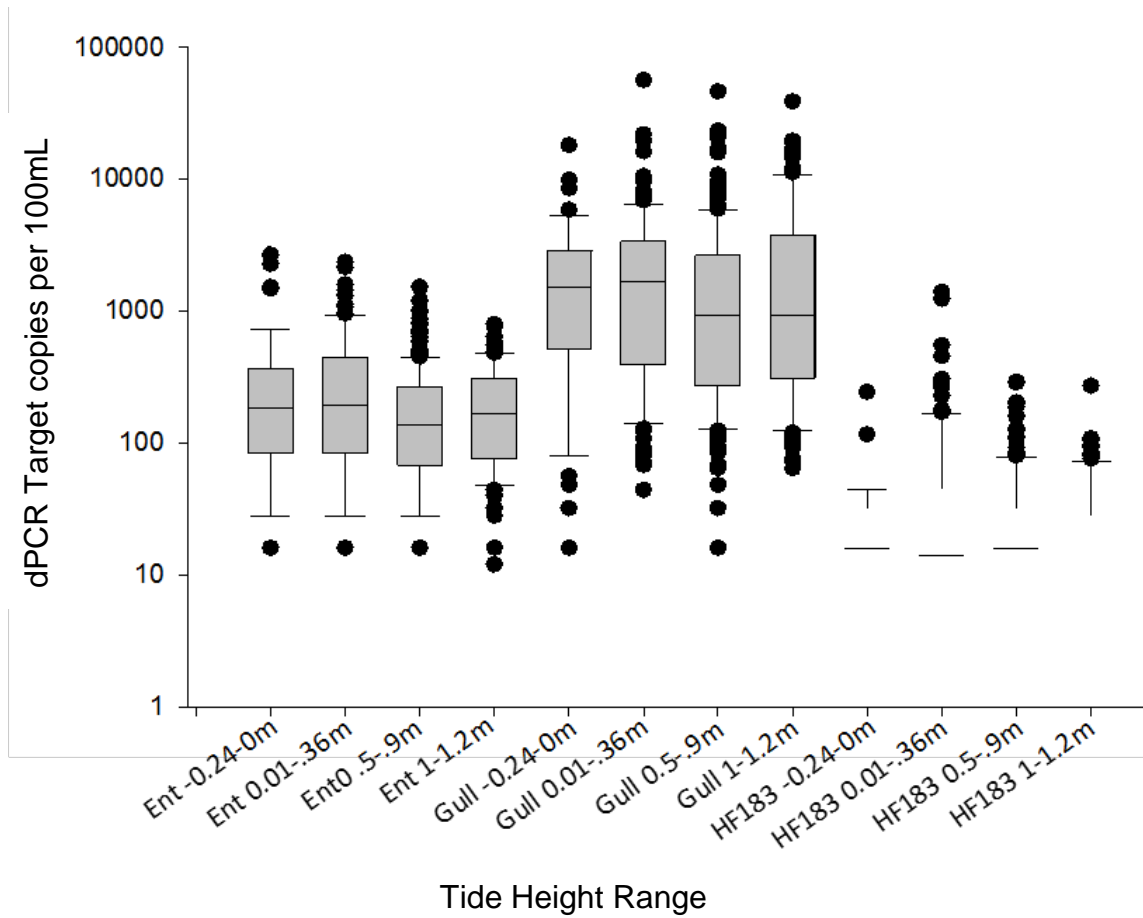


Figure 11. Concentrations of *Enterococcus*, Gull-specific marker, and Human-specific marker in water samples binned by tide heights (from MLLW) at the time of sampling. See Figure 2 for box plot descriptions. Sample size for tide height range -0.24 – 0 m = 49, 0.01 – 0.36 m = 126, 0.5 – 0.9 m = 175, 1.0 – 1.2 m = 126.

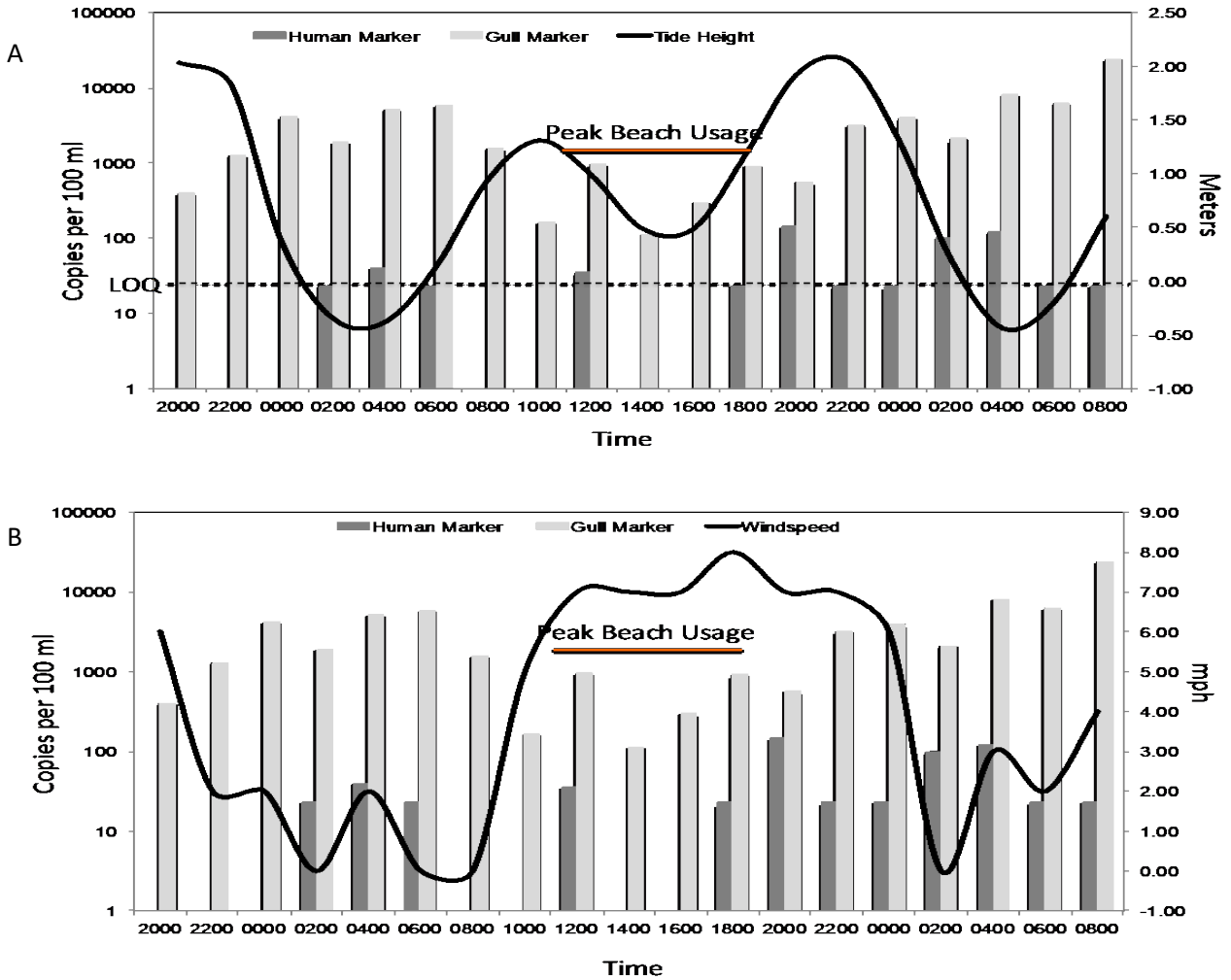


Figure 12. Concentrations of Gull-specific marker and Human-specific marker in water samples collected at site 3 (CB02) over 36 hours during a spring tide. Source markers (bars) are plotted across time with tide height (A) and windspeed (B) co-plotted (lines).

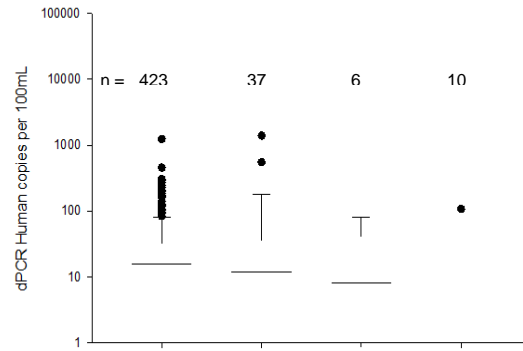
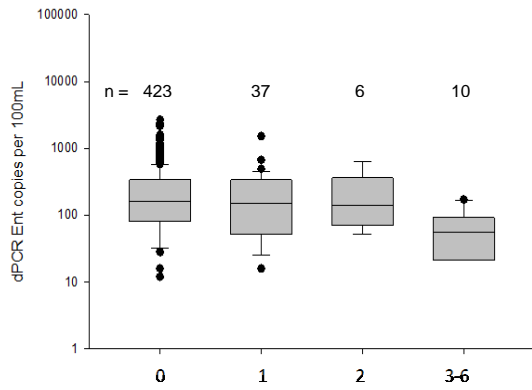
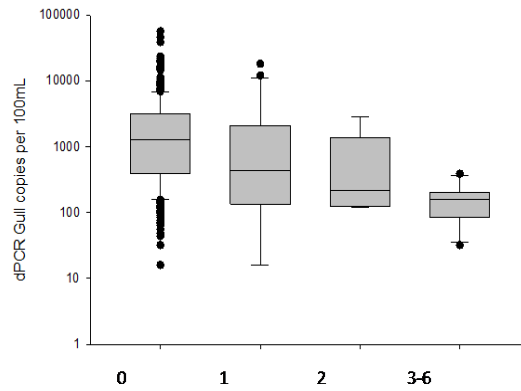


Figure 13. Box plots showing concentrations of A) *Enterococcus* genetic marker copies, B) Human genetic marker, and C) Gull genetic marker, and in daily water samples grouped by number of people in the water. See Figure 2 for box plot descriptions.



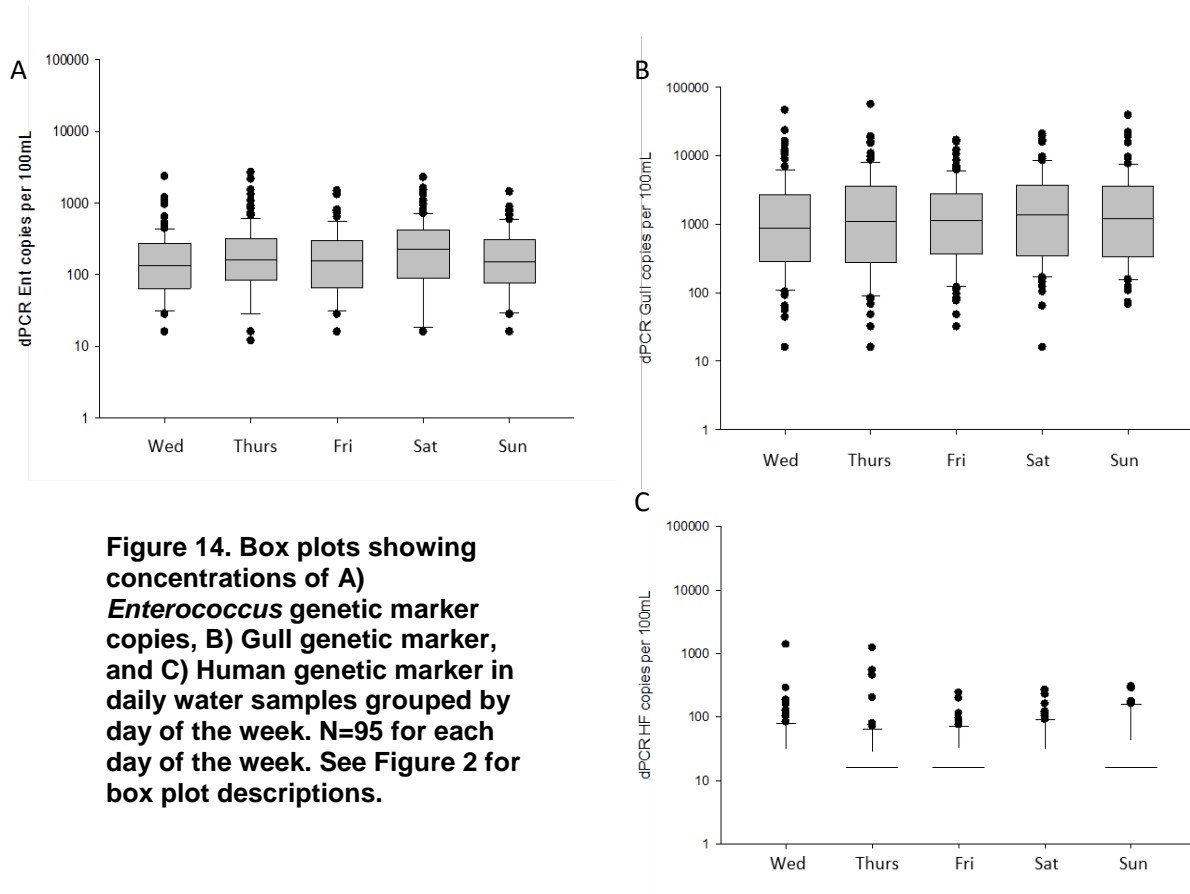


Figure 14. Box plots showing concentrations of A) *Enterococcus* genetic marker copies, B) Gull genetic marker, and C) Human genetic marker in daily water samples grouped by day of the week. N=95 for each day of the week. See Figure 2 for box plot descriptions.

Conclusions from Beach Monitoring

The beach monitoring to identify sources in summer 2016 identified four conclusions:

- **There was a consistent spatial pattern in the extent of fecal indicator bacteria in the water and sand across Inner Cabrillo Beach.**

Enterococcus concentrations peaked at the middle of Inner Cabrillo Beach, co-located with the compliance site CB02, where water quality objective exceedances occurred in 28% of the 88 samples collected at that site. Concentrations and frequency of exceedance decreased moving away from this site along the beach. Cumulatively, across the 602 water samples collected at all sites, 15% of samples exceeded water quality objectives for *Enterococcus*. *Enterococcus* concentrations in sand samples followed a nearly identical spatial pattern as the water samples.

- **Birds are a primary source of fecal pollution to Inner Cabrillo Beach.**

Gull-specific markers were measured in nearly 100% of the water samples collected from Inner Cabrillo Beach. Concentrations of gull-specific markers in water followed the same spatial pattern as, and were significantly correlated to, *Enterococcus* concentrations in water.

- **Human markers of fecal pollution were present at relatively frequent, but low levels.**

Human-specific markers were detected in 52% of the 602 water samples collected at Cabrillo Beach. Concentrations of human-specific genetic marker were generally quite low; approximately half of these detectable samples were below the limit of quantification. Human-specific markers were not statistically different between sites and were not correlated to *Enterococcus* or gull-specific marker concentrations.

- **Beach conditions such as day, wind, tide, and swimmer population did not affect *Enterococcus* or marker concentrations.**

Enterococcus, gull-specific and human-specific markers did not significantly vary between days of the week, weekends vs. weekdays, wind speeds or tide level. An intensive 36-hour study sampling every 2 hours across a large tidal cycle did not indicate strong changes in concentrations at minimum low or maximum high tides. There was a modest indication that *Enterococcus* and gull-specific marker concentrations decreased with increasing number of swimmers based on daily data, providing additional evidence to support the linkage between birds and fecal indicator bacteria.

Taken together, the data from this source tracking study suggests that human sources are not a major contributor to *Enterococcus* at Inner Cabrillo Beach. The low level, diffuse human signal suggests a more distributed source, rather than a concentrated source, such as the avian source on the beach identified by the spatial pattern in the *Enterococcus* and correlated gull-specific marker concentrations.

Since there is no surface water discharge at the beach, diffuse non-point sources for human fecal material at Inner Cabrillo Beach are only possible from two potential sources: contaminated groundwater exfiltrating at the beach or from harbor sources further offshore the beach. Groundwater contamination could arise either from on the beach infrastructure, or from the

urban areas upland of the beach. Scientists have observed the transport of wastewater contaminated groundwater to other southern California beaches including Avalon Beach (Boehm et al. 2004) and Malibu Surfrider Beach (Izbicki et al. 2012).

Offshore sources of human-specific marker could be transported to the beach via harbor currents. The origin of these sources could include illegal discharges from ships, recreational or fishing vessels, and/or dry weather runoff discharges. While conceptually possible, all of these sources are generally small, sporadic, or distant. The largest potential source of the human-associated marker offshore, located 4 km east of the beach, is the Terminal Island Wastewater Reclamation Plant that discharges an average 16 million gallons per day of tertiary treated and disinfected effluent.

The next step in the QMRA is pathogen loading, a key factor for the dose portion of the risk model for swimmers. Measuring pathogens in the beach water will not currently suffice for estimating this dose term because concentrations that can lead to swimming-related illness may not be detectable using available methodology for even the newest technology such as digital PCR. Ideally, by measuring pathogens closer to their source, concentrations will be large enough for current methodology to confidently measure pathogens and estimate risk.

The next step recommended in this project to support QMRA is to conduct source confirmation, determining whether human-specific markers are emanating from contaminated groundwater or from offshore harbor sources. Once sources are confirmed, then assessment of pathogen loading can begin.

HARBOR MONITORING FOR HUMAN MARKER IN 2017

One potential explanation of the human-specific marker along the beach is contaminated outer harbor waters coming ashore at ICB. This is of particular concern since the Terminal Island WRP is located approximately 4 km east of ICB and discharges up to 16 million gallons per day (mgd) of tertiary treated effluent. To assess the potential of harbor waters coming ashore and contaminating ICB, a screening level study was designed and implemented to look for gradients between the WRP and ICB. This screening study was meant to indicate if there was a potential for cross-harbor contamination. A more rigorous study could be designed and implemented to confirm cross-harbor contamination should the screening level study indicate a strong potential.

There have been several studies examining the circulation within the Los Angeles Harbor and at ICB. For example, current meter studies indicate that water circulation patterns at ICB favor a south to north current along the beach face, from the accretion beach towards the Cabrillo Marina. Superimposed on this current circulation pattern at ICB are wind driven vertical circulation patterns (Evans Hamilton 2004). During the quiescent morning, little water movement is measurable in the vertical direction. As westerly winds predictably increase mid-day during the summer, surface layer water is pushed offshore ICB, entraining deeper (5 m) water to the beach.

More recently, the Harbor Water Resources Action Plan (WRAP) model was used to address harbor water circulation patterns (Port of Los Angeles and Port of Long Beach 2009). Harbor water circulation is complex. During flood tides, harbor water flows either east or west from Angels Gate, located approximately halfway between ICB and the WRP (Figure 15).

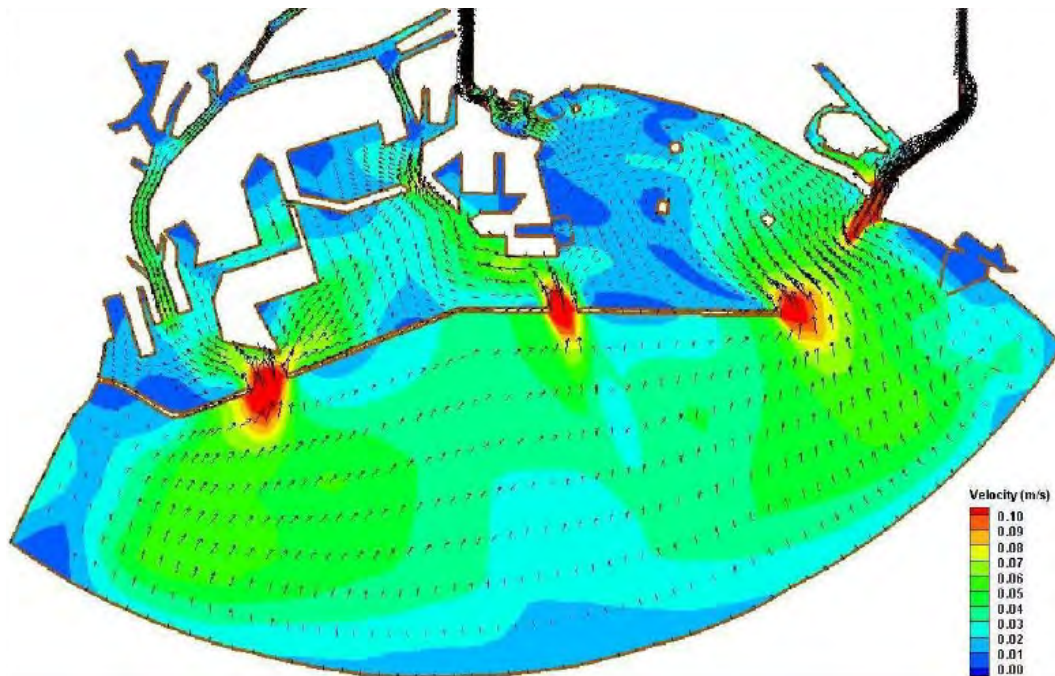


Figure 15. Modeled harbor water circulation patterns using the Harbor Water Resources Action Plan (WRAP) during flood tides (Port of Los Angeles and Port of Long Beach 2009). Arrows indicate strength and direction of current flow.

Methods

A full description of the study design and methods can be found in the Sampling and Analysis Plan (SCCWRP 2017).

Surface and near-bottom water samples were collected from 20 sites located between the Terminal Island WRP outfall and ICB to identify if human genetic marker (HF183) was present in the Los Angeles Harbor (Figure 16). In addition to the compliance sites at ICB (CB02), a sampling grid was developed to collect harbor waters near the Terminal Island WRP outfall, the Main Channel, the Cabrillo Marina, Los Angeles (LA) Outer Harbor, and the entrance to the Harbor, just offshore ICB, and at Outer Cabrillo Beach. Samples were analyzed for Total Coliform, *E. coli*, and *Enterococcus* using IDEXX culture methods and HF183 human-specific marker utilizing methods described in the Sampling and Analysis Plan (SCCWRP 2017) and Appendix A. Water samples were collected during spring tide conditions between 9:40 AM and 4:20 PM on May 25, 2016 and during neap tide conditions between 8:49 AM and 2:51 PM on June 2, 2017. In addition, a 24-hour composite sample of the WRP final effluent was also tested on each sampling date. In total, 80 water samples were collected across 20 sites during spring and neap tide conditions, plus one effluent sample from the WRP. 100% of water samples were successfully collected, processed and analyzed.



Figure 16. Map of Los Angeles Harbor showing sample locations sampled May and June, 2017. Surface and bottom water samples were taken at all offshore locations, with surface water only taken at Cabrillo Beach sites (CB01, CB02, and SDS7).

To address concerns about methodological comparability, split samples at every site and time were run for HF183 genetic marker at Weston Laboratories (by qPCR), at SCCWRP Laboratories (by ddPCR), and at the University of North Carolina Laboratories (using ddPCR). Finally, CTD casts to measure conductivity (salinity), temperature, and depth were collected at a subset of sites to establish if the harbor water column was stratified, preventing the mixing of bottom and surface waters.

Los Angeles Harbor Spatial Survey

The HF183 human-specific marker was detected much more frequently during spring tide than neap tide (Figure 17). Ninety three percent (93%) of all samples contained detectable HF183 during spring tide compared to 24% of samples during neap tide. In addition, spring tide conditions had far more (37%) quantifiable samples compared to neap tide conditions (12%). During both tide conditions, *Enterococcus* concentrations were either at or below the detection limit in every sample tested except three (Appendix B). All three of these samples were measured during spring tide conditions and located at ICB (CB02, CB01, SD7); the highest concentration was at CB02, the TMDL compliance site at ICB.

Spatial gradients of HF183 human-specific marker between the WRP and ICB were not readily apparent during neap tide (Figure 17). The greatest concentrations of HF183 human genetic marker during neap tide was observed near the Terminal Island WRP outfall (216 copies per 100 ml). Of the five sites with quantifiable HF183 concentrations during neap tide, the four samples with the largest concentrations were located at the surface and adjacent to the outfall terminus. No HF183 was detected at ICB during this neap tide screening survey, consistent with the diminished HF183 detections and *Enterococcus* exceedances during neap tides within the 2016 intensive beach sampling. HF183 was detected, but below the limits of quantification, at three additional surface water samples extending from ICB to just inside the Angel's Gate entrance to LA Harbor (about halfway to the outfall). However, no detectable HF183 marker was observed in surface or bottom water samples between Angel's Gate and the WRP outfall. Thus, a gradient between the WRP and ICB cannot be assumed during neap tides.

Spatial gradients of HF183 human-specific marker between the WRP and ICB were not obvious during spring tide (Figure 17). The greatest concentrations of HF183 human genetic marker was observed in the beach sample from CB02 (560 copies per 100 ml) during spring tide. The site with the second greatest concentration of HF183 human genetic marker during spring tide was observed at site HW29 (508 copies per 100 ml) located North of ICB and south of Watchorn Basin. Focusing just on bottom water, the HF183 human genetic marker was found in 83% of all non-beach samples (15 of 18 samples) with concentrations ranging from 52 to 508 copies per 100 mL across the sampling grid. Concentrations of HF183 human genetic marker were lower near the Terminal Island WRP (80 copies per 100 ml) than at ICB. Spatial gradients of HF183 concentrations in bottom water during spring tide were insufficient to identify where the human genetic marker was originating.

The CTD casts did not illustrate strong water column stratification during either neap or spring tides (Figure 18). This indicates that a buoyant WRP plume, although discharged near the bottom of the harbor, could surface. While bottom water samples did not detect HF183 human-specific

markers nearest the outfall, surface water samples did detect HF183 and these could have included the surfacing treated wastewater plume. This also indicates that the signal during spring tide must have been relatively large relative to neap tides, contaminating both surface and bottom waters, and in a somewhat similar spatial gradient.

The spatial gradients observed during the harbor survey appeared independent of measurement method (Figure 19). Regardless of laboratory or analytical method (qPCR or ddPCR), spring tides had greater frequencies of detection and magnitude of concentrations than neap tides for the HF183 human-specific marker. Regardless of laboratory or analytical method (qPCR or ddPCR), stations closest to the WRP outfall had the highest concentrations of HF183 during neap tides and CB02 had the highest concentration of HF183 human-specific marker during spring tides. HF183 was highly correlated amongst all three methods. Comparisons between the laboratories performing ddPCR assays, SCCWRP and UNC, had a slope of 0.83 and $r^2=0.76$. The qPCR and ddPCR relationships were also highly correlated; assays had a slope of 2.99 and $r^2=0.84$ (SCCWRP and Weston) and a slope of 2.71 and $r^2=0.68$. While the two ddPCR labs were quite similar (slope near unity), the qPCR lab tended to have higher HF183 concentrations relative to ddPCR (slopes near 3) regardless of laboratory.

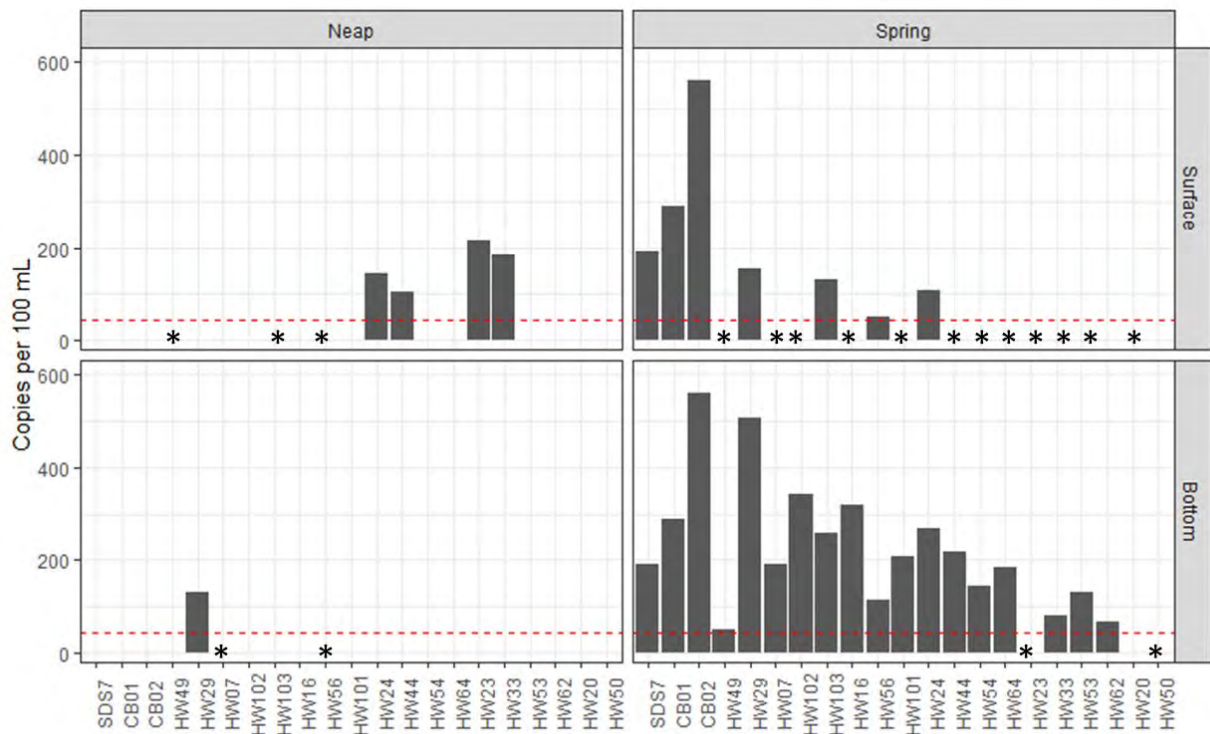


Figure 17. Human-specific marker concentration, measured by SCCWRP, for bottom and surface samples taken during Spring (May 25, 2017) and Neap tide events (June 2, 2017) during the 2017 spatial survey. Site on the x-axis and concentration on the y-axis. Bars represent quantifiable human-specific marker concentrations. Asterisks represent human-specific marker detected but not quantified. Dashed line represents the limit of quantification. Beach samples (CB02, CB01, SDS7), plotted as both surface and bottom water.

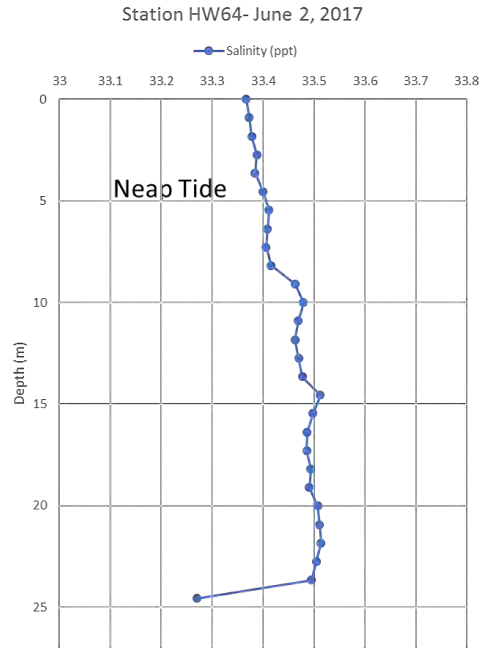
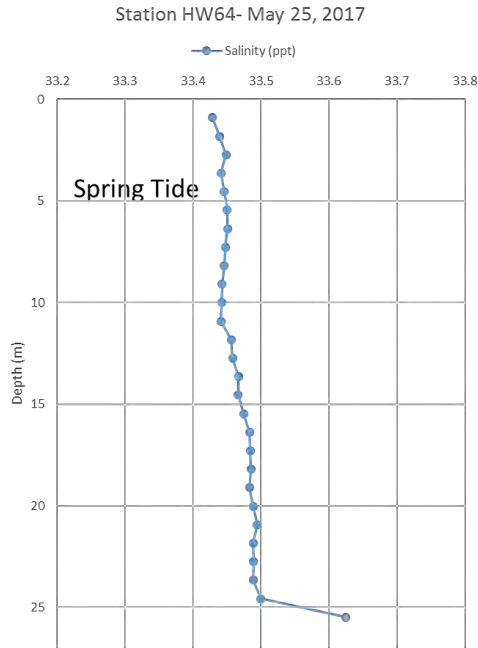


Figure 18. CTD casts showing salinity at station HW64 (outer harbor between ICB and WRP) during neap tide and spring tide sampling events. Strong water column density stratification was not apparent during either tide cycle.

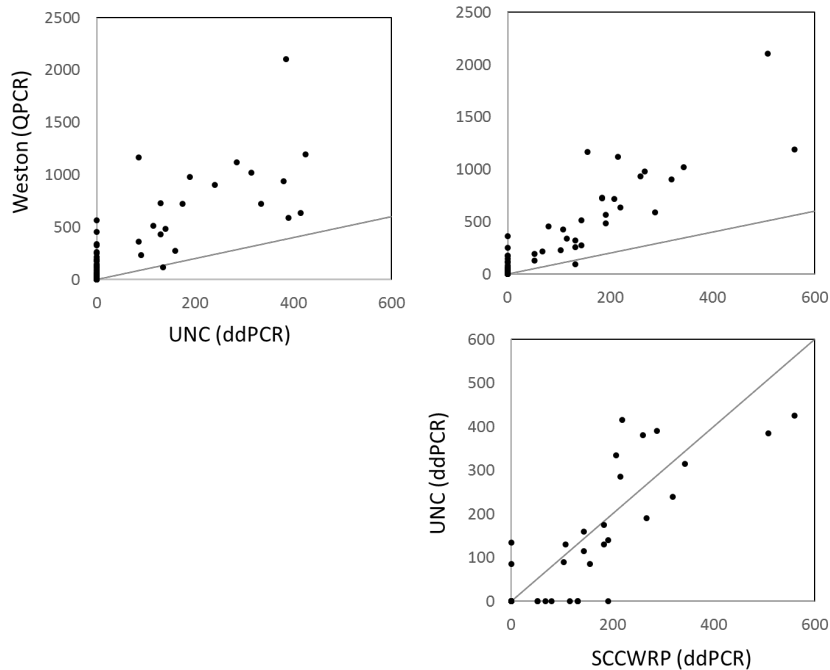


Figure 19. Comparisons among methods and laboratories including QPCR (Weston) and ddPCR (SCCWRP and UNC). 1:1 lines are shown in each plot. The QPCR laboratory estimated higher concentrations than either ddPCR laboratory.

Conclusions from the Harbor Spatial Survey

Three conclusions were drawn from the results of the harbor spatial survey:

- **No consistently clear pattern of HF 183 human-specific marker was observed; however some localized patterns of HF 183 were detected**

The greatest concentration of HF183 human-specific marker occurred at station CB02 at ICB. The second greatest concentration of HF183 human-specific marker occurred at station HW29 north of ICB and south of Watchorn Basin. Concentrations of HF183 human-specific marker also occurred in the array of stations by the WRP outfall.

- **Concentrations of the HF183 human-specific marker varied between two tidal conditions**

During spring tide, concentrations of HF 183 were detectable at most stations. The greatest concentration of the HF183 human-specific marker occurred at stations CB02 at ICB and HW29 north of Cabrillo Marina and south of Watchorn Basin, decreasing moving towards the outer harbor. During neap tide, much fewer stations detected the HF183 human-specific marker with the greatest concentrations detected closest to the Terminal Island WRP outfall.

- **Spatial patterns were independent of HF183 measurement method**

Regardless of method, split harbor water samples indicated the same concentration patterns, including the differences between spring and neap tides. In general, the lab conducting the qPCR method had greater concentrations than either lab conducting ddPCR.

While some localized patterns were observed during the harbor screening survey, caution is advised when interpreting the results. This was just a screening survey and more information would be required before making any confirmations about sources and transport. However, the preliminary results obtained from this screening survey were sufficient for the Advisory Committee to not recommend additional harbor water investigations, effectively moving the WRP low on the list of sources impacting ICB.

CONFIRMING HUMAN SOURCE REMOVAL AT ICB IN 2018

Up to this point, this project had identified birds as a major source of *Enterococcus* to ICB (see previous section). However, there was also a persistent, low-level source of HF183 human-specific marker at ICB. The source of the HF183 appeared not to be originating from the Terminal Island WRP outfall (see previous section). Therefore, the HF183 human-specific marker observed at ICB may potentially be from onshore sources, possibly through resurfacing groundwater, or from nearby offshore sources.

To assess the potential for sewage infrastructure as a source of fecal contamination, the Port of Los Angeles conducted a sewage infrastructure inspection and repair program starting December 2016. The inspection activities included: visual inspections of sewer lines (camera), clarifiers, and lift stations; static pressure testing of sewer lines; and visual inspections of the storm drain system. The static testing indicated no failures in the sewer system; however, the Port of Los Angeles moved forward with repair of several items identified by the visual inspections. These items were completed by March 30, 2018 (with the exception of the storm drain diversion valves, which were replaced by June 30, 2018). The repairs included:

- Internal patch repairs to sewer pipes
- Replacing 300 feet of cast iron pipe with ABS piping
- Minor patches to inlet and outlet pipes in clarifiers
- Replacing valves in the storm drain diversion

These repairs are in addition to the numerous infrastructure repairs completed as part of the TMDL starting as early as 2004 (DMJM Harris 2006, SCCWRP 2016a). Additional repairs identified during the inspection program, but yet to be completed include replacing another cast iron pipe and repair/replacement of the central lift station.

The goal for this portion of the study was to resample ICB at the sites and times when the HF183 human-specific marker was most prevalent during the 2016 summer survey. Theoretically, if the sewage infrastructure was the source HF183 at the beach, then resampling in 2018 would return little to no detectable HF183 human-specific marker. If similar levels were found between 2018 and 2016, then the repaired sewage infrastructure was not the source of HF183 observed in the beach waters.

Methods

The study design mimicked the design in 2016 with the following exceptions: 1) 2018 focused on a subset of sites with the highest HF183 concentrations in 2016; 2) 2018 focused around spring tides when the highest HF183 concentrations occurred in 2016; and 3) 2018 focused on *Enterococcus* and HF183 measurements, but did not include all of the non-human markers (i.e., bird) analyzed in 2016. Details can be found in the Sampling and Analysis Plan (SCCWRP 2018).

Field and Laboratory Methods

Between June 11 and August 29, 2018 grab water samples were collected once-per-day across a gradient of four sites along the beach and one site on Outer Cabrillo Beach (matching five of the seven sites sampled during 2016). Spring tide conditions were targeted with sampling occurring over six spring tide events. Samples were collected on seven consecutive days per event, the day with the greatest spring tide, plus three days on either side. Water samples were collected between 6:30 and 8:00 AM and transported to SCCWRP for FIB analysis and filtered for bacterial DNA. Sites sampled are presented in Figure 20, excluding Sites ICB2 and ICB4, which were not re-sampled in 2018.



Figure 20. Map of Inner and Outer Cabrillo beach indicating sampling locations at ICB. All sites were sampled in 2016; all sites except ICB2 and ICB4 were sampled again in 2018.

Summer 2018 sampling was designed to only sample spring tide conditions. Spring tide conditions were defined by finding the largest tidal ranges ($T_{\max}-T_{\min}$) and then sampling a 7-day window surrounding that event (± 3.5 days).

For data analysis, 2016 results were divided into spring tide and non-spring tide conditions based on the spring tide sampling design from 2018. Summer 2018 sampling targeted spring tide conditions only, but 2016 was sampled in both spring tide and non-spring tide conditions. To assess if spring tide conditions were comparable between the two years, tidal height at the time of sampling was compared between the two years (Figure 21). There was no significant difference between average tidal height during spring tide sampling for 2016 vs 2018 ($p > 0.2$). Tidal height at the time of sampling during 2016 non-spring tide conditions was significantly lower than spring tide sampling height ($p < 0.05$).

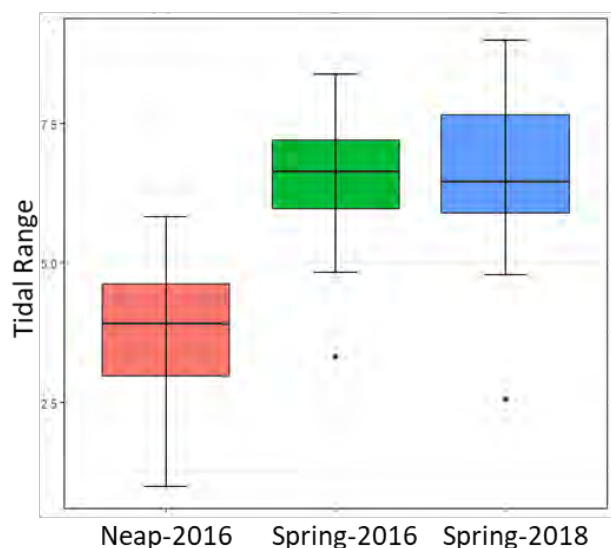


Figure 21. Comparison between tidal range for classified Spring and Neap tide events summer 2016 and summer 2018.

Results

A total of 210 water samples were collected across the 5 study sites over six spring tide events (Table 8). 100% of samples were successfully collected. 100% of water samples were successfully processed and analyzed for DNA. There were 5 samples (2%) which did not meet QA/QC objectives for holding times or incubation temperatures for the IDEXX kits. These five samples were excluded from further analysis. All samples met QA/QC requirements for DNA analysis.

Comparison Between Summer 2016 and Summer 2018

The exceedance rate of water quality objectives for *Enterococcus* during spring tides in 2018 was slightly greater, but not significantly greater, than the rate of *Enterococcus* exceedances during spring tides in 2016 (Table 9). The average increase in exceedance rate across all four inner beach sites was 17%. Non-spring tide exceedance rates for *Enterococcus* water quality objectives in 2016 were generally lower, but not significantly lower, than exceedance rates during spring tide conditions in 2016.

Spatial patterns of *Enterococcus* concentrations were consistent during spring tides in 2016 versus 2018 (Figure 22). *Enterococcus* concentrations were greater at sites 3, 5, and 6. Lower concentrations were observed at sites 1 and 7. The differences in mean concentrations between these two groups of sites during spring tide were significantly different regardless of year (all p-values < 0.05). The differences in mean concentrations between individual sites during spring tide conditions in 2016 vs 2018 were not significantly different (all p-values > 0.2).

The HF183 human-specific marker did not display a strong spatial pattern in concentration during spring tides for either 2016 or 2018 (Figure 23). No significant differences in HF183 concentrations were observed between sites during either summer 2016 or summer 2018 (p > 0.2). Likewise, no significant differences in HF183 concentrations were observed between years at any single site (p > 0.2).

No substantial difference in the spatial pattern or frequency of detection was observed at ICB sites in 2018 compared to 2016 (Table 9, Figure 23). The HF183 human-specific marker was detected in the majority of samples during spring tides in 2016 and 2018 (Table 9). HF183 was detected in 62% of spring tide samples collected across all inner beach sites during 2016 and 53% of the spring tide samples collected across the inner beach sites during 2018. Despite the high frequency of detection, the concentrations were rather low; 19% and 28% of the samples detected HF183 above the limit of quantification (HF Quantifiable > ~40 gene copies/100 ml) during 2016 and 2018 spring tides, respectively.

Table 8. Percent sampling and processing success from the Source Tracking Study. N₂₀₁₆=68 per site; N₂₀₁₈=42 per site; NA=no samples collected.

Site	Fecal Indicator Bacteria		HF183 Human Marker	
	2016	2018	2016	2018
ICB 1	99%	98%	100%	100%
ICB 2	99%	NA	100%	NA
ICB 3 (CB02)	99%	98%	100%	100%
ICB 4	99%	NA	100%	NA
ICB 5	99%	98%	100%	100%
ICB 6	99%	98%	100%	100%
ICB 7 (SDS7)	99%	98%	100%	100%

Table 9. Percent of days with water samples exceeding *Enterococcus* water quality objectives at Cabrillo Beach in 2018 compared to 2016. Samples divided into spring and non-spring tidal conditions. N_{2016 Non-springTide}=35 per site; N_{2016 SpringTide}=33 per site; N_{2018SpringTide}=42 per site.

Site	% Days Exceeding <i>Enterococcus</i> Objectives (104 MPN per 100 mL)		
	2016 Non-spring tide	2016 Spring tide	2018 Spring tide
ICB 1	9%	6%	15%
ICB 3 (CB02)	23%	30%	44%
ICB 5	17%	30%	44%
ICB 6	0%	9%	39%
All Inner Beach	12%	19%	36%
7 (Outer)	0%	0%	0%

Table 10. Percent of water samples with genetic markers detected by digital PCR at Cabrillo Beach. N_{2016 Non-springTide}=35 per site; N_{2016 SpringTide}=33 per site; N_{2018SpringTide}=42 per site.

Site	HF183 Human Marker Detection		
	2016 Non-Spring tide	2016 Spring tide	2018 Spring tide
ICB 1	51%	73%	62%
ICB 3 (CB02)	51%	45%	52%
ICB 5	49%	55%	43%
ICB 6	60%	76%	55%
All Inner Beach	53%	62%	53%
ICB 7 (Outer Beach)	23%	27%	40%

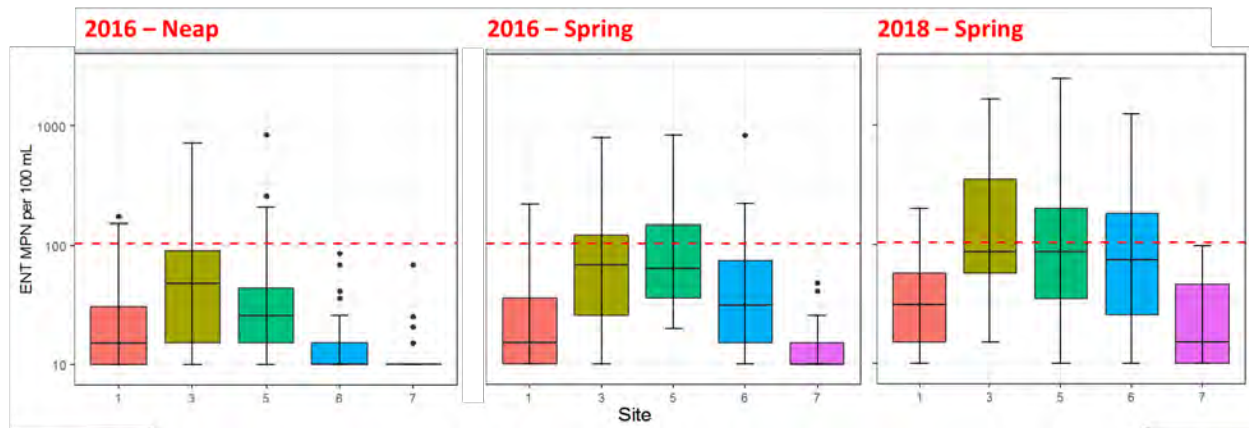


Figure 21. Concentrations of *Enterococcus* during different tidal conditions at each site at ICB. See table 10 for sample size.

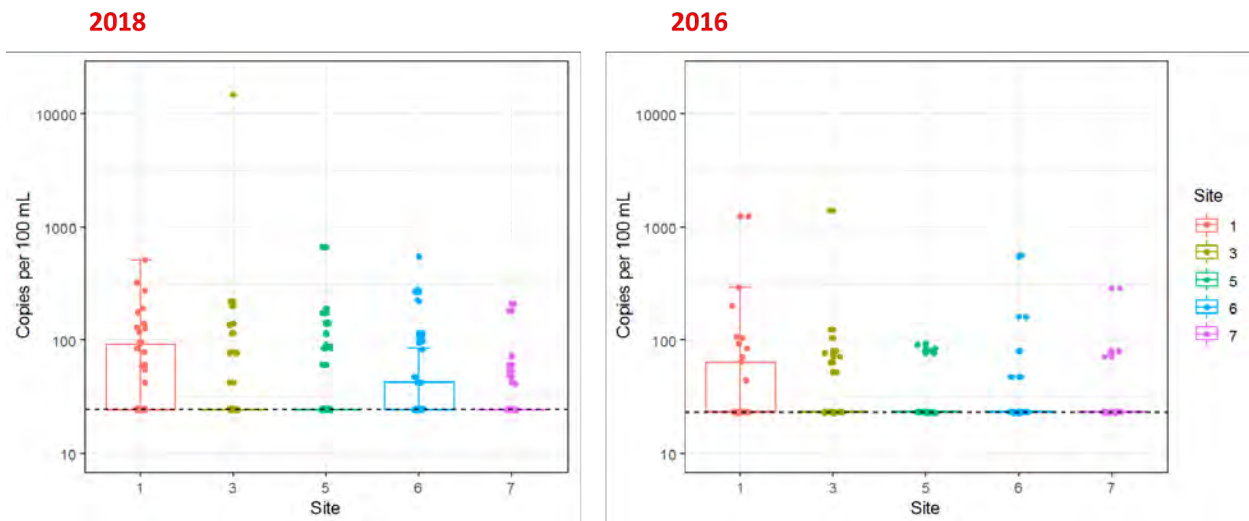


Figure 22. Box plots of HF183 human-specific marker concentrations at ICB during spring tides in 2016 versus 2018. For site locations see Figure 20

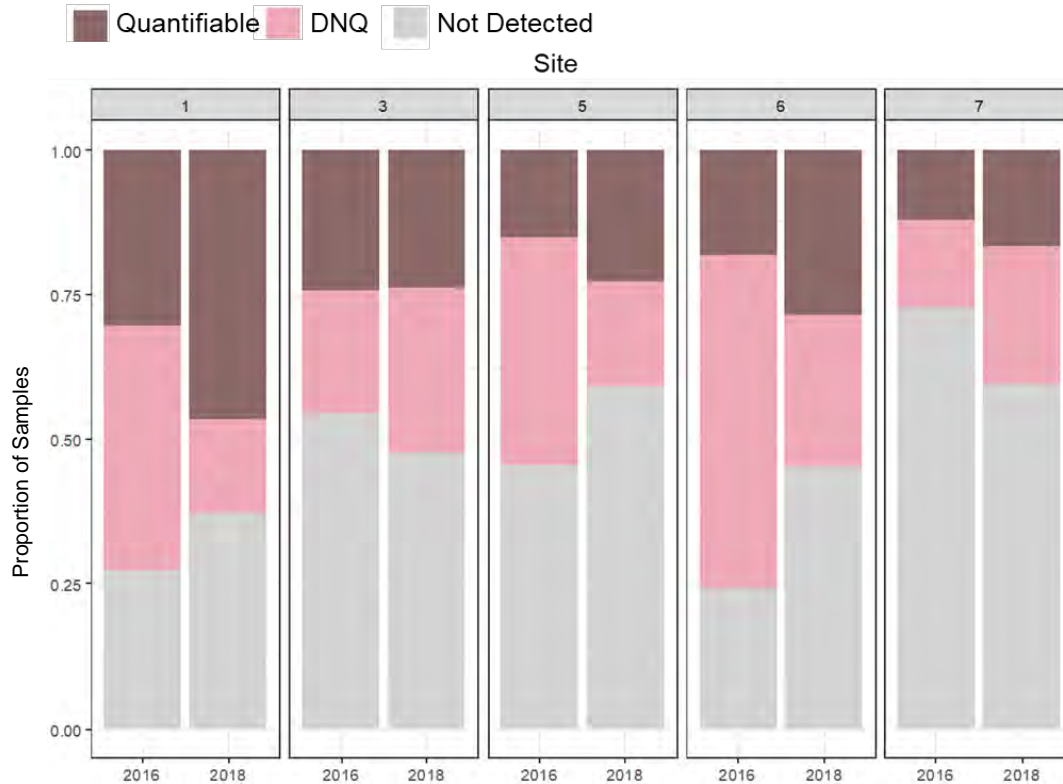


Figure 23. Proportion of human-specific genetic marker (HF183) in daily water samples at each site. Detection categories are the quantifiable range (Quantifiable), below the limit of quantification (DNQ), and non-detects (Not Detected).

Discussion

The overall concentrations, distribution among sites, and detection frequencies of HF183 human specific marker were generally comparable between 2016 and 2018. Interestingly, this was not true for *Enterococcus*, which had significant spatial patterns and water quality exceedance rates both between sites and between years. This adds to the evidence that the *Enterococcus* and HF183 human-specific marker arise from different sources. In both studies, the low level, diffuse human signal suggests a distributed rather than a concentrated source.

The lack of change in HF183 detection frequencies and concentrations indicates that the inspections and repairs performed by the Port of Los Angeles to their sewage infrastructure at ICB did not eliminate the human fecal source to ICB. It is possible that sewage infrastructure is not a source, not all necessary repairs were made, insufficient time was given for any contamination remaining at ICB to dissipate, or other sources are present at ICB.

We do not think the lack of change observed at ICB was an artifact in sampling or analysis because the same laboratory, analytical methods and instruments, and staff were used in 2016 and 2018. Moreover, the exceedance rates for *Enterococcus* during 2016 and 2018 were comparable to exceedance rates during similar months observed in previous years.

Conclusions

The final conclusion from the confirmation study was:

- **There was little difference in HF183 human-specific marker concentrations during spring tides in 2016 compared to 2018, indicating little change in the human source(s) to ICB.**

The HF183 human-specific markers were detected in 52% and 50% of the 602 and 210 water samples collected at Cabrillo Beach during summer of 2016 and 2018, respectively. However, approximately half of these detectable samples were below the limit of quantification. Although the Port of Los Angeles inspected and repaired the sewage infrastructure at ICB, HF183 human-specific marker concentrations were not statistically different between sites or between years, indicating the repaired sewage infrastructure was not the source of HF183 observed in the beach waters.

FINAL QMRA DETERMINATION AND NEXT STEPS

Because there are still low but persistent levels of HF183 at ICB that has yet to be identified, implementing a comprehensive, precedent-setting QMRA is problematic. Without knowing the origin of the source(s) of HF183 human-specific genetic marker, scientists are unable to assess what pathogens might be present at ICB that could lead to illness. The final determination at ICB was that a comprehensive QMRA could not be completed with the existing information.

Among the potential next steps for moving towards a QMRA at ICB, there are two that the Advisory Committee discussed at length identifying the advantages and disadvantages:

2) *Quantify groundwater for human fecal contamination.*

One potential origin of human fecal contamination at ICB is conveyance through groundwater that surfaces at or just offshore ICB. Groundwater is targeted because there are no other surface water discharges at ICB and the harbor survey did not indicate a strong gradient of HF183 human-specific genetic marker impacting ICB from offshore. Specifically, the goal of measuring human fecal contamination in groundwater is to quantify pathogen loading, a critical step in the QMRA process.

One advantage of measuring groundwater for pathogen loading is the ability to measure inputs to ICB before mixing and diluting with beach receiving waters. If groundwater is the primary conveyance of human fecal contamination, then both HF183 human-genetic marker and difficult-to-detect pathogens should be more concentrated than after mixing in receiving waters. This concentrated discharge enhances the opportunity to quantify pathogens for loading estimates. The approach of looking closer to sources for pathogen loading is consistent with the QMRA process identified by the USEPA.

Although the technology exists for sampling and measuring groundwater, there are also unique challenges to measuring groundwater for pathogen loading. For example, providing sufficient measurements that this conveyance is well-quantified for pathogen concentrations and groundwater flow will likely require substantial effort and will be fiscally burdensome.

The potential outcomes from measuring groundwater are four-fold based on the presence or absence of pathogens in groundwater and resulting human health risk assessment (table 11). Two of the outcomes support pursuing site-specific objectives when the QMRA indicates human health risk is acceptable. Two of the outcomes support additional source tracking when the human health risk is unacceptable or cannot be estimated. The Advisory Committee did not agree upon acceptable risk levels, but there was discussion that the presence of human fecal contamination did not automatically rule out site-specific objectives if the risk was acceptably low.

If the pathogen loading from groundwater is the selected option, then it could be accomplished in a two-step process. First, HF183 and pathogens should be measured in multiple groundwater wells and at multiple depths per well, at ICB. More than a single sampling event is recommended. If HF183 and pathogens are found, then groundwater

flow into nearshore beach waters will be required to complete pathogen loading for risk modeling.

Table 11. If HF183 human-specific genetic marker at ICB persists, there are four potential outcomes from a groundwater investigation for pathogen loading to support Quantitative Microbial Risk Assessment (QMRA).

	Resulting risk acceptable	Resulting risk unacceptable or cannot be estimated
Pathogens detected in groundwater	QMRA would be used to set site-specific objectives	QMRA would be used to justify source tracking in groundwater to identify human contamination source(s)
Pathogens not detected in groundwater	QMRA would be used to set site-specific objectives	No QMRA and initiate source tracking to identify distant human contamination source(s) through harbor transport

3) *Measure the beach water for pathogens using low level detection limits.*

A second option to pursue is to forego pathogen loading altogether and measure pathogens in (REC-1) beach water. This greatly simplifies the QMRA process providing a direct measure of swimmer exposure.

The advantage of direct pathogen measurement means foregoing measuring all sources or conveyances, both human and non-human. This option also removes uncertainty associated with estimating fate and transport once sources or conveyances enter the receiving water, which is sometimes a challenge when considering swimmer exposure. If low detection limits could be achieved, then this would help not just ICB, but all other beaches considering QMRA.

The disadvantage of direct pathogen measurement in marine receiving waters is that current methodology is not capable of quantifying many pathogens at levels sufficiently low enough to estimate risk; pathogen concentrations below detection limits could still result in substantial swimmer illness. This is particularly true for highly infective viral pathogens such as norovirus, one of the most common etiological agents of swimming-related gastrointestinal illness.

New technology such as ddPCR provides the opportunity to start testing new low level viral detection methods. However, selecting this option will require research to develop and validate the method before it could be used as a QMRA tool. The research will need to include both positive and negative controls for both DNA and RNA viruses along each step of the laboratory process using spiked material of known concentration in both clean and native matrices. In addition to the research necessary to develop and validate the method, a receiving water monitoring program will need to be developed for applying the new method and assess swimmer exposure. Knowing that low concentrations will be targeted means that many samples will likely be necessary for estimating swimmer exposure with satisfactory levels of confidence.

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APPENDIX A – LABORATORY METHODS

Cultivable FIB

To measure cultivable FIB in water, sand, and eelgrass, total coliform bacteria and *E. coli* were analyzed using IDEXX Colilert-18 kits, and *Enterococci* were analyzed using the Quantitray 2000™ system (IDEXX, Westbrook, ME), as per the manufacturer's instructions, with two dilutions covering a 10,000-fold range of concentrations for each sample. Equipment blanks were collected and tested for FIB contamination in the same manner as regular samples. Laboratory blanks were performed using sterile phosphate buffered saline solution.

Filtration and Extraction of DNA

Briefly, 100 ml of water was filtered in triplicate on a vacuum manifold through 47 mm diameter, 0.4 µm polycarbonate filters (Millipore Type HTTP, Millipore, Bedford, MA) to capture bacterial DNA. The filters were folded and placed into microcentrifuge tubes. When collected, wet sand (40 g) and eelgrass wrack (10 g) were measured out and shaken with filtered, autoclaved phosphate buffered saline for a final concentration of wash from 10 g sand and 5 g eelgrass per 100 ml. This wash was filtered onto a 0.4 µm polycarbonate filter for DNA, flash frozen in liquid N₂, and stored at -80° C. A filter blank was also collected for every sampling event as follows: autoclaved PBS solution was filtered, flash frozen in liquid nitrogen and stored at -80° C until extraction.

DNA was extracted using GeneRite kits according to the protocol outlined in the State Source Tracking Manual (Griffith et al. 2013) using commercial kits (DNA EZ ST1, GeneRite, Mammoth Junction, NJ, USA). Halophile DNA was added to the lysis buffer prior to extraction as an external extraction and inhibition control. Negative Extraction Controls (NEC) containing only lysis buffer and Halophile DNA were processed for every extraction in the same manner as the samples.

MST Marker Assays

Genetic markers for *Enterococcus* (EnterolA) and human-associated *Bacteroidetes* (HF183) were measured using a duplex digital PCR assay following a previously published protocol (Cao et al. 2015). Gull-associated *Catellibococcus* spp. (LeeSeaGull: Lee et al. 2013) was also quantified in 2016 samples only. All genetic markers were quantified using digital PCR. The limit of quantification for the digital PCR assays was approximately 50 gene copies/100 ml (46 copies/100 ml: 2016 and 49 copies/100 ml: 2018), or a minimum of 3 positive droplets above the baseline threshold. Results meeting this threshold were designated quantifiable. Assays with one or two positive droplets above baseline were designated below the limit of quantification (DNQ). For data analysis, these samples were assigned a value of 0.5*LOQ (approximately 24 copies per 100 ml). Assays with 0 positive droplets above baseline were designated as non-detects.

Quality Assurance

Field and equipment blanks detected no microbial targets. All laboratory blanks did not detect microbial targets and all duplicate samples met the data quality objective of < 25% reproducible percent difference. For MST markers, all non-template controls contained zero positive droplets.

APPENDIX B: SPLIT SAMPLE ANALYSIS WITH CITY OF LOS ANGELES ENVIRONMENTAL MONITORING DIVISION FOR FECAL INDICATOR BACTERIA

In order to assess the comparability of *Enterococcus* results between the City of Los Angeles and SCCWRP, split samples were collected at five different sites from Inner Cabrillo Beach on four different days between August 30 and September 3, 2015. From these 20 split samples, with each laboratory using IDEXX methods, *Enterococcus* concentrations were significantly correlated (Figure B1, $r^2=0.42$). This relationship is consistent with blind split sample intercalibrations amongst multiple southern California laboratories using IDEXX. Noble et al. (2003) found that 50% of the variance in *Enterococcus* measurements from multiple laboratories could be attributed to interlaboratory variation. The remaining 50% variance was due to within laboratory variation and, on average, laboratories were within 0.5 log units when analyzing split samples. The average difference between City:SCCWRP split samples was 0.3 log units, although SCCWRP results were biased high relative to City results ($y= 1.6x + 15.7$).

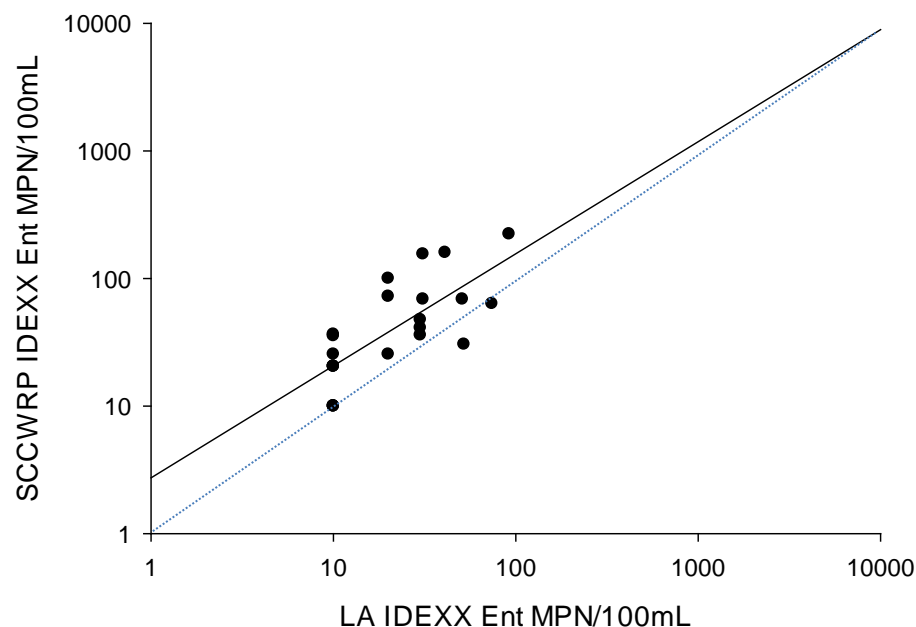


Figure B1. Split sample results between the routine monitoring laboratory at Inner Cabrillo Beach (City of Los Angeles) and the source tracking laboratory used in this study (SCCWRP). Solid line is the linear regression. Dotted line is the 1:1 line.

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APPENDIX C: RAW DATA RESULTS FROM THE HARBOR SCREENING SURVEY

Table C1. Human-specific marker concentrations (gene copies per 100ml) from samples collected during the 2017 spatial survey. Human-specific marker was run on split samples by SCCWRP (ddPCR), Weston (qPCR), and UNC (ddPCR). DNQ is below limit of quantification, BLOD is below limit of detection, ND is not detected.

Station	Water Column Location	May 25, 2017- Spring Tide			June 2, 2017- Neap Tide		
		SCCWRP	Weston	UNC	SCCWRP	Weston	UNC
HW07	Surface	ND	BLOD	ND	ND	BLOD	ND
	Bottom	192	486	140	ND	BLOD	ND
HW16	Surface	ND	DNQ	ND	ND	BLOD	ND
	Bottom	320	905	240	ND	ND	ND
HW20	Surface	ND	BLOD	ND	ND	ND	ND
	Bottom	ND	DNQ	ND	ND	BLOD	ND
HW23	Surface	ND	DNQ	ND	216	1,121	285
	Bottom	ND	DNQ	ND	ND	ND	ND
HW24	Surface	108	429	130	144	273	160
	Bottom	268	979	190	ND	ND	ND
HW29	Surface	156	1,166	85	ND	BLOD	ND
	Bottom	508	2,106	385	132	BLOD	ND
HW33	Surface	ND	BLOD	ND	184	729	130
	Bottom	80	456	ND	ND	BLOD	ND
HW44	Surface	ND	BLOD	ND	104	231	90
	Bottom	220	637	415	ND	BLOD	ND
HW49	Surface	ND	DNQ	135	ND	BLOD	ND
	Bottom	52	DNQ	ND	ND	ND	ND
HW50	Surface	ND	BLOD	ND	ND	BLOD	ND
	Bottom	ND	BLOD	ND	ND	ND	ND
HW53	Surface	ND	DNQ	ND	ND	BLOD	ND
	Bottom	132	DNQ	ND	ND	ND	ND
HW54	Surface	ND	ND	ND	ND	BLOD	ND
	Bottom	144	512	115	ND	ND	ND
HW56	Surface	52	DNQ	ND	ND	BLOD	ND
	Bottom	116	DNQ	ND	ND	ND	ND
HW62	Surface	ND	BLOD	ND	ND	ND	ND
	Bottom	68	DNQ	ND	ND	ND	ND
HW64	Surface	ND	ND	ND	ND	ND	ND
	Bottom	184	723	175	ND	BLOD	ND
HW101	Surface	ND	DNQ	ND	ND	BLOD	ND
	Bottom	208	721	335	ND	ND	ND
HW102	Surface	ND	362	85	ND	BLOD	ND
	Bottom	344	1,023	315	ND	BLOD	ND
HW103	Surface	132	DNQ	ND	ND	ND	ND
	Bottom	260	937	380	ND	BLOD	ND
CB01	Surface	288	588	390	ND	ND	ND
CB02	Surface	560	1,193	425	ND	ND	ND
SDS7	Surface	192	566	ND	ND	BLOD	ND
TIWRP	Effluent	6400	21,617	6,650	21,360	77,603	24,000

SCCWRP ND is < 50 copies per 100 ml

UNC ND is < 85 copies per 100 ml

Weston BLOQ was < 353 copies per 100ml, DNQ was 95-353 copies per 100 ml, ND < 95 copies per 100 ml

APPENDIX D: GRANT TASK DESCRIPTIONS AND BUDGETING

Background

The goal of this project was to conduct a Quantitative Microbial Risk Assessment (QMRA), a recreational water contact health risk model, for setting dry weather site-specific water quality objectives contaminated by non-human sources of fecal contamination. However, a dry weather QMRAs has not previously been conducted at a beach in California for setting site-specific objectives. The aim was to establish a case study precedent for technical QMRA implementation for use at other beaches in California. This would ensure a sufficiently rigorous technical foundation was built to support policy discussions and decision making for creating fecal indicator bacteria site-specific water quality objectives or total maximum daily load (TMDL) numeric targets based on quantified swimmer health risk.

Project Description

This QMRA project was comprised of five basic technical steps:

- 6) Beach selection
- 7) Source identification
- 8) Pathogen loading
- 9) Risk assessment
- 10) Sensitivity analysis

Summary of Work Completed

A summary table of the tasks and work completed is as follows:

ITEM	DESCRIPTION	CRITICAL DUE DATE	ESTIMATED DUE DATE	COMPLETION DATE	PERCENT COMPLETION
EXHIBIT A – SCOPE OF WORK – WORK TO BE PERFORMED BY THE GRANTEE					
A.	PLANS AND GENERAL COMPLIANCE REQUIREMENTS				
1.	GPS information for Project site and monitoring locations	Day 90	August 1, 2015 November 1, 2015	October 5, 2015	100%
2.	Monitoring and Reporting Plan				100%
2.1	Project Assessment and Evaluation Plan (PAEP)	Day 90	August 1, 2015 November 1, 2015	October 5, 2015	100%
2.2	Monitoring Plan (MP)	Day 90	August 1, 2015	March 31, 2016	100%

ITEM	DESCRIPTION	CRITICAL DUE DATE	ESTIMATED DUE DATE	COMPLETION DATE	PERCENT COMPLETION
EXHIBIT A – SCOPE OF WORK – WORK TO BE PERFORMED BY THE GRANTEE					
			May 1, 2016		
2.3	Quality Assurance Project Plan (QAPP)	Day 90	August 1, 2015 May 1, 2016	March 31, 2016	100%
2.4	Proof of Water Quality Data Submission to CEDEN	Before Final Invoice		March 31, 2019	100%
3.	Copy of final CEQA/NEPA Documentation	Day 30	June 1, 2015 November 1, 2015	October 5, 2015	100%
7.	Public Agency Approvals, Entitlements or Permits		As needed		NA
B.	PROJECT-SPECIFIC REQUIREMENTS				
1.	Project Management				
1.2	Meeting, Workshop, and Training notification		As needed		100%
2.	Advisory Panel				
2.1	Final List and their roles and responsibilities	June 30, 2015 November 1, 2015		October 5, 2015 Updated Jan 20, 2016	100%
2.2	Agendas, Meeting minutes, and Sign in lists		As needed	March 31, 2019	100%
3.	Beach confirmation				
3.2	Water quality monitoring plan for any limited monitoring		July 2015 April 1, 2016	March 31, 2016	100%
3.4	Summary of results and conclusions	December 31, 2015 August 1, 2016		March 31, 2016	100%
4.	Watershed characterization and hypothesis formation				
4.2	Hypothesis and MST sampling and analysis plan	March 31, 2016 May 1, 2016		April 6, 2016	100%
5.	MST				
5.2	Source identification report		December 2016 March 31, 2017	March 31, 2017	100%
6.	Pathogen load				
6.1	Pathogen load sampling and analysis plan	March 31, 2017		March 31, 2017	100%
6.3	Pathogen load technical memo		December 2017 December 2018		100%
7	Risk modeling				

ITEM	DESCRIPTION	CRITICAL DUE DATE	ESTIMATED DUE DATE	COMPLETION DATE	PERCENT COMPLETION
EXHIBIT A – SCOPE OF WORK – WORK TO BE PERFORMED BY THE GRANTEE					
7.3	Risk modeling technical report		December 2018	N/A	20%
8.	Sensitivity analysis				
8.2	Sensitivity analysis technical report		December 2018	N/A	20%
9	Beach water quality workshop meeting				
9.1	Presentation material and summary of feedback		As needed	March 31, 2019	100%
EXHIBIT B – INVOICING, BUDGET DETAIL, AND REPORTING PROVISIONS					
A.	INVOICING		As needed		
G.	REPORTS				
1.	Progress Reports within forty-five (45) days following the end of the calendar quarter (March, June, September, and December)		Quarterly	April 10, 2019	100%
2.	Annual Progress Summaries		Annually by 9/30	October 20, 2018	100%
3.	Natural Resource Projects Inventory (NRPI) Survey Form	Before Final Invoice		NA	NA
4.	Draft Project Report	January 31, 2019		January 31, 2019	100%
5.	Final Project Report	March 31, 2019		March 31, 2019	100%
6.	Final Project Summary	Before Final Invoice		March 31, 2019	100%
7.	Final Project Inspection and Certification	Before Final Invoice		March 31, 2019	100%

Task Completion Narrative

In December 2012, the US EPA promulgated new national beach water quality criteria based solely on the fecal indicator bacteria *Enterococcus*. Part of this criteria included an option for creating site-specific objectives when little to no human sources of *Enterococcus* exist using a Quantitative Microbial Risk Assessment (QMRA). However, a QMRA has not been conducted at a marine beach in California, or any marine beach nationwide, for the purpose of site-specific objectives. The goal of this project is to evaluate EPA's QMRA framework at a marine beach in California and assess its applicability for regulatory-based management decision making, including its potential for site-specific objectives or natural source exclusion.

The project was contractually delayed and, after adjusting scheduling to begin sampling in summer 2016, SCCWRP focused the remaining 2015 effort on compiling and reviewing historical information and data. This compilation achieved several project milestones:

- Creating a beach confirmation report suggesting to the Advisory Committee that sufficient historical information existed such that preliminary sampling was not necessary to confirm that Inner Cabrillo Beach was an appropriate beach for a QMRA
- Creating a Hypothesis and MST Sampling and Analysis Plan
- Holding an Advisory Committee meeting to review and approve the Beach Confirmation Report and the Sampling and Analysis Plan
- Creating a Quality Assurance Project Plan for the Sampling and Analysis Plan

During Calendar year 2016, SCCWRP completed the summer MST sampling campaign to identify sources of *Enterococcus*. More than 600 samples were collected and analyzed according to the Sampling and Analysis Plan and Quality Assurance Project Plan. This data was compiled, analyzed, and presented to the project Advisory Committee in November 2016.

During Calendar year 2017, SCCWRP completed the Source Identification Report, completed the Sampling and Analysis Plan (SAP), completed the sampling and analysis detailed in the SAP. In fall, the project stalled awaiting the Port of Los Angeles (POLA) to complete its inspection and repairs of beach infrastructure. POLA found numerous infrastructure repair needs and estimated at least one year to complete these repairs. The Advisory Committee recommended to delay the pathogen loading from beach sources until the repairs are completed, significantly delaying the product for task 6.3.

During Calendar year 2018, SCCWRP held three Advisory Committee meetings: an in-person meeting March 14th, a conference call April 18th, and a second in person meeting October 30th. The goal of the first two meetings was to confirm the pathogen loading SAP for summer 2018. Based on these meetings, the Advisory Committee recommended repeating the summer sampling from 2016. This repeated monitoring confirmed if repairs conducted by the Port of Los Angeles were effective at reducing HF183 concentration and frequency of detection in beach receiving waters. To support the summer 2018 monitoring campaign, SCCWRP drafted a Sampling and Analysis Plan, which was reviewed and approved by the Advisory Committee and Contract Manager in June 2018.

The Advisory Committee met to review results from the summer sampling campaign on October 30, 2018. The results from 2016 and 2018 were similar, indicating that the repairs the Port pursued did not remediate the source of human fecal pollution at Inner Cabrillo Beach. After discussing the next steps in the QMRA – final source identification and pathogen loading from that source, along with associated risk modeling and sensitivity analysis – the Advisory Committee recommended to end the grant funded portion of the project if additional funding from the SWRCB did not materialize. SCCWRP was directed to complete any outstanding activities associated with the sampling campaign and prepare the project draft final report. This action was confirmed with SWRCB staff at the Division of Financial Assistance and the Los Angeles RWQCB via phone call on December 18, 2018. Explicitly discussed was acknowledgement that the risk modeling and sensitivity analysis (Tasks 7 and 8) will not be completed.

SCCWRP completed the draft Final Report and distributed it to the Advisory Committee for Review. This review occurred on February 26, 2019. SCCWRP received both written and oral comment and made these changes to the Final Report. SCCWRP completed its presentation to

the Beach Water Quality Work Group on February 27, 2019. SCCWRP submitted project monitoring data to the California Environmental Data Exchange (CEDEN). Finally, SCCWRP completed the required project summary and project inspection and certification letter.

Project Costs

This Project did not use all of the grant funds. Because human sources were not remediated and QMRA modeling was not finalized, only \$1,264,615 of the total grant \$1,557,224 was expended (81.2%). These funds were to be spent completing Tasks 6, 7, and 8.

Project costs were allocated as follows:

Item	Grant Allotment	Total Expenditures	% of Line Item Spent
Direct project administration	\$398,146	\$397,197	99.9%
Planning/Design/Engineering/Environmental	\$324,650	\$309,004	95.2%
Monitoring and performance	\$788,898	\$525,212	66.6%
Education/Outreach	\$45,530	\$32,482	71.3%
TOTALS	\$1,557,224	\$1,264,615	81.2%