

Lovers Point Beach Pilot Microbial Source Tracking Study

Conducted by Stanford University¹
as part of the Source Identification Protocol Project (SIPP)

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Disclosure statement

Funding for this project has been provided in full or in part through an agreement with the State Water Resources Control Board. The contents of this document do not necessarily reflect the views and policies of the State Water Resources Control Board, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

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Background

Lovers Point Beach (LP) was chosen for a small, pilot microbial source tracking study site as part of SIPP due to the results of reconnaissance sampling that showed the highest levels of human marker of SIPP reconnaissance beaches sampled in the Bay Area. Additionally, previous sampling at LP indicated it has elevated fecal indicator bacteria (FIB) concentrations.

LP is located in Pacific Grove, Monterey, CA (Lat: 36.625204, Long: -121.916613). The beach is located adjacent to a bluff and a jetty (Figure 1). It is sheltered from most waves and thus is believed to have low circulation. It is popular among families with small children, divers, and kayakers. A small business rents dive and kayaking equipment daily in the summer. There are marine mammals that haul out near and adjacent to the beach. There is a storm drain that discharges to the beach on the south side of the cliffs (Figure 1). During the dry season, flow from the storm drain is diverted to the sanitary sewer through an urban runoff diversion system. During the wet season, the storm drain flows to the beach.

Several characteristics of LP are similar to other California beaches, and thus it is hoped that the approaches used in the present study may inform microbial source tracking efforts in other locales. These characteristics include: popularity among families; limited surf zone circulation near a jetty or breakwater; storm drain inputs containing urban runoff in wet weather; large populations of marine mammals; and aging sewer infrastructure.

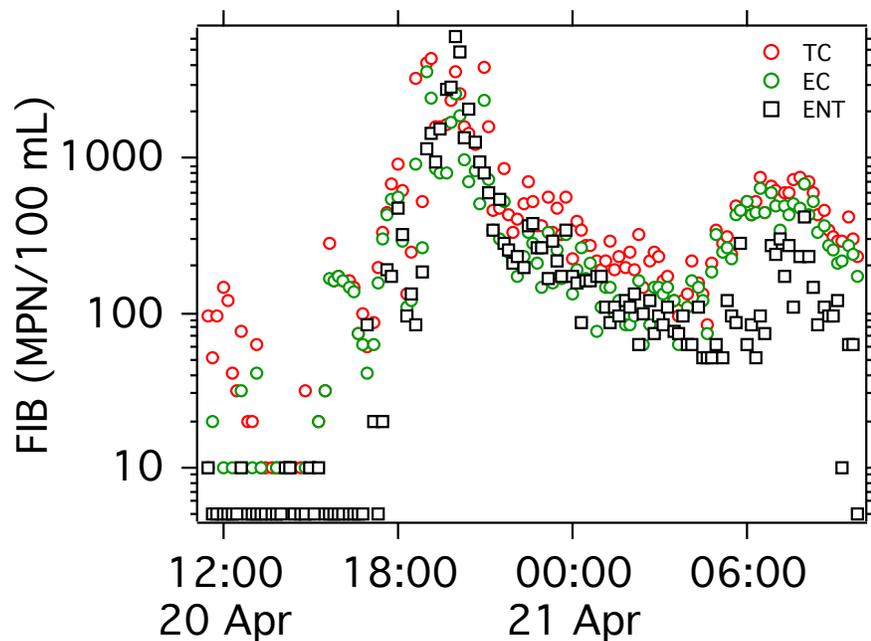


Figure 1. A portion of the map of sewage and storm drain infrastructure at Lovers Point provided by the City of Pacific Grove. The beach is located in the sheltered cove at the red circle. The storm drain is adjacent to the blue square.

Previous Work

Stanford University students have been studying water quality at Lovers Point for nearly a decade and they have published several peer-reviewed papers on water quality at the beach. This work has been carried out by PhD students as well as students in Dr. Boehm's course "The California Coast: Science, Policy, and Law." Data from a recent sampling effort by her class is shown in Figure 2 and is representative of dry weather water quality conditions at the beach. Insights obtained from previously published work are summarized below:

1. Water quality is strongly affected by the tide level and sunlight (Yamahara et al. 2007¹, Boehm 2007²). FIB tend to be higher during high tide and lower during sunlit hours.
2. The sand at Lovers Point contains a population of enterococci that seem to be a source to the ocean during high tide and also appear to grow in the sand when it is wetted by tidal action (Yamahara et al. 2007¹, Yamahara et al. 2009³). Note that *E. coli* in sand here has not been studied.
3. The human marker in *Bacteroidales* (HF183) was detected in the storm drain discharge and in the sand in single samples taken on one day in 2007 (Yamahara et al. 2007¹).
4. A single sample of sand taken from Lovers Point in 2011 was found to be negative for *Salmonella* and *Campylobacter* (Yamahara et al. 2012⁴).



¹ K. M. Yamahara, B. A. Layton, A. E. Santoro, A. B. Boehm. 2007. Beach sands along the California coast are diffuse sources of fecal bacteria to coastal waters. *Environmental Science & Technology*, 41, 4515-4521.

² A. B. Boehm. 2007. Enterococci concentrations in diverse coastal environments exhibit extreme variability. *Environmental Science & Technology*, 41, 8227-8232

³ K. M. Yamahara, S. P. Walters, and A. B. Boehm. 2009. Growth of enterococci in unaltered, unseeded beach sands subjected to tidal wetting. *Applied and Environmental Microbiology*, 75, 1517-1524.

⁴ K. M. Yamahara, L. M. Sassoubre, K. D. Goodwin, A. B. Boehm. 2012. Occurrence and persistence of human pathogens and indicator organisms in beach sands along the California coast. *Applied and Environmental Microbiology*, 78, 1733-1745.

Figure 2. Total coliform (TC), *E. coli* (EC), and enterococci (ENT) at Lovers Point in April 2013. Samples were collected every 10 minutes for 24 hours as part of Boehm’s class “The California Coast: Science, Policy, and Law.”

In addition to the knowledge documented in these peer-reviewed studies, sampling and analysis were conducted at Lovers Point for human specific *Bacteroidales* (HF183Taqman) and FIB as part of the SIPP reconnaissance study. The goal of the reconnaissance study was to sample a source of FIB at 5+ beaches in the Bay area, as well as other beaches around the State to identify a beach for follow-up MST projects under SIPP. To this end, we took triplicate samples of water flowing from the storm drain of Lovers Point on three days in May 2011. During this period of time, discharge was not being diverted due to recent rain events. The *E. coli* and enterococci concentrations measured by IDEXX were high (Table 1). The measured concentration of human marker (Table 1) were the highest levels observed from samples collected at all the reconnaissance beaches in the Bay area.

Table 1. Summary of SIPP reconnaissance sampling results from the flowing drain at Lovers Point.

Sampling Date	mean ENT MPN/100 ml	mean EC MPN/100 ml	HF183Taqman copies/100 ml
3 May 2011	4301	22749	39000
9 May 2011	850	1581	27000
16 May 2011	557	582	10000

Potential Sources of Dry Weather Contamination

Given the previous work at LP, it is clear that the flowing storm drain is a source of contamination to the beach and the human marker results indicate storm drain discharge contains some sewage contamination. However, since 2003 the City has implemented a diversion system to eliminate dry weather discharges from the storm drain system during the months of April through October. A small trickle flows from the drain in the dry season, but it was tested and contains very low levels of FIB (non-detect or 10 MPN/100 mL). No malfunctions of the diversion at Lovers Point have been observed by city staff. Because the storm drain effluent is diverted in the dry season, it cannot explain the high concentrations of FIB that persist at the beach in dry weather. Interestingly, the concentration of enterococci appears to be higher at the beach since the diversion was put in place based on historical data collected by the County of Monterey during its AB411 sampling (Figure 3). Thus, the dry weather source of contamination remains unknown, although the sand seems to be a reservoir of enterococci that contributes enterococci to the nearshore during high tides¹. The origin of the enterococci in the sand remains unknown. The origin of the *E. coli* in the water at the beach has not been studied.

Having ruled out the storm drain, the remaining potential dry weather sources of fecal contamination to the beach include 1) contaminated sand, 2) contaminated groundwater, and 3) direct deposition of animal feces including seals and gulls. Because we know that sand contamination is prevalent at this beach, we should consider possible sources of contamination in the sand. The contamination in the sand could come from 1) direct deposition of feces on the sand, 2) growth of the fecal bacteria in sand³, and 3) accumulation from discharge from the storm drain in the wet season, or 4) accumulation from contaminated seawater. The potential connection between storm drain discharge and sand contamination suggests a potential connection between wet and dry season contamination.

Stakeholder Involvement

Monterey Bay Aquarium, Hopkins Marine Station, City of Pacific Grove, Monterey Bay National Marine Sanctuary, and the State Water Board participated in one stakeholder meeting on the design and

implementation of the pilot project. If additional work is conducted in the future, Monterey County should be engaged.

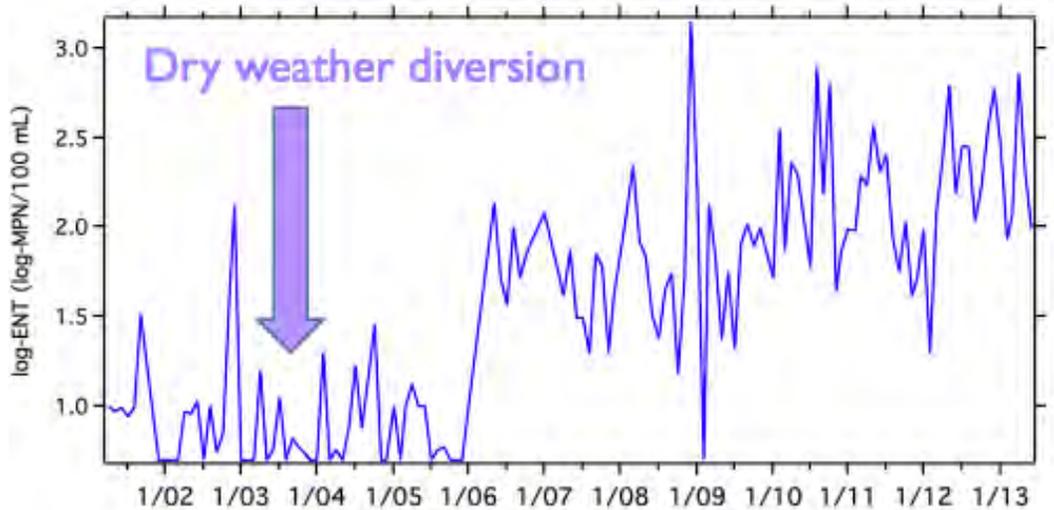


Figure 3. Monthly log-mean concentration of enterococci at Lovers Point as measured by the County of Monterey for their AB411 sampling. The time when the dry weather diversion was begun is shown with the purple arrow.

Hypotheses

The hypotheses that guided the data collection during the pilot MST study at Lovers Point are as follows:

- H1: Human and gull-specific fecal markers remain specific and sensitive in the presence of seal feces.
- H2: Sand at Lovers Point is contaminated with FIB, human fecal material, and bacterial pathogens (*Salmonella* and *Campylobacter*).
- H3: Groundwater at Lovers Point is contaminated with FIB and human fecal material.
- H4: FIB and bacterial pathogens in seawater are higher near the seal haul-out beach adjacent to Lovers Point.
- H5: Dry weather runoff in the storm sewer and discharge from the storm drain (while very low flow) contains human fecal material.

Project Approach

To test H1, seal feces were collected by personnel at Hopkins Marine Station and used to test the specificity of the human and gull-specific assays. This was necessary because there are many seals in the area. According to the SIPP MST manual, we need to consider potential cross reactivity of qPCR markers if the watershed contains fecal sources not tested in the SIPP method evaluation study. Therefore, this specificity testing was necessary before any pilot MST work could be conducted.

To test H2, sand samples were collected weekly for 8 weeks from 3 sites: Lovers Point, a beach to the north (called 'pristine') and a wildlife-dominated (seal) beach to the south near Hopkins Marine Station (Figure 4, Table 2). Sand samples (~ 20 g) were tested for *E. coli*, enterococci, human-specific Bacteroidales (HF183Taqman), and cultivatable *Campylobacter* and *Salmonella*.



Figure 4. Left panel. Map of beaches studied for the project during the 8 weeks of sampling. Right panel. Maps of sampling locations along the storm sewer that connects to the drain at Lovers Point. The storm sewer is diverted during the dry season.

Table 2. Sampling sites.

Site ID	Site Type	Latitude	Longitude	Site Description	Notes
C10	MS4	36° 37.340'N	121° 55.210'W	Pacific Grove storm drain	
C14	MS4	36°37'10.18"N	121°55'14.90"W	Pacific Grove storm drain	
CSD	MS4	36° 37.482'N	121° 55.027'W	Pacific Grove storm drain	
S2	MS4	36° 37.481'N	121° 55.119'W	Pacific Grove storm drain	
S5	MS4	36° 37.326'N	121° 55.213'W	Pacific Grove storm drain	
S6	MS4	36° 37.240'N	121° 55.253'W	Pacific Grove storm drain	
S7	MS4	36° 37.431'N	121° 55.164'W	Pacific Grove storm drain	
S9	MS4	36°37'10.18"N	121°55'14.90"W	Pacific Grove storm drain	
LP	Ocean, sand, groundwater, storm drain	36° 37.509'N	121° 54.984'W	Lovers Point Beach	also monitored by Monterey County
SB	Ocean, sand	36° 57.798'N	122° 1.591'W	Beach at Hopkins Marine Station used as marine mammal haul out site	only sampled when no animals present
NB	Ocean, sand	36° 57.798'N	122° 1.591'W	Beach to the north of LP	

To test H3, groundwater was sampled weekly from Lovers Point for 8 weeks. Each sample was assayed for enterococci, *E. coli*, total coliform, and HF183Taqman.

To test H4, seawater was collected from 4 sites weekly for 8 weeks: the north/pristine beach, the LP jetty, LP, and south/seal beach. Samples were tested for *E. coli*, enterococci, HF183Taqman, and cultivatable *Campylobacter* and *Salmonella*. For the pathogen assays, 500 ml of water was tested.

To test H5, samples were collected from the storm sewer leading to the drain at LP and analyzed for FIB and human-specific *Bacteroidales* (Figure 4, Table 2). In addition, the storm drain discharge (the small drip) at LP was sampled weekly for 8 weeks and tested for FIB, HF183 Taqman and cultivatable *Campylobacter* and *Salmonella*.

Project Outcomes

Extraction blanks, field blanks, and no template controls were all negative.

Regarding H1, we found that there is no cross reactivity between human fecal marker HF183Taqman and seal feces, which means this marker can be used with confidence in this marine mammal-dominated area. There is some cross reactivity between the LeeSeaGull assay and seal feces so care must be taken in interpreting data on concentrations of this marker at the site if it is used (Figure 5).

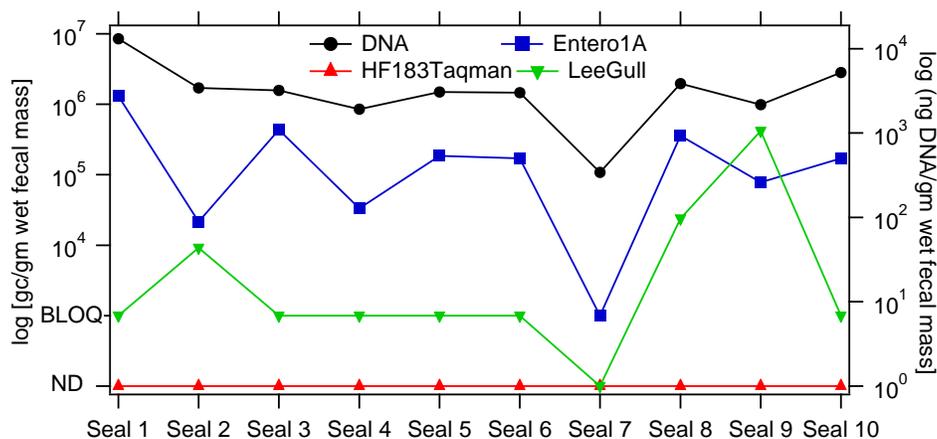


Figure 5. DNA and three qPCR assay concentration normalized to wet fecal mass

Regarding H2 (sand contamination), sand quality was generally good at Lovers Point during the study. Plots of the log-mean of the three FIB in the sand samples are shown in Figure 6. The human marker was detected in 0/8 sand samples at LP, 3/8 sand samples at North Beach, and 1/8 sand samples at Seal Beach. All detections were Below the Limit of Quantification (BLOQ). No *Campylobacter* or *Salmonella* was detected in the sand samples.

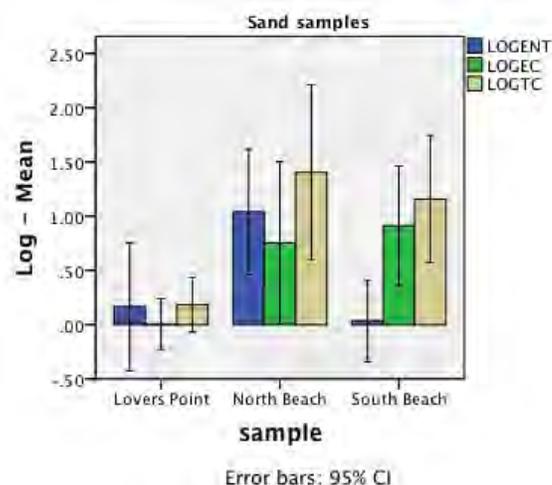


Figure 6. Log-mean concentrations of three FIB in sand during the study.

Regarding H3 (groundwater contamination), we found that groundwater samples from Lovers Point had low FIB concentrations (usually below or at the IDEXX detection limit of 10 MPN/100 mL). The human marker was detected in 1/8 groundwater samples at BLOQ.

Regarding H4 (influence of seals on seawater quality), water quality was generally good at Lovers Point during the study, while water quality at Seal Beach was generally poor. Plots of the log-mean of the three FIB in the ocean samples are shown in Figure 7. We sampled before the sun rose, but this time of day was also a time of low tide. We know from our previous work that water quality is worse at LP during high tide. The human marker was detected in 1/8 LP ocean water samples, 1/8 jetty ocean water samples, 1/8 North Beach ocean water samples, and 3/8 Seal Beach ocean water samples. All human marker detections were BLOQ. *Campylobacter* was detected at Seal Beach in 3/8 water samples, 1/8 LP ocean samples, and 1/8 North Beach ocean samples. *Salmonella* was not detected in any of the environmental samples.

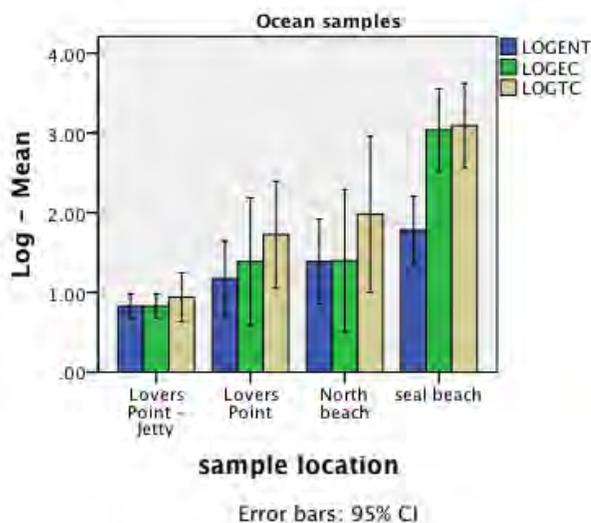


Figure 7. Log-mean concentrations of three FIB in ocean water during the study.

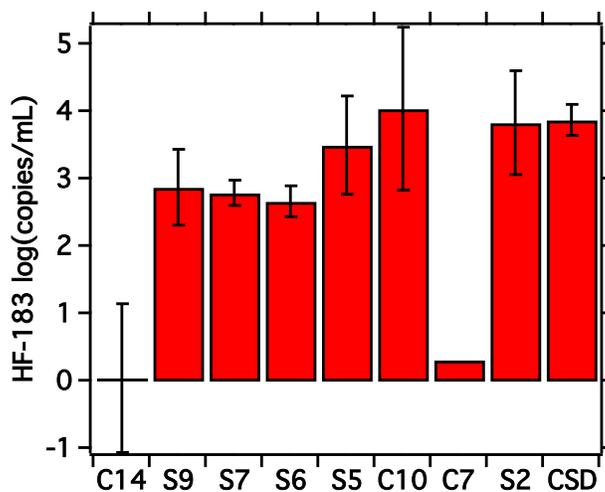


Figure 8. Concentration of HF183 Taqman in storm drain water sampled in the summer of 2013. Note that this water was being diverted to the sanitary sewer during the summer. Sample locations to the right are closer to the beach while sampling sites to the left are further up-watershed.

Regarding H5, we discovered that the storm drain system in Lovers Point is grossly contaminated with sewage, as revealed by the high levels of FIB and human marker (human marker results in Figure 8). The water in this drain has been diverted for years during the dry season, yet based on historical data collected

by the County and Boehm at Stanford University, dry weather water quality problems at Lovers Point persist. However, the flow rate from the storm drain was very low during our pilot study (about 14 liters per hour) and the discharge generally had very low FIB (≤ 30 MPN/100 mL enterococci and *E. coli*). The human marker was detected in 1/8 LP storm drain discharge samples at levels BLOQ. Given these disparate findings, further study is needed to determine the impact of discharge from this drain on water quality during dry weather.

Lessons Learned

In retrospect, we should have targeted a high tide condition for sampling, or included one in our 8 week sampling campaign. We know water quality is worse during high tide. We focused on sampling when the sun was not out, in the early morning and this generally occurred during a low tide. A future full-scale MST study will need to consider both sun and tide in the sampling plan.

Next Steps

The 8 weeks of sampling during the summer of 2013 did not provide enough information to finish the source tracking study at Lovers Point. Using these pilot data along with the previous historical knowledge of the site, a CBI proposal could be developed for a full MST study.

The City applied for a CBI Implementation Grant to fund these capital improvements to the MS4, as well as to make additional sewer system repairs in the Lovers Point drainage area. If funded, the start date for this project will likely be late summer/early fall of 2014, and under the best-case scenario, construction will take about one year.