Arroyo Burro Beach Microbial Source Tracking Study

Conducted by the University of California Santa Barbara (UCSB)
as part of the Source Identification Protocol Project (SIPP)

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Background

The lower Arroyo Burro (AB) watershed (Figures 1 & 2) is located on California's central coast in Santa Barbara County (34°24'9.44"N, 119°44'35.72"W). The entire watershed area is approximately 25 km^2, with residential, commercial, agricultural, and open space land uses[1]. Furthest downstream, at the Santa Barbara Channel, is the Arroyo Burro (AB) Lagoon, which is within Arroyo Burro Beach Park. Downstream of the AB Lagoon is Arroyo Burro Beach (a.k.a. Hendry's Beach) with annual visitation of over one million people[2], and where dogs are allowed in off- and on-leash areas. The outlet of the AB Lagoon has a sand berm that periodically breaches, allowing discharge to AB Beach and the surf zone (Figure 1). AB Creek, which carries the combined flow of its upper stem and Las Positas Creek, discharges into the AB lagoon, as does Mesa Creek (Figure 1). Lands adjacent to the upstream creeks are primarily roads, open spaces, or residential developments with public and private infrastructure including sanitary sewers, storm drains, and septic systems (Figure 2). Arroyo Burro Beach Park has a sewered restaurant and bathroom (Figure 3). Other features in the study area include a closed municipal solid waste landfill, a showground, and two golf courses (Figures 1 & 2).

Arroyo Burro Beach was selected as a microbial source tracking (MST) field site for the State of CA Clean Beach Initiative (CBI) Source Identification Protocol Project (SIPP), due to historically poor water quality as indicated by an "F" score on Heal the Bay's 2011 Annual Beach Report Card and the #7 ranking on California's Top Ten Beach Bummers List[3]. AB Beach consistently had the highest fecal indicator bacteria (FIB) levels of any beach monitored in Santa Barbara County, with single sample exceedance rates of greater than 15% each year from 2008-2012 and greater than 30% in 3 of those years. Human waste markers were previously detected in the AB Lagoon and in surf zone samples[4], and surf zone FIB exceedances were shown to correlate with the lagoon being open[5]. Steps were previously taken to discover and remediate watershed fecal sources[4,6], including diverting (during the AB411 season) an upstream human contaminated storm drain to the sanitary sewer (since 2007), and later discovering and repairing a leaking sewer that was likely the main source of contamination. Also, a restoration project for lower Mesa Creek and the upper AB Lagoon was completed in 2008. However, past actions had not resulted in significantly improved water quality, and MST was needed to identify ongoing sources of fecal contamination affecting AB Beach.

Project Approach

This MST study was conducted in accordance with guidelines set forth in *The California Microbial Source Identification Manual* developed as part of the SIPP project[7], which recommends beginning MST by evaluating historical information to guide field watershed reconnaissance and to support MST field study design. Reconnaissance for the AB watershed was performed during 2011 and included reviewing historical FIB and other water quality data, compiling infrastructure maps (sanitary sewers, storm drains, and septic systems), visually inspecting and walking the beach, lagoon and creeks, preliminarily sampling

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[1] Steets and Holden. A Mechanistic Model of Runoff-associated Fecal Coliform Fate and Transport through a Coastal Lagoon. *Water Research* **2003**, *37*, 589–608.
[2] Menzies, J. Parks Divison, County of Santa Barbara, CA. Personal Communication, October 2012.
[3] Griesbach et al. *Heal the Bay's 2010-2011 Annual Beach Report Card*; 2011; p. 98.
[4] Sercu et al. Storm Drains Are Sources of Human Fecal Pollution During Dry Weather in Three Urban Southern California Watersheds. *Environmental Science & Technology* **2009**, *43*, 293–298.
[5] Murray et al. Water Quality Research and Monitoring Program Fiscal Year FY10 Annual Report.
[6] Sercu et al. Sewage Exfiltration as a Source of Storm Drain Contamination During Dry Weather in Urban Watersheds. *Environmental Science & Technology* **2011**, *45*, 7151–7.
[7] Griffith et al. *The California Microbial Source Identification Manual: A Tiered Approach to Identifying Fecal Pollution Sources to Beaches*; 2013.

of lagoon and creek waters, and consulting with stakeholders and others having local watershed knowledge. During this study phase, the City of Santa Barbara used closed-circuit television (CCTV) to investigate storm drains identified as at risk for infiltration from sanitary sewers to determine if there was visual evidence of sewage inflow during dry weather (Figure 2). Based on reconnaissance and stakeholder consultation, potential fecal sources were identified and hypotheses were developed to guide MST.

Fecal Sources Investigated

Evidence from historical FIB data suggested that the AB Lagoon harbored FIB that were being discharged into the surf zone when the lagoon outlet was open. However, the sources of FIB into the AB Lagoon were uncertain. There were a few possible sources of FIB into the AB Lagoon: upstream creeks (AB and Mesa) that discharge into the lagoon; storm drains, dogs, birds, or other direct sources internal to the lagoon; surf zone contamination from dogs or birds that returned FIB to the lagoon with the incoming tide. It was also possible that sources directly at the beach were responsible for surf zone FIB exceedances. Thus, there were three geographic "regions" requiring MST: creeks upstream of the lagoon, the lagoon itself, and the beach. The MST study plan was therefore organized by region because, while the creeks, lagoon, and surf zone are hydrologically connected, a systematic evaluation of potential sources within each of these three regions provided the best opportunity to discover major FIB sources that could then be remedied. Potential fecal sources identified during information discovery and field watershed reconnaissance were investigated within these three geographic regions. The following list summarizes hypothesized fecal sources:

1) Fecal Sources directly at the Beach

    a. Humans: Potential sources of human fecal contamination at the beach included sanitary sewers from the restaurant and bathrooms at AB Beach and failing septic systems from homes to the west of AB Beach (Figure 2). As there were no obvious surface water (other than the lagoon) or flowing pipe outfall connections to the beach during dry weather for these possible sources, such sources would have migrated via groundwater to the beach.

    b. Birds & Dogs: Dogs are allowed at AB Beach, with an on-leash area west of the lagoon and an off-leash area to the east. Birds including gulls and pigeons were commonly observed at AB Beach. Thus these sources would have been through direct deposition at the beach.

    c. Sand & Wrack: FIB, arising from dogs or birds at the beach, may be harbored or amplified on sand and wrack and later released to the surf zone by tides and wave action.

2) Fecal Sources into the Lagoon

    a. Humans: Besides possibly contaminating the surf zone via groundwater transport, sanitary sewers running from the restaurant and bathrooms at AB Beach to the sewer main were determined by geographic information system (GIS) analysis of pipe elevations and locations to cross directly over a storm drain that discharges into the AB Lagoon (Figure 3). Thus, if the sanitary sewers were leaking in the vicinity of the storm drain, infiltration of sewage into the storm drain and then conveyance directly into the lagoon was possible[8].

    b. Birds & Dogs: Birds and dogs were observed in and around the lagoon. Thus, these sources could have been directly depositing fecal material into AB Lagoon.

[8] Sercu et al. Sewage Exfiltration as a Source of Storm Drain Contamination During Dry Weather in Urban Watersheds. *Environmental Science & Technology* **2011**, *45*, 7151–7.

3) Upstream Fecal Sources

a. Humans: Potential sources of human fecal contamination along upstream creeks included homeless encampments, leaking sanitary sewers, and failing septic systems. Homeless encampments would have involved direct deposition of fecal material into the creeks, sewers could have been exfiltrating into storm drains that then conveyed sewage directly to creeks, and septic systems could have been contaminating groundwater that then exchanged with creek baseflow.

b. Dogs: Domestic dogs living at residences adjacent to upstream creeks, and wild canines (coyote and fox) known to inhabit the creeks, were both potential FIB sources. The route of entry for domestic dog fecal material most likely would have been improper disposal of pet waste into the creek, while wild canines may have been directly depositing feces near creeks resulting in creek water contamination.

c. Horses: Storm drains at the Earl Warren Showgrounds flow directly to upper Las Positas Creek. Over 20 horse shows are hosted at the showgrounds each year. If horse waste were improperly managed at the showground, these storm drains could have been carrying contamination directly to upstream Las Positas Creek.

Stakeholder Involvement

Stakeholders for this MST study included members of the California State Water Resources Control Board, the City of Santa Barbara Creeks Division, the County of Santa Barbara Project Clean Water, Santa Barbara County Environmental Health Services, the Clean Beach Task Force, and Heal the Ocean. Multiple stakeholder meetings were held throughout the study to aide in the planning, implementation, and communication of results. Stakeholder involvement was an essential component of successful MST in the AB watershed.

Stakeholders from the City and County of Santa Barbara provided historical sampling data collected in the AB watershed, as well as maps with the location of sanitary sewers, storm drains, and septic systems both within and outside the Santa Barbara city limits. This information was critical for hypothesizing sources of contamination and planning MST field sampling. Additionally, the City of Santa Barbara Creeks Division used CCTV to investigate locations identified as at-risk for exfiltration of sewage to storm drains based on the age, material, and proximity of infrastructure[9]. Thus, the City of Santa Barbara applied traditional source tracking tools in several areas to investigate possible major sewage leaks, which were ruled out as contamination sources before MST tools were implemented. During the second year of MST, the Creeks Division also assisted by performing door-to-door education regarding proper pet waste disposal to residents along creeks where dog fecal inputs had been identified during the prior year using MST field sampling and analysis methods. The coordination of MST sampling with the education program was integral to identifying a significant source of fecal contamination in this watershed.

[9] City of Santa Barbara Creeks Division Source Tracking Protocol Development Project http://www.santabarbaraca.gov/civicax/filebank/blobdload.aspx?BlobID=16724.

Hypotheses

Based on the potential sources described above, we developed the following hypotheses for each of the three geographic regions.

H1: Fecal sources contribute FIB directly to the surf zone at AB Beach.

- H1.1: Human waste is directly contributed, from coastal septic systems or AB Beach Park restroom and sewers, via groundwater discharging into the surf zone.
- H1.2: Dogs and birds are contributing FIB directly to the surf zone at AB Beach.
- H1.3: Sand and wrack harbor FIB that are washed into the surf zone by tides and wave action.

H2: Fecal sources contribute FIB directly to the AB Lagoon, which are then released to the surf zone when the lagoon breaches.

- H2.1: FIB directly enter the AB Lagoon, where they may amplify, and are released directly into the surf zone when the lagoon is open.
- H2.2: Human FIB are being transported via the storm drain into the lagoon from sanitary sewers located adjacent to the AB Lagoon.
- H2.3: Dogs and birds are contributing FIB directly into the AB Lagoon.

H3: Fecal sources contribute FIB to upstream creeks, which persist into the lagoon and are then released to the surf zone when the lagoon breaches.

- H3.1: FIB from human fecal contamination are entering creeks from leaking sewers that exfiltrate into storm drains, and/or from failing septic systems (via groundwater exchanging with creek surface water) along upstream creeks in the AB watershed.
- H3.2: FIB from horse fecal contamination are entering Las Positas Creek from the Earl Warren Showgrounds.
- H3.3: FIB are deposited directly into the creek in conjunction with homeless encampments on upper Las Positas Creek.

Field Sampling in Support of 2012 MST

A 10-week study was performed during the 2012 AB411 period to test for hypothesized sources of fecal contamination in the AB watershed. Surface water samples were collected once per week for 10 consecutive weeks from 15 locations throughout the AB watershed (Figure 1; Table 1). Sampling sites were selected based on hypotheses about potential fecal sources (described above) and included 5 surf zone (sites 1-5), 4 lagoon (sites 6-9), and 6 upstream creek (sites 10, 11 & 13-16) locations. Surf zone locations were distributed across 400m of the beach centered at the lagoon outlet. These locations were chosen to evaluate if humans (H1.1) or dogs and birds (H1.2) were direct sources at the beach and if FIB were discharging into the surf zone when the lagoon breached (H2). Sand and wrack samples were also collected from 3 beach locations (sites 2-4) to determine if FIB were being harbored and washed into the surf zone (H1.3). Lagoon sampling sites were chosen to determine if FIB were amplifying in the lagoon (H2.1), if a storm drain was contributing FIB from sewage (H2.2), and if dogs and birds were contributing FIB directly to the lagoon (H2.3). Creek sampling sites were chosen downstream of hypothesized fecal sources to determine if areas of homes on septic systems (H3.1), the showgrounds (H3.2), or homeless

encampments (H3.3) were contributing FIB into the lagoon. To further understand the impact of the lagoon breaching on surf zone water quality (H2), an experiment was conducted in which samples were collected from 8 locations (sites 1-6, W & E) before and after a single lagoon breach event on July 31st, 2012.

Initial Project Outcomes from 2012 MST[10]

Surf zone FIB levels (Figure 4) were correlated with lagoon outlet flow rate ($p<0.04$), and the breaching of the AB Lagoon was shown to have a dramatic effect on surf zone water quality (see Appendix A). FIB levels were high in lagoon samples, as well as in AB Creek (Figure 4), suggesting upstream FIB inputs were affecting downstream lagoon and surf zone water quality. FIB levels were low in sand and wrack samples (see Appendix A) taken at the beach, showing these were not major sources of FIB to the surf zone during the study period.

Human markers (see Appendix A) were quantified in upstream Las Positas Creek (site 15, Figure 1) near a homeless encampment, but these markers attenuated before reaching downstream sampling locations. Human markers were also found at low levels in the surf zone (sites 1-5, A, B, C & W, Figure 1) and in upstream AB Creek (sites 16 & 17, Figure 1), both near areas with homes on septic systems. No human markers were detected in lagoon samples, showing that infrastructure near the lagoon was not a significant source of human fecal contamination to the lagoon during the study period. Further, the CCTV results showed that sanitary sewers near the lagoon were not exfiltrating to the storm drain (Figure 3).

Dog markers were quantified in the surf zone (Figure 5) and were correlated with surf zone FIB levels and lagoon outlet flow rate ($p<0.03$). Dog markers were also quantified in the AB Lagoon (sites 6-9, Figure 1), in AB Creek at sites upstream from the lagoon (sites 11-13, Figure 1), and in Las Positas Creek (site 14, Figure 1). Dog markers quantified in both creeks occurred downstream from areas where residential backyards were adjacent to the creek. Lagoon dog markers were higher in the upper lagoon at site 9 and in the lower lagoon at sites 6 & 7 compared to the middle lagoon site 8, suggesting that dog marker inputs may be occurring both into the upper lagoon from AB Creek and into the lower lagoon either directly from dogs or from the surf zone during high tides. Although dog markers were detected in the surf zone even when the lagoon was closed, these inputs from dogs directly at the beach did not result in FIB exceedances on days when the lagoon was closed.

Gull markers were frequently detected in the surf zone and lagoon (Figure 5), but did not correlate with FIB concentrations in the surf zone or lagoon ($p>0.05$) during the study period. No evidence of horse fecal contamination was found downstream from the Earl Warren Showgrounds in Las Positas Creek (site 15, Figure 1).

Refined Hypotheses

Based on the results of the 2012 MST field program and with additional stakeholder consultation, the following "targeted" hypotheses were added for study in 2013, to further investigate potential fecal sources:

- H1.4: Groundwater impacted by improperly functioning septic systems and/or the restaurant and bathroom at AB Beach is contributing human markers to the surf zone.

[10] For detailed analysis and discussion of project results, see Appendix A: Technical Report.

- H3.3: Fecal material from domestic dogs is entering lower AB Creek from improper pet waste disposal, and is causing elevated levels of dog-associated fecal markers, as well as an increase in FIB in this portion of the creek which flows directly into the AB Lagoon.
- H3.4: Groundwater impacted by improperly functioning septic systems near the upper branch of AB Creek is a source of human fecal contamination into the Creek.

Field Sampling in Support of 2013 Targeted MST

Sampling was performed during the 2013 AB411 period to further investigate sources of fecal contamination identified during 2012 MST. Surf zone and submarine groundwater samples were collected on 3 dates at 5 sites (A, B, C, W & E, Figure 1) to determine if human fecal bacteria were entering the surf zone via groundwater from possibly failing septic systems to the west of AB Beach, or from leaking sanitary sewers at the beach restaurant and bathroom (H1.4). Surface water samples were collected on 10 dates from 5 upstream locations (sites 11-13, 16 & 17, Figure 1). Sites 12 and 17 were added in 2013 to increase the spatial resolution of sampling along AB Creek. Samples collected from sites 11-13 along lower AB Creek were coordinated with a pet waste disposal education program conducted by the City of Santa Barbara to determine if domestic dog waste was entering the creek from adjacent homes (H3.3). Five samples were taken over a 3-week period, followed by 2-weeks in which resident education was performed. Another 5 samples were then taken over the 3-week period following education. Water samples were also collected on 10 dates from upstream AB Creek at sites 16 & 17 to determine if human fecal bacteria were entering the creek from possible septic to groundwater exchange (H3.4).

Sample Analyses

With each surface water sample, field measurements were taken, including: water temperature, dissolved oxygen, electrical conductivity, and flow rate (where possible). All samples were analyzed by defined substrate culture methods (IDEXX) for enterococci, *E. coli* and total coliform FIB. Based on hypothesized sources, DNA extracted from water samples was analyzed by qPCR for the HF183Taqman and HumM2 human fecal markers and the DogBact (dog) and Gull2 (seabird) animal fecal markers. These MST methods were selected based on the results of the SIPP methods evaluation study[11].

Overall Project Outcomes[12]

FIB in the surf zone at AB Beach (Figure 4) were strongly correlated with lagoon outlet flow rate ($p<0.04$). Thus, flow from the AB Lagoon, when open, caused elevated levels and exceedances of FIB measured in the surf zone. While the effect of lagoon outlet flow was observed primarily to the east (down-current) side of the lagoon outlet, the lagoon breach experiment (see Appendix A) showed that the entire beach area can be significantly affected when a large lagoon breach occurs.

A source of human fecal contamination from a homeless encampment was discovered in upper Las Positas Creek. Quantifiable levels of both human-associated markers were detected at site 15 (Figure 1), but markers did not persist at downstream sampling locations (see Appendix A). This signaled that the encampment waste did not significantly affect downstream water quality. The City of Santa Barbara was immediately contacted to remove the homeless encampment and clean the area.

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[11] Boehm et al. Performance of Forty-one Microbial Source Tracking Methods: A Twenty-seven Lab Evaluation Study. *Water Research* **2013**, *47*, 6812–6828.
[12] For detailed analysis and discussion of project results, see Appendix A: Technical Report.

Human markers were detected at low levels in the surf zone at AB Beach and along upper AB Creek (see Appendix A). Although the human markers were detected in the vicinities of homes on septic systems, further study would be needed to pinpoint failing septic systems, or to determine if there were other (e.g. up-or down-coast, and transported along shore) sources unrelated to the immediate area. Samples taken from submarine groundwater on the west side of AB Beach did not show any evidence of human contamination in the shallow groundwater (see Appendix A), suggesting that there was no shallow subsurface flow of human markers into the surf zone at these locations. This result suggests that septic-served homes near the sampling were not a source of human markers to the surf zone.

Dog markers were detected at quantifiable levels in the surf zone (Figure 5) and were strongly correlated with surf zone FIB and lagoon outlet flow rate ($p<0.03$). While fecal contamination from domestic dogs directly at the beach was observed and detected, this did not result in FIB exceedances during the study period. Rather, an important source of dog markers entering the surf zone was from the AB Lagoon when breached. Sources of dog markers to the AB Lagoon included both domestic dogs and wild canines along lower AB Creek, as well as possible direct inputs to the lagoon and input from the surf zone during high tides. A pet waste education program implemented by the City of Santa Barbara was effective in reducing dog fecal input from domestic dogs living adjacent to lower AB Creek (Figure 6, see Appendix A).

Lessons Learned

- When coastal lagoons are FIB-contaminated, their periodic breach into the surf zone releases fecal bacteria, causing FIB exceedances during dry weather.

- Multiple FIB fluxes into coastal lagoons are possible, including creek inflows, direct inputs, and surf zone FIB exchange during high tides. While it may not be possible to accurately quantify the relative contribution of all FIB sources into the lagoon, animal and human markers can identify sources, and thus indicate management strategies for improving water quality. In this case, improper management of pet waste from domestic dogs living along creeks upstream of the lagoon can be solved by homeowner education. Further, dog waste at the beach and lagoon can be controlled by increasing education of beachgoers regarding pet waste pickup. Using both of these strategies, the entire lower AB watershed can be managed to improve surf zone water quality.

- A two-year MST program is ideal, as it allows for comprehensive testing of initial hypotheses regarding FIB sources and their relative importance during year 1, then follow-up during year 2 to probe findings in more detail and test refined hypotheses.

- Cooperation in project planning, implementation, and in communicating results between researchers and stakeholders is needed throughout for successful MST. That cooperation may involve a real partnership in research, for example with the agency leading homeowner education regarding proper pet waste management, and the researchers sampling before and after such education to learn that the education program has resulted in creek water quality improvement.

Next Steps

For the upper AB watershed, it will be necessary to continuously and vigilantly monitor and remove homeless encampments near the creeks. Fecal inputs from only a few individuals can have a large impact on creek water quality.

Further investigation of human markers identified in this study is needed. Low levels of human markers were identified in the surf zone at AB Beach and along upstream AB Creek, but further study is needed to determine the source(s).

Expanded education on the impacts of domestic dog feces on water quality in both the creek and at the beach and lagoon is needed, targeting residents and beachgoers in the AB watershed and throughout the Santa Barbara area. Since short-term targeted education of homes along lower AB Creek resulted in a significant reduction in dog marker, expanding resident and beachgoer education to the entire community could have wider benefits to area creek, lagoon, and beach water quality.

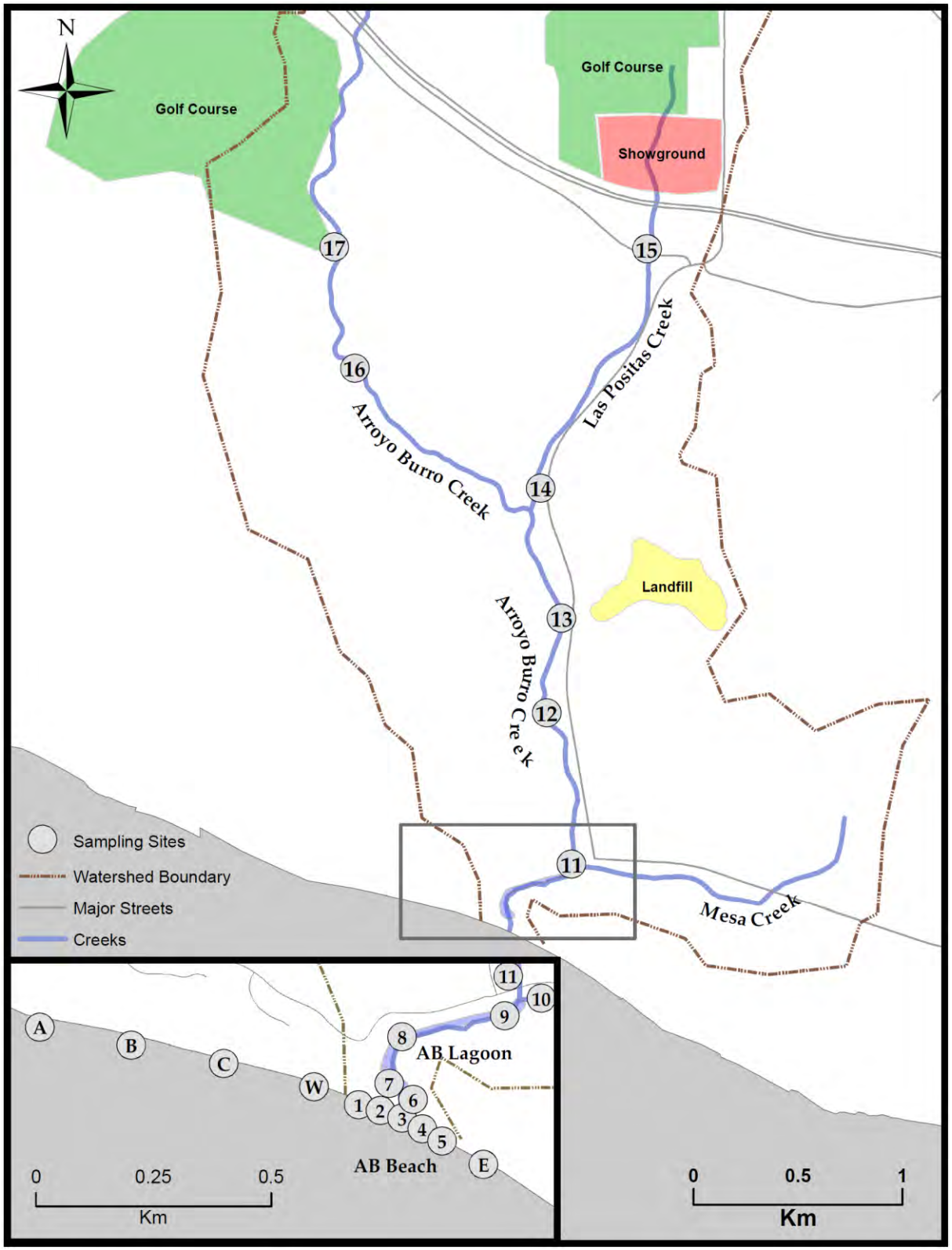

Figure 1. Map of the lower Arroyo Burro watershed with the Arroyo Burro (AB) beach and lagoon area (inset). Surf zone, lagoon, and creek sampling locations are shown as grey-filled circles enclosing sampling site numbers or letters.

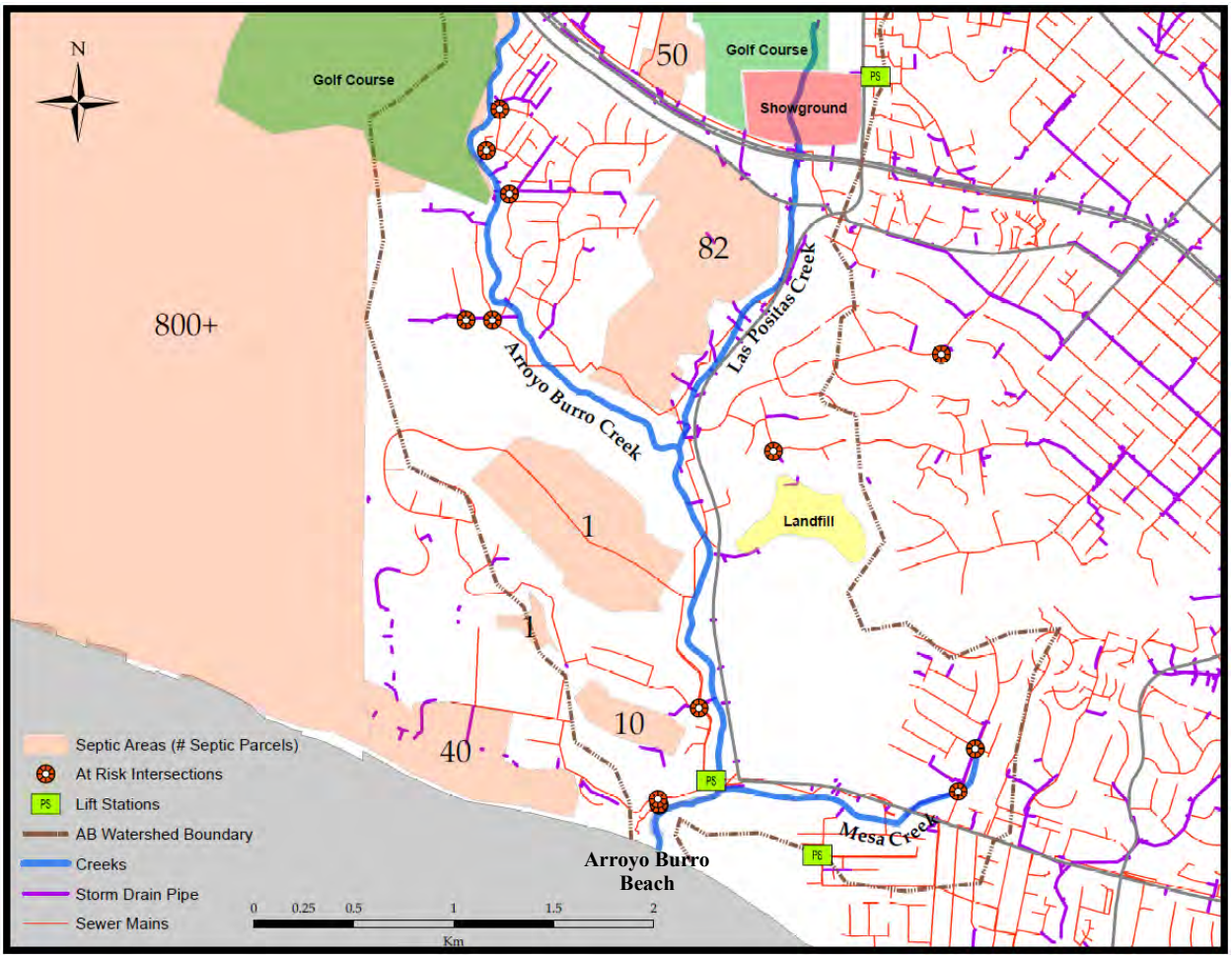

Figure 2. Map of the lower Arroyo Burro watershed with GIS-based information showing the location of storm drains, sanitary sewers, and septic parcels[13]. The identified "At Risk Intersections" were determined based on the proximity of sanitary sewers to storm drains along with their age and material. The City of Santa Barbara Creeks Division investigated these storm drains using CCTV[14].

[13] Hantzsche et al. *Septic System Sanitary Survey for Santa Barbara County California*; 2003; p. 204.
[14] City of Santa Barbara Creeks Division Source Tracking Protocol Development Project http://www.santabarbaraca.gov/civicax/filebank/blobdload.aspx?BlobID=16724.

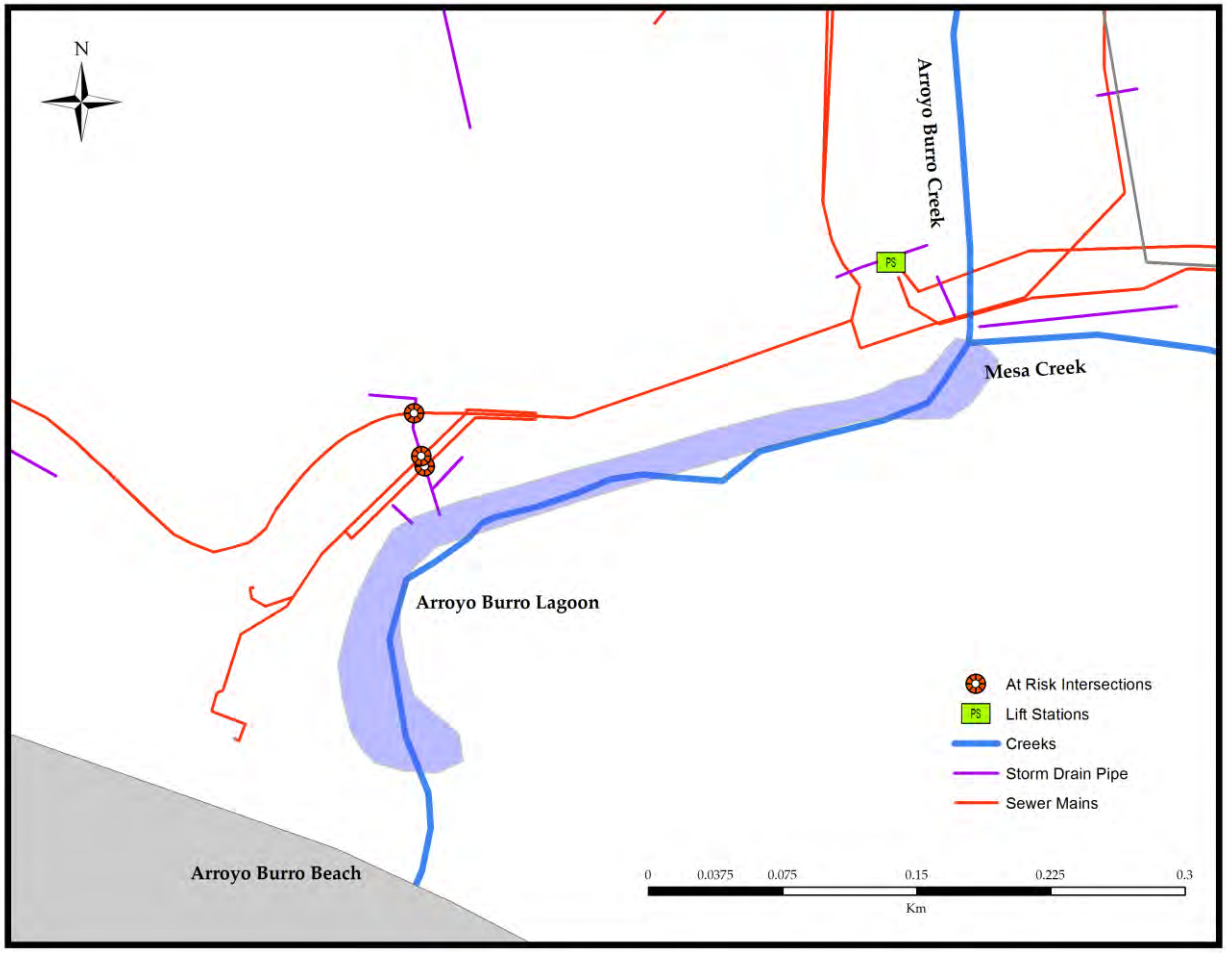

Figure 3. Map of the Arroyo Burro Beach and Lagoon area with GIS-based information showing the location of storm drains and sanitary sewers.

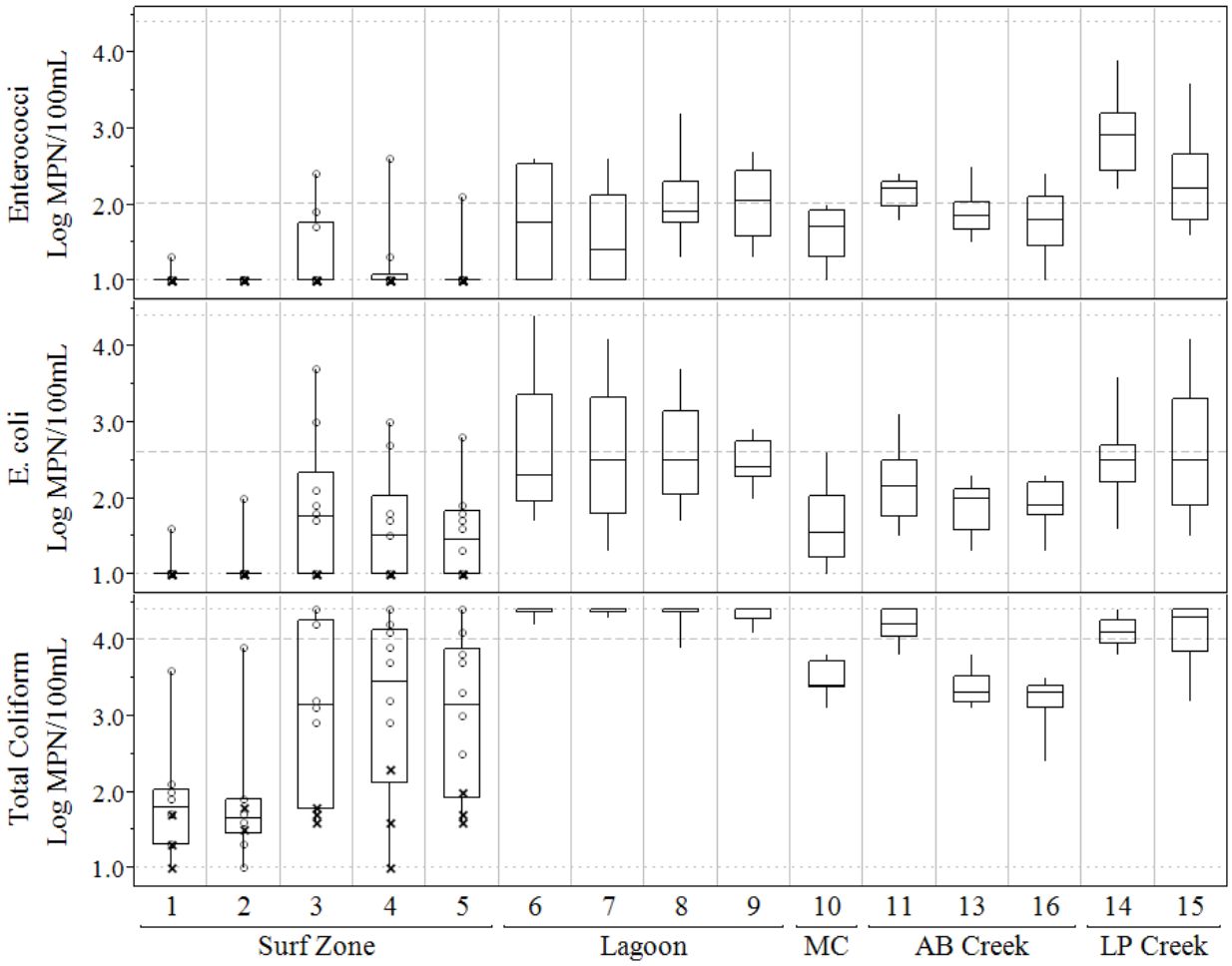

Figure 4. FIB concentrations at surface water locations sampled in 2012. Box plots represent the 25th, 50th, and 75th percentiles; whiskers represent the range (n=10-13). For surf zone samples, data points are shown representing samples collected when the lagoon was open (o) and closed (x). Dotted lines represent the upper and lower limits of quantification; data outside the quantifiable range are plotted at the limits of quantification. Dashed lines represent the CA single sample surf zone FIB limits[15]. MC = Mesa Creek, AB = Arroyo Burro, LP = Las Positas. Site numbers correspond to Figure 1.

[15] California Department of Health Services: Regulations for Ocean Beaches and Ocean Water-Contact Sports Areas Pursuant to AB 411 http://www.cdph.ca.gov/HealthInfo/environhealth/water/Documents/Beaches/Regulations-OceanBeaches.pdf.

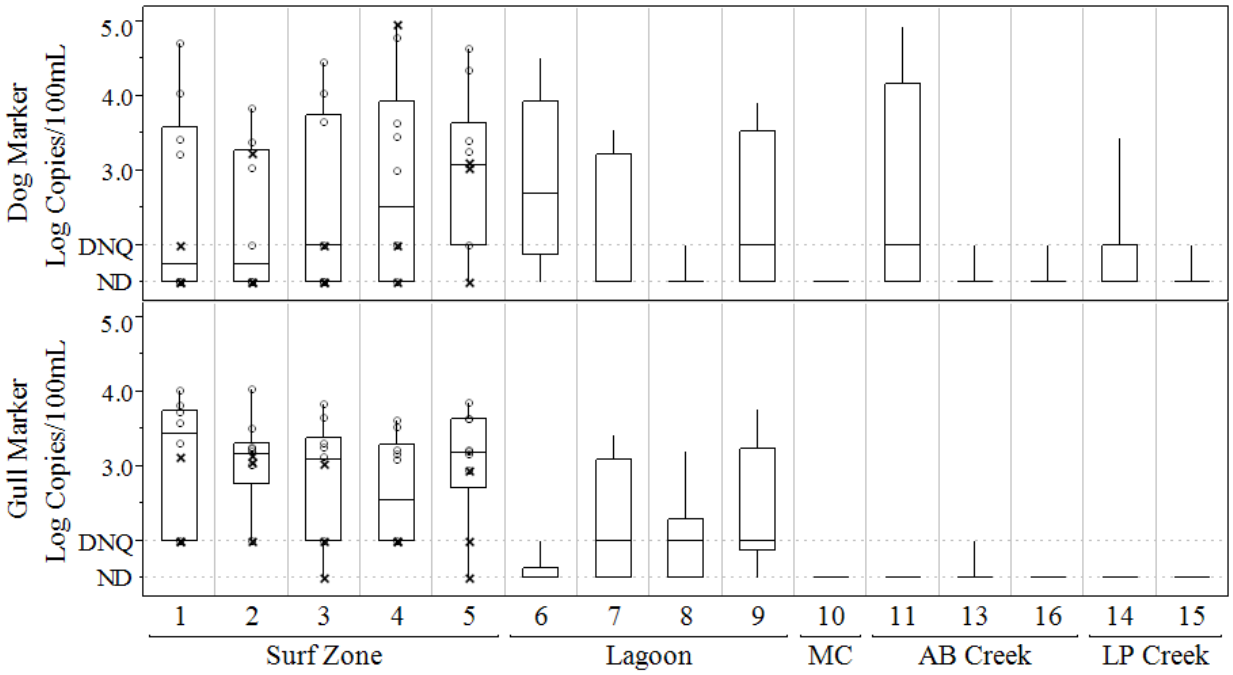

Figure 5. Dog (top) and Gull (bottom) fecal marker concentrations at surface water locations sampled in 2012. Box plots represent the 25th, 50th, and 75th percentiles; whiskers represent the range (n=10-13). For surf zone samples, data points are shown representing samples collected when the lagoon was open (o) and closed (x). DNQ = Detected but Not Quantifiable, ND = Not Detected, MC = Mesa Creek, AB = Arroyo Burro, LP = Las Positas. Site numbers correspond to Figure 1.

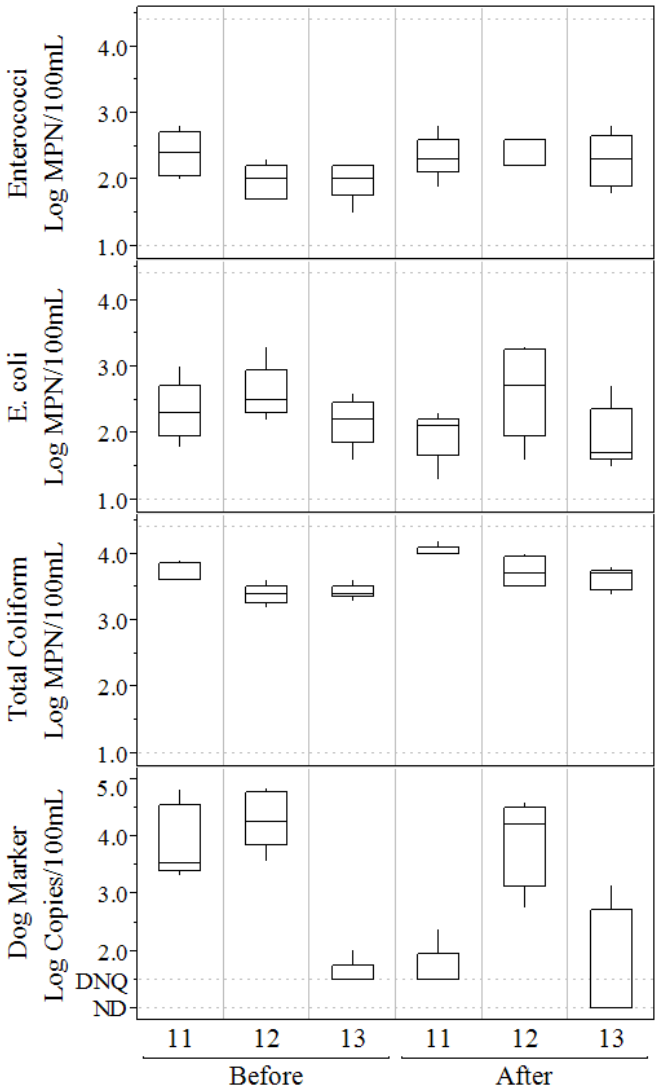

Figure 6. FIB and dog fecal marker concentrations at creek sampling locations collected before and after public education in 2013. Box plots represent the 25th, 50th, and 75th percentiles, whiskers represent the range (n=5). DNQ = Detected but Not Quantifiable, ND = Not Detected. Site numbers correspond to Figure 1.

Table 1. Detailed description of sampling sites. Site numbers correspond to Figure 2. LB = Lagoon Breach Experiment, AB = Arroyo Burro, LP = Las Positas.

Site	Sample Type	Study Phase(s)	Latitude	Longitude	Site Description	Notes
1	Surf Zone	2012, LB	N 34°24.164'	W 119°44.626'	AB Beach, 100m west of lagoon outlet	Location varied based on tide
2	Surf Zone	2012, LB	N 34°24.157'	W 119°44.595'	AB Beach, 50m west of lagoon outlet	Location varied based on tide
3	Surf Zone	2012, LB	N 34°24.149'	W 119°44.565'	AB Beach, surf zone at lagoon outlet	Location varied based on lagoon status & tide
4	Surf Zone	2012, LB	N 34°24.137'	W 119°44.537'	AB Beach, 50m east of lagoon outlet	Location varied based on tide, County weekly monitoring site
5	Surf Zone	2012, LB	N 34°24.123'	W 119°44.509'	AB Beach, 100m east of lagoon outlet	Location varied based on tide
6	Lagoon	2012, LB	N 34°24.164'	W 119°44.556'	AB Lagoon, lagoon outlet	Location varied based on lagoon status
7	Lagoon	2012	N 34°24.189'	W 119°44.584'	AB Lagoon, lower lagoon	
8	Lagoon	2012	N 34°24.242'	W 119°44.567'	AB Lagoon, middle lagoon	Near storm drain outlet
9	Lagoon	2012	N 34°24.282'	W 119°44.412'	AB Lagoon, upper lagoon	
10	Creek	2012	N 34°24.289'	W 119°44.386'	Mesa Creek, upstream of AB Lagoon	
11	Creek	2012, 2013	N 34°24.290'	W 119°44.411'	AB Creek, upstream of AB Lagoon	
12	Creek	2013	N 34°24.616'	W 119°44.475'	AB Creek, upstream of homes along creek	
13	Creek	2012, 2013	N 34°24.817'	W 119°44.437'	AB Creek, downstream of AB & LP confluence	
14	Creek	2012	N 34°25.092'	W 119°44.491'	LP Creek, upstream of AB & LP confluence	
15	Creek	2012	N 34°25.600'	W 119°44.217'	LP Creek, downstream of showground	
16	Creek	2012, 2013	N 34°25.347'	W 119°44.970'	AB Creek, upstream of AB & LP confluence	
17	Creek	2013	N 34°25.604'	W 119°45.022'	AB Creek, downstream of septic area	
A	Surf Zone	2013	N 34°24.246'	W 119°45.070'	AB Beach, 800m west of lagoon outlet	Location varied based on tide
B	Surf Zone	2013	N 34°24.227'	W 119°44.943'	AB Beach, 600m west of lagoon outlet	Location varied based on tide
C	Surf Zone	2013	N 34°24.208'	W 119°44.814'	AB Beach, 400m west of lagoon outlet	Location varied based on tide
W	Surf Zone	LB, 2013	N 34°24.183'	W 119°44.688'	AB Beach, 200m west of lagoon outlet	Location varied based on tide
E	Surf Zone	LB	N 34°24.097'	W 119°44.451'	AB Beach, 200m east of lagoon outlet	Location varied based on tide
A	Groundwater	2013	N 34°24.251'	W 119°45.046'	AB Beach, 800m west of lagoon outlet	
B	Groundwater	2013	N 34°24.235'	W 119°44.910'	AB Beach, 600m west of lagoon outlet	
C	Groundwater	2013	N 34°24.216'	W 119°44.805'	AB Beach, 400m west of lagoon outlet	
W	Groundwater	2013	N 34°24.190'	W 119°44.681'	AB Beach, 200m west of lagoon outlet	
1	Groundwater	2013	N 34° 24.174'	W 119° 44.621'	AB Beach, 100m west of lagoon outlet	
2	Sand, Wrack	2012	N 34°24.157'	W 119°44.595'	AB Beach, high tide line west of lagoon outlet	Location varied based on presence of beach wrack
3	Sand, Wrack	2012	N 34°24.149'	W 119°44.565'	AB Beach, high tide line near lagoon outlet	Location varied based on presence of beach wrack
4	Sand, Wrack	2012	N 34°24.137'	W 119°44.537'	AB Beach, high tide line east of lagoon outlet	Location varied based on presence of beach wrack

Appendix A: Technical Report[16]

Microbial Source Tracking in a Coastal Suburban Watershed Reveals Canines as Significant yet Controllable Sources of Fecal Contamination

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ABSTRACT

Elevated levels of fecal indicator bacteria (FIB), including *E. coli* and enterococci, trigger coastal beach advisories and signal public health risks. Solving FIB pollution in suburban coastal watersheds is challenging, as there are many potential sources. The Arroyo Burro Watershed in Santa Barbara, CA is an example, with its popular, but chronically FIB-contaminated beach. To address, a microbial source tracking (MST) study was performed, beginning with historical data evaluation and field reconnaissance. Surface waters and beach sand, wrack, and groundwater were then sampled over two years. FIB were quantified, and DNA was analyzed for host-associated fecal markers. Surf zone FIB were only elevated when the coastal lagoon was discharging. Among the fecal sources into the lagoon, including upstream human sources and coastal birds, canines were the most important. Canine sources included input via upstream creek water, which significantly decreased after creek-side residences were educated about proper pet waste disposal, and direct inputs to the lagoon and surf zone, where dog waste could have been tidally exchanged with the lagoon. Based on this study, canine waste can be an influential, yet controllable, fecal source to suburban coastal beaches.

1. INTRODUCTION

Culturable fecal indicator bacteria (FIB), including *E. coli* and enterococci, are monitored to assess surf zone microbiological water quality and to protect public health at recreational beaches. Elevated FIB levels have been correlated with an increased risk of illness[1–3]. However, FIB can be from many sources such as leaking sanitary sewers[4,5], domestic animals[6–8], wildlife[9,10], and birds[11–13]. FIB may also persist in sediments and on beach wrack[13–17]. The health risk from dogs, birds, and other animal sources is lower than for human sources[18]. Therefore, discerning human from non-human FIB sources is needed to inform water quality management.

Microbial source tracking (MST) is used to identify fecal sources contaminating recreational waters. Analysis of historical data and a sanitary survey are first conducted to hypothesize possible FIB sources and to inform field study design. Field samples are then acquired, and laboratory analyses are performed, including the use of DNA-based molecular markers that indicate fecal contamination from specific hosts[19]. Although source-associated markers do not exist for all hosts[20], there are fecal markers that are both sensitive to low levels of contamination, and specific to hosts, e.g. for humans, dogs, and gulls[21]. MST in suburban coastal watersheds is challenging given the variety of land uses[22] and the many potential fecal sources. Lagoons and estuaries can release FIB into the surf zone[22–24], but identifying the sources of FIB to suburban lagoons requires investigation upstream into the watershed.

In this study, MST was performed for a popular coastal beach and its upstream watershed, because of historically-elevated surf zone FIB levels. MST revealed that surf zone FIB primarily originated from an upstream creek and coastal lagoon that periodically discharges to the ocean. An important fecal source to the creek was found to be canines. A hypothesis that creek-side residences contributed dog fecal contamination to the creek was tested by sampling before and after a door-to-door education program regarding proper pet waste disposal. While

fecal contamination from domestic dogs defecating directly on the beach has been described previously[7,8], this study suggested that bacteria from dogs along upstream creeks may also impact downstream lagoon and surf zone water quality. Additionally, this study indicated that a low investment education program can contribute to managing surface water contamination.

2. MATERIALS AND METHODS

2.1 Study Area

The lower Arroyo Burro Watershed (Figure 1) is located on California's central coast in Santa Barbara County (34°24'9.44"N, 119°44'35.72"W). The entire watershed area is 25.6 km^2, with residential, commercial, agricultural, and open space land uses[22]. Furthest downstream, on the Santa Barbara Channel, is Arroyo Burro Beach Park with annual visitation of over one million[25] people, and where dogs are allowed on the beach. Arroyo Burro Beach scored an "F" on Heal the Bay's 2011 Annual Beach Report Card and the #7 spot on California's Top Ten Beach Bummers List[26]. The outlet of Arroyo Burro Lagoon (Figure 1) has a sand berm that periodically breaches, allowing discharge to the surf zone. Lower Arroyo Burro Creek, which carries the combined flow of its upper stem and Las Positas Creek, discharges into the lagoon, as does Mesa Creek (Figure 1). During summer months when beach water quality is regulated, there is little flow upstream of sites 17 and 15 in upper Arroyo Burro and Las Positas Creeks, respectively (Figure 1). Downstream from these sites, the creeks flow year-round through mostly naturally-vegetated channels except for a 600-meter trapezoidal concrete reach on Las Positas Creek upstream of site 14 (Figure 1). Most lands adjacent to the creeks are roads, open spaces, or residential developments with public and private infrastructure including sanitary sewers, storm drains, and septic systems. Other features in the study area include a closed municipal solid waste landfill, a showground, and two golf courses (Figure S1).

2.2 Evaluation of Historical Information and Watershed Reconnaissance

To aid in designing the MST study, reports of prior environmental sampling in the study area were collected and evaluated, and field reconnaissance including preliminary sampling was performed. Surf zone and creek FIB data were acquired from the City[27] and County[28] of Santa Barbara to understand surf zone FIB exceedance rates above California coastal water quality standards[29]. Water quality data were reviewed from research publications[4,5,22,30] and from available groundwater, septic survey, and landfill monitoring reports[31–35]. An analysis of GIS data (e.g. location, depth, material and age) for sanitary sewers and storm drains had been performed by the City of Santa Barbara, following a protocol for identifying storm drains that were at-risk for sewage infiltration[36]. During this study, the City of Santa Barbara televised storm drain locations for visual evidence of sewage infiltration during dry weather (Figure S1; see SI for details). Visual inspection for possible fecal sources was performed along creeks and around the lagoon, beach, and showgrounds (Figure 1). Water samples were collected on three days, in triplicate each day, from Arroyo Burro and Mesa creeks (sites 10 & 11), the lagoon (site 8), and Las Positas Creek (site 15) (Figure 1). Samples were analyzed for FIB and DNA-based human

markers using methods described for MST (see below). Based on our results and stakeholder consultation, potential fecal sources were identified and hypotheses were developed to guide MST.

2.3 MST Field Sampling

Overall, across two field seasons, surface water was collected during the dry season (May to October) from 22 locations in the lower Arroyo Burro Watershed including; 10 beach, 4 lagoon, and 8 creek sites (Figure 1). In the summer of 2012, fifteen locations (sites 1-11 & 13-16) were sampled weekly for 10 consecutive weeks. Additional water samples were collected from Los Positas Creek (sites 14 & 15) during 3 horse shows at the showgrounds (Figure 1). Additional surf zone samples were collected before and after a lagoon breach in 2012 (see SI for details). Also in 2012, sand and wrack at Arroyo Burro Beach were sampled to assess if they were FIB reservoirs (see SI for details).

In the summer of 2013, Creek waters were sampled at sites 11, 12, and 13 along Arroyo Burro Creek on 5 dates over a 3-week period before, and after, a 2-week period in which education, but not sampling, was performed. No samples were collected in 2013 at sites 14 and 15 in Las Positas Creek because, based on apparent FIB attenuation between sites 14 and 13, downstream dog waste inputs (between sites 11 and 13) would have the greater potential impact on surf zone water quality. Creek samples were also acquired from sites 16 and 17 on the same 10 dates as downstream creek samples (sites 11-13) in the summer of 2013, to further investigate possible human fecal sources indicated by the 2012 field study. Similarly, to further investigate possible human contamination in the surf zone, sites A, B, C, W, and 1 were sampled three times during three separate weeks in the summer of 2013, in conjunction with submarine groundwater sampling on the beach (see SI for details).

Surface water samples were collected in sterile 2-L or 4-L polypropylene bottles, stored on ice and processed within 6 hours of collection. Dissolved oxygen, conductivity, and temperature were measured at each site, using an HQ40d multi-parameter meter equipped with conductivity and LDO probes (Hach, Loveland, OH). Creek and lagoon outlet water velocities were measured, using an FP111 velocity probe (Global Water, Gold River, CA). The channel dimensions (depth and width) were measured simultaneously for calculating flow rate from the product of average velocity and channel cross-sectional area.

Feces from coyote, fox, and deer were also collected to evaluate their possible cross-reactivity with DNA-based dog markers. The collection was not in the field, but rather from captured animals, so that field contamination (e.g. soil, sediments, or other feces) was avoided (see SI for details).

2.4 Education Program

An education program was conducted because of the results of the 2012 field and laboratory research that indicated dog feces as important contamination sources in lower Arroyo Burro and Las Positas Creeks. The City of Santa Barbara planned and conducted the door-to-

door education program in mid-summer 2013, at residential properties (mostly single-family) adjacent to Arroyo Burro (26 homes between sites 11 and 12) and Las Positas (22 homes between sites 14 and 15) Creeks (Figure 1). Sampling in 2013 was timed to coordinate with the education program, such that baseline sampling preceded the education, and subsequent sampling occurred after education ended. During the education program, residents were asked about the number of dogs at each home and their dog waste disposal methods. They were informed of the potential impact that dog feces could have on water quality and asked to prevent dog waste from entering the creek from their properties. Residents were also asked to share this information with co-residents and with contractors and gardeners that may also dispose of pet waste.

2.5 Laboratory Analyses

Samples were analyzed by defined substrate culture methods (IDEXX, Westbrook, ME) for enterococci, *E. coli* and total coliform bacteria[4]. For water samples collected in 2012, several 200mL aliquots were filtered through 0.4µM polycarbonate (PC) membranes (EMD Millipore, Billerica, MA). Filters were immediately frozen and stored (-20°C) until DNA extraction. DNA was extracted from each of two filters using the DNA-EZ ST1 kit (GeneRite, North Brunswick, NJ), and the extracts were pooled. Methods were modified for 2013 to allow for filtering larger volumes to increase analyte detection sensitivity (Table S1). For water samples collected in 2013, up to 2-L were filtered through a single 0.2 µM Supor MicroFunnel (Pall, Port Washington, NY), and DNA was extracted using the PowerWater DNA isolation kit (Mo-Bio, Carlsbad, CA). DNA concentrations were quantified using the Quant-iT dsDNA broad-range assay kit (Life Technologies, Carlsbad, CA) (Table S2).

DNA extracts were analyzed by quantitative polymerase chain reaction (qPCR) for the HF183Taqman[37] and HumM2[38] human-associated fecal markers, and DogBact[39,40] (dog) and Gull2Taqman[40,41] (gull) fecal markers. Selected samples were also analyzed for the routine PCR HoF597[39] (horse) and the qPCR Entero1A[42] (enterococci) markers. Markers were selected based on their performance in a comprehensive methods evaluation study[21,43–45]. Inhibition during qPCR was assessed using a spiking and dilution procedure[46]. Filter and extraction blanks were analyzed to assess contamination during sample filtration and DNA extraction, respectively[21]. PCR reactions were run in triplicate; separate plasmid DNA standards were PCR amplified as before[21]. Samples with at least two replicates amplifying within the range of the standard curve were considered within the range of quantification (ROQ) and were quantified. Samples with replicates amplifying below the concentration of the lowest standard were considered detected but not quantifiable (DNQ), and samples with one or zero replicates amplifying were considered not detected (ND), as described previously[44,45].

2.6 Statistical Analyses

FIB exceedances were based on California single sample surf zone regulations for total coliform (10,000 MPN/100ml), *E. coli* (400 MPN/100ml), and enterococci (104 MPN/100ml)[29]. Exceedances for fecal to total coliform ratio were not calculated. Statistical analyses of FIB and

marker data including Spearman's Rank Correlations, Mann-Whitney U Tests, Kruskal-Wallis Tests, and Wilcoxon Signed-Rank Tests were performed using JMP 10 (SAS, Cary, NC). The non-parametric tests accommodated censored data[47] which arose for FIB and qPCR markers. Mann-Whitney and Kruskal-Wallis Tests were used to compare groups of data between sites or dates, while the Wilcoxon Signed-Rank Test was used for paired data collected on the same date. FIB and marker loads were calculated by multiplying analyte concentration by water flow rate. Spatial information and maps were produced using ESRI ArcMap ver. 10.1 (Redlands, CA).

3. RESULTS

3.1 Historical Data Evaluation and Watershed Reconnaissance

Based on historical Arroyo Burro surf zone FIB data, greater than 15% of samples in each of the past five years, and at least 30% of samples in three of the past five years, exceeded California AB411 water quality criteria[29] for at least one indicator. Previous studies had shown a relationship between elevated levels of FIB in the surf zone and the lagoon outlet being open or closed [22,48]. Human markers had been previously detected in the surf zone and in upstream creeks, but this contamination was traced to sewage exfiltrating into a storm drain which has been diverted to the sanitary sewer during dry weather since 2007[4,49]. During reconnaissance, dogs and birds were observed at the beach and in the lagoon. Drains were observed to be discharging into the concretized channel of Las Positas Creek between sites 14 and 15, and a homeless encampment was discovered downstream of the showgrounds on Las Positas Creek (site 15, Figure 1). Storm drains suspected for sanitary sewer infiltration were televised (Figure S1), but no significant leaks were discovered. Previously published reports[31–35] (e.g. watershed groundwater, septic survey, and landfill monitoring well) did not indicate fecal sources other than those investigated in this study.

Surface waters sampled during reconnaissance showed relatively low FIB levels (Table S3) for Mesa Creek entering the lagoon (site 10), compared to the lagoon (site 8), lower Arroyo Burro Creek (site 11), and upstream Las Positas Creek (site 15) (Figure 1). No human markers were detected in the lagoon or in downstream Arroyo Burro or Mesa Creeks during reconnaissance sampling, but quantifiable concentrations of both human markers were detected in upstream Las Positas Creek (site 15, Table S4). Overall, based on reconnaissance, we hypothesized that human markers could enter creeks via either failing septic systems (Figure S1) and concomitant ground-to-surface water exchange along upper Arroyo Burro (sites 16 & 17) and Las Positas Creeks (sites 14 & 15), or from a homeless encampment near upper Las Positas Creek (site 15); we also hypothesized that birds and dogs at the beach could impact surf zone water quality, that sand and wrack could harbor FIB, and that the showgrounds (Figure 1) could periodically contribute FIB from horse events. MST was thus designed to test these hypotheses, and to determine the source(s) of fecal pollution to the lagoon and surf zone.

3.2 MST FIB Results

Surf zone FIB (Figure 2, Table S5) exceeded the CA single sample limit in 18%, 10%, and 6% of samples collected in 2012 for total coliform, *E. coli* and enterococci, respectively. All exceedances were associated with samples acquired either at or to the east of the lagoon outlet (sites 3-5), and only on days when the lagoon was discharging into the surf zone (Figure 2, Table S6). When the lagoon outlet was not flowing, all surf zone samples were at or below the detection limit for *E. coli* and enterococci (Figure 2). Nearshore currents flowed primarily from the west, based on observed wind and wave directions. Thus, FIB discharging from the lagoon were advected in an easterly direction alongshore. Lower conductivity readings east of the lagoon outlet (sites 3-5, Table S7) confirmed the influence of freshwater. No surf zone FIB exceedances were measured in 2013 (Table S5). Median FIB concentrations in beach sand and wrack were low, with enterococci and *E. coli* at or below the quantifiable range in most sand samples (Table S8). Further, no significant difference was observed in surf zone FIB concentrations based on tide status (flood/ebb, Table S6) (Mann-Whitney U Test, $p>0.05$), suggesting that neither sand nor wrack significantly contributed FIB to the surf zone. This result is consistent with a prior analysis of surf zone data that found no significant correlation between FIB exceedance and tidal status at this beach[48].

Surf zone FIB concentrations were significantly correlated with the FIB load discharging from the lagoon in 2012 (Spearman's Rank Correlation, $p<0.05$), and this correlation was more significant for samples taken at and to the east of the lagoon outlet (sites 3-5, $p<0.01$). Additional surf zone samples collected before and after a single lagoon breach showed a dramatic increase in FIB, with no surf zone exceedances detected before the breach, and exceedances in 86% of samples collected after the breach (Figure S2). These results are consistent with a prior analysis of historical surf zone data at this beach that found a significant correlation between FIB exceedances and the lagoon being open, with a seven-fold greater chance of enterococci exceedance when the lagoon was open[48]. Lagoon FIB concentrations were high relative to the surf zone, with 98%, 40%, and 40% of samples higher than the CA single sample limit for total coliform, *E. coli*, and enterococci, respectively. Although FIB levels fluctuated throughout the lagoon (sites 6-9, Figure 2), no significant differences were found between the load of FIB (Table S9) entering (site 11) versus exiting (site 6) the lagoon (Wilcoxon Signed-Rank Test, $p>0.05$). The FIB load (Table S9) was significantly greater at site 11 versus site 10 (Mann-Whitney U Test, $p<0.01$), showing the primary upstream FIB source into the lagoon was from Arroyo Burro Creek (site 11) and not Mesa Creek (site 10). Within the lagoon, total coliform concentrations were significantly higher at sites 6-8 versus those entering the lagoon from site 11 (Kruskal-Wallis Test, $p<0.05$), suggesting additional sources or growth of total coliform bacteria in the lagoon. No significant differences were observed for *E. coli* and enterococci concentrations within the lagoon (sites 6-9) compared to site 11 (Kruskal-Wallis Test, $p>0.05$).

FIB concentrations (Figure 2, Table S5) along Arroyo Burro Creek fluctuated, but were generally greater at downstream site 11, versus upstream sites 13 and 16, suggesting that downstream inputs were more significant to lagoon and beach water quality. FIB concentrations

were relatively high in Las Positas Creek (sites 14 & 15). Due to the low flow rate at site 15, the FIB load (Table S9) from site 14 was significantly higher than for upstream site 15 (Wilcoxon Signed-Rank Test, $p<0.01$). The FIB load from Las Positas Creek at site 14 was also significantly higher than the load in upstream Arroyo Burro Creek at site 16 for both total coliform and enterococci (Mann-Whitney U Test, $p<0.05$), suggesting that Las Positas Creek discharged more FIB than upper Arroyo Burro Creek. *E. coli* loads were not significantly different at sites 14 and 16 (Mann-Whitney U Test, $p>0.05$).

3.3 DNA-Based Markers of Human Fecal Contamination

The human marker HF183Taqman was detected at DNQ levels in 18% of surf zone samples collected in 2012 (sites 1-5, Table S10). These detections were not correlated with FIB levels or lagoon outlet flow rate (Spearman's Rank Correlation, $p>0.05$) and occurred in the surf zone even on days when the lagoon was closed (Table S6). No human markers were detected in lagoon samples (Table S10), suggesting the lagoon was not a source of human markers to the surf zone. Additional surf zone sampling completed in 2013 on the west side of the beach also revealed HF183Taqman and HumM2 markers in 60% and 20% of samples, respectively (Table S10). The west side of the beach is popular for swimming and is also located near an adjacent watershed with over 800 homes on septic systems (Figure S1). Submarine groundwater sampled at the beach (sites A, B, C, W & 1) showed low FIB levels (Table S5) and no human marker detections, suggesting shallow groundwater was not a source of human markers to the surf zone. Because these human marker data from 2012 and 2013 suggested sources outside the study area, and because of the lack of correlation with surf zone FIB at AB beach, no further research was towards determining the origins of low-level human markers in the surf zone for this MST study.

No human markers were detected in downstream Arroyo Burro or Mesa Creeks (sites 10 & 11), indicating that human markers were not entering the lagoon from the upstream creeks. Both human markers were quantified in upstream Las Positas Creek at site 15 in 2012 (Table S10), likely related to an upstream homeless encampment that was observed (including toilet paper and feces near the creek). However, although significant concentrations of both human markers were detected, it is unlikely that human marker loads were impacting downstream sites, due to the low creek flow rate at site 15. Consistently, the HF183Taqman marker was detected only once at downstream site 14, and no HumM2 marker was detected at this site (Table S10).

On upper Arroyo Burro Creek, the HF183Taqman marker was detected at DNQ levels in 20% of site 16 samples in 2012 (Table S10). HF183Taqman and HumM2 human markers were also detected at DNQ levels in 35% of samples at sites 16 and 17 in 2013 (Table S10). Similarly to results from Las Positas Creek, human markers did not persist as there were no human markers detected at site 13. While these upper watershed indicators of human waste contamination may have been related to nearby septic systems, the indication of downstream human waste marker attenuation suggested that such contamination was unlikely to cause FIB contamination at the beach. Thus, emphasis in MST was placed on downstream FIB sources that appeared influential to lagoon and beach water quality.

3.4 DNA-Based Markers of Animal Fecal Contamination

Dog and gull fecal markers were frequently detected and quantified in the surf zone and lagoon (Figure 3, Table S11). The gull marker was detected in 96% of surf zone samples collected in 2012, with 68% at a quantifiable level. However, no significant correlations were observed between gull markers versus FIB in the surf zone or lagoon (Spearman's Rank Correlation, $p>0.05$). The gull marker was detected in 55% of lagoon samples, with similar concentrations at sites 7-9 compared to surf zone sites 1-5 (Figure 3). The gull marker at the lagoon outlet (site 6) had only 2 DNQ detections, which was significantly lower than other lagoon and surf zone sites (Mann-Whitney U Test, $p<0.05$), suggesting that gull marker inputs occurred at the beach and within the lagoon. The gull marker was detected in only one creek sample (site 13, Figure 3), showing that upstream gull marker inputs were low.

The horse marker was analyzed for samples collected from Las Positas Creek (sites 14 & 15) downstream from the showground (Figure S1). No horse markers were detected during regular 2012 sampling or in samples taken during three horse shows.

The dog marker was detected in 64% of surf zone samples collected in 2012, with 42% at a quantifiable level (Figure 3, Table S11). Although the median dog marker concentration increases from sites 3 through 5 (Figure 3), there was no significant difference between these sites (Kruskal-Wallis Test, $p>0.05$). The dog marker concentration in the surf zone was significantly correlated with FIB and lagoon outlet flow rate (Spearman's Rank Correlation, $p<0.05$), suggesting that dog waste may be related to surf zone FIB, and that FIB and dog markers were entering the surf zone from the lagoon. Surf zone dog marker concentration was also significantly correlated with lagoon outlet dog marker load for sites 3-5 (Spearman's Rank Correlation, $p=0.02$). The dog marker was detected in the surf zone when the lagoon was closed (Figure 3), and many dogs were counted at the beach (Table S6), showing that fecal input from dogs at the beach could be an important source. However, there were no corresponding FIB exceedances on days when the lagoon was closed (Table S6), suggesting that fecal inputs from defecation directly at the beach, although measurable, were not directly responsible for FIB exceedances. The dog marker was detected in 45% of lagoon samples, with significantly higher levels at sites 6 and 9 compared to site 8 (Kruskal-Wallis Test, $p<0.05$). While dog marker decay in the lagoon was not studied, this spatial pattern suggests multiple sources may have been present, including creek discharge to the upper lagoon and direct inputs or tidal exchange to the lower lagoon. Less frequent detection at site 8 could also have been due to "short-circuiting" between the upper and lower lagoon[22]. Dog markers were detected in 70% of downstream Arroyo Burro Creek (site 11) samples collected in 2012, and no dog marker was detected in Mesa Creek (site 10). The dog marker concentrations entering the lagoon from Arroyo Burro Creek (site 11) were not significantly different from those exiting the lagoon (site 6) in paired 2012 samples (Wilcoxon Signed-Rank Test, $p>0.05$), and this also held for the loads of dog markers at these sites (Table S9). Dog markers were detected at all upstream creek sites sampled in 2012, though only one DNQ was detected at sites 13, 15, and 16 (Figure 3). The dog marker

concentration significantly increased from site 13 to site 11 in paired 2012 samples (Wilcoxon Signed-Rank Test, $p=0.02$), suggesting a source between these sites.

3.5 FIB and DNA-Based Water Quality Related to Outreach and Education

Creek sampling in 2013 was timed to coordinate with an education program to investigate the impact of domestic dog feces on FIB levels, and to determine if the impacts were controllable through education. During education, contact was made with 25 of 26 residents along lower Arroyo Burro Creek (between sites 11 & 12), where 25 dogs were reported. Contact was also made with 18 of 22 residents along Las Positas Creek (between sites 14 & 15), where 18 dogs were reported (Figure 1). The dog marker was detected in 90% of samples collected from sites 11-13 (Figure 4, Table S11). Dog marker concentrations significantly increased from upstream site 13 to downstream sites 12 and 11 before education (Kruskal-Wallis Test, $p<0.01$), showing a dog marker source between these sites, similarly to 2012. No significant difference in dog marker concentration was observed between sites 12 and 11 before education (Mann-Whitney U Test, $p>0.05$), but after education there was a significant decrease from upstream site 12 to downstream site 11 (Mann-Whitney U Test, $p<0.01$). A significant decrease in dog marker concentration was also observed at site 11 when comparing before versus after education (Mann-Whitney U Test, $p<0.01$). However, there were no significant decreases in dog marker concentrations at upstream sites 12 or 13 before versus after education (Mann-Whitney U Test, $p>0.05$). These results indicate that education between sites 11 and 12 was effective in reducing the input of dog markers to this creek reach. Although an associated decrease in FIB was not observed at site 11 (Figure 4), dog markers were significantly correlated with both the Entero1A marker and *E. coli* (Spearman's Rank Correlation, $p<0.01$) across sites 11-13 in 2013, suggesting dog fecal inputs along this entire reach of the creek were impacting FIB. Consistently, cultured enterococcus and *E. coli* concentrations were significantly correlated to dog markers when data from both years were combined for sites 11-13 (Spearman's Rank Correlation, $p<0.05$). Although dog markers were quantified in Las Positas Creek at site 14 during 2012, and education was conducted along Las Positas Creek in 2013, no samples were collected in this creek for 2013 due to decreased FIB and dog markers between sites 14 and 13 (Figures 2 & 3).

3.6 Dog Marker Cross-Reaction

To further evaluate the host origins of dog marker between sites 12 and 13 (where there are no homes with domestic dogs), feces from wild animals were collected and analyzed. During education, residents noted to City employees that coyote and fox were frequently observed near the creek. DNA extracted from coyote and fox fecal samples harbored dog markers in concentrations that were similar to that of domestic dogs (Figure S3). Only a few fecal samples for each animal type were collected, but this shows that wild canines could be contributing to the fecal bacteria measured in this creek. However, although input from wild canines may be occurring, the decrease in dog marker at site 11 following education indicates that domestic dogs were an influential source of dog marker to the downstream creek.

4. DISCUSSION

The relationship between lagoon outlet flow rate and FIB level in the surf zone was consistent with other studies showing that lagoons and estuaries can significantly impact beach water quality by releasing FIB to the surf zone[23,24]. Such results were predicted through a model of FIB storage and discharge[22], and had been demonstrated by a statistical analysis of historical lagoon status and FIB exceedance[48]. Although no surf zone FIB exceedances were detected in weekly Santa Barbara County sampling[28], multiple exceedances were measured during this study. FIB persistence on beach sand and wrack can impact surf zone waters, but were not found to significantly contribute FIB at this beach, as compared to lagoon outlet flow. This is consistent with the findings of another MST study showing that FIB-contaminated wrack was not a major source to the surf zone[16].

The observed fluctuations in FIB levels measured along Arroyo Burro and Los Positas Creeks suggest that multiple inputs were likely present, but that FIB were attenuating between input locations. Human fecal markers were detected in both creeks upstream, but these markers also appeared to attenuate before reaching downstream sampling locations. Given that these markers are used to indicate the presence of human waste, the inference is that human waste from upstream sources was unlikely to influence beach water quality. This is consistent with other MST studies that have shown human markers decaying rapidly under environmental conditions[50]. Still unknown, however, is the presence of pathogens associated with human sources which may decay at different rates compared to source-associated markers and FIB[51,52], potentially creating a public health concern. Low-level human markers detected in upstream creeks and in the surf zone near septic-served areas could imply improperly functioning septic systems. While submarine groundwater sampling results showed that shallow groundwater at the beach had no human markers, it is possible that deep groundwater or contamination up coast of the sampling area are sources. Studies in other watersheds have linked septic-served areas to human markers in recreational waters[53,54], but further research would be required to determine if septic systems are a source of human fecal markers at this beach.

Dog markers identified in the surf zone originated both at the beach and from the Arroyo Burro Lagoon, and a significant input to the lagoon was identified along a downstream reach of Arroyo Burro Creek. Although dog markers were also detected further upstream, domestic dog waste input along this creek reach was shown to impact downstream water quality. Few studies have identified dog waste as a significant source of fecal bacteria to recreational beaches[7,8,11], and only minor inputs of dog waste along upstream creeks have previously been reported[10]. Based on dog marker results from a methods evaluation study[21], the estimated mass of fresh dog feces required to achieve the measured loads of dog marker exiting the creek to the lagoon was as little as 24 grams of feces per day (see SI for details). This is approximately the mass of a single fecal event for a small dog[7]. Based on FIB levels measured in fresh dog feces[21], this input to the lagoon could potentially account for FIB exceedances in the surf zone. These estimates are consistent with studies that have quantified markers[55] and FIB[7] in dog feces, and demonstrate the impact that a small number of dogs could have on water quality.

Results from sampling coordinated with residential education about pet waste management further supported that domestic dogs were most likely a significant source of fecal bacteria to the creek; education also indicated how to control the source. Although FIB did not decrease after the education program, FIB levels in environmental waters are known to fluctuate over time[9,56] and may persist in the environment even after their source has been removed[57,58]. Therefore, even with the significant reduction in dog fecal input along the creek, downstream FIB concentrations may not be immediately affected. Upstream fecal sources and sources directly to the lagoon and beach that were not affected by education may also be impacting water quality, particularly if there is increased FIB persistence compared to source-associated markers.

The host-associated MST dog fecal marker did cross-react with DNA extracted from wild canine feces. Therefore, the dog marker source cannot be attributed solely to domestic dogs. Similarly, human markers could be from septage, sewage, or homeless encampments, and further research is needed to distinguish these sources. However, regardless of the cross-reaction with wild canines, results before versus after education suggest domestic dogs as an influential source of dog marker to the creek which may then impact the lagoon and surf zone. Further research is needed to determine if dog markers from the creek persist through the lagoon to the surf zone. Education results also suggest that conducting similar efforts near the lagoon and at the beach could improve water quality in the lagoon and surf zone.

In summary, MST was performed using DNA-based human- and animal-associated fecal markers to identify and locate sources of fecal contamination affecting a coastal suburban watershed in Santa Barbara, CA. The main findings may be common suburban scenarios: lagoon discharges greatly impact beach water quality, and improper pet waste management alongside creeks could impact downstream water quality. Dog waste was a concern due to the popularity of the beach as a local dog park. However, while waste from dogs at the beach was detected, this did not appear to be directly responsible for beach warnings during this study. An important source of dog waste to the creek and lagoon was from domestic dogs along an upstream creek. Thus, even if dogs are not common at the beach, waste from dogs along creeks that are tributary to coastal zones could still be impactful. Additionally demonstrated is how an education program can be effective in reducing dog waste input to a coastal creek.

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FIGURES

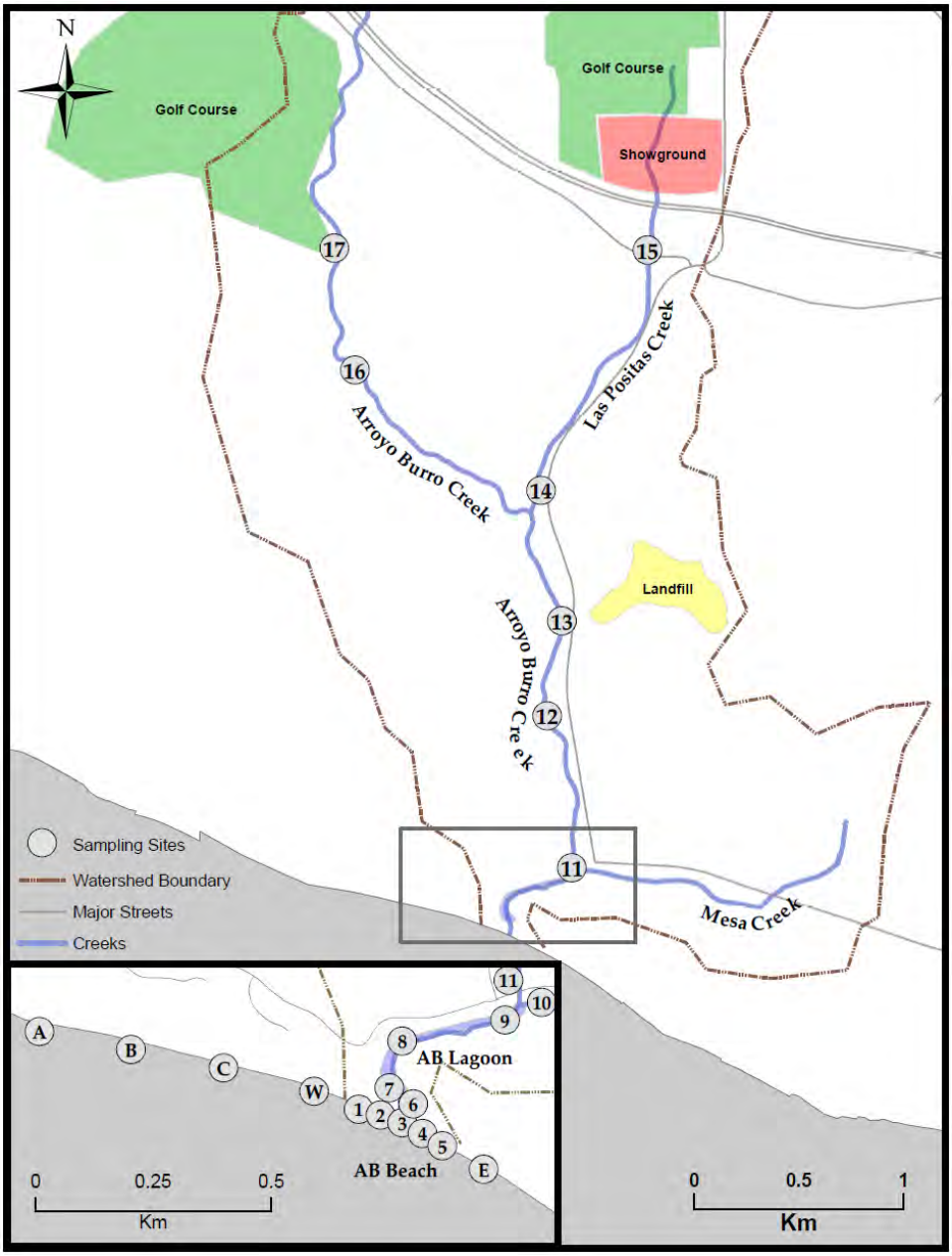

Figure 1. Map of the lower Arroyo Burro Watershed with the Arroyo Burro (AB) beach and lagoon area (inset). Surf zone, lagoon, and creek sampling locations are shown as grey-filled circles enclosing sampling site numbers or letters.

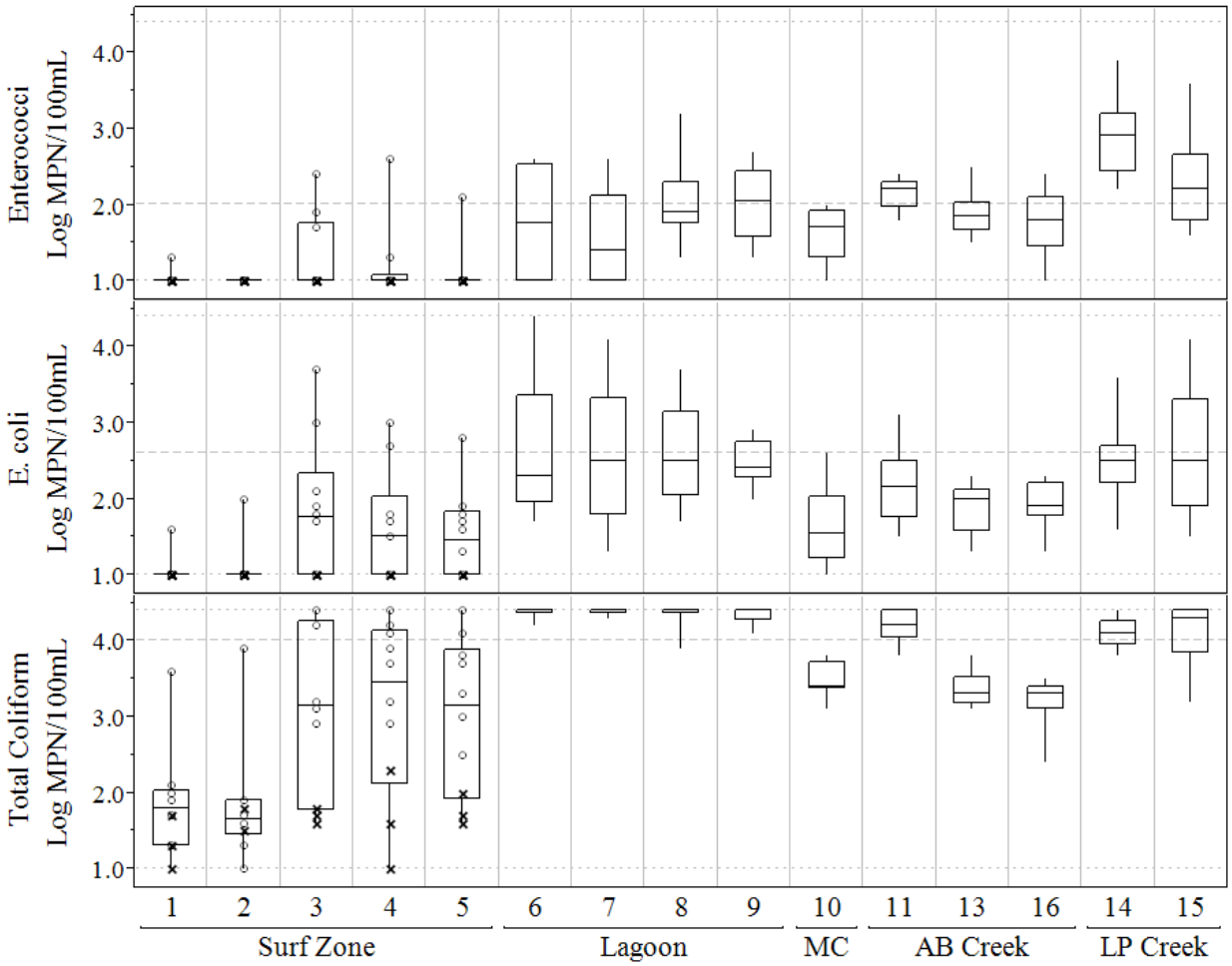

Figure 2. FIB concentrations at surface water locations sampled in 2012. Box plots represent the 25th, 50th, and 75th percentiles; whiskers represent the range (n=10-13). For surf zone samples, data points are shown representing samples collected when the lagoon was open (o) and closed (x). Dotted lines represent the upper and lower limits of quantification; data outside the quantifiable range are plotted at the limits of quantification. Dashed lines represent the CA single sample surf zone FIB limits[29]. MC = Mesa Creek, AB = Arroyo Burro, LP = Las Positas. Site numbers correspond to Figure 1.

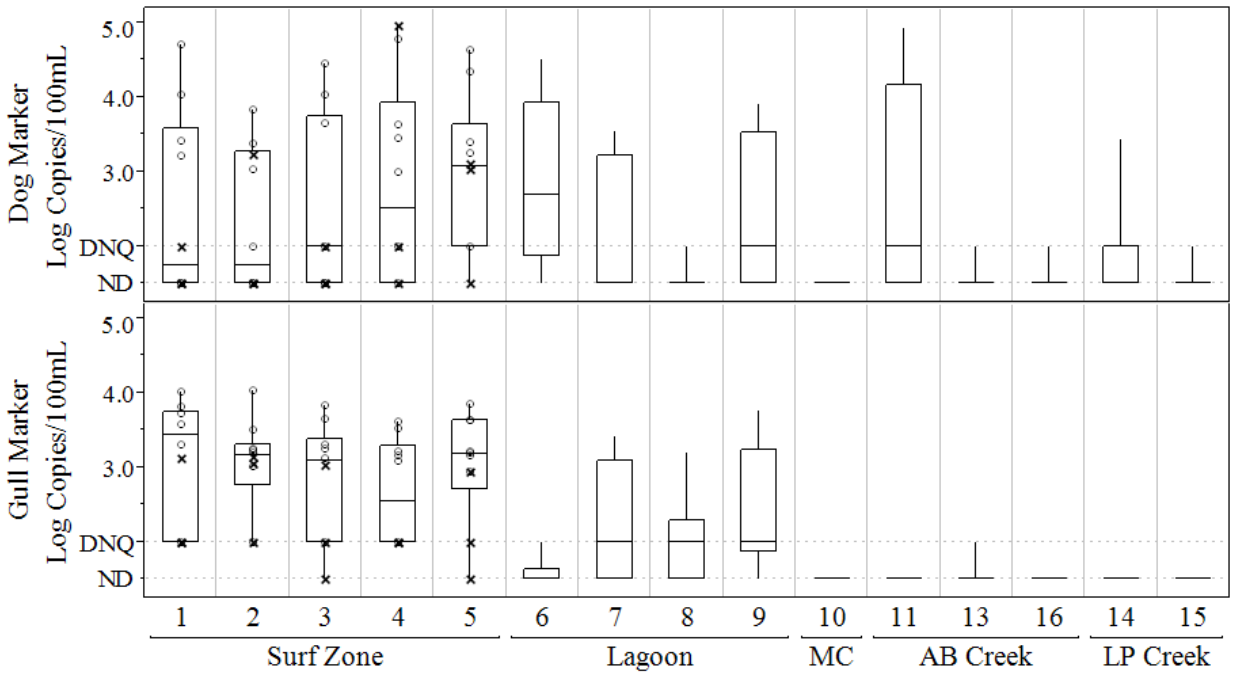

Figure 3. Dog (top) and Gull (bottom) fecal marker concentrations at surface water locations sampled in 2012. Box plots represent the 25th, 50th, and 75th percentiles; whiskers represent the range (n=10-13). For surf zone samples, data points are shown representing samples collected when the lagoon was open (o) and closed (x). DNQ = Detected but Not Quantifiable, ND = Not Detected, MC = Mesa Creek, AB = Arroyo Burro, LP = Las Positas. Site numbers correspond to Figure 1.

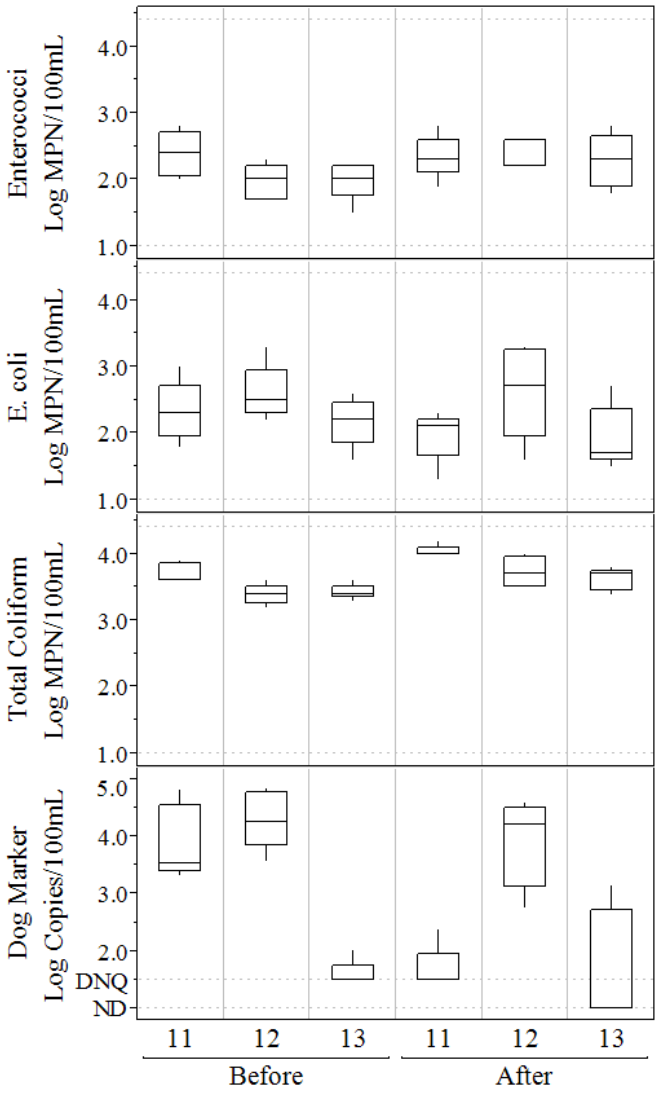

Figure 4. FIB and dog fecal marker concentrations at creek sampling locations collected before and after public education in 2013. Box plots represent the 25th, 50th, and 75th percentiles, whiskers represent the range (n=5). DNQ = Detected but Not Quantifiable, ND = Not Detected. Site numbers correspond to Figure 1.

SUPPORTING INFORMATION

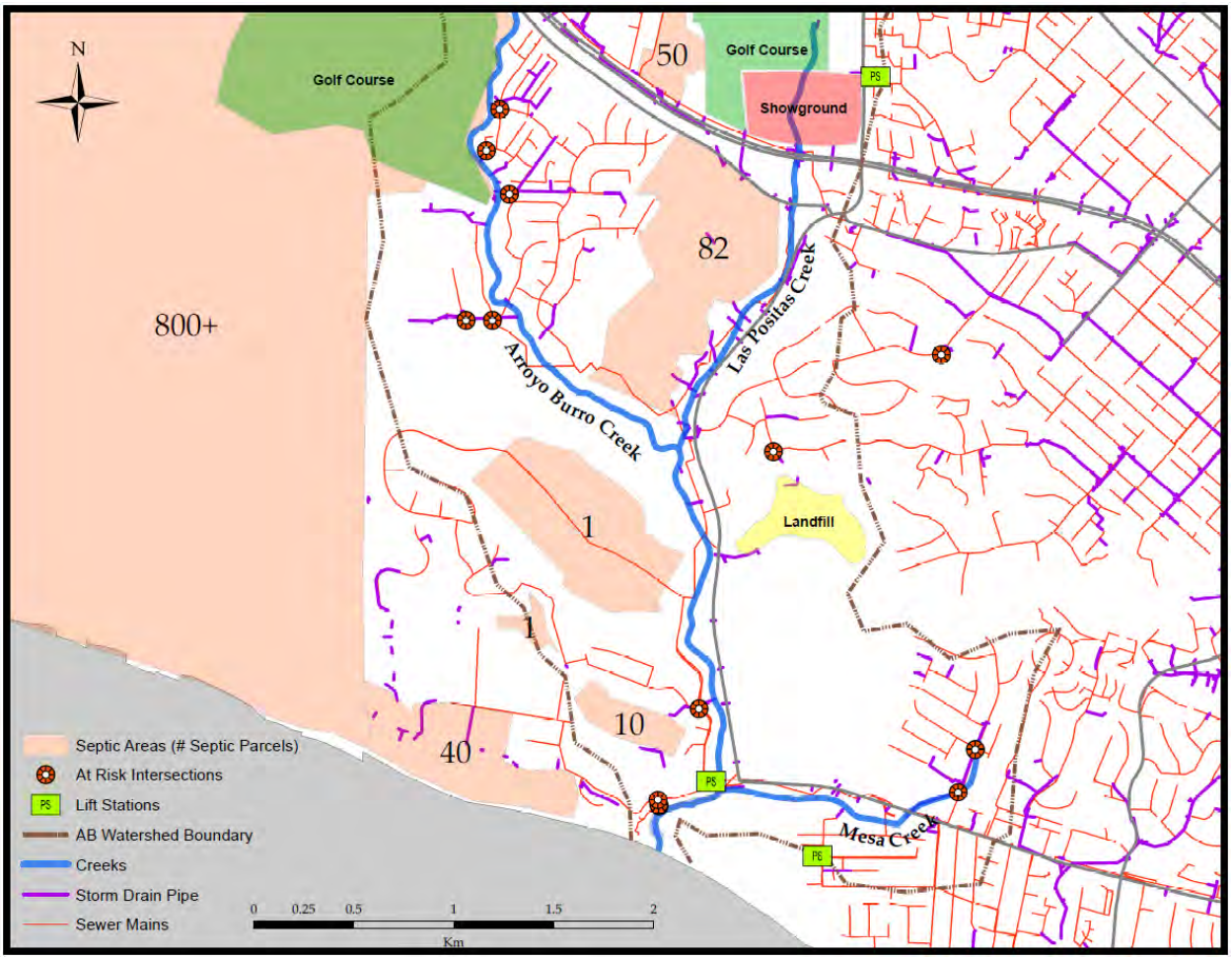

Figure S1. Map of the lower Arroyo Burro Watershed with GIS-based information related to hypothesized fecal sources[1]. The identified "At-Risk Intersections" were determined based on the proximity of sanitary sewers to storm drains along with their age and material. The City of Santa Barbara Creeks Division investigated these storm drains using CCTV[2].

Sand and Wrack Sampling

Sand and wrack samples were collected from 3 beach locations extending across 200m of the beach centered at the lagoon outlet. Sites were located on the beach as close as possible to perpendicular to where surf zone samples were taken at sites 2, 3 and 4 (Figure 1). Samples were collected weekly for 10 consecutive weeks during the summer of 2012. Still moist samples from near the previous high tide line were collected in 50 mL Falcon tubes (BD Biosciences, Franklin Lakes, NJ) for sand and Whirl-Pak bags (Nasco, Fort Atkinson, WI) for wrack and stored on ice. A subset of each sand and wrack sample was dried at 105 °C for 24 hours to determine moisture content. For sand samples, 50 g of wet sand were added to 500 mL of DI water in a sterile 2-L bottle. For wrack samples, 50 g of wet wrack were added to 500 mL of autoclaved phosphate buffered saline plus magnesium chloride solution (0.085 g KH2PO4 + 0.19 g anhydrous MgCl2 per L)[3]. Sand and wrack samples were then hand shaken for 3 minutes and allowed to settle for 30 seconds. The supernatant was filtered as described in the methods for surface water samples collected in 2012 with 100 mL per filter for sand and 25 mL per filter for wrack.

Lagoon Breach Experiment

Samples were collected from the surf zone before and after a single lagoon breach event on July 31st, 2012. Locations sampled included those sampled during the 2012 10-week study (sites 1-5), as well as one additional location 100m further to the east and to the west of the lagoon outlet (sites W & E). Results from this experiment showed that concentrations of FIB in the surf zone increased dramatically after the lagoon breached (Figure S2) and support the previously observed correlations between lagoon outlet flow and FIB concentration.

Groundwater Sampling

In 2013, submarine groundwater samples were collected from 5 locations extending 800m west of the lagoon outlet. Sites were located on the beach perpendicular to where surf zone samples were collected at sites A, B, C, W, and 1 in Figure 1. Samples were collected on three dates during July and August of 2013 during a low neap tide. Preliminary sampling revealed that sampling at a low neap tide allowed for the maximum proportion of fresh water in submarine groundwater samples. Submarine groundwater was collected by drilling a hole into the sand with a 3-1/4 inch soil auger (AMS, American Falls, ID) and then pumped to the surface through sterile tubing with a portable peristaltic pump (Geotech, Denver, CO). Samples were pre-filtered through sterile Miracloth (EMD Millipore, Billerica, MA) to remove sand and allow for flow through the pump. FIB and DNA analysis were then completed as described in the methods for surface water collected during 2013 field sampling.

Collection and Analysis of Wild Animal Feces

Fresh feces from coyote, fox and deer were analyzed to determine if there was cross-reaction with the dog fecal marker (DogBact). Three coyote, one composite fox (from two individuals), and one deer fecal sample was collected in August 2013 from the Animal Rescue Team in Solvang, CA. Each fecal sample was homogenized and approx. 0.25g was extracted using the PowerSoil DNA isolation kit (Mo-Bio, Carlsbad, CA). DNA extracts were tested for PCR inhibition as previously described (see Methods), and all filter and extraction blanks were within acceptable limits. DNA was analyzed using the Entero1A and DogBact markers (Figure S3) as previously described (see Methods). Additionally, DNA archived from several individual dog and raccoon samples collected from the Santa Barbara area in 2010 was also analyzed. The results from a dog composite sample from the SIPP study (composite of 12 dogs from across California) are also included for comparison.

Estimated Dog Fecal Input to Lower AB Creek

Based on the measured concentration of dog markers and flow of AB Creek at Site 11 during the 2012 sampling, the load of dog marker to the lagoon (geomean of 4 quantifiable samples) was approximately 2.7E6 copies/s (Table S9) or 2.4E11 copies/day. The concentration of the dog marker (DogBact) measured for a fresh composite sample from 12 dogs during the SIPP methods evaluation study was approximately 1E8 copies/filter or 1E10 copies/g[4,5]. Taking the creek load measured in this study and dividing by the SIPP study dog marker concentration for fresh dog feces yields an estimated dog fecal input of 24 g/day. Based on a study of the microbial load from animals, this is approximately the fecal load from a single event for a small dog and could account for elevated levels of FIB, which were measured at over 1E8 CFU/g dog feces for enterococci in some dogs[6]. This is also consistent with FIB levels measured in dog feces during the SIPP study with an estimated concentration of 8.8E7 CFU/g for enterococci[5]. Depending on the fate and transport (i.e. dilution and aging) of these fecal bacteria as they travel through the lagoon and are then released into the surf zone, these estimates for the load from domestic dogs to lower AB Creek could potentially account for elevated levels of dog marker and FIB measured in the lagoon and surf zone.

Table S1. Comparison of different filtering and extraction methods used in this study performed on replicate subsamples from a single creek sample at site 11.

Method	Sample	Volume Filtered (mL)	DNA (ng/100mL)	DogBact (Copies/100mL)	Entero1A (Copies/100mL)
Supor filter / Mo-Bio	11A	1130	363	267	1732
	11B	1180	366	207	1798
	11C	1120	397	294	1168
PC filter / GeneRite	11D	200	49	DNQ	961
	11E	200	45	DNQ	1446
	11F	200	18	ND	ND

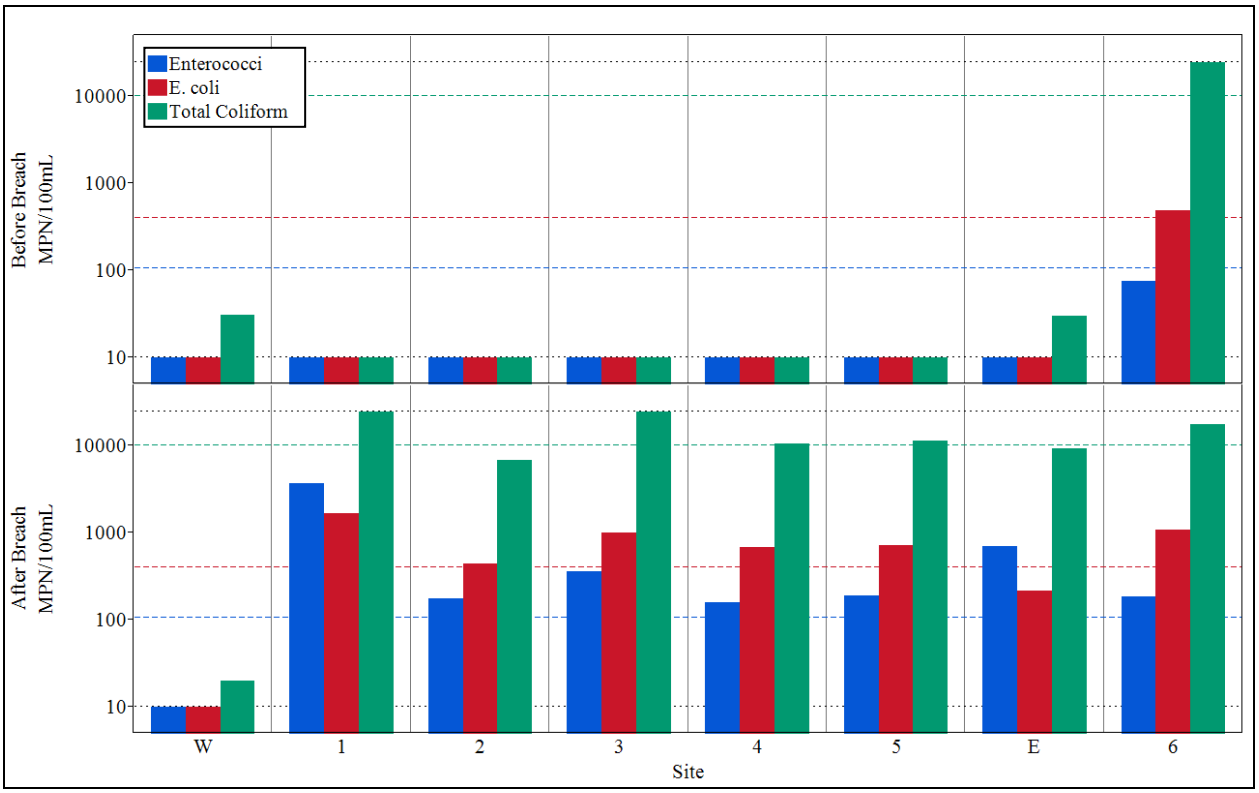

Figure S2. FIB concentrations at 7 surf zone sampling locations and the lagoon outlet (site 6) before (top) and after (bottom) a lagoon breach on 7/31/12. Dotted black lines represent the upper and lower limits of quantification and dashed colored lines represent the California single sample surf zone FIB limits[3]. Site numbers correspond to Figure 1.

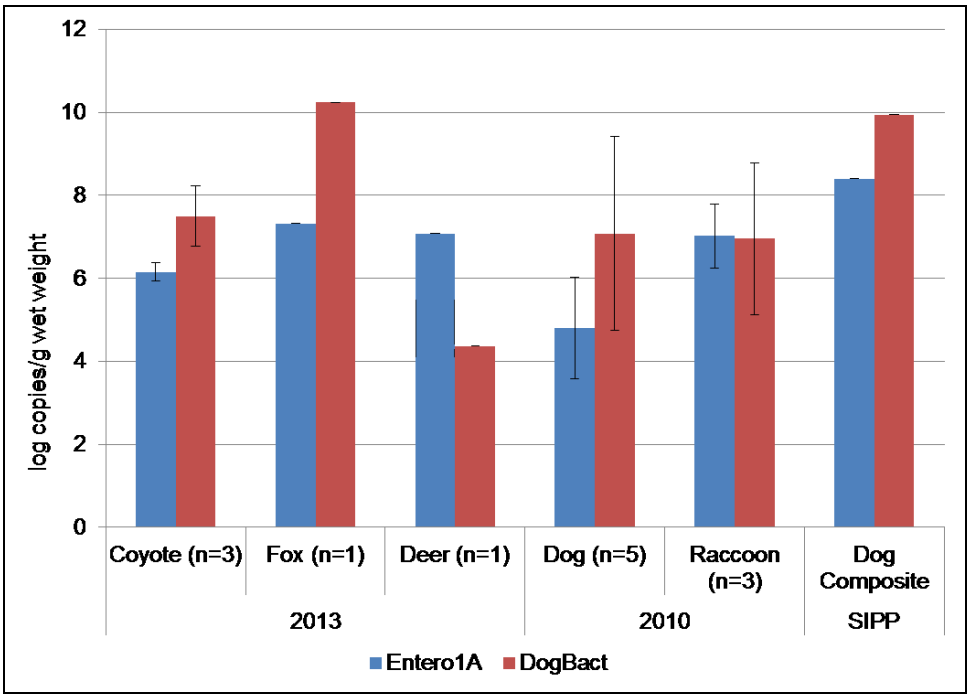

Figure S3. Concentration of Entero1A and DogBact markers in animal fecal samples. Geomean is shown for samples with n>1 and error bars represent the 95% confidence interval.

Table S2. Volume filtered and DNA yield for all water samples collected. Samples denoted "*" were collected in 2013, while all other samples were collected during the 2012 sampling. Site numbers correspond to Figure 1.

Location	Site #	n*	Volume Filtered (mL)		DNA (ng/100mL)	
			Mean	Range	Mean	Range
Surf Zone	A	3*	1330	1190 - 1510	348	146 - 551
	B	3*	1247	1140 - 1370	544	377 - 628
	C	3*	1197	1160 - 1250	581	527 - 631
	W	3*	1200	1140 - 1290	498	315 - 597
	1	10	200	200 - 200	722	314 - 1443
		3*	1210	1150 - 1300	664	518 - 779
	2	10	200	200 - 200	824	507 - 1836
	3	10	190	100 - 200	1166	529 - 3371
	4	10	195	150 - 200	1087	400 - 2724
	5	10	200	200 - 200	857	493 - 1650
Lagoon	6	10	155	100 - 200	4002	1329 - 16486
	7	10	165	100 - 200	2187	814 - 4843
	8	10	145	100 - 200	2955	1048 - 5848
	9	10	155	50 - 200	5292	536 - 17029
Mesa Creek	10	10	200	200 - 200	166	107 - 271
Arroyo Burro Creek	11	10	200	200 - 200	334	143 - 557
		10*	1176	1060 - 1330	393	287 - 495
	12	10*	1373	1190 - 1510	284	201 - 359
	13	10	200	200 - 200	134	114 - 157
		10*	1430	1290 - 1620	282	108 - 371
Las Positas Creek	14	13	188	100 - 200	355	179 - 586
	15	13	181	100 - 200	663	200 - 1500
Arroyo Burro Creek	16	10	200	200 - 200	190	121 - 307
		10*	874	680 - 990	1213	786 - 1927
	17	10*	1292	1140 - 1390	445	70 - 642
Submarine Groundwater	A	3*	1843	1830 - 1870	65	49 - 95
	B	3*	1850	1820 - 1870	64	37 - 86
	C	3*	1863	1840 - 1900	128	86 - 153
	W	3*	1857	1850 - 1860	90	81 - 94
	1	3*	1463	970 - 1780	279	97 - 576

Table S3. FIB abundance for samples collected during watershed reconnaissance. MPN = Most Probable Number. Site numbers correspond to Figure 1.

Location	Site #	n	Total Coliform (MPN/100mL)		*E. coli* (MPN/100mL)		Enterococci (MPN/100mL)	
			Median	Range	Median	Range	Median	Range
Arroyo Burro Lagoon	8	3	15531	14136 - 19863	1334	146 - 1334	336	161 - 373
Mesa Creek	10	9	6867	3725 - 11199	31	10 - 75	96	41 - 201
Arroyo Burro Creek	11	9	11199	6015 - >24196	146	41 - 262	404	173 - 581
Las Positas Creek	15	6	>24196	8664 - >24196	941	292 - >24196	390	86 - >24196

Table S4. Human marker results for samples collected during reconnaissance. ND = Not Detected, DNQ = Detected But not Quantifiable, ROQ = Range of Quantification. "-" denotes that no quantifiable results were obtained. Site #'s correspond to Figure 1.

Location	Site #	n	HF183Taqman				HumM2			
			%ND	%DNQ	%ROQ	ROQ (Copies/100mL)	%ND	%DNQ	%ROQ	ROQ (Copies/100mL)
Arroyo Burro Lagoon	8	3	100	0	0	-	100	0	0	-
Mesa Creek	10	9	100	0	0	-	100	0	0	-
Arroyo Burro Creek	11	9	100	0	0	-	100	0	0	-
Las Positas Creek	15	6	0	0	100	2040, 2846, 3140, 8351, 10074, 18787	17	50	33	745, 789

Table S5. Median and range of FIB concentrations for all water samples collected. MPN = Most Probable Number. "*" denotes samples collected in 2013, all other samples were collected in 2012. Site numbers correspond to Figure 1.

Location	Site #	n*	Total Coliform (MPN/100mL)		*E. coli* (MPN/100mL)		Enterococci (MPN/100mL)	
			Median	Range	Median	Range	Median	Range
Surf Zone	A	3*	51	41 - 63	10	<10 - 10	<10	<10 - 10
	B	3*	63	63 - 74	<10	<10 - <10	<10	<10 - <10
	C	3*	122	109 - 142	10	<10 - 31	<10	<10 - 10
	W	3*	30	30 - 63	<10	<10 - <10	<10	<10 - 10
	1	10	62	10 - 3609	<10	<10 - 41	<10	<10 - 20
		3*	31	30 - 109	10	<10 - 10	<10	<10 - 10
	2	10	47	<10 - 7701	<10	<10 - 97	<10	<10 - <10
	3	10	1433	41 - >24196	57	<10 - 5475	<10	<10 - 259
	4	10	3318	10 - >24196	31	<10 - 988	<10	<10 - 408
	5	10	1480	41 - >24196	31	<10 - 581	<10	<10 - 135
Arroyo Burro Lagoon	6	10	>24196	17329 - >24196	213	51 - >24196	63	<10 - 426
	7	10	>24196	19863 - >24196	305	20 - 12997	25	<10 - 383
	8	10	>24196	7215 - >24196	325	51 - 4884	75	20 - 1607
	9	10	>24196	12997 - >24196	246	98 - 839	121	20 - 480
Mesa Creek	10	10	2464	1201 - 6867	35	10 - 373	51	10 - 110
Arroyo Burro Creek	11	10	15733	5794 - >24196	156	31 - 1314	157	63 - 275
		10*	8866	3654 - 15531	134	20 - 906	208	74 - 663
	12	10*	3365	1455 - 9804	360	41 - 2014	164	52 - 364
	13	10	2205	1336 - 5794	97	20 - 216	69	31 - 331
		10*	2964	1918 - 6131	116	31 - 465	128	30 - 591
Las Positas Creek	14	13	12033	5748 - >24196	301	41 - 3654	754	148 - 7701
	15	13	19863	1539 - >24196	309	31 - 12033	145	41 - 3654
Arroyo Burro Creek	16	10	1912	259 - 2987	85	20 - 218	63	10 - 243
		10*	3873	1785 - >24196	86	10 - 7556	75	20 - 538
	17	10*	2604	1081 - 5794	279	75 - 1918	122	41 - 359
Submarine Groundwater	A	3*	30	<10 - 63	<10	<10 - <10	<10	<10 - <10
	B	3*	<10	<10 - 52	<10	<10 - <10	<10	<10 - <10
	C	3*	<10	<10 - 30	<10	<10 - <10	<10	<10 - <10
	W	3*	75	<10 - 645	<10	<10 - 10	<10	<10 - <10
	1	3*	<10	<10 - 20	<10	<10 - <10	<10	<10 - <10

Table S6. Lagoon outlet flow rate, tide status, FIB exceedance, marker detection and dogs counted at the beach by sampling date for 2012 surf zone samples (sites 1-5, Figure 1). %Exceedance is based on California single sample surf zone FIB limits[7]. Dogs on the beach were counted three times each sampling day and the count shown is the sum.

Sample Date	n	Lagoon Outlet Flow Rate (L/s)	Tide Status	%Exceedance			%Detection			Dog Count
				Total Coliform	*E. coli*	Enterococci	HF183Taqman	DogBact	Gull2	
5/21/2012	5	0	Ebb	0	0	0	0	0	100	38
5/29/2012	5	18	Flood	40	0	0	60	60	100	37
6/4/2012	5	87	Ebb	20	0	0	0	100	100	6
6/12/2012	5	23	Flood	20	0	0	20	80	100	20
6/18/2012	5	404	Ebb	60	60	60	0	60	100	18
6/25/2012	5	15	Flood	0	0	0	0	80	100	38
7/2/2012	5	0	Ebb	0	0	0	60	40	100	46
7/9/2012	5	20	Flood	0	0	0	0	20	100	40
7/16/2012	5	0	Ebb	0	0	0	0	100	60	26
7/23/2012	5	16	Flood	40	40	0	40	100	100	29

Table S7. Electrical conductivity, dissolved oxygen and temperature of water samples. "*" denotes samples collected in 2013, all other samples were collected in 2012. Site numbers correspond to Figure 1.

Location	Site #	n*	Electrical Conductivity (mS/cm)		Dissolved Oxygen (mg/L)		Temperature (°C)	
			Mean	Range	Mean	Range	Mean	Range
Surf Zone	A	3*	53.3	52.9 - 53.8	10.1	9.9 - 10.3	16.4	14.8 - 17.5
	B	3*	53.4	53 - 53.9	10.2	9.9 - 10.4	16.2	14.9 - 17.5
	C	3*	53.6	53.3 - 53.9	10.2	10 - 10.3	16.4	15.6 - 17.3
	W	3*	53.3	53 - 53.9	10.1	10 - 10.4	16.4	15.1 - 17.2
	1	10	52.5	51.1 - 53.2	9.2	8.8 - 9.8	19.0	17 - 21
		3*	53.5	53.2 - 53.8	10.1	10 - 10.3	16.6	15.3 - 17.4
	2	10	52.7	51.8 - 53.1	9.3	8.9 - 9.8	18.5	16.9 - 20.5
	3	10	48.0	20 - 52.9	9.6	8.9 - 10.8	18.9	16.9 - 21.1
	4	10	51.6	47.7 - 52.9	9.3	9.1 - 9.8	18.8	17.5 - 20
	5	10	50.9	38.2 - 52.7	9.3	9 - 9.7	18.8	17.3 - 20.4
AB Lagoon	6	10	15.1	8.4 - 34.4	12.0	5.5 - 22	21.7	19.4 - 27.8
	7	10	11.7	8.1 - 25.4	12.0	6.2 - 19.6	20.8	18.7 - 22.9
	8	10	8.0	5.2 - 11	11.6	5.8 - 17.5	19.5	17.5 - 21.2
	9	10	6.2	3.5 - 9.8	11.7	3.2 - 22	18.8	15.7 - 21.5
Mesa Creek	10	10	3.8	3.5 - 6.6	6.2	5.1 - 10	16.7	15.9 - 17.3
AB Creek	11	10	2.5	2.4 - 2.5	8.0	7.4 - 10	17.3	16.3 - 19.1
		10*	2.3	2.3 - 2.4	6.7	6 - 7.5	18.2	17.1 - 19.4
	12	10*	1.9	1.9 - 1.9	7.6	7.1 - 8.1	17.8	16.7 - 19.3
	13	10	1.8	1.8 - 1.8	8.6	8.1 - 9.3	17.2	16.6 - 18.3
		10*	1.7	1.7 - 1.7	8.4	8 - 8.8	18.1	16.7 - 19.8
LP Creek	14	13	1.6	1.5 - 1.8	6.8	6.3 - 7.4	17.7	16.4 - 20.1
	15	13	2.4	1.8 - 2.8	2.5	1.8 - 3.4	16.8	15.5 - 19.2
AB Creek	16	10	1.8	1.8 - 1.9	8.3	7.8 - 8.8	17.6	16.7 - 18.6
		10*	1.9	1.8 - 1.9	9.0	8.5 - 9.4	18.5	16.7 - 20.3
	17	10*	2.0	1.9 - 2	6.0	4.9 - 6.8	18.1	16.5 - 19.9
Submarine Groundwater	A	3*	14.8	2.5 - 36.3	2.3	1.9 - 2.6	21.6	20.5 - 22.7
	B	3*	54.5	54 - 54.9	2.7	2.2 - 3	18.7	12.4 - 22.7
	C	3*	22.2	13.9 - 34.3	2.8	2.3 - 3.9	21.4	20.9 - 22
	W	3*	50.2	46.3 - 52.9	2.5	1.4 - 3.3	20.9	20.6 - 21.2
	1	3*	2.2	1.1 - 3.9	2.6	2 - 3.1	20.0	19.4 - 20.4

Table S8. FIB concentrations in sand and wrack samples. MPN = Most Probable Number. Values are shown on a wet weight basis. The quantifiable range was from 1 to 2420 MPN/g. Site numbers correspond to Figure 1.

Location	Site #	n	Total Coliform (MPN/g)		*E. coli* (MPN/g)		Enterococci (MPN/g)	
			Median	Range	Median	Range	Median	Range
Sand	2	10	<1	<1 - >2420	<1	<1 - 2	<1	<1 - <1
	3	10	18	2 - 93	2	<1 - 30	<1	<1 - 2
	4	10	3	<1 - 178	1	<1 - 130	1	<1 - 579
Wrack	2	10	278	28 - >2420	27	6 - >2420	4	<1 - 75
	3	10	1733	10 - >2420	156	5 - >2420	2	<1 - >2420
	4	10	244	5 - >2420	26	2 - >2420	1	<1 - 19

Table S9. Summary of FIB and Marker loads at sampling sites where flow was measured. Geomean and Range values are shown for quantifiable samples only. "*" denotes samples collected in 2013, all other samples were collected in 2012. "-" denotes that no quantifiable results were obtained. Site numbers correspond to Figure 1.

Location	Site #	n	Flow (L/s)		Total Coliform (MPN/s)		*E. coli* (MPN/s)		Enterococci (MPN/s)		HF183Taqman (Copies/s)		HumM2 (Copies/s)		DogBact (Copies/s)	
			Mean	Range	Geomean	Range	Geomean	Range	Geomean	Range	Geomean	Range	Geomean	Range	Geomean	Range
Lagoon Outlet	6	10	58	0 - 404	3670368	2723525 - 4460074	81767	9438 - 574059	23592	1556 - 1720963	-	-	-	-	5720124	531731 - 130462475
Mesa Creek	10	10	2.2	1.0 - 4.1	62759	27616 - 221738	843	193 - 15123	927	195 - 3451	-	-	-	-	-	-
Arroyo Burro Creek	11	10	18	14 - 24	2491999	1232254 - 5819066	26966	6121 - 315897	25078	9101 - 50618	-	-	-	-	2740447	425985 - 16049565
		10*	8.7	4.8 - 12	628509	414120 - 1007883	11315	1818 - 96672	18615	6642 - 67349	-	-	-	-	417333	17257 - 7403354
	12	10*	8.3	6.2 - 12	287029	141416 - 640521	32966	3034 - 224919	11644	5818 - 23468	-	-	-	-	1000111	35216 - 7989004
	13	10	14	11 - 18	332604	153487 - 936745	10930	2618 - 25877	10934	3671 - 55189	-	-	-	-	-	-
		10*	9.0	6.1 - 12	283832	190079 - 495159	9840	2999 - 51339	11987	3522 - 36019	-	-	-	-	25544	12212 - 87110
Las Positas Creek	14	13	2.0	1.0 - 4.2	195772	129332 - 387327	5374	753 - 61989	13914	3700 - 321902	-	-	-	-	55344	49333 - 62087
	15	13	0.07	0.01 - 0.15	6360	255 - 28376	241	4 - 8595	114	5 - 2383	5092	1113 - 18874	802	509 - 1187	-	-
Arroyo Burro Creek	16	10	5.9	4.8 - 8.3	91162	13316 - 146589	5023	1243 - 18002	3314	513 - 12554	-	-	-	-	-	-
		10*	2.7	2.4 - 3.5	115557	52071 - 263652	3950	292 - 208532	1920	509 - 14125	-	-	-	-	N/A	N/A
	17	10*	2.0	1.3 - 3.5	47269	14237 - 152970	5488	954 - 50633	2722	529 - 9013	-	-	-	-	N/A	N/A

Table S10. Human fecal marker results in water samples. ND = Not Detected, DNQ = Detected But not Quantifiable, ROQ = Range of Quantification. Samples denoted "*" were collected in 2013, while all other samples were collected in 2012. "-" denotes that no quantifiable results were obtained. Site numbers correspond to Figure 1.

Location	Site #	n	HF183Taqman				HumM2			
			%ND	%DNQ	%ROQ	ROQ (Copies/100mL)	%ND	%DNQ	%ROQ	ROQ (Copies/100mL)
Surf Zone	A	3*	33	33	33	81	67	33	0	-
	B	3*	67	33	0	-	100	0	0	-
	C	3*	33	67	0	-	100	0	0	-
	W	3*	33	67	0	-	67	33	0	-
	1	10	80	20	0	-	100	0	0	-
		3*	33	67	0	-	67	33	0	-
	2	10	90	10	0	-	100	0	0	-
	3	10	70	30	0	-	100	0	0	-
	4	10	90	10	0	-	100	0	0	-
	5	10	80	20	0	-	100	0	0	-
Arroyo Burro Lagoon	6	10	100	0	0	-	100	0	0	-
	7	10	100	0	0	-	100	0	0	-
	8	10	100	0	0	-	100	0	0	-
	9	10	100	0	0	-	100	0	0	-
Mesa Creek	10	10	100	0	0	-	100	0	0	-
Arroyo Burro Creek	11	10	100	0	0	-	100	0	0	-
		10*	100	0	0	-	80	20	0	-
	12	10*	80	20	0	-	50	50	0	-
	13	10	100	0	0	-	100	0	0	-
		10*	100	0	0	-	100	0	0	-
	16	10	80	20	0	-	100	0	0	-
		10*	50	50	0	-	60	40	0	-
	17	10*	80	20	0	-	70	30	0	-
Las Positas Creek	14	13	92	8	0	-	13	0	0	-
	15	13	8	38	54	775, 1420, 2831, 13626, 23950, 37737, 49831	38	31	31	979, 1162, 2804, 3262

Table S11. Dog and gull fecal markers results in water samples. ND = Not Detected, DNQ = Detected But not Quantifiable, ROQ = Range of Quantification. Samples denoted "*" were collected in 2013, while all other samples were collected during the 2012 sampling. N/A = Not Analyzed. Site numbers correspond to Figure 1.

Location	Site #	n	DogBact Marker					Gull2 Marker				
			%ND	%DNQ	%ROQ	Copies/100mL		%ND	%DNQ	%ROQ	Copies/100mL	
						Median	Range				Median	Range
Surf Zone	1	10	50	10	40	ND/DNQ	ND - 51383	0	30	70	2894	DNQ - 10377
	2	10	50	10	40	ND/DNQ	ND - 6794	0	20	80	1490	DNQ - 10865
	3	10	40	30	30	DNQ	ND - 57815	10	30	60	1443	ND - 6936
	4	10	30	20	50	DNQ/1009	ND - 92775	0	50	50	DNQ/1219	DNQ - 4065
	5	10	10	30	60	1172	ND - 42672	10	10	80	1498	ND - 7105
Arroyo Burro Lagoon	6	10	20	30	50	DNQ/2277	ND - 31735	80	20	0	ND	ND - DNQ
	7	10	70	0	30	ND	ND - 3527	40	20	40	DNQ	ND - 2544
	8	10	90	10	0	ND	ND – DNQ	40	40	20	DNQ	ND - 1541
	9	10	40	20	40	DNQ	ND - 8126	20	40	40	DNQ	ND - 5924
Mesa Creek	10	10	100	0	0	ND	-	100	0	0	ND	-
Arroyo Burro Creek	11	10	30	30	40	DNQ	ND - 85402	100	0	0	ND	-
		10*	0	40	60	1185	DNQ - 65324	N/A	N/A	N/A	N/A	N/A
	12	10*	0	0	100	17032	571 - 71543	N/A	N/A	N/A	N/A	N/A
	13	10	90	10	0	ND	ND - DNQ	90	10	0	ND	ND - DNQ
		10*	30	40	30	DNQ	ND - 1429	N/A	N/A	N/A	N/A	N/A
	16	10	90	10	0	ND	ND - DNQ	100	0	0	ND	-
		10*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	17	10*	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Las Positas Creek	14	13	62	23	15	ND	ND - 2726	100	0	0	ND	-
	15	13	92	8	0	ND	ND - DNQ	100	0	0	ND	-

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