Microbial Source Tracking in a Coastal California Watershed Reveals Canines as Controllable Sources of Fecal Contamination

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Supporting Information

ABSTRACT: Elevated levels of fecal indicator bacteria (FIB), including Escherichia 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 study was performed. Surface waters were sampled over 2 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 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 Escherichia 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 nonhuman sources is estimated to be lower than that from human sources.18 Therefore, discerning human from nonhuman FIB sources is needed to inform water quality management.

Microbial source tracking (MST) is used to identify fecal sources contaminating ambient 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 closely associated with hosts, e.g., for humans, dogs, and gulls.21 MST in suburban coastal watersheds is challenging given the variety of land uses22 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. Although 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 km², with residential, commercial, agricultural, and open space land uses.22 Furthest downstream, on the Santa Barbara Channel, is Arroyo Burro Beach Park with an annual visitation of over 1 million people,25 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-m 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 1).

2.2. 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, 15 locations (sites 1−11 and 13−16) were sampled weekly for 10 consecutive weeks. Additional water samples were collected from Los Positas Creek (sites 14 and 15) during 3 horse shows at the showgrounds (Figure 1). Additional samples were also collected from the surf zone (sites 1−5, E and W) before and after a lagoon breach on July 31, 2012.

In the summer of 2013, creek waters were sampled along Arroyo Burro Creek (sites 11−13) 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 in Las Positas Creek (sites 14 and 15) because, based on apparent FIB attenuation between sites 14 and 13, downstream fecal inputs (between sites 11 and 13) would have the greater potential impact on surf zone water quality. Samples were also acquired from upstream Arroyo Burro Creek (sites 16 and 17) on the same 10 dates as downstream creek samples (sites 11−13) in the summer of 2013, to investigate fecal sources indicated by the 2012 field study.

To further investigate fecal sources in the surf zone, surf zone waters were sampled (sites A, B, C, W, and I, Figure 1) three times during three separate weeks in the summer of 2013. Submarine groundwater samples were collected on the beach perpendicular to each surf zone sample. Samples were collected during a low neap tide based on preliminary sampling, which indicated that this allowed for the maximum proportion of fresh water in groundwater samples. Submarine groundwater was collected by drilling a hole into the sand with a 3−1/4 in. soil auger (AMS, American Falls, ID) and then pumping to the surface through sterile tubing with a portable peristaltic pump (Geotech, Denver, CO). Samples were prefilled through sterile Miracloth (EMD Millipore, Billerica, MA) to remove sand and allow flow through the pump.

Samples were collected in sterile 2 L or 4 L polypropylene bottles, stored on ice, and processed within 6 h of collection. Dissolved oxygen, electrical conductivity, and temperature were measured at each site using an HQ40d multiparameter meter equipped with conductivity and luminescent dissolved oxygen (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.

2.3. 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 midsummer 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 coresidents and with contractors and gardeners that may also dispose of pet waste.

2.4. Laboratory Analyses. Samples were analyzed by defined substrate culture methods (IDEXX, Westbrook, ME) for enterococci, E. coli and total coliform bacteria. For water samples collected in 2012, several 200 mL aliquots were filtered through 0.4 μM 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. 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). A comparison of triplicate samples processed by both methods shows that DNA recoveries were not significantly different (t-test, p = 0.44), but sensitivity was increased (Table S1, Supporting Information). DNA concentrations were quantified using the Quant-iT dsDNA broad-range assay kit (Life Technologies, Carlsbad, CA) (Table S2, Supporting Information).

DNA extracts were analyzed by quantitative polymerase chain reaction (qPCR) for the HF183Tagman27 and HumM28 human-associated fecal markers, and DogBact29,30 (dog) and Gull2Tagman30,31 (gull) fecal markers. Selected samples were also analyzed for the routine PCR HoF59729 (horse) and the qPCR Entero1A32 (enterococci) markers. Markers were selected based on their performance in a comprehensive methods evaluation study.25,33−35 Inhibition during qPCR was assessed using a spiking and dilution procedure.36 Filter and extraction blanks were analyzed to assess contamination during sample filtration and DNA extraction, respectively.21 PCR reactions were performed in triplicate with three no-template controls included in each 96-well plate; separate plasmid DNA standards were PCR amplified in triplicate as before.21 Samples with at least two replicates amplifying within the range of the standard curve were considered to be 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.34,35

2.5. Collection and Analysis of Animal Feces. Feces from coyote, fox, and deer were collected to evaluate their possible cross-reactivity with DNA-based dog markers. Fecal samples from three coyote, one composite fox (from two individuals), and one deer were collected in August 2013 from the Animal Rescue Team in Solvang, CA. Each fecal sample was homogenized and approximately 0.25 g was extracted using the PowerSoil DNA isolation kit (Mo-Bio, Carlsbad, CA). DNA was analyzed using the Entero1A and DogBact markers as previously described. 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 sample (composite of 12 dogs from across California) analyzed as part of a recent methods evaluation study are included for comparison. Human fecal samples, as well as septage and sewage were also tested for cross-reactivity with the DogBact marker as part of this study.

2.6. Statistical Analyses. FIB exceedances were based on California single sample surf zone regulations for total coliform (10,000 MPN/100 mL), E. coli (400 MPN/100 mL), and enterococci (104 MPN/100 mL). 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 nonparametric tests accommodated censored data that arose for FIB and qPCR markers. Mann–Whitney and Kruskal–Wallis tests were used to compare groups of data between sites or dates, whereas 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. MST FIB Results. Surf zone FIB (Figure 2, Table S3, Supporting Information) 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 S4, Supporting Information). 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 S5, Supporting Information) confirmed the influence of lagoon water. No surf zone FIB exceedances were measured in 2013 (Table S3, Supporting Information).

Surf zone FIB concentrations were correlated with the FIB load discharging from the lagoon in 2012 (Spearman’s rank correlation, p < 0.05), and these correlations were 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 S1, Supporting Information). These results are consistent with a prior analysis of historical surf zone data at Arroyo Burro Beach that found a correlation between FIB exceedances and the lagoon being open, with a 7-fold greater chance of enterococci exceedance when the lagoon was open. Lagoon FIB concentrations (Figure 2) 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. Dissolved oxygen and temperature were also higher on average in the lagoon (sites 6–9) compared to other surface water sites (Table S5, Supporting Information). The FIB load (Table S6, Supporting Information) entering the lagoon was greater from Arroyo Burro Creek (site 11) compared to Mesa Creek (site 10) (Mann–Whitney U test, p < 0.01). Within the lagoon, total coliform concentrations were higher at sites 6–8 versus those entering...
the lagoon from site 11 (Kruskal–Wallis test, \(p < 0.05\)). 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 S3, Supporting Information) along Arroyo Burro Creek fluctuated, but were generally greater at downstream site 11, versus upstream sites 13 and 16. FIB concentrations were relatively high in Las Positas Creek (sites 14 and 15).

### 3.2. qPCR Quality Assurance and Controls.

All master calibration curves were of acceptable quality (\(R^2 > 0.98, E > 0.89\), Table 1), and no amplification inhibition was detected based on the spiking and dilution procedure (e.g., diluted spike was within \(\pm 1\) \(C\) of expected). DNA contamination was low with 99.2% of no-template controls below the detection limit (\(n = 127\)), and 98.4% and 96.5% of filter blanks (\(n = 64\)) and extractions blanks (\(n = 114\)) below detection, respectively.

#### Table 1. qPCR Standard Curve Statistics for DNA-based Fecal Markers

<table>
<thead>
<tr>
<th>assay</th>
<th>slope</th>
<th>(y)-intercept</th>
<th>(R^2)</th>
<th>efficiency(^a)</th>
<th>ROQ (log copies/reaction)</th>
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</thead>
<tbody>
<tr>
<td>HF183Taqman</td>
<td>−3.60</td>
<td>40.3</td>
<td>0.988</td>
<td>0.894</td>
<td>1.3 to 5.3</td>
</tr>
<tr>
<td>HumM2</td>
<td>−3.50</td>
<td>40.5</td>
<td>0.989</td>
<td>0.929</td>
<td>1.3 to 5.3</td>
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<tr>
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<td>−3.43</td>
<td>42.0</td>
<td>0.989</td>
<td>0.957</td>
<td>1.3 to 5.3</td>
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<tr>
<td>Gull2Taqman</td>
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<td>41.8</td>
<td>0.995</td>
<td>0.929</td>
<td>1.3 to 5.3</td>
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<tr>
<td>Entero1A</td>
<td>−3.53</td>
<td>39.2</td>
<td>0.996</td>
<td>0.920</td>
<td>1.3 to 7.3</td>
</tr>
</tbody>
</table>

\(^a\)Efficiency = \(10^{(−1/\text{slope})}−1\).

#### Table 2. DNA-based Fecal Marker Results for Water Samples Collected in 2012\(^b\)

<table>
<thead>
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<th>location</th>
<th>site #</th>
<th>(\pi)</th>
<th>human markers</th>
<th>non-human markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HF183Taqman</td>
<td>HumM2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>%DNQ</td>
<td>%ROQ</td>
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<tr>
<td>surf zone</td>
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<tr>
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<td>10</td>
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<tr>
<td></td>
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<td>20</td>
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<td>Arroyo Burro Lagoon</td>
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<tr>
<td></td>
<td>15</td>
<td>13</td>
<td>38</td>
<td>54</td>
</tr>
</tbody>
</table>

\(^b\)DNQ = detected but not quantifiable, ROQ = range of quantification. Site numbers correspond to Figure 1.

![Figure 3](https://example.com/figure3.png)

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.
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 2). 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 S4, Supporting Information). 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 S7, Supporting Information). Submarine groundwater sampled at the beach (sites A, B, C, W, and 1) showed low FIB levels (Table S3, Supporting Information) and no human marker detections (Table S7, Supporting Information). No human markers were detected in lagoon samples (sites 6–9) or in downstream Arroyo Burro or Mesa Creek samples (sites 10 and 11) collected in 2012 (Table 2). Both human markers were quantified in upstream Las Positas Creek at site 15 (Table 2), likely related to an upstream homeless encampment that was observed (including toilet paper and feces near the creek). However, although quantifiable concentrations of both human markers were detected, it is unlikely that human marker loads were impacting downstream sites (Table S6, Supporting Information). The HF183Taqman marker was detected only once at downstream site 14, and no HumM2 marker was detected at this site (Table 2).

On upper Arroyo Burro Creek, the HF183Taqman marker was detected at DNQ levels in 20% of site 16 samples in 2012 (Table 2). HF183Taqman and HumM2 human markers were also detected at DNQ levels in 35% of samples at sites 16 and 17 in 2013 (Table S7, Supporting Information). Similarly to results from Las Positas Creek, human markers did not persist, as there were no human markers detected at site 13 (Tables 2 and S7, Supporting Information). Although these upper watershed indicators of human waste contamination may have been related to nearby septic systems, the absence of downstream human waste markers suggested that such contamination was unlikely to cause elevated FIB levels 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 Non-Human Fecal Contamination. Dog and gull fecal markers were frequently detected and quantified in the surf zone and lagoon (Figure 3). The gull marker was detected in 96% of surf zone samples, 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 had only two DNQ detections at the lagoon outlet (site 6), and was detected in only one creek sample (site 13, Figure 3).

The horse marker was analyzed for samples collected from Las Positas Creek (sites 14 and 15) downstream from the showground (Figure 1). 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, with 42% at a quantifiable level (Figure 3). Although the median dog marker concentration increased from sites 3–5 (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 correlated with FIB and lagoon outlet flow rate (Spearman’s rank correlation, \( p < 0.05 \)). Surf zone dog marker concentration was also 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 S4, Supporting Information). However, there were no corresponding surf zone FIB exceedances on days when the lagoon was closed (Table S4, Supporting Information). The dog marker was detected in 45% of lagoon samples, with higher levels at sites 6 and 9 compared to site 8 (Kruskal–Wallis test, \( p < 0.05 \)). This spatial pattern suggests multiple dog marker 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). 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 increased from site 13 to site 11 in paired 2012 samples (Wilcoxon signed-rank test, \( p = 0.02 \)).

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 dog marker and 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 and 12), where 25 dogs were reported. Contact was also made with 18 of 22 residents along Las Positas Creek (between sites 14 and 15), where 18 dogs were reported (Figure 1). Before education in 2013, dog marker concentrations increased from upstream site 13 to downstream sites 12 and 11 (Figure 4; Kruskal–Wallis test, \( p < 0.01 \)), 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 decrease from upstream site 12 to downstream site 11 (Mann–Whitney U test, \( p < 0.01 \)). A decrease in dog marker concentration was also observed at site 11 when before versus after education data were compared (Mann–Whitney U test, \( p < 0.01 \)). 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 reach of Arroyo Burro Creek. Although an associated decrease in FIB was not observed at site 11 (Figure 4), dog markers were correlated with both the Entero1A marker and E. coli (Spearman’s rank correlation, \( p < 0.01 \)) across sites 11–13 in 2013. 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 Las Positas creek for 2013 due to the pattern of decreased FIB and dog markers between sites 14 and 13 (Figures 2 and 3).

3.6. Estimated Dog Fecal Input to Lower Arroyo Burro Creek. On the basis of the measured dog marker concentrations and flows of Arroyo Burro Creek at site 11 during the 2012 samplings, the load of dog marker to the lagoon (geomean of 4 quantifiable samples) was approximately

shows that wild canines and other animals could have been contributing to the dog markers measured in Arroyo Burro Creek. However, although there may have been fecal inputs from wild animals, the decrease in dog marker concentration at site 11 following education (Figure 4) suggests that domestic dogs were an influential source of dog marker to downstream Arroyo Burro Creek. Human fecal samples analyzed as part of a related study showed only low level cross-reaction with the dog marker used in this study.35 High concentrations of human waste would be required to cause nonspecific amplification with the DogBact primers at the dog marker levels measured, but no quantifiable human markers were detected in Arroyo Burro Creek (sites 11–13, Tables 2 and S7, Supporting Information).

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 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.40 Although no surf zone FIB exceedances were detected in weekly Santa Barbara County sampling,42 multiple exceedances were measured during this study, showing the impact that sample timing and location can have on beach warnings.

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.43 Still unknown, however, is the presence of pathogens associated with human sources that may decay at different rates compared to source-associated markers and FIB,44,45 potentially creating a public health concern.

Dog markers identified in the surf zone originated both at the beach and from the Arroyo Burro Lagoon, and an input to the lagoon was measured along a downstream reach of Arroyo Burro Creek. Although dog markers were also detected further upstream, canine waste input along lower Arroyo Burro Creek was shown to impact downstream water quality. Few studies have identified dog waste as a quantifiable 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 Here we show quantitatively, based on relating DNA-based dog marker concentrations in creek water to those in fresh dog feces, that the fecal material from just one dog could be enough to impact surface water quality including FIB concentrations.

Results from sampling coordinated with residential education about pet waste management further supported that domestic dogs were a source of fecal bacteria to Arroyo Burro 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 time5,46 and may persist in the environment even after their source has been removed.47,48 Therefore, even with the demonstrated reduction

2.7 × 10^6 copies s^{−1} (Table S6, Supporting Information) or 2.4 × 10^{11} copies day^{−1}. The concentration of the dog marker (DogBact) measured for a fresh composite sample from 12 dogs during a prior study was approximately 1 × 10^{10} copies per gram wet feces.35,41 Dividing the Arroyo Burro Creek dog fecal load measured in this study by the dog marker concentration for fresh dog feces yields an estimated dog fecal input of 24 g per day. This is approximately the fecal load from a single event for a small dog^7 and could account for elevated levels of FIB, which have been measured at over 1 × 10^{8} CFU per gram dog feces for enterococci in individual dogs^7 and 8.8 × 10^{10} CFU per gram wet weight for a dog composite sample.41 An input of 24 g per day of fresh wet dog feces with an enterococci concentration of 8.8 × 10^{10} CFU per gram gives an estimated concentration of 136 CFU per 100 mL in the creek, based on an average creek flow at site 11 of 18 L per second (Table S6, Supporting Information), which is above the State of California single sample surf zone limit for enterococci.38

3.7. Dog Marker Cross-Reaction. To further evaluate the host origins of dog markers between sites 12 and 13 (where there were no homes with domestic dogs), feces from relevant wild animals were collected and analyzed. During education, residents noted to City employees that coyote and fox were observed near Arroyo Burro Creek. DNA extracted from coyote and fox fecal samples harbored dog markers in concentrations that were similar to that of domestic dogs (Figure S2, Supporting Information). Fecal samples collected from deer and raccoon also resulted in quantifiable dog markers. Only a few fecal samples for each animal type were collected, but this
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 have been contributing FIB.

The host-associated dog fecal marker did cross-react with DNA extracted from wild canine feces (coyote and fox), suggesting that this marker may be more useful as a genus-level canine-associated marker. Cross-reaction of the dog marker with other wild animals including deer and raccoon could also be problematic in source identification. Therefore, the dog marker source cannot be attributed solely to domestic dogs. Future development of a dog marker that is specific to domestic dogs could be useful in microbial source tracking, and markers need to be thoroughly validated against closely related animals. Regardless of the cross-reaction with wild animals, results before versus after education support that domestic dogs may be an influential source of dog marker to the creek which could impact the lagoon and surf zone. Education results suggest that conducting similar efforts near the lagoon and at the beach could improve water quality in the lagoon and surf zone. Water quality managers of other beaches and surface waters may also be able to reduce dog fecal inputs through similar education programs.

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 watershed with its popular beach in Santa Barbara, CA. The main findings may comprise a common suburban scenario: lagoon discharges greatly impact beach water quality, and improper pet waste management alongside creeks could introduce fecal contamination into the lagoon. Dog waste was a concern due to the popularity of the beach as a local dog park. However, although waste from dogs at the beach was detected, breaching of the lagoon was the dominant factor causing surf zone water quality violations 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 and estuaries 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.

ASSOCIATED CONTENT

Supporting Information
Comparison of filtration and extraction methods, FIB concentrations during lagoon breach, concentration of markers in animal fecal samples, volume filtered and DNA yield, median and range of FIB concentrations, data by sampling date for 2012 surf zone samples, electrical conductivity, dissolved oxygen and temperature, FIB and host fecal marker loads, and human markers for samples collected in 2013. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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Fecal Pollution Sources to Beaches


