Mahaulepu and Waikomo Watersheds PhyloChip Source Tracking Study, Hawaii

Final Report

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I. Executive Summary

Counts of fecal indicator bacteria (enterococci and *Clostridium perfringens*) are frequently high in the drainage system of Mahaulepu Valley on the island of Kauai. A sanitary survey completed by Hawaii Department of Health (DOH) concluded that high bacteria levels are attributable to sources other than human sewage, due to the absence of human sewage sources in Mahaulepu Valley. The DOH noted several alternative sources of fecal indicator bacteria (FIB), including avian wildlife, feral pigs and chickens, domesticated animals, and environmental sources such as sediments. The DOH also expressed concern about human wastewater contamination from the approximately 120 injection wells and 1600 cesspools found in the Koloa and Poipu area that may be contaminating groundwater and surface waters in the Waikomo Watershed, and possibly impacting water quality in the Mahaulepu Valley as well.

The goals of this study were to use PhyloChip microbial source tracking to measure the influence of human, animal and environmental inputs of FIB on water quality in both Mahaulepu and Waikomo watersheds. PhyloChip is a DNA microarray that identifies over 59,000 different types of bacteria in a single sample. This technology enables a comprehensive survey of bacterial diversity in a sample and provides a genetic fingerprint of the microbial community. Unique community signatures based on thousands of DNA markers are used to very accurately detect and classify multiple fecal sources in a single test (Dubinsky et al. 2012, Cao et al. 2013, Dubinsky et al. 2016).

We used PhyloChip to characterize the microbial compositions of potential sources of fecal indicator bacteria in the Mahaulepu and Waikomo watersheds, including cesspools, avian wildlife, chickens, cattle, sheep, horses, pigs and sediments. We then used this information to look for fecal DNA signatures upstream and downstream of potential contamination sources in both Waiopili Ditch and Waikomo Stream, and in coastal seeps in Poipu, during wet and dry periods. Goals of this project were to 1) use PhyloChip to identify both fecal and environmental sources of fecal indicator bacteria in Waiopili Ditch and Waikomo Stream during wet and dry periods, 2) determine if high counts of enterococci and *C. perfringens* could be explained by human, animal or environmental sources of bacteria, 3) determine if fecal sources of bacteria are transmitted through Makauwahi Cave to Waiopili Ditch, and 4) determine if coastal seeps in the resort area of Poipu are impacted from human contamination from nearby wastewater injection wells.

Results showed that high concentrations of FIB in both Waiopili Ditch and Waikomo Stream were not likely caused by human or animal fecal contamination. Most samples with high FIB concentrations had nominal human and animal fecal signals. There was no association between any fecal sources and FIB concentrations in Waiopili Ditch. In Waikomo Stream, the highest concentrations of FIB during rainy weather sampling contained weak fecal signals from ruminants, feral pigs or humans, indicating these sources may impact Waikomo Stream during high runoff conditions even if they are not the primary sources of FIB. In Waiopili Ditch, one sample contained a strong ruminant signal and one sample contained a strong pig signal. One sample in Waikomo Stream was strongly positive for pigs. Fecal contamination from these

sources occurs sporadically and cannot explain the frequently high concentrations of FIB observed in both drainage systems.

Strong human fecal signal was found in a coastal seep along the beachfront of the Poipu resort area. The strength of the human signal was comparable in magnitude and microbial composition to injection wells in the Waikomo watershed. This finding indicates that coastal seeps in Poipu can be impacted by nearby injection wells.

No fecal contamination was found in stream sediments despite high sediment FIB concentrations. Patterns of similarity between surface water and sediment samples did not indicate that FIB found in surface waters originated directly from sediment sources. No fecal contamination was found in Makauwahi Cave sediments or water, indicating that subterranean transport of fecal bacteria between Waikomo and Mahaulepu watersheds was not likely at the time of sampling.

II. Introduction

Many freshwater and coastal water bodies in Hawaii are impaired by high counts of fecal indicator bacteria. Sources of fecal indicator bacteria must be identified in order to determine the need for beach advisories and closures, and ultimately contain the sources of bacterial contamination from human and agricultural activities. Non-point sources of fecal indicator bacteria are challenging to identify because enterococci bacteria that are measured to assess water quality are ubiquitous in human sewage, domesticated and agricultural animals, and wildlife. Further confounding the issue in Hawaii is the widespread environmental occurrence of these bacteria in pristine soils and sediments (Hardina and Fujioka 1991).

In south-central Kauai, the Hawaii Department of Health (DOH) conducted a sanitary survey of Waiopili Ditch in the Mahaulepu watershed to determine the source of high background concentrations of enterococci (ENT) and *Clostridium perfringens* (CP) that are frequently detected in this drainage system and where it enters the ocean at Mahaulepu Beach (DOH 2015). Part 1 of the sanitary survey confirmed the presence of elevated levels of ENT and CP in Waiopili Ditch. DOH conducted a literature search and physical surveys of the Mahaulepu Watershed and found no evidence that biosolids, wastewater treatment plants, central sewage systems, sewer lines, septic systems, or stormwater systems were significant contributors (DOH 2015). Supporting this conclusion, the USGS multi-tracer study conducted as part of the sanitary survey found no persistent pharmaceuticals and no isotopic tracers of wastewater influence in Waiopili Ditch samples. In the microbial source tracking study presented in this report, we looked for direct evidence of human fecal contamination by measuring microbial DNA fingerprints found in human feces and sewage.

The sanitary survey also noted that the adjacent Waikomo watershed may have contamination problems from on-site sewage disposal systems (cesspools) and wastewater injection wells in the heavily used resort areas surrounding Poipu (Figure 1), but no testing was conducted to identify

human or non-human sources in this watershed. DOH also raised the possibility that injection well and cesspool effluent may flow in the subsurface from developed areas in Koloa and Poipu into the coastal zone, and may be transported though subterranean lava tubes that connect to Waiopili Ditch via Makauwahi Cave.

The sanitary survey found possible non-human sources in the watershed that may contribute significant ENT and CP including animal agriculture, wildlife and birds (DOH 2015). Healthy populations of feral pigs, chickens, ducks, nene goose and sheep were observed in the area. High concentrations of ENT and CP were also found in streambed sediments in the drainage system. These sediments may act as a reservoir for bacterial regrowth and distribution, and might supply ENT and CP to the water column (DOH 2015). In the microbial source tracking study presented in this report, we investigate whether microbial DNA fingerprints from potential animal and sediment sources are associated with high concentrations of ENT and CP.

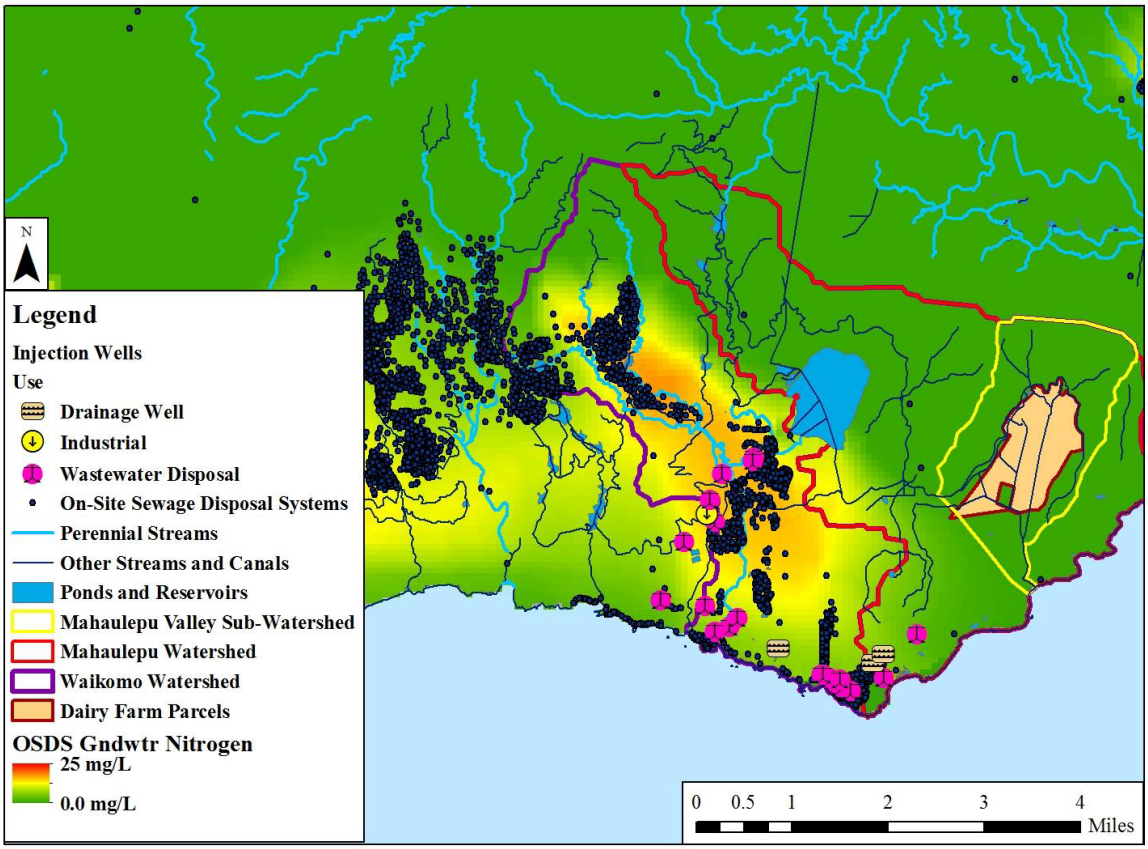

Figure 1. Injection Wells and on-site sewage disposal systems in Waikomo and Mahaulepu Watersheds (DOH 2015)

In this project, the Andersen lab at UC Berkeley (Berkeley Lab) used PhyloChip microarray analysis to provide more conclusive identification of animal and human fecal sources associated enterococci and *Clostridium perfringens* in Mahaulepu and Waikomo watersheds. The Berkeley Lab developed a PhyloChip microarray test that can fingerprint multiple animal and human sources with a single test (Dubinsky et al. 2012, Dubinsky et al. 2016). PhyloChip DNA

microarray contains 1.1 million probes that capture representatives of all known, nearly complete 16S rRNA genes in public databases. The PhyloChip can detect over 59,000 bacterial taxa in a single sample by targeting variations in the 16S rRNA gene. The 16S rRNA gene is universally present in all microbes and small sequence variations within the gene can be used as a "barcode" for bacteria and archaea identification. The analysis quantifies changes in relative abundance of each gene sequence and corresponding bacterial taxa among samples. The usefulness and performance of this technology for microbial source tracking has been evaluated in marine and freshwater systems (Dubinsky et al. 2012, Cao et al. 2013, Dubinsky et al. 2016).

Every microbial source has a unique combination of hundreds to thousands of different bacterial species that can be used for source tracking. PhyloChip detects thousands of diagnostic genetic markers from each source, in contrast to conventional source tracking methods that rely on a single molecular marker for detection and classification. Thousands of diagnostic DNA markers in each source establish unique DNA signatures that can be classified accurately with machine-learning analysis (Dubinsky et al. 2016). Samples from local fecal sources, such as native wildlife and proximal wastewater systems, can be used to train the analysis for improved classification and tracking of sources that are not accurately detected by single-marker methods.

In this project, PhyloChip was used to probe for suspected sources of fecal indicator bacteria in stream water, coastal seeps and sediments. The primary goals were to 1) identify both fecal and environmental sources of fecal indicator bacteria in Waiopili Ditch and Waikomo Stream during wet and dry periods, 2) determine if high counts of enterococci and *C. perfringens* could be explained by human, animal or environmental sources of bacteria, 3) determine if fecal sources of bacteria are transmitted through Makauwahi Cave to Waiopili Ditch, and 4) determine if coastal seeps in the resort area of Poipu are impacted from human contamination from nearby wastewater injection wells.

Berkeley Lab analyzed microbial community data from samples of stream water, coastal seeps, sediments, injection wells, cesspools and animal feces from both Mahaulepu and Waikomo watersheds. Local fecal samples from suspected animal and wastewater sources were characterized to provide Hawaii-specific reference samples for more accurate source identification. Diagnostic DNA profiles were developed for human fecal sources (stool, sewage), livestock (cattle, sheep), feral pigs and chickens, avian wildlife (ducks, nene goose, cattle egret) and horses. Water samples were collected along major drainages at locations that were upstream of, adjacent to, and downstream from areas with high densities of cesspools, injection wells, or agricultural activity.

III. Methods

Sampling

Hawaii DOH conducted field sampling of surface waters, sediments, injection wells and fecal sources. In the Mahaulepu Valley, surface water samples were collected from 13 locations that

were previously sampled for the DOH monitoring program (Figure 2, Appendix 1) and an additional location between Sites 11 and 12 (Site 11.5). These stations were established from the head of Mahaulepu Valley, down through Warner Dam, to the Bridge to Makauwahi Cave.

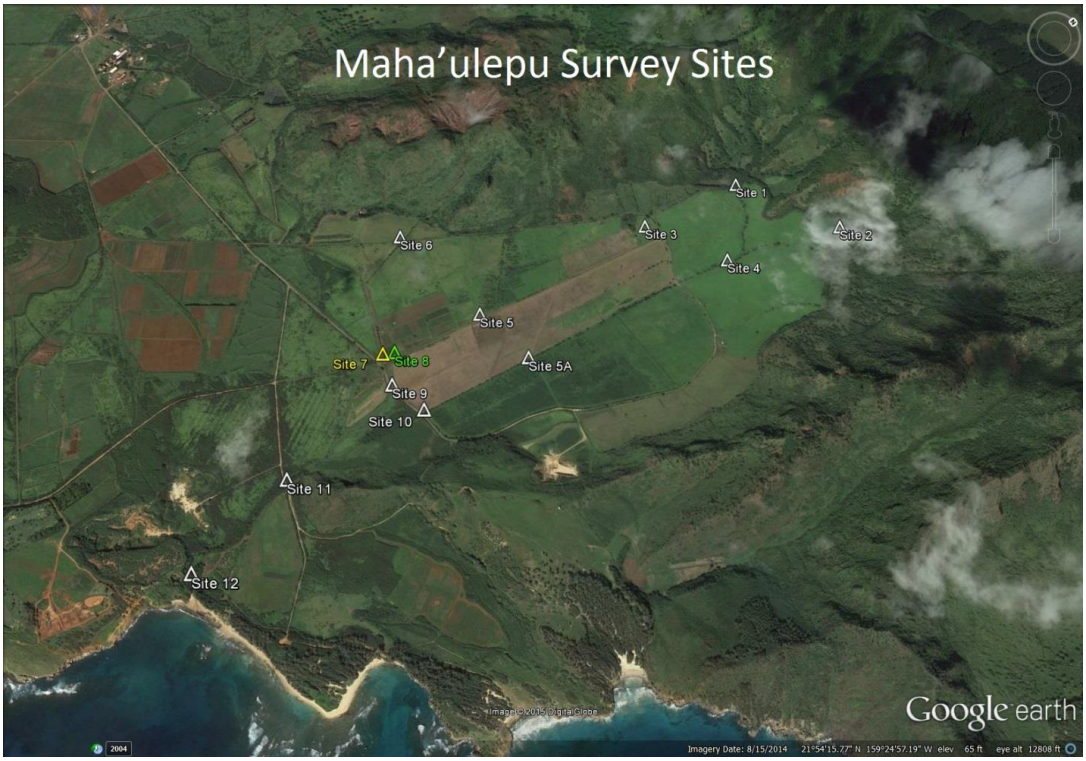

Figure 2. Sampling sites in the Waiopili Ditch drainage, Mahaulepu Watershed.

In the Waikomo watershed, surface water samples were collected at five locations along the Waikomo Stream near Koloa and Poipu, and upstream of Omao above human settlements with on-site sewage disposal systems (Figure 3, Appendix 1). In addition, samples were collected from three coastal seeps along the resort area beachfront in Poipu (Figure 3, Appendix 1). Wastewater injection wells were sampled from six different locations around Poipu (Figure 4).

Figure 3. Sampling sites in Waikomo Stream and tributaries.

Figure 4. Location of Poipu wastewater injection well samples.

All stream and seep locations were sampled on four different sampling dates during both dry periods (9/27/16-9/28/16 and 10/18/16-10/19/16) and following heavy rains (3/1/17-3/2/17 and 4/26/17-4/27/17) to capture variations in runoff conditions and stream water flow. Some sites intermittently discharge after rainfall and therefore lacked samples during dry periods. Coastal seeps were sampled during low tide when seeps were exposed and undiluted by ocean water. At

the time of sampling, DOH measured water quality parameters in all surface waters including salinity, temperature, dissolved oxygen, pH and turbidity (Appendix 2). Sediment samples were collected on 5/10/17 from Waiopili Ditch sites. Equipment blank samples were collected on all sample dates.

Fecal samples from suspected animal sources were collected to provide local reference samples for accurate source identification. Samples of composite droppings were collected from distinct populations of pigs, cattle, horses, sheep, chickens, nene goose, Muscovy ducks and cattle egrets (Table 1). Human wastewater samples were collected from the liquid layer of six different cesspools in the Waikomo Watershed (Table 1).

Field samples of water, sediments and equipment blanks were collected by DOH using standard collection and handling procedures for Fecal Indicator Bacteria (FIB) tests. At the DOH laboratory on Kauai, each sample was split and subsampled for FIB tests (enterococci and *C. perfringens*) and PhyloChip analysis. For PhyloChip, 100 ml of sample was vacuum filtered through a 47 mm, 0.45 μM polycarbonate membrane filter. Filters were placed in 2 ml microtube using sterile forceps and immediately frozen for storage in a -80ºC freezer until shipping to Berkeley Lab for DNA extraction and analysis. At least 20 g of sediment and animal droppings were collected and immediately frozen for storage and shipping.

Table 1. Hawaii fecal sources used as reference samples

Source Type	Location	Sample date	Sample ID	Source category	*C. perfringens* (CFU/100mL)	Enterococcus (MPN/100mL)	Notes
Cattle Egret	Hokuala	11/18/16	Egret_11-18-16	avian wildlife	NA	NA	
Muscovy Duck	Mahaulepu	11/17/16	Duck_11-17-16	avian wildlife	NA	NA	
Nene	Hokuala	11/18/16	Nene(Hokuala)_11-18-16	avian wildlife	NA	NA	
Nene	Kiahuna	11/17/16	Nene(Kiahuna)_11-17-16	avian wildlife	NA	NA	
Nene	Mahaulepu	11/17/16	Nene(Mahaulepu)_11-17-16	avian wildlife	NA	NA	
Chicken	Hokuala	1/31/17	Chicken_1-31-17	chicken	NA	NA	
Chicken	Lawa	6/8/17	Chicken_6-8-17	chicken	NA	NA	
Chicken	?	7/11/17	Chicken_7-11-16	chicken	NA	NA	
Horse	CJM Stables	11/17/16	Horse(CJM)_11-17-16	horse	NA	NA	
Horse	Kaneshiro Farms	11/16/16	Horse(KaneShiro)_11-16-16	horse	NA	NA	
Cesspool	Poipu	6/7/17	Cesspool_1	human	>50000	>2005	
Cesspool	Poipu	6/7/17	Cesspool_2	human	3700	>2005	
Cesspool	Omao	6/7/17	Cesspool_3	human	500	178	
Cesspool	Omao	6/7/17	Cesspool_4	human	6400	>2005	
Cesspool	Koloa	6/7/17	Cesspool_5	human	<1	624	
Cesspool	Koloa	6/7/17	Cesspool_6	human	<1	>2005	
Pig	Kaneshiro	11/16/16	Pig(KA)_11-16-16	pig	NA	NA	
Pig	Mahaulepu	11/17/16	Pig(Mahaulepu)_11-17-16	pig	NA	NA	
Pig	Waimano	12/23/16	Pig(Wailava)_12-23-16	pig	NA	NA	
Cattle	Kaneshiro Farms	11/16/16	Cattle(Kaneshiro)_11-16-16	ruminant	NA	NA	PCR failed
Cattle	Mahaulepu	11/17/16	Cattle(Mahauleph)_11-17-16	ruminant	NA	NA	
Cattle	Wailua	11/18/16	Cattle(Wailava)_11-18-16	ruminant	NA	NA	
Sheep	Haragumi Farm	11/17/16	Sheep(Haragumi)_11-17-16	ruminant	NA	NA	
Sheep	Kaneshiro Farms	11/16/16	Sheep(KaneShiro)_11-16-16	ruminant	NA	NA	
Sheep	Wailua	11/16/16	Sheep(Wailava)_11-16-16	ruminant	NA	NA	

DNA Extraction and PCR amplification

Genomic DNA was extracted from all water filters, feces and sediments using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Solon, OH). DNA was quantified by a fluorometric assay for total DNA concentration (QuBit; Invitrogen). No quantifiable genomic DNA was detected from equipment blanks. Bacterial 16S rRNA gene was amplified from genomic DNA using PCR with universal bacterial primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). Each PCR reaction contained 1× Ex Taq buffer (Takara Bio Inc., Japan), 0.025 units/µl Ex Taq polymerase, 0.8 µM dNTP mixture, 1.0 µg/µl BSA, 300 nM each primer and 1 ng DNA (genomic DNA) as template. Each sample was amplified in 4 replicate 25 µl reactions spanning annealing temperatures ranging from 50-56°C to create a more comprehensive amplification of all community members. PCR conditions are 95°C (3 min), followed by 25 cycles 95°C (30 s), 50-56°C (25 s), 72°C (2 min), followed by a final extension 72°C (10 min). Amplicons from each reaction were pooled for each sample, purified with the QIAquick PCR purification kit (Qiagen, Valencia, CA), and eluted in 50 µL elution buffer.

PhyloChip Analysis

Detailed descriptions of PhyloChip design, validation and laboratory procedures are described elsewhere (DeSantis et al., 2007; Hazen et al., 2010). Purified PCR products were purified then fragmented with DNAaseI; the fragmented products were then labeled with biotin followed by hybridization overnight onto the PhyloChip microarray (Second Genome, South San Francisco, CA); the microarray was then stained and scanned to provide raw PhyloChip data in the form of fluorescent image files. Probe intensities were background-subtracted and scaled to quantitative standards (non-16S spike-ins) and outliers were identified as described in Hazen et al. (2010). Fluorescent image files following array scanning were used to evaluate hybridization intensities for each of the PhyloChip's 1,015,124 oligonucleotide probes, and evaluate targeted sequences that matched in the sample. PhyloChip results were output as lists of detected probe quartets (Probst et al. 2014) and operational taxonomic units (OTUs) with their hybridization scores with associated taxonomic information.

Source tracking analysis

Probe-quartet profiles measured in the 27 fecal reference samples were used to define a subset of DNA targets useful for source identification. The subset consisted of probes that targeted Bacteroidales and Clostridiales taxonomic orders. Bacteroidales and Clostridiales were selected because the vast majority of fecal bacteria are found in these groups, and many of these obligately anaerobic bacteria are found exclusively in these fecal sources, unlike other more ubiquitous bacterial taxa that are found in both fecal and environmental sources. Additionally, sequence targets needed to be present in at least two different fecal samples, but no more than 50% of all fecal samples, to be recruited for source tracking analysis. This filter eliminated targets that were common to most fecal sources and less useful for classification. Out of 121,229 target probes present in the dataset, 9887 were recruited to the diagnostic subset used for source tracking analysis. In addition, 563 additional target probes that are universally found in water and

sediment samples, but almost never found in fecal samples (<=1 sample), were added to the subset as background controls.

Source tracking analysis was conducted using the SourceTracker2 package (v. 2.0.1) under default parameter settings with no rarefaction depth restriction. Fecal training samples were grouped into six fecal source types: avian wildlife (nene geese, ducks, cattle egrets), chickens, horses, humans (cesspools, human stool), pigs and ruminants (cattle, sheep). Four composite human stool samples from Berkeley Lab's reference library were added to the training set to increase specificity for human feces detection (Dubinsky et al. 2016). Five stream water samples with low FIB concentrations were added to the training set for background controls.

The predictive performance of the classifier for source tracking was evaluated by leave-one-out cross-validation of fecal source samples. PhyloChip data from an independent dataset of 64 mixtures of different fecal mixtures (Dubinsky et al. 2016) was analyzed using Hawaiian sources as the training set. The tradeoff between specificity (true positive rate) and sensitivity (true negative rate) was evaluated over a range of source signal thresholds. Positive likelihood ratios [LR^+ = Sensitivity / (1 – Specificity)] associated with each test result were used to interpret the signal strength using conventional guidelines for diagnostic tests as reviewed by Grimes and Schulz (2005): strong signal ($LR^+ > 10$), moderate signal ($5 <= LR^+ <= 10$) and nominal signal ($LR^+ < 5$). Using these LR^+ definitions, the categorical probability values from SourceTracker that defined signal strength were >= 0.2 for strong signal and >=0.1 to <0.2 for moderate signal.

IV. Results

Fecal source classification

A total of 336,694 PhyloChip probe features (quartets) matched their targets, detecting a total of 39,004 different bacterial taxa (OTUs) in the dataset. A subset of 10,450 diagnostic probe quartets matching Bacteroidales and Clostridiales found in Hawaiian fecal sources was used for source tracking analysis. Comparison of diagnostic 16S rRNA gene compositions of individual fecal sources showed different source types grouped into distinct clusters based on similarity in bacterial community compositions (Figure 5). Samples clustered into three main groups: human waste, domesticated and wild mammals, and birds. Human waste further clustered by human stool and cesspool samples, and non-human mammals grouped distinctly into ruminants (cattle, sheep) and feral pigs and horses. These results are consistent with previous work that found similar clustering patterns in California sources (Dubinsky et al. 2012).

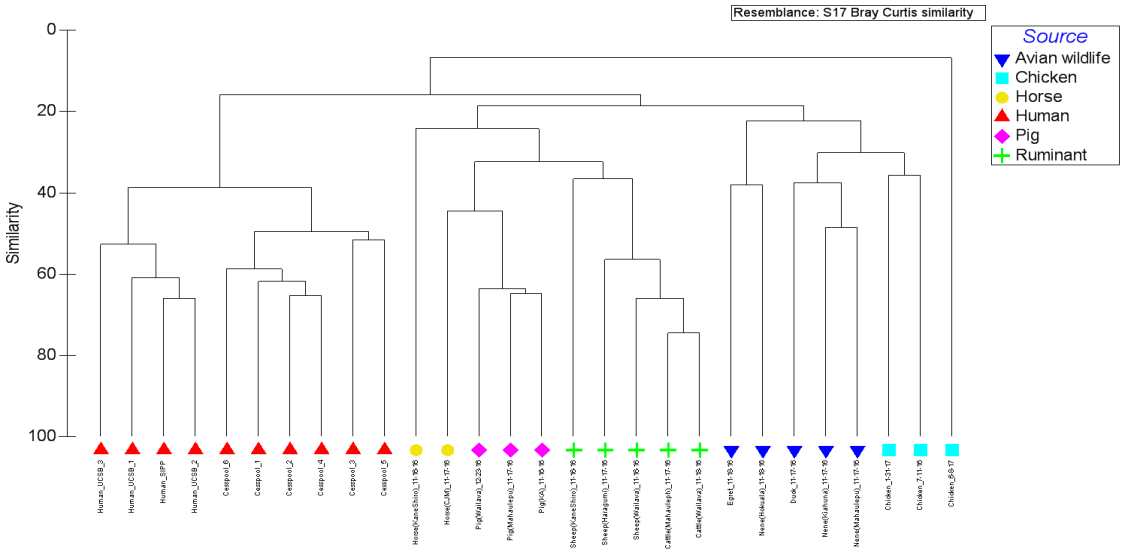

Figure 5. Relatedness of diagnostic bacterial communities among fecal sources. Inter-profile dissimilarity was calculated with the Bray-Curtis metric and analyzed with hierarchical cluster analysis using the Primer E (v. 7.0).

Diagnostic source quartets were used to train the SourceTracker algorithm to classify DNA signatures from six fecal source types: human (human stool, cesspools), ruminants (cattle, sheep), avian wildlife (ducks, cattle egret, nene goose), chickens, feral pigs and horses. Through leave-one-out cross-validation, SourceTracker achieved excellent performance in source prediction for each of the fecal training samples (Figure 6). Average prediction ratios for the correct source category ranged from 0.93 to 0.98 for each source type (1.0 is perfect classification), indicating robust source identification with the training set and classification method. One cesspool sample (cesspool 3) was excluded from the training set due to the high signal from background water in this sample.

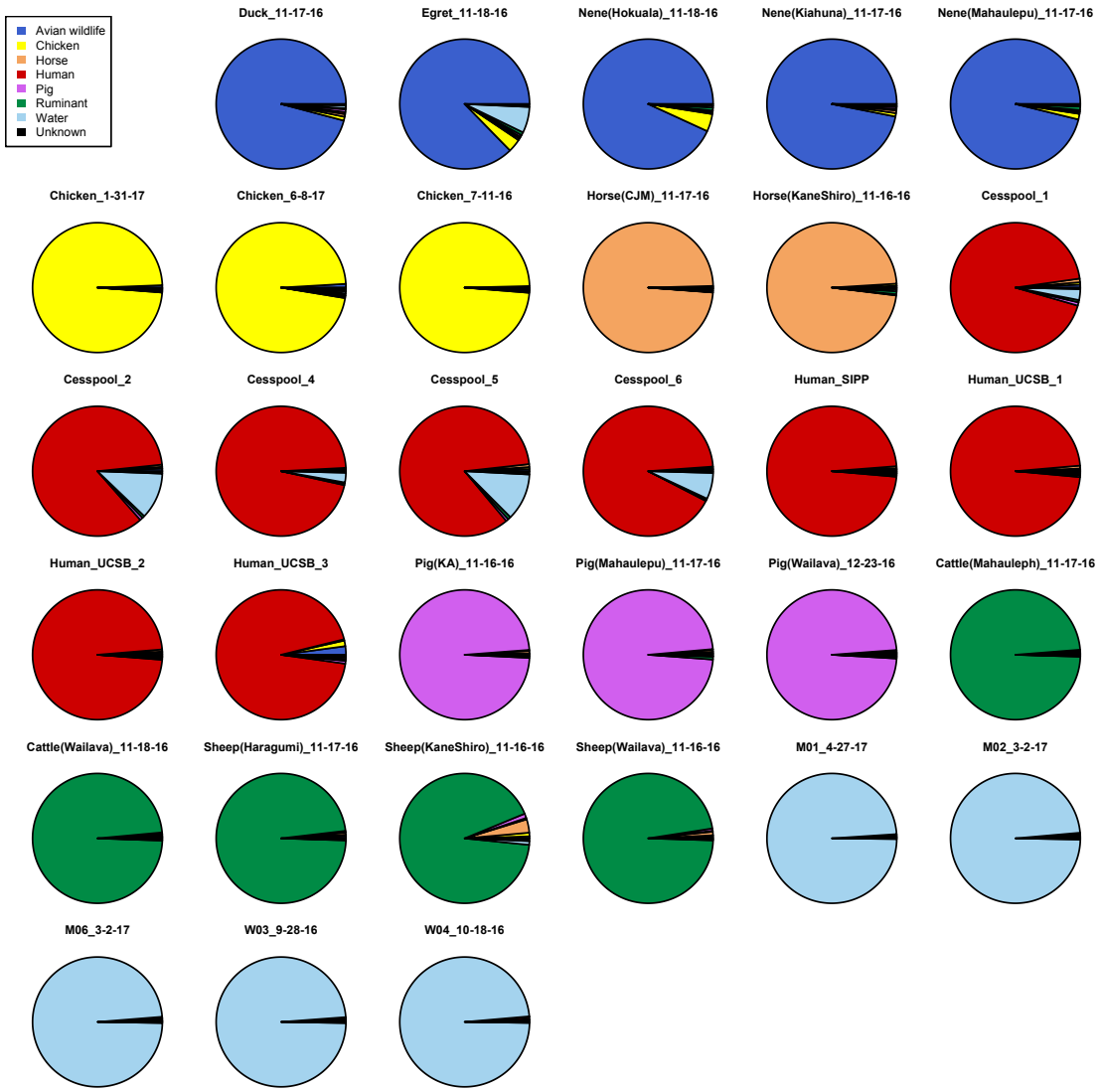

Figure 6. Validation of source classification of Hawaii sources samples. Prediction ratios for each source type were generated through leave-one-out cross-validation implemented in SourceTracker2.

Mahaulepu source tracking

In the Mahaulepu watershed, 43 total surface water samples from Waiopili Ditch were analyzed using PhyloChip quartet detection with SourceTracker classification (Figure 7, Appendix 1). Strong fecal signals were found in two samples collected from the Waiopili Ditch. A strong ruminant fecal signal (0.30) was detected in April at Site 1, and strong feral pig signal (0.27) was detected in March 2017 at Site 4. No other surface water samples showed evidence of contamination from ruminants, pigs, horses, chickens or avian wildlife. Moderate human signal was detected in two samples from September (Sites 5a, 10) and two from April (Sites 4, 10). We

have lower confidence that moderate signals represent true positives, however moderate signals are above typical background values and may be caused by weak or degraded source influences.

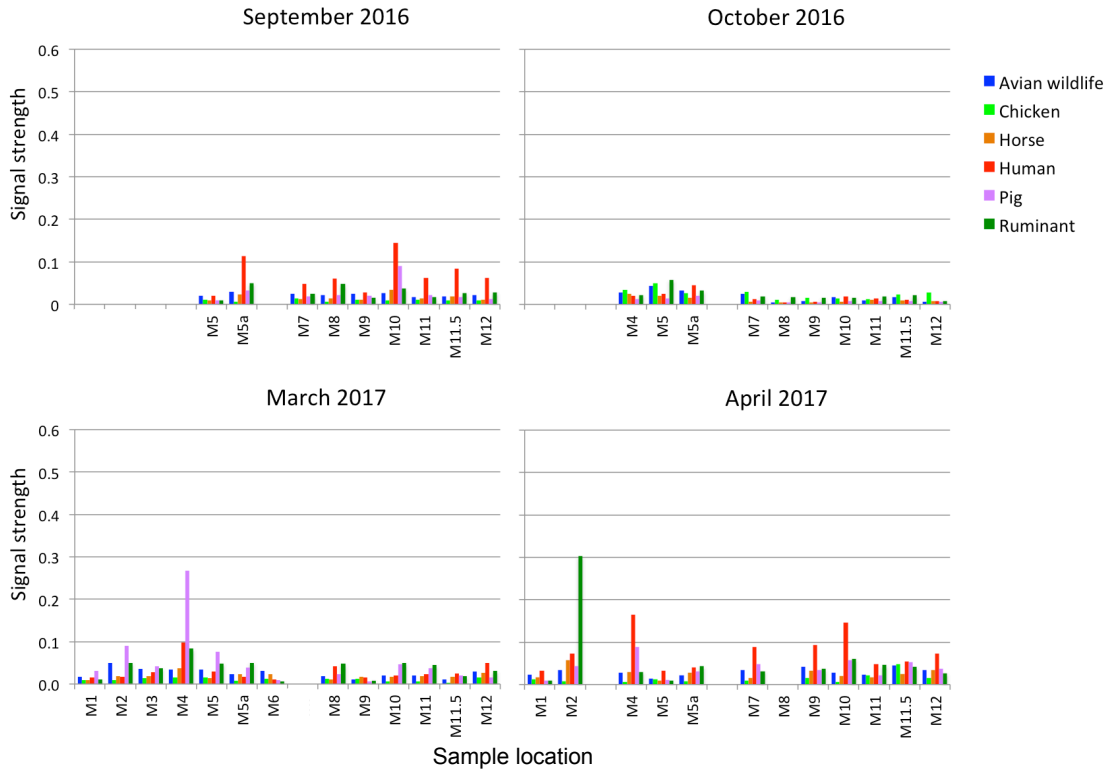

Figure 7. Fecal source detection in Waiopili Ditch surface water during dry (September and October) and wet (March and April) sampling events.

There was no correspondence between the strength of fecal signals in Waiopili Ditch and concentrations of CP and ENT (Figure 8, Appendix 1). CP and ENT concentrations spanned three orders of magnitude; yet source signals were mostly nominal (<0.10) across this range of FIB concentrations, even in samples with the highest FIB counts. Enterococci exceeded the recommended U.S. EPA threshold value of 130 MPN/100 ml in 36 of 43 samples (84%), but fecal signals were rare in these samples; only one sample (2%) had a strong fecal signal for ruminant, one had a strong signal for pigs (2%), and three had a moderate signal for humans (7%). *C. perfringens* exceeded 50 CFU / 100 ml in 49% of Waiopili samples, but none of these samples was associated with a strong fecal signal for any fecal source, and only one sample (2%) had a moderate signal for humans. Notably, the only two samples in Waiopili Ditch with strong fecal signals had CP values of 1 and 4 CFU/100 ml, respectively (Figure 8, Appendix 1).

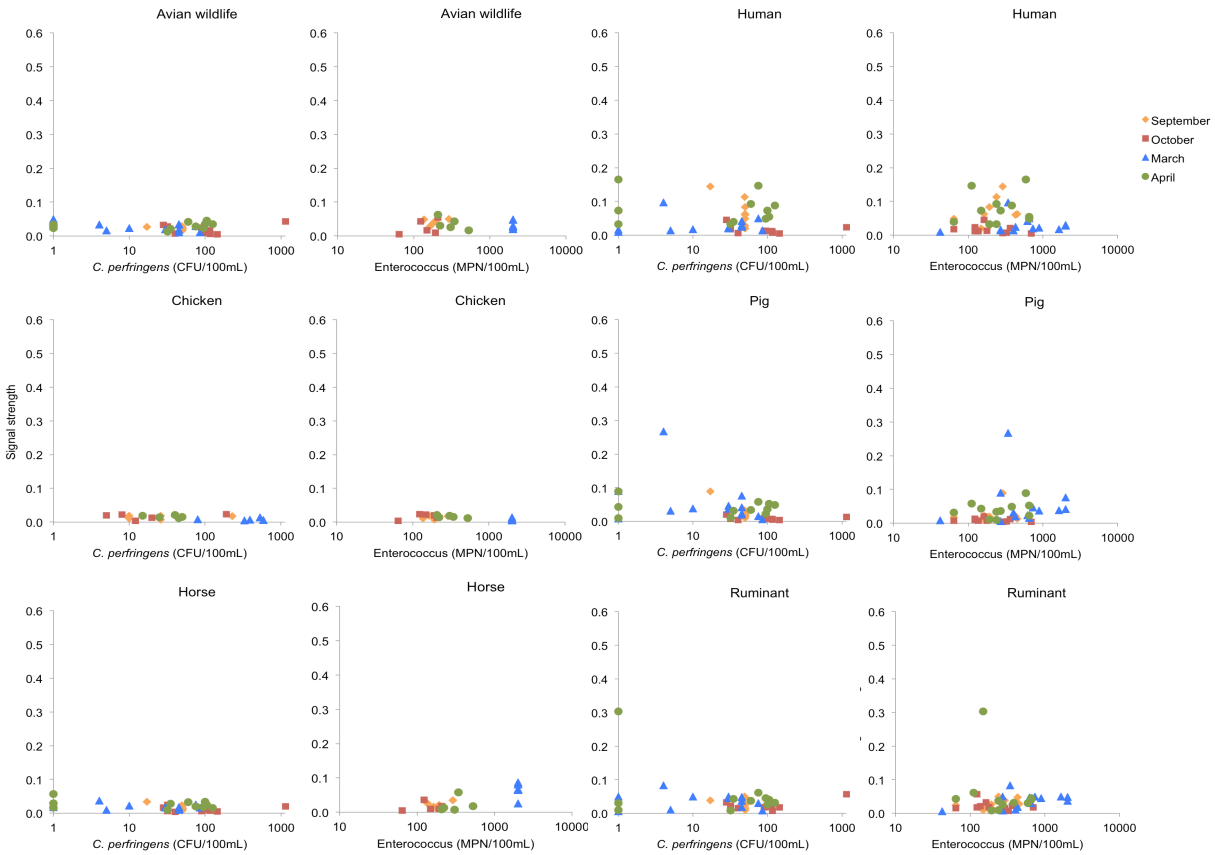

Figure 8. Relationships between fecal indicator bacteria and detection of potential fecal sources in Waiopili Ditch.

No fecal signals were detected in Makauwahi Cave sediments or water, indicating that the cave system is unlikely a conduit for wastewater bacteria from the Koloa/Poipu area (Appendix 1). No fecal signals were detected in Waiopili Ditch sediments in spite of the high numbers of CP and ENT measured in these sediments (Figure 9, Appendix 1).

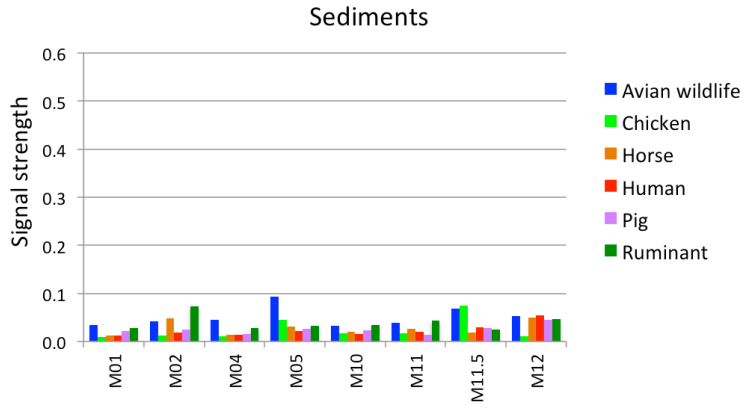

Figure 9. Fecal source detection in Waiopili Ditch sediments.

Sediments in both Waiopili Ditch contain high concentrations of CP and ENT, and may themselves be a direct environmental source of CP and ENT to overlying water, particularly if sediments are disturbed and sediment bacteria are suspended in the water column. To assess the influence of sediments, we compared the bacterial community composition of each water sample to the bacterial community composition of each sediment sample using the Bray-Curtis index of similarity. Samples with more shared taxa have a higher similarity index. Higher sediment similarity values in water column samples with high concentrations of CP or ENT would be expected if sediment particles were the primary source for these bacteria. Sediment similarity varied with sampling date and location, but it was uncorrelated with FIB concentrations in Waiopili Ditch (Figure 10). Thus there was no indication that FIB in surface waters were supplied directly by bacterial inputs from suspended streambed sediments.

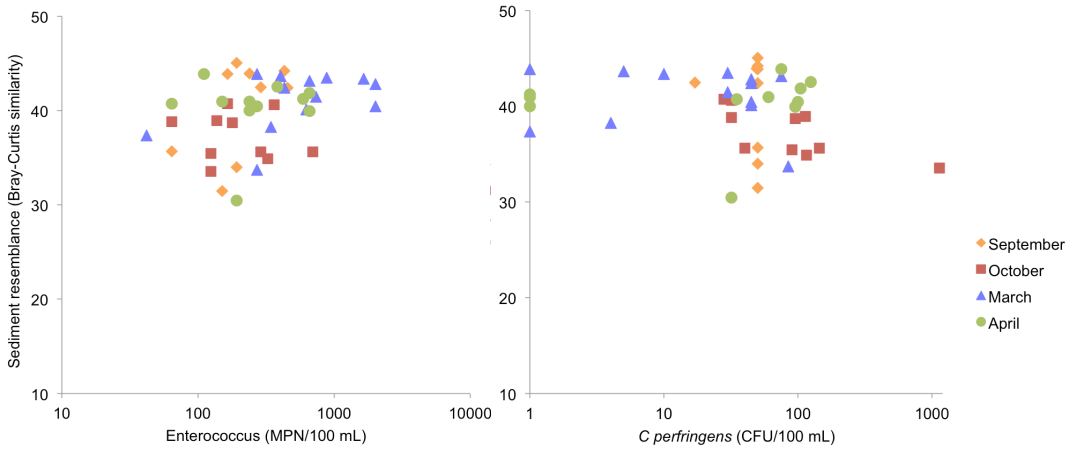

Figure 10. Relationships between fecal indicator bacteria and sediment signatures in Waiopili Ditch.

Waikomo source tracking

In Waikomo Stream, none of the 20 samples collected had strong human signal, and only one had a moderate human signal (Figure 11). A strong feral pig signal (0.28) was detected in April at the most upstream site sampled in the watershed (W5), and moderate feral pig signal was detected at this site in both October and March. No strong fecal signals from other animals were detected in any Waikomo Stream samples. In March, moderate signal from ruminants was detected in two samples (W2 and W3) (Figure 11).

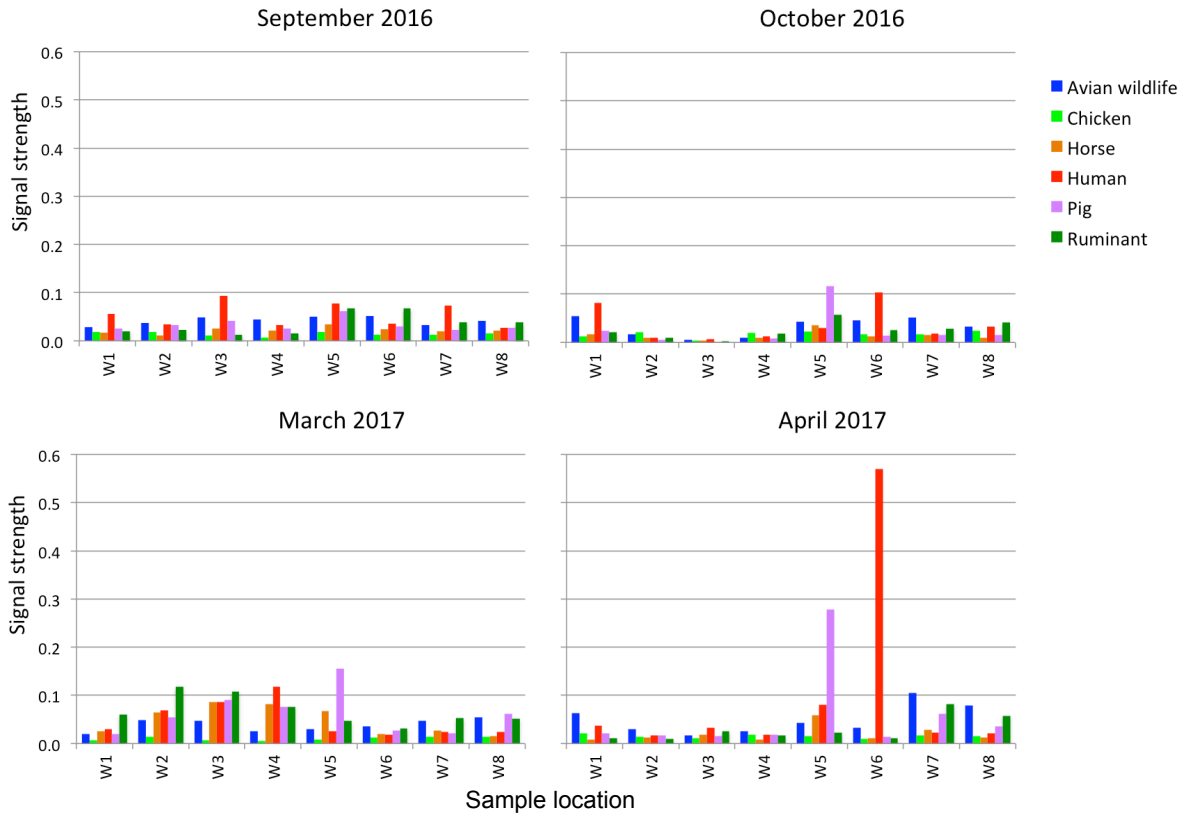

Figure 11. Fecal source detection in Waikomo Watershed during dry (September and October) and wet (March and April) sampling events. Sites W1-W5 were from Waikomo Stream and upstream tributaries. Sites W6-W8 were coastal seeps.

A strong human signal (0.57) was detected in the coastal seep near Kapili Resort (Site W6) in April, and moderate human signal was detected at this site in October (Figure 11). Seep samples were collected during low tide and largely undiluted by seawater (salinity = 2.5 and 3.7 ppt, respectively) (Appendix 2). The strong human signal was within the range of values found in the six Poipu injection wells (0.52 – 0.82) (Figure 12) and was similar in bacterial composition to these injection wells (Figure 13). The ordination plot in Figure 13 compares the bacterial composition between seep samples and possible sources of human contamination (injection wells, cesspools and human stool). Samples that are similar in composition appear closer together on the plot. The contaminated seep sample from Site W6 with a strong human signal was most similar in composition to injection well samples, indicating that nearby injection wells are the likely source of human fecal contamination. Figure 4 shows the locations of the contaminated seep (seep #3) and sampled injection wells.

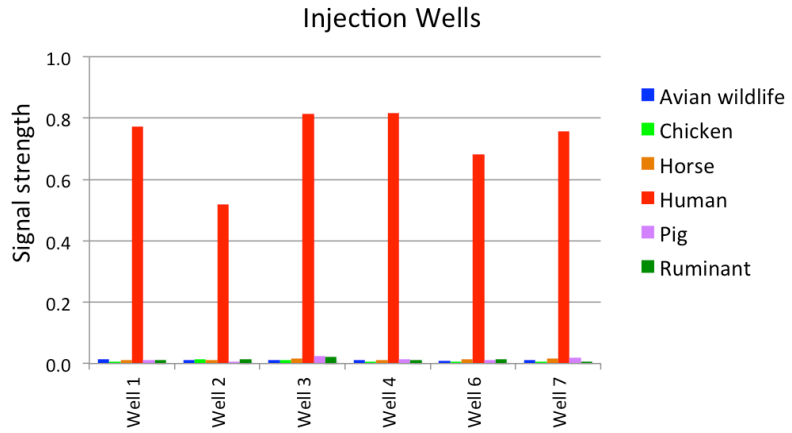

Figure 12. Fecal source detection in injection wells.

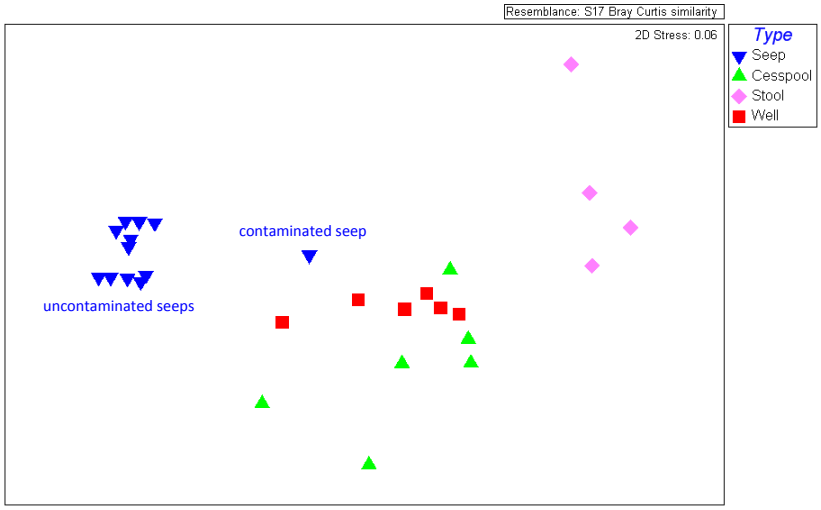

Figure 13. Similarity among diagnostic bacterial communities from coastal seeps and human source samples. Human source samples included cesspools, wastewater injection wells and human stool. The contaminated seep sample was collected at Site W6 in April 2017. Dissimilarity between bacterial community compositions (presence/absence) was calculated with the Bray-Curtis metric and analyzed by nonmetric multidimensional scaling (NMDS) using Primer E (v. 7.0).

Oddly, the FIB concentrations in the W6 sample with strong human signal were low (CP=1, ENT=23 MPN/100 ml). Similarly, one injection well sample had low ENT concentrations (20 MPN/100 ml) (Appendix 1) and two cesspool samples (5 and 6) had undetectable numbers of *C. perfringens* (<1 CFU/100 ml) (Table 1), in spite of the near certainty that these samples contained human fecal contamination. These low ENT and CP values in samples with known human wastewater contamination are false negatives.

In Waikomo Stream, high FIB concentrations were not associated with strong fecal signals (Figure 14). However, moderate fecal signals for pigs (2 samples), ruminants (2 samples) and humans (1 sample) were detected where CP and ENT concentrations were high during wet

sampling events (Figure 14). Of the seven samples with the highest enterococci concentrations, five had moderate signal for one of these sources. *C. perfringens* exceeded 50 CFU / 100 ml in 8 of 20 samples (40%), and six of these samples (30%) had moderate fecal signal. However, these fecal sources did not explain the high concentrations of FIB observed in almost every Waikomo Stream sample, both upstream and downstream of human settlements. Ninety percent of water samples exceeded the U.S. EPA recommended threshold for enterococci, yet only 25% had any fecal signal. In addition, the fecal signals were relatively weak (with the exception of the strong pig signal at W5), and they were not consistently detected from one source type. The detection of only one sample with a moderate human signal indicates that cesspools and injection wells around Koloa and Poipu were not significantly contributing to the high FIB counts observed in Waikomo Stream.

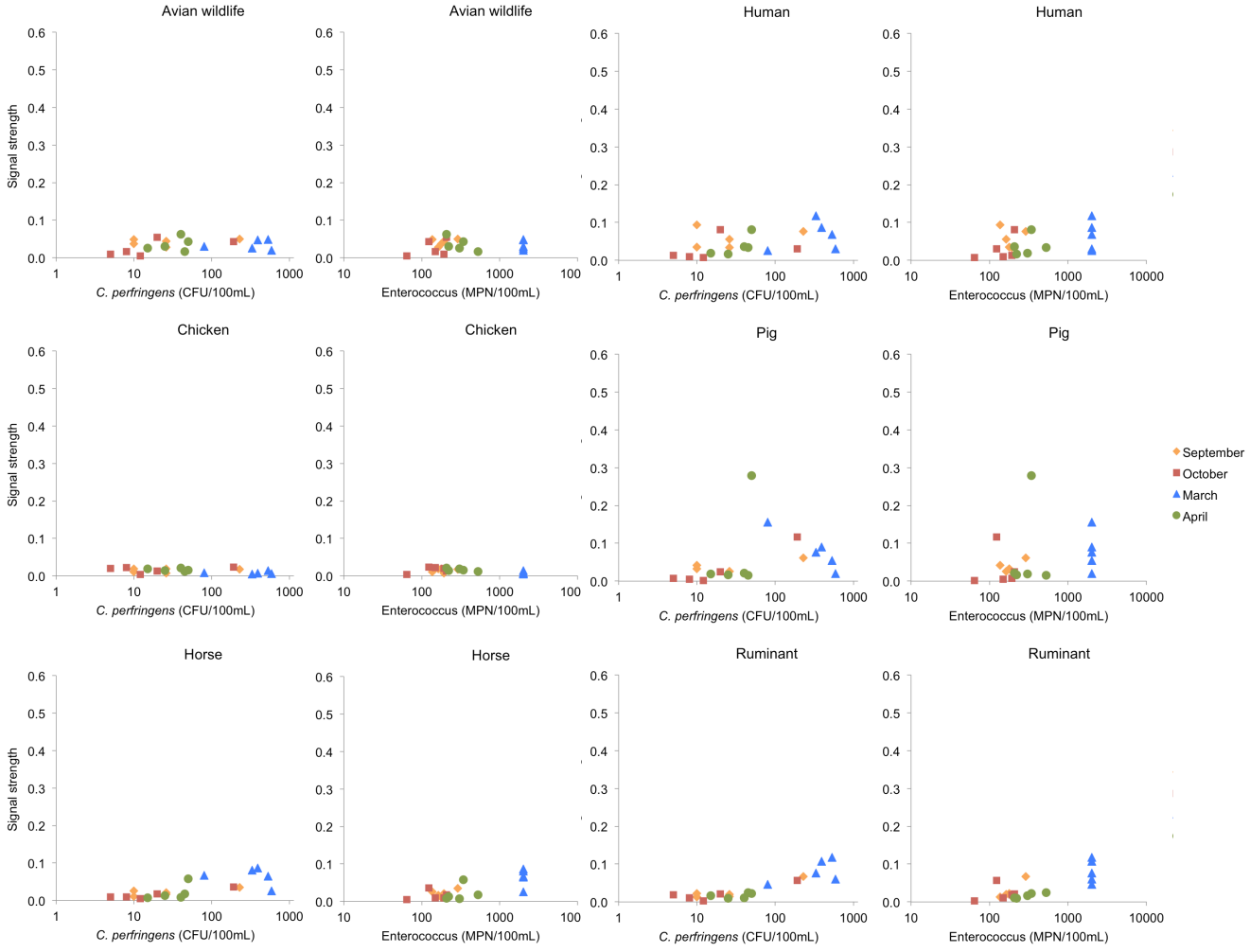

Figure 14. Relationships between fecal indicator bacteria and sediment signatures in Waikomo Stream.

Stream sediments were not clearly linked to high FIB concentrations in Waikomo Stream surface water. Sediment similarity in the water column was uncorrelated with FIB concentrations (Figure

15). Some of the lowest counts CP in the water column (<= 15 CFU / 100 ml) were also the most dissimilar to sediment microbial communities, but samples with CP concentrations ranging from 15 to >1000 CFU / 100 ml had comparable similarity to sediments. Higher resemblance to sediment microbial communities would be expected in the highest CP and ENT samples if sediment particles were a direct source of these indicator bacteria.

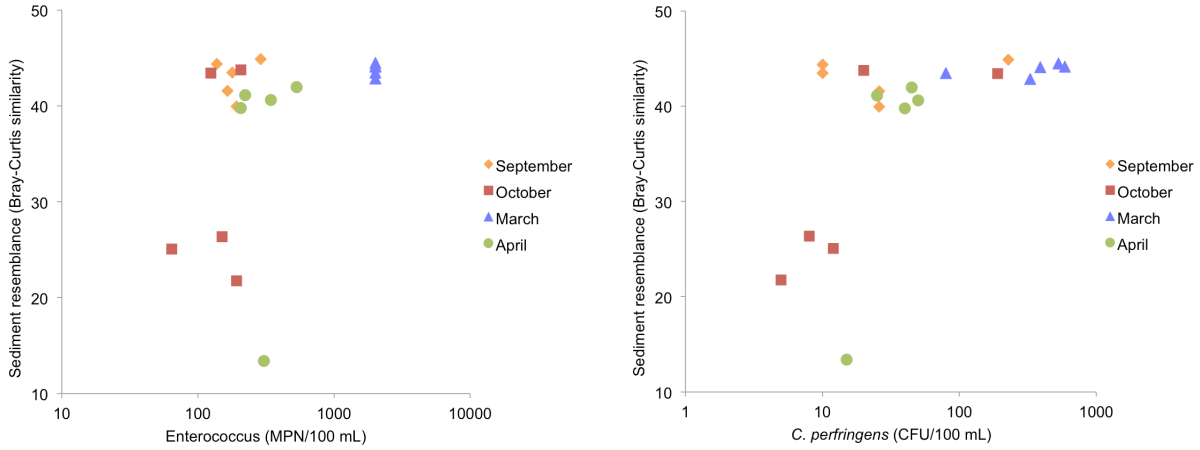

Figure 15. Relationships between fecal indicator bacteria and sediment signatures in Waikomo Stream.

V. Discussion

Variability in ENT and CP counts in both Waikomo and Mahaulepu streams was not linked to bacterial inputs from human or animal feces (Figures 8 and 14). The occurrence of abundant ENT and CP in the absence of DNA signatures from fecal sources indicates that the majority of FIB in Mahaulepu and Waikomo streams was likely sourced from the surrounding environment or growing *in situ*. Both enterococci and *C. perfringens* naturally occur in a variety of environmental habitats such as soils, sediments, beach sands, and a variety of aquatic vegetation (Badgley et al. 2010, Byappanahalli et al. 2012, Byamukama et al. 2005, Hardina and Fujioka 1991; Yamahara et al. 2009). Streambed sediments do not appear to be the primary source for FIB in Waiopili Ditch and Waikomo Stream (Figures 10 and 15). Hardina and Fujioka (1991) concluded that soil was the primary source of FIB in the environment in Hawaii and that soil-bound fecal indicator bacteria were transported by precipitation events into pristine streams and rivers.

C. perfringens is commonly found in tropical stream water and soils that are free from anthropogenic influences (Mushi 2018). In a study of tropical catchments in Tanzania, a strong correlation was found between stream water and catchment soils that erode into the stream. It is possible that soils near Waikomo and Mahaulepu streams are the primary sources of CP and ENT. CP or closely related *Clostridia* species may survive in sediment pore waters or soils that have low oxygen, and their persistent spores may be transported into the water column via soil runoff or erosion (Byamukama et al. 2005, Davies et al. 1995).

In the majority of samples, CP and ENT were unassociated with molecular evidence of fecal contamination, and thus the potential health risks indicated by these bacteria are likely overestimated if they are assumed to be fecal in origin. Conversely, a few samples with strong human fecal signatures and known wastewater origins, including samples from cesspools and wastewater injection wells, had unexpectedly low CP or ENT concentrations and were clearly false negatives.

More routine confirmation of FIB tests is needed with modern methods of fecal source detection. The emergence of high-resolution molecular tools enables the measurement of unambiguous DNA signatures that are exclusive to human waste and other potential pathogen sources, and not ubiquitous in the environment like enterococci, *E. coli* and *C. perfringens*. More routine fecal source detection would avoid confusion and alarm caused by FIB from non-fecal sources, particularly in subtropical and tropical environments where FIB are naturally abundant.

V. Conclusions

- High concentrations of FIB in both Waiopili Ditch and Waikomo Stream were not caused by human or animal fecal contamination. Most samples with high FIB concentrations had no observable human or animal fecal signals.

- Ruminants and feral pigs sporadically contaminate Waiopili and Waikomo Streams. There was infrequent detection of weak human signal in some stream samples. There was no evidence for contamination by avian wildlife, chickens or horses in any samples.

- Strong human fecal signal was found in a coastal seep along the beachfront of the Poipu resort area. The strength of the human signal was comparable in magnitude and microbial composition to injection wells in Poipu.

- No fecal contamination was found in Makauwahi Cave sediments or water, indicating that subterranean transport of fecal bacteria between Waikomo and Mahaulepu watersheds was not likely at the time of sampling.

- No fecal contamination was found in stream sediments despite high sediment FIB concentrations.

- Inputs of bacteria from bulk streambed sediments do not appear to be causing high CP or ENT concentrations in stream waters.

- Some samples with strong fecal signal, including samples taken directly from human cesspools and injection wells, had abnormally low concentrations of *C. perfringens* or enterococci. CP and ENT tests may be giving false negative results in some cases. This issue warrants further investigation.

VI. References

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Appendix 1. Table of PhyloChip source tracking results. Source signal is the proportion of PhyloChip signal attributed to each source type by SourceTracker analysis: Values >=0.2 (red) indicate strong source signal (source contamination likely); values >=0.1 and <0.2 (yellow) indicate marginal source signal (source contamination possible); values <0.1 are within expected background values (source contamination unlikely). Fecal indicator bacteria counts provided by DOH.

					Source Signal						Fecal Indicators		
Watershed	**Site**	**Sample ID**	**Sample date**	**Type**	**Avian wildlife**	**Chicken**	**Horse**	**Human**	**Pig**	**Ruminant**	***C. perfringens* (CFU/100mL)**	**Enterococcus (MPN/100mL)**	**Notes**
Mahulepu	M-05	M05_9-27-16	9/27/16	stream water	0.020	0.011	0.009	0.020	0.010	0.009	>50	150	
Mahulepu	M-05a	M05a_9-27-16	9/27/16	stream water	0.029	0.006	0.024	0.113	0.033	0.050	49	238	
Mahulepu	M-07	M07_6-27-16	9/27/16	stream water	0.024	0.013	0.013	0.048	0.018	0.024	>50	64	
Mahulepu	M-08	M08_9-17-16	9/27/16	stream water	0.022	0.006	0.014	0.061	0.021	0.048	>50	429	
Mahulepu	M-09	M09_9-27-16	9/27/16	stream water	0.025	0.010	0.011	0.028	0.020	0.015	>50	192	
Mahulepu	M-10	M10_9-27-16	9/27/16	stream water	0.027	0.010	0.033	0.145	0.089	0.038	17	288	
Mahulepu	M-11	M11_9-27-16	9/27/16	stream water	0.016	0.010	0.014	0.063	0.022	0.016	>50	164	
Mahulepu	M-11.5	M11.5_9-27-16	9/27/16	stream water	0.018	0.010	0.019	0.083	0.017	0.027	>50	192	
Mahulepu	M-12	M12_9-27-16	9/27/16	stream water	0.022	0.009	0.011	0.062	0.013	0.028	>50	453	
Mahulepu	M-04	M04_10-19-16	10/19/16	stream water	0.028	0.034	0.025	0.021	0.012	0.022	32	364	
Mahulepu	M-05	M05_10-19-16	10/19/16	stream water	0.043	0.049	0.020	0.024	0.013	0.057	1140	124	
Mahulepu	M-05a	M05a_10-19-16	10/19/16	stream water	0.033	0.026	0.016	0.045	0.020	0.032	28	164	
Mahulepu	M-07	M07_10-19-16	10/19/16	stream water	0.024	0.030	0.006	0.012	0.010	0.018	90	124	
Mahulepu	M-08	M08_10-19-16	10/19/16	stream water	0.005	0.011	0.005	0.005	0.004	0.018	144	697	
Mahulepu	M-09	M09_10-19-16	10/19/16	stream water	0.007	0.015	0.004	0.006	0.004	0.016	40	288	
Mahulepu	M-10	M10_10-19-16	10/19/16	stream water	0.017	0.013	0.007	0.018	0.008	0.016	32	64	
Mahulepu	M-11	M11_10-19-16	10/19/16	stream water	0.010	0.012	0.011	0.013	0.007	0.019	95	178	
Mahulepu	M-11.5	M11.5_10-19-16	10/19/16	stream water	0.017	0.023	0.009	0.011	0.008	0.021	114	137	
Mahulepu	M-12	M12_10-19-16	10/19/16	stream water	0.007	0.027	0.008	0.008	0.006	0.008	116	324	
Mahulepu	M-01	M01_3-2-17	3/2/17	stream water	0.017	0.009	0.009	0.015	0.032	0.011	5	406	
Mahulepu	M-02	M02_3-2-17	3/2/17	stream water	0.050	0.009	0.018	0.016	0.090	0.050	1	271	
Mahulepu	M-03	M03_3-2-17	3/2/17	stream water	0.035	0.013	0.018	0.028	0.042	0.037	45	>2005	
Mahulepu	M-04	M04_3-2-17	3/2/17	stream water	0.034	0.015	0.037	0.097	0.267	0.084	4	344	
Mahulepu	M-05	M05_3-2-17	3/2/17	stream water	0.034	0.015	0.013	0.030	0.076	0.049	45	2005	
Mahulepu	M-05a	M05a_3-2-17	3/2/17	stream water	0.024	0.008	0.023	0.018	0.038	0.050	10	1652	
Mahulepu	M-06	M06_3-2-17	3/2/17	stream water	0.030	0.013	0.023	0.010	0.008	0.006	<1	42	
Mahulepu	M-07	M07_3-2-17	3/2/17	stream water	NA	NA	NA	NA	NA	NA	20	192	insufficient DNA
Mahulepu	M-08	M08_3-2-17	3/2/17	stream water	0.019	0.013	0.010	0.042	0.024	0.048	45	624	
Mahulepu	M-09	M09_3-2-17	3/2/17	stream water	0.010	0.013	0.016	0.015	0.006	0.008	85	271	
Mahulepu	M-10	M10_3-2-17	3/2/17	stream water	0.020	0.006	0.017	0.020	0.046	0.050	30	738	
Mahulepu	M-11	M11_3-2-17	3/2/17	stream water	0.020	0.007	0.019	0.023	0.037	0.045	30	885	
Mahulepu	M-11.5	M11.5_3-2-17	3/2/17	stream water	0.011	0.003	0.018	0.025	0.020	0.019	45	429	
Mahulepu	M-12	M12_3-2-17	3/2/17	stream water	0.029	0.016	0.026	0.050	0.016	0.031	75	659	
Mahulepu	M-01	M01_4-27-17	4/27/17	stream water	0.024	0.013	0.016	0.033	0.010	0.010	1	238	
Mahulepu	M-02	M02_4-27-17	4/27/17	stream water	0.034	0.007	0.057	0.072	0.044	0.302	<1	150	
Mahulepu	M-04	M04_4-27-17	4/27/17	stream water	0.028	0.006	0.029	0.164	0.089	0.030	<1	591	
Mahulepu	M-05	M05_4-27-17	4/27/17	stream water	0.013	0.012	0.009	0.032	0.011	0.009	32	192	
Mahulepu	M-05a	M05a_4-27-17	4/27/17	stream water	0.022	0.008	0.028	0.040	0.032	0.043	35	64	
Mahulepu	M-07	M07_4-27-17	4/27/17	stream water	0.035	0.009	0.016	0.088	0.049	0.032	125	384	
Mahulepu	M-09	M09_4-27-17	4/27/17	stream water	0.042	0.016	0.033	0.093	0.034	0.038	60	238	
Mahulepu	M-10	M10_4-27-17	4/27/17	stream water	0.028	0.006	0.020	0.146	0.058	0.061	75	111	
Mahulepu	M-11	M11_4-27-17	4/27/17	stream water	0.023	0.021	0.018	0.049	0.022	0.046	95	659	
Mahulepu	M-11.5	M11.5_4-27-17	4/27/17	stream water	0.045	0.049	0.025	0.054	0.053	0.041	105	659	

					Source Signal						Fecal Indicators		
Watershed	**Site**	**Sample ID**	**Sample date**	**Type**	**Avian wildlife**	**Chicken**	**Horse**	**Human**	**Pig**	**Ruminant**	***C. perfringens* (CFU/100mL)**	**Enterococcus (MPN/100mL)**	**Notes**
Mahulepu	M-12	M12_4-27-17	4/27/17	stream water	0.035	0.016	0.034	0.072	0.037	0.026	100	271	
Mahulepu	M-01	S01_5-10-17	5/10/17	sediment	0.035	0.009	0.013	0.013	0.022	0.028	500	306	
Mahulepu	M-02	S02_5-10-17	5/10/17	sediment	0.043	0.013	0.048	0.019	0.025	0.073	180	591	
Mahulepu	M-04	S04_5-10-17	5/10/17	sediment	0.045	0.010	0.014	0.014	0.016	0.028	45	87	
Mahulepu	M-05	S05_5-10-17	5/10/17	sediment	0.093	0.045	0.031	0.022	0.026	0.033	1600	478	
Mahulepu	M-10	S10_5-10-17	5/10/17	sediment	0.033	0.018	0.020	0.016	0.023	0.035	210	2880	
Mahulepu	M-11	S11_5-10-17	5/10/17	sediment	0.039	0.017	0.026	0.020	0.013	0.043	160	1652	
Mahulepu	M-11.5	S11.5_5-10-17	5/10/17	sediment	0.069	0.074	0.019	0.029	0.027	0.025	160	324	
Mahulepu	M-12	S12_5-10-17	5/10/17	sediment	0.053	0.011	0.049	0.054	0.044	0.046	220	306	
Makawahi	cave	Makawahi_water	10/18/16	stream water	0.031	0.019	0.019	0.040	0.011	0.023	1	75	
Makawahi	cave	Makawahi_sediment	10/19/16	sediment	0.049	0.032	0.025	0.024	0.026	0.042	N/A	N/A	
Waikomo	W-01	W01_9-28-16	9/28/16	stream water	0.028	0.019	0.016	0.055	0.025	0.020	26	164	
Waikomo	W-02	W02_9-28-16	9/28/16	stream water	0.038	0.019	0.011	0.035	0.033	0.022	10	178	
Waikomo	W-03	W03_9-28-16	9/28/16	stream water	0.049	0.011	0.025	0.094	0.042	0.013	10	137	
Waikomo	W-04	W04_9-28-16	9/28/16	stream water	0.044	0.007	0.021	0.033	0.026	0.015	26	192	
Waikomo	W-05	W05_9-28-16	9/28/16	stream water	0.050	0.018	0.035	0.077	0.062	0.068	228	288	
Waikomo	W-01	W01_10-18-16	10/18/16	stream water	0.055	0.013	0.017	0.081	0.025	0.022	20	207	
Waikomo	W-02	W02_10-18-16	10/18/16	stream water	0.017	0.022	0.010	0.010	0.005	0.010	8	150	
Waikomo	W-03	W03_10-18-16	10/18/16	stream water	0.005	0.004	0.005	0.008	0.002	0.003	12	64	
Waikomo	W-04	W04_10-18-16	10/18/16	stream water	0.010	0.021	0.009	0.013	0.008	0.018	5	192	
Waikomo	W-05	W05_10-18-16	10/18/16	stream water	0.043	0.023	0.036	0.030	0.116	0.057	190	124	
Waikomo	W-01	W01_3-1-17	3/1/17	stream water	0.020	0.006	0.025	0.030	0.020	0.060	590	>2005	
Waikomo	W-02	W02_3-1-17	3/1/17	stream water	0.049	0.014	0.064	0.069	0.054	0.118	530	>2005	
Waikomo	W-03	W03_3-1-17	3/1/17	stream water	0.048	0.007	0.086	0.086	0.090	0.107	390	>2005	
Waikomo	W-04	W04_3-1-17	3/1/17	stream water	0.025	0.005	0.082	0.117	0.077	0.076	330	>2005	
Waikomo	W-05	W05_3-1-17	3/1/17	stream water	0.030	0.009	0.067	0.025	0.156	0.047	80	>2005	
Waikomo	W-01	W01_4-26-17	4/26/17	stream water	0.063	0.021	0.009	0.036	0.022	0.011	40	207	
Waikomo	W-02	W02_4-26-17	4/26/17	stream water	0.030	0.014	0.013	0.016	0.017	0.010	25	222	
Waikomo	W-03	W03_4-26-17	4/26/17	stream water	0.016	0.012	0.018	0.033	0.015	0.025	45	531	
Waikomo	W-04	W04_4-26-17	4/26/17	stream water	0.026	0.019	0.008	0.019	0.019	0.016	15	306	
Waikomo	W-05	W05_4-26-17	4/26/17	stream water	0.043	0.015	0.058	0.081	0.279	0.023	50	344	
Waikomo	W-06	W06_9-28-16	9/28/16	seep	0.051	0.013	0.024	0.036	0.030	0.067	<1	<10	
Waikomo	W-07	W07_9-28-16	9/28/16	seep	0.032	0.012	0.020	0.074	0.023	0.039	<1	<10	
Waikomo	W-08	W08_9-28-16	9/28/16	seep	0.042	0.016	0.021	0.026	0.027	0.039	6	<10	
Waikomo	W-06	W06_10-18-16	10/18/16	seep	0.045	0.017	0.012	0.103	0.014	0.026	1	2.3	
Waikomo	W-07	W07_10-18-16	10/18/16	seep	0.051	0.016	0.015	0.018	0.016	0.028	<1	2.3	
Waikomo	W-08	W08_10-18-16	10/18/16	seep	0.033	0.024	0.010	0.033	0.015	0.041	1	10	
Waikomo	W-06	W06_3-1-17	3/1/17	seep	0.035	0.012	0.019	0.018	0.026	0.031	1	2.3	
Waikomo	W-07	W07_3-1-17	3/1/17	seep	0.048	0.014	0.028	0.025	0.021	0.052	1	42	
Waikomo	W-08	W08_3-1-17	3/1/17	seep	0.054	0.014	0.016	0.024	0.061	0.052	150	478	
Waikomo	W-06	W06_4-26-17	4/26/17	seep	0.033	0.010	0.012	0.569	0.014	0.012	<1	23	
Waikomo	W-07	W07_4-26-17	4/26/17	seep	0.106	0.018	0.028	0.023	0.062	0.082	1	23	
Waikomo	W-08	W08_4-26-17	4/26/17	seep	0.080	0.016	0.012	0.021	0.035	0.058	26	42	
Waikomo	Well-1	Well-1_6-29-17	6/29/17	injection well	0.014	0.006	0.011	0.772	0.010	0.011	>500	20	
Waikomo	Well-2	Well-2_6-29-17	6/29/17	injection well	0.012	0.013	0.012	0.519	0.007	0.014	>500	>2005	
Waikomo	Well-3	Well-3_6-29-17	6/29/17	injection well	0.012	0.011	0.016	0.814	0.023	0.022	570	>2005	
Waikomo	Well-4	Well-4_6-29-17	6/29/17	injection well	0.012	0.006	0.012	0.817	0.015	0.012	>500	>2005	

					Source Signal						Fecal Indicators		
Watershed	**Site**	**Sample ID**	**Sample date**	**Type**	**Avian wildlife**	**Chicken**	**Horse**	**Human**	**Pig**	**Ruminant**	***C. perfringens* (CFU/100mL)**	**Enterococcus (MPN/100mL)**	**Notes**
Waikomo	Well-6	Well-6_6-29-17	6/29/17	injection well	0.008	0.005	0.014	0.682	0.010	0.013	>500	>2005	
Waikomo	Well-7	Well-7_6-29-17	6/29/17	injection well	0.010	0.006	0.017	0.757	0.020	0.007	750	>2005	

Appendix 2. Water quality parameters and fecal indicator bacteria in surface water samples collected from streams, seeps and caves.

					Water Quality Parameters						Fecal Indicators	
Watershed	**Site**	**Sample ID**	**Sample date**	**Type**	**Salinity (ppt)**	**Temp (°C)**	**Dissolved oxygen (mg/L)**	**Dissolved oxygen (%)**	**pH**	**Turbidity (NTU)**	***C. perfringens* (CFU/100mL)**	**Enterococcus (MPN/100mL)**
Mahulepu	M-05	M05_9-27-16	9/27/16	stream water	0.11	26.12	3.71	46.6	6.64	5.71	>50	150
Mahulepu	M-05a	M05a_9-27-16	9/27/16	stream water	0.20	25.01	6.81	82.5	7.61	15.10	49	238
Mahulepu	M-07	M07_6-27-16	9/27/16	stream water	0.07	25.77	7.48	91.8	7.07	104.00	>50	64
Mahulepu	M-08	M08_9-17-16	9/27/16	stream water	0.11	26.90	1.48	18.6	6.76	16.40	>50	429
Mahulepu	M-09	M09_9-27-16	9/27/16	stream water	0.07	27.15	7.13	89.8	7.07	69.40	>50	192
Mahulepu	M-10	M10_9-27-16	9/27/16	stream water	0.20	24.43	6.88	83.0	7.44	20.00	17	288
Mahulepu	M-11	M11_9-27-16	9/27/16	stream water	0.08	26.72	7.09	88.8	7.63	42.40	>50	164
Mahulepu	M-11.5	M11.5_9-27-16	9/27/16	stream water	0.08	25.23	7.58	92.4	7.69	43.70	>50	192
Mahulepu	M-12	M12_9-27-16	9/27/16	stream water	0.08	24.81	7.43	89.7	7.77	45.30	>50	453
Mahulepu	M-04	M04_10-19-16	10/19/16	stream water	0.14	23.12	4.50	34.2	6.74	20.70	32	364
Mahulepu	M-05	M05_10-19-16	10/19/16	stream water	0.12	25.17	2.51	30.9	6.93	36.70	1140	124
Mahulepu	M-05a	M05a_10-19-16	10/19/16	stream water	0.20	24.46	6.14	74.3	7.57	9.20	28	164
Mahulepu	M-07	M07_10-19-16	10/19/16	stream water	0.07	25.49	7.03	85.9	7.38	45.00	90	124
Mahulepu	M-08	M08_10-19-16	10/19/16	stream water	0.07	25.02	7.42	89.9	7.36	42.30	144	697
Mahulepu	M-09	M09_10-19-16	10/19/16	stream water	0.07	25.52	7.45	91.2	7.45	43.00	40	288
Mahulepu	M-10	M10_10-19-16	10/19/16	stream water	0.20	25.14	6.66	80.4	7.87	12.80	32	64
Mahulepu	M-11	M11_10-19-16	10/19/16	stream water	0.08	25.77	6.93	85.2	7.83	37.60	95	178
Mahulepu	M-11.5	M11.5_10-19-16	10/19/16	stream water	0.08	25.16	7.39	89.9	7.94	34.20	114	137
Mahulepu	M-12	M12_10-19-16	10/19/16	stream water	0.08	25.01	7.21	87.5	7.99	41.30	116	324
Mahulepu	M-01	M01_3-2-17	3/2/17	stream water	0.11	20.84	8.33	93.2	7.44	25.90	5	406
Mahulepu	M-02	M02_3-2-17	3/2/17	stream water	0.09	21.22	8.38	94.5	7.41	11.00	1	271
Mahulepu	M-03	M03_3-2-17	3/2/17	stream water	0.09	22.04	6.61	76.9	7.00	208.00	45	>2005
Mahulepu	M-04	M04_3-2-17	3/2/17	stream water	0.10	21.72	7.91	90.1	6.53	23.30	4	344
Mahulepu	M-05	M05_3-2-17	3/2/17	stream water	0.11	22.66	5.93	68.8	6.34	134.00	45	2005
Mahulepu	M-05a	M05a_3-2-17	3/2/17	stream water	0.12	21.63	5.58	63.7	6.58	25.60	10	1652
Mahulepu	M-06	M06_3-2-17	3/2/17	stream water	0.20	23.02	8.29	96.7	5.91	445.00	<1	42
Mahulepu	M-07	M07_3-2-17	3/2/17	stream water	0.10	24.27	7.79	93.3	6.20	349.00	20	192
Mahulepu	M-08	M08_3-2-17	3/2/17	stream water	0.15	22.53	1.90	22.1	6.48	90.00	45	624
Mahulepu	M-09	M09_3-2-17	3/2/17	stream water	0.13	23.34	6.48	76.6	6.56	431.00	85	271
Mahulepu	M-10	M10_3-2-17	3/2/17	stream water	0.13	21.50	5.33	60.6	6.74	51.70	30	738
Mahulepu	M-11	M11_3-2-17	3/2/17	stream water	0.13	22.35	6.69	77.6	6.51	435.00	30	885
Mahulepu	M-11.5	M11.5_3-2-17	3/2/17	stream water	0.13	22.68	7.89	91.8	6.78	410.00	45	429
Mahulepu	M-12	M12_3-2-17	3/2/17	stream water	0.13	23.12	7.86	93.1	6.66	490.00	75	659
Mahulepu	M-01	M01_4-27-17	4/27/17	stream water	0.13	22.51	6.33	73.1	7.06	7.03	1	238
Mahulepu	M-02	M02_4-27-17	4/27/17	stream water	0.12	23.50	3.90	46.0	6.70	1.74	<1	150
Mahulepu	M-04	M04_4-27-17	4/27/17	stream water	0.13	23.62	6.42	75.9	6.54	4.25	<1	591
Mahulepu	M-05	M05_4-27-17	4/27/17	stream water	0.14	23.56	6.52	77.4	7.08	6.41	32	192
Mahulepu	M-05a	M05a_4-27-17	4/27/17	stream water	0.18	23.76	2.53	30.0	6.61	60.10	35	64

					Water Quality Parameters						Fecal Indicators	
Watershed	**Site**	**Sample ID**	**Sample date**	**Type**	**Salinity (ppt)**	**Temp (°C)**	**Dissolved oxygen (mg/L)**	**Dissolved oxygen (%)**	**pH**	**Turbidity (NTU)**	***C. perfringens* (CFU/100mL)**	**Enterococcus (MPN/100mL)**
Mahulepu	M-07	M07_4-27-17	4/27/17	stream water	0.06	24.59	7.66	92.3	6.92	20.20	125	384
Mahulepu	M-09	M09_4-27-17	4/27/17	stream water	0.06	24.91	7.99	96.7	6.99	19.60	60	238
Mahulepu	M-10	M10_4-27-17	4/27/17	stream water	0.13	23.58	7.10	84.0	7.00	5.96	75	111
Mahulepu	M-11	M11_4-27-17	4/27/17	stream water	0.09	24.36	7.67	91.7	7.26	21.00	95	659
Mahulepu	M-11.5	M11.5_4-27-17	4/27/17	stream water	0.09	24.50	7.69	91.9	7.37	27.10	105	659
Mahulepu	M-12	M12_4-27-17	4/27/17	stream water	0.09	23.97	7.68	91.3	7.34	29.80	100	271
Mahulepu	Makawahi cave	Makawahi_water	10/18/16	stream water	1.89	23.72	2.91	34.9	7.23	3.03	1	75
Waikomo	W-01	W01_9-28-16	9/28/16	stream water	0.08	23.23	8.26	96.8	8.00	5.33	26	164
Waikomo	W-02	W02_9-28-16	9/28/16	stream water	0.08	23.33	7.41	86.9	7.83	4.68	10	178
Waikomo	W-03	W03_9-28-16	9/28/16	stream water	0.08	23.39	7.54	88.6	7.66	5.36	10	137
Waikomo	W-04	W04_9-28-16	9/28/16	stream water	0.08	23.26	5.71	67.0	7.56	7.20	26	192
Waikomo	W-05	W05_9-28-16	9/28/16	stream water	0.05	23.79	4.44	52.6	7.19	13.50	228	288
Waikomo	W-01	W01_10-18-16	10/18/16	stream water	0.08	24.08	8.18	97.4	8.11	3.98	20	207
Waikomo	W-02	W02_10-18-16	10/18/16	stream water	0.08	24.27	7.15	85.5	8.16	4.37	8	150
Waikomo	W-03	W03_10-18-16	10/18/16	stream water	0.08	24.15	7.26	86.5	8.08	6.72	12	64
Waikomo	W-04	W04_10-18-16	10/18/16	stream water	0.08	23.82	5.06	60.1	7.72	7.35	5	192
Waikomo	W-05	W05_10-18-16	10/18/16	stream water	0.09	23.58	4.07	48.3	7.46	11.10	190	124
Waikomo	W-01	W01_3-1-17	3/1/17	stream water	0.01	20.38	8.75	97.0	7.05	186.00	590	>2005
Waikomo	W-02	W02_3-1-17	3/1/17	stream water	0.07	20.56	8.13	90.8	6.99	147.00	530	>2005
Waikomo	W-03	W03_3-1-17	3/1/17	stream water	0.07	20.51	7.02	78.0	6.98	176.00	390	>2005
Waikomo	W-04	W04_3-1-17	3/1/17	stream water	0.07	20.40	3.45	38.3	6.87	108.00	330	>2005
Waikomo	W-05	W05_3-1-17	3/1/17	stream water	0.06	20.65	8.03	90.0	8.46	328.00	80	>2005
Waikomo	W-01	W01_4-26-17	4/26/17	stream water	0.07	23.45	8.33	98.0	7.65	4.95	40	207
Waikomo	W-02	W02_4-26-17	4/26/17	stream water	0.07	23.86	7.86	93.0	7.52	5.39	25	222
Waikomo	W-03	W03_4-26-17	4/26/17	stream water	0.07	23.61	7.57	89.3	7.47	4.35	45	531
Waikomo	W-04	W04_4-26-17	4/26/17	stream water	0.08	23.40	5.74	57.6	7.41	4.02	15	306
Waikomo	W-05	W05_4-26-17	4/26/17	stream water	0.08	22.93	6.00	70.0	7.09	50.00	50	344
Waikomo	W-06	W06_9-28-16	9/28/16	seep	3.19	25.54	5.88	73.4	7.09	0.60	<1	<10
Waikomo	W-07	W07_9-28-16	9/28/16	seep	15.64	26.07	5.67	78.7	7.27	1.15	<1	<10
Waikomo	W-08	W08_9-28-16	9/28/16	seep	3.53	24.56	6.18	75.9	7.37	2.62	6	<10
Waikomo	W-06	W06_10-18-16	10/18/16	seep	3.71	25.50	6.03	75.7	6.93	0.36	1	2.3
Waikomo	W-07	W07_10-18-16	10/18/16	seep	12.78	26.27	5.59	76.6	6.94	0.80	<1	2.3
Waikomo	W-08	W08_10-18-16	10/18/16	seep	4.72	26.62	6.58	84.6	7.40	1.83	1	10
Waikomo	W-06	W06_3-1-17	3/1/17	seep	3.11	24.48	6.65	81.3	6.54	0.41	1	2.3
Waikomo	W-07	W07_3-1-17	3/1/17	seep	21.64	24.46	6.46	88.6	7.33	0.76	1	42
Waikomo	W-08	W08_3-1-17	3/1/17	seep	1.08	22.82	7.01	82.2	6.95	14.70	150	478
Waikomo	W-06	W06_4-26-17	4/26/17	seep	2.47	25.27	5.90	73.0	7.10	0.54	<1	23
Waikomo	W-07	W07_4-26-17	4/26/17	seep	8.37	25.66	6.39	82.6	6.88	0.91	1	23
Waikomo	W-08	W08_4-26-17	4/26/17	seep	3.07	26.20	5.21	65.8	7.17	1.99	26	42