APPENDIX A

MICROBIAL SOURCE TRACKING TO IDENTIFY FECAL CONTAMINATION SOURCES IN THE RIO FERNANDO DE TAOS

TAOS, NM

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1.0 INTRODUCTION AND BACKGROUND

In 2007 Amigos Bravos initiated a monitoring program with Water Sentinels – Rios de Taos (Sierra Club) to develop a baseline of information on the Rio Fernando, with a special focus on the *E. coli* concerns. In 2016 Amigos Bravos received a Clean Water Act (CWA) 319 grant to develop the Watershed-Based Plan (WBP). The primary impetus for developing the Rio Fernando de Taos WBP derives from the abundance of *E. coli* sampling that highlighted an on-going water quality and public health concern from 2007-2016.

The most current Total Maximum Daily Load (TMDL) report for the Upper Rio Grande basin, which includes the Rio Fernando de Taos, identified *E. coli* exceedances affecting the rivers "primary contact" designated use. The cause of impairment in the Rio Fernando de Taos is identified as *E. coli* in the 2012 TMDL document for Upper Rio Grande. However, neither the 2009 study nor the interpretation methods that led to development of the TMDL was designed to identify specific sources of impairment. While results from the WBP sampling identified several hotspots, more information was needed to pinpoint sources. Hotspots are locations with multiple *E. coli* exceedances (>235 CFU/100ml) during sampling periods. To pinpoint *E. coli* sources, Amigos Bravos chose five sites from the WBP sampling where microbial source tracking (MST) data would be beneficial to decreasing the *E. coli* load on the Rio Fernando.

MST is a set of methods using genetic biomarkers from several strains of *Bacteroidetes* bacteria to identify the animal sources of fecal contamination. Similar to *E. coli* bacteria, *Bacteroidetes* are found in the intestines of warm-blooded animals and are used as indicators of possible fecal contamination in surface waters. The presence of *Bacteroidetes* in water is an indicator of recent fecal contamination.

A grant was acquired from the EPA to conduct the MST project with the goal of including it in the final approved WBP. Source Molecular Laboratory was hired to conduct the MST upon consultation with partners (https://www.sourcemolecular.com/).

2.0 PROJECT GOALS

The goal of this project was to determine what sources of *E. coli* bacteria (i.e. human, dog, cow, elk, bird, beaver) were most prevalent at the five specific locations on the Rio Fernando. The information will provide a basis for management practices that may reduce the concentration and loading of *E. coli* bacteria to the Rio Fernando de Taos. To achieve this goal, the following objectives and associated tasks were conducted:

1. Use previously collected data and GIS files to pinpoint hotspots.
    a. Use for sample location selection for MST sampling.

2. Identify the major contributing sources of fecal contamination to the Rio Fernando.
    a. Use microbial source tracking methods to identify the source of fecal contamination.
    b. Test for *E. coli* levels (enumeration) at the same time using the IDEXX method.
    c. Collect fecal validation samples per consultation with Source Molecular Labs.

3. Coordinate with the Town of Taos, Taos County, the Rio Fernando Revitalization Collaborative, and others to reduce *E. coli* bacteria levels in the Rio Fernando by implementing the Watershed-Based Plan.

3.0 METHODS

3.1 Sample Site Selection

This study was conducted from March 26, 2019 – September 23, 2019 using *E. coli* enumeration and MST analysis to identify the sources of fecal contamination in the Rio Fernando. A total of five sample sites were chosen based on the *E. coli* enumeration results of the Rio Fernando Watershed-Based Plan sampling from 2016-2018.

Due to dry conditions observed once at site F16, a different urban site was sampled resulting in F16 not being sampled as frequently as planned. The single-sample site (F26) is included in the table and map but was only sampled once and will not be included in the graphs.

Figures 1 and 2: The Rio Fernando De Taos Placement Maps

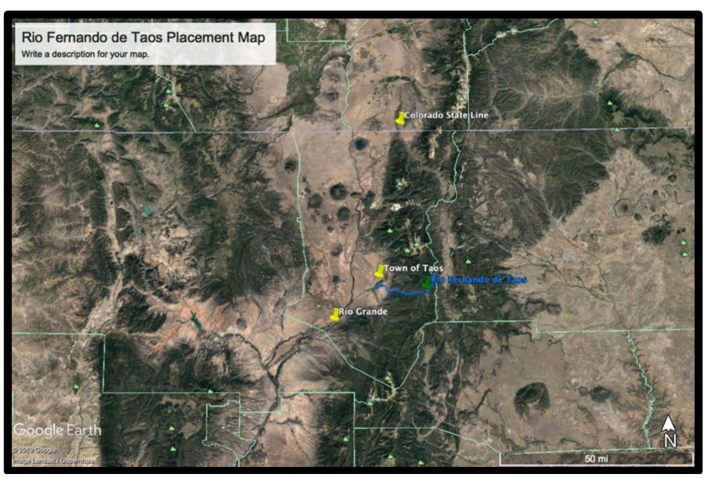

Figure 3: Rio Fernando MST Sample Site Map. Map of MST sampling locations. F26 was only sampled once. The other five sites were sampled 7-14 times based on water availability. Sites chosen indicate there has been an on-going *E. coli* problem in that location and more information about the source was needed.

Figure 4: Site locations and description.

Site	Location Description	Lat/Long
FLJ	La Jara, at base of Forest Road 5	36.418273; -105.34331
FRE	Riparian Exclosure	36.403791; -105.34512
F16	At the end of Santistevan lane near the center of Taos	36.400167; -105.58355
F4	Fred Baca Park near the bridge	36.399578; -105.58931
PS3	Merris spring after it crosses Upper Ranchitos Road by the Good News Church	36.404810; -105.59808
F26	Downstream of Angladas building where the Rio Pueblo connected acequia comes in.	36.391217; -105.56445

3.2 Microbial Source Tracking Methods – Source Molecular Laboratory

We used six species markers based on probable sources identified in the Watershed-Based Plan and lab consultations. Sources thought most likely to be contributing bacteria in the Rio Fernando: humans, cattle, elk, dogs, birds (including chickens, waterfowl, and other wild birds), and beaver. The study utilized adaptive management to make mid-study changes and hone in on the most likely contributors to fecal pollution. For example, after half the sample results were received, a bird marker was added to three more of the sites because it was showing up frequently as another site.

Each water sample submitted was filtered through 0.45 micron membrane filter(s). Each filter was placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample was homogenized for 1 minute and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus Real-Time Thermal Cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays were run in duplicate.

Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers. For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

3.3 Sample Collection

A total of 61 samples were collected over 17 days. Sampling occurred between March 26, 2019 and September 23, 2019. *E. coli* levels (enumeration) were collected at the same time as MST samples. A total of 216 tests were run by Source Molecular on the 61 samples sent in according to the methods in section 3.2 above.

Field parameters and stream discharge were collected along with grab samples. Field parameters (pH, specific conductivity, dissolved oxygen, and water temperature) were measured to look for differences in water quality conditions between sites that could support identification of areas of incoming wastewater contamination.

3.4 Quality Assurance/Quality Control

Field meters were calibrated regularly using standard reference solutions to assure the meters were working properly. Quality control samples for *E. coli* enumeration consisted of one duplicate and one blank sample collected at random from one of the sampling sites for *E. coli* bacteria analysis. Due to the cost associated of MST analysis, duplicate and blank samples were not collected. Quality control criteria for duplicate and field blank samples are:

- Relative Percent Difference of duplicates = <25%
- All field blank results must be < Reporting Limit

The *E. coli* bacteria levels were quantified in the Amigos Bravos lab using standard IDEXX methods. See Amigos Bravos *E. coli* Quality Assurance Project Plan for full methods (Appendix B).

4.0 RESULTS

4.1 Species Markers Used at Each Site:

Figure 5 shows which species were tested for at each site. Species tested for varied by site and were chosen based on results from the Watershed-Based Plan. Species tested were also adjusted mid-way through the study in response to earlier results. For example, we increased the number of sites where bird markers were tested based on frequent bird markers observed in the only location where it was tested. Samples submitted had been frozen by the lab for potential future testing such as this.

Site	Events	Human	Cow	Elk	Dog	Bird	Beaver
F16	7	X			X	X	
F4	14	X	X		X	X	X
FLJ	14	X	X	X	X		
FRE	14	X	X	X	X	X	
PS3	12	X	X		X	X	
F26	1	X	X		X		

Figure 5: X's indicate host markers that were tested for at each site. Blank cells indicate that the species was not sampled for at that site.

4.2 Fecal Validation Summary and Results

Discriminating between pollution from cattle and elk populations was of concern in some of the drainages under investigation in this study. Representative fecal samples were collected from individual cows and elk to investigate the performance of tests designed to detect fecal bacteria from both cows and elk. Bacterial DNA from each fecal sample was extracted and analyzed for the cow and elk-associated markers using qPCR. The results were used to calculate sensitivity and specificity of the markers.

Sensitivity and specificity of each host marker were calculated as follows:

•Sensitivity = Number of target individuals tested positive/total number of target individuals.
•Specificity = Number of non-target individuals tested negative/total number of non-target individuals.

Figure 6: Sensitivity and specificity of each host marker

Fecal Host	Samples Tested	Cow-associated marker detected	Elk-associated marker detected
Cow	8	8	0
Elk	11	0	9

Host-Associated Marker	Sensitivity	Specificity
Cow	100%	100%
Elk	82%	100%

4.3 E. coli Bacteria

E. coli values ranged from 2 to >2419.6 cfu/100 mL. The state water quality standard for *E. coli* in summer months is 235 cfu/100 mL. There were exceedances at all sites except for Site FRE, the Riparian Pasture in the upper Rio Fernando. Just upstream of this at FLJ, we found the highest number of *E. coli* exceedances at 50%. See Figure 7 for the number and percentage of exceedances at each site. Overall, 26.23% of the samples (16 of 61 samples) exceeded the 235 CFU/100ml water quality standard for primary contact.

Figure 7: *The number of samples collected at each site, the number of E. coli exceedances at each site, and the frequency of those exceedances.*

# of Samples with *E. coli* over 235 CFU/100ml				
FLJ	FRE	F16	F4	PS3
7	0	1	4	4

# of Samples Collected				
FLJ	FRE	F16	F4	PS3
14	14	7	14	12

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Frequency of Exceedances (percent)				
FLJ	FRE	F16	F4	PS3
50.00%	0.00%	14.29%	28.57%	33.33%

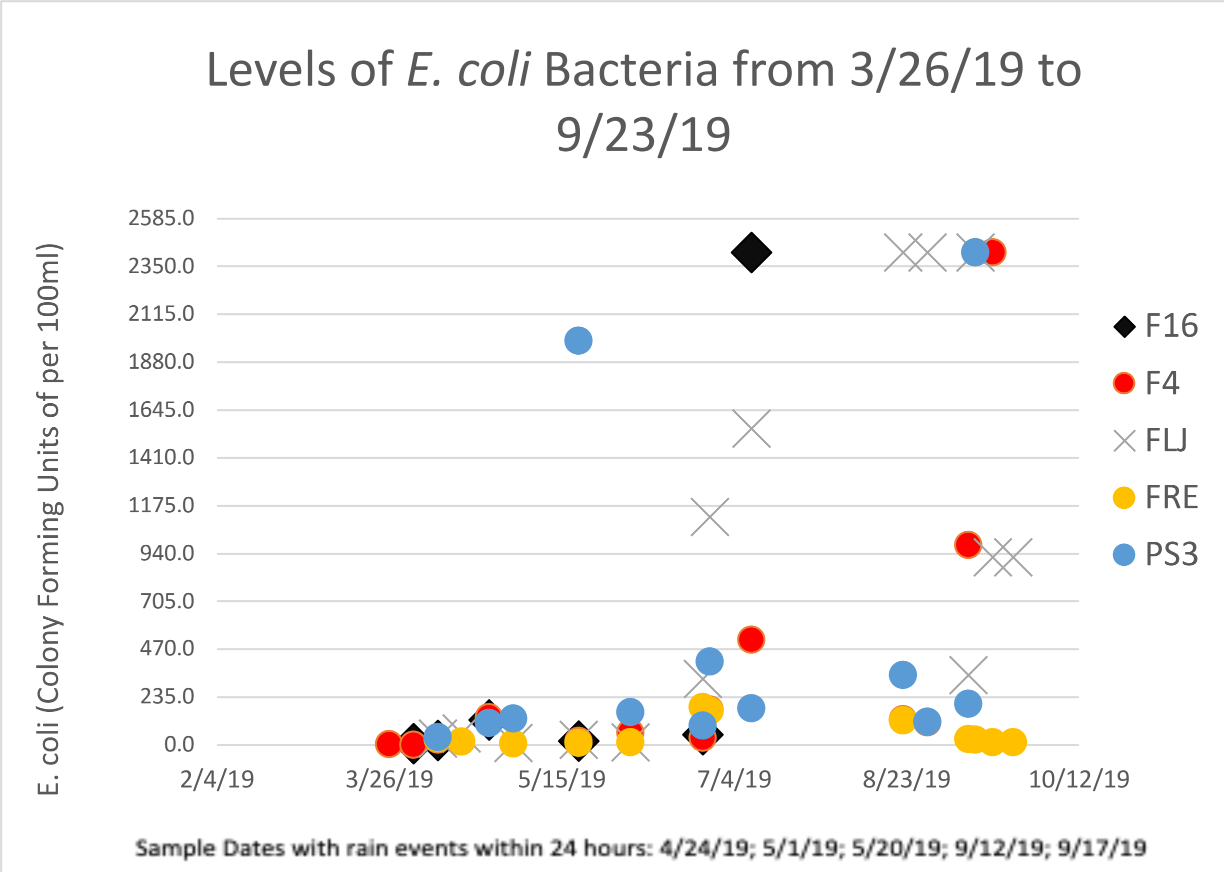


Figure 8: Shows the levels of E. coli found from each sample day. There were 17 samples day and 61 total samples.

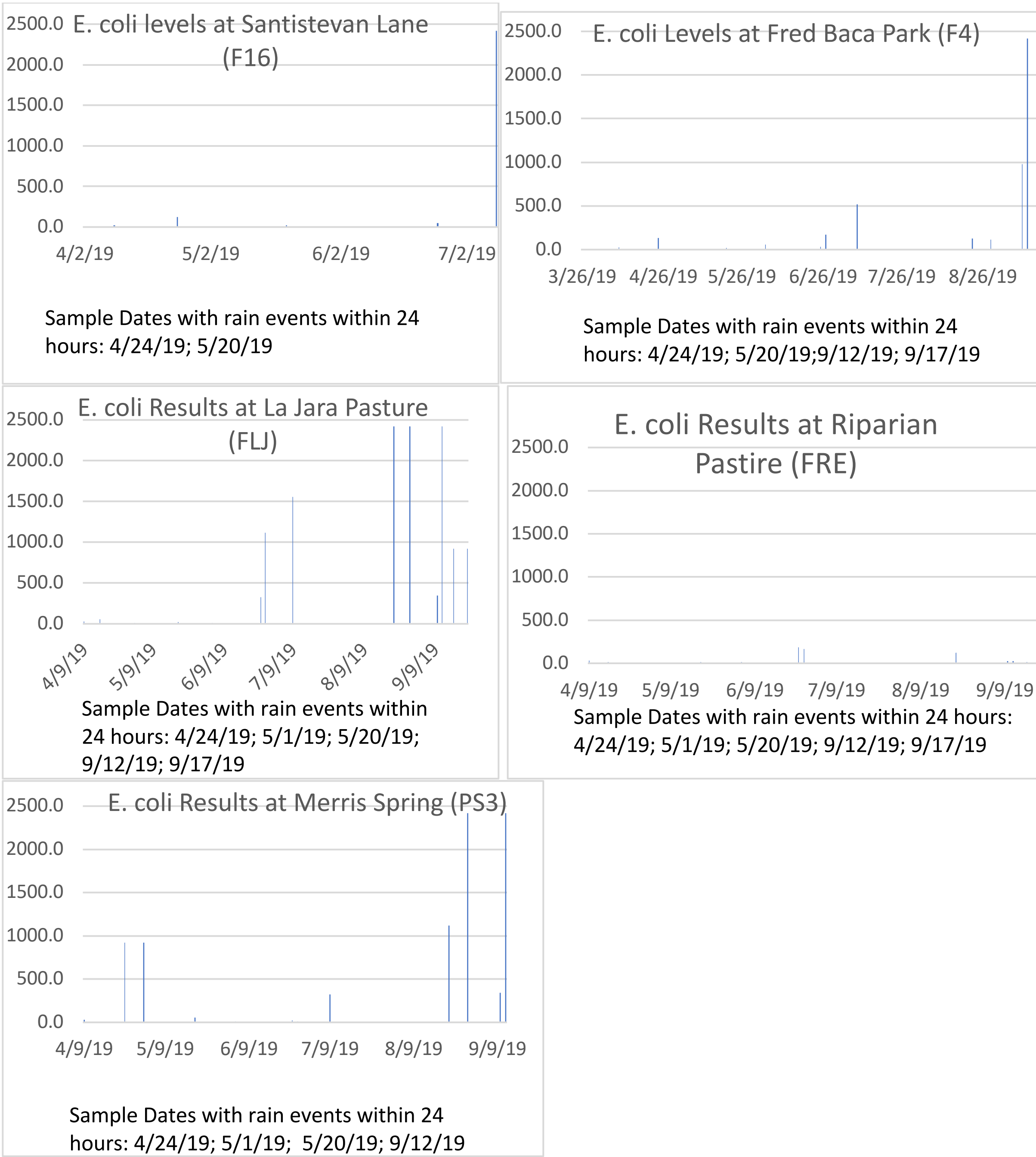


Figure 9: The E. coli levels found at each site on each sample day. The X-axis are the dates of sample events, the Y-axis is the level of E. coli found using IDEXX methods.

FRE is the only site that did not have any exceedances (Figure 9). While our data period excludes fall and winter, Figure 9 indicates that *E. coli* bacteria levels increased at F4, PS3, and FLJ in August and September. This timing coincides with low water levels, high recreational activity, high agricultural activity, and cattle grazing in the watershed. During the sampling period there were rain events with-in 24 hours of sampling on 4/24/19, 5/1/19, 5/20/19, 9/12/19, 9/17/19. There was not a clear pattern of *E. coli* exceedances at every site following rain events.

Site PS3 showed exceedances following the 4/24/19, 5/20/19 and the 9/12/19 rain but not following the 5/1/19 rain event. La Jara pasture and Fred Baca Park showed exceedances following the fall rain events but not the spring rain events. The Riparian Pasture did show exceedances at all, or any increases after any rain events.

4.4 Microbial Source Tracking Results

Proportional Frequencies of sources (# of detections / # sample events) is shown in Figure 11. Site F16 was sampled seven times, due the frequent dry periods at the site. Site F26 was sampled once after a rain event and was tested for human, cow, and dog. Results indicated human and dog markers to be present at F26 from this single sample. Figure 11 shows detection frequencies as a percent. Bird markers were found most frequently, followed by dog, cow, and human, depending on the site (Figures 10 and 11). Beaver was only tested for at the site known to have beaver (Fred Baca Park, F4)

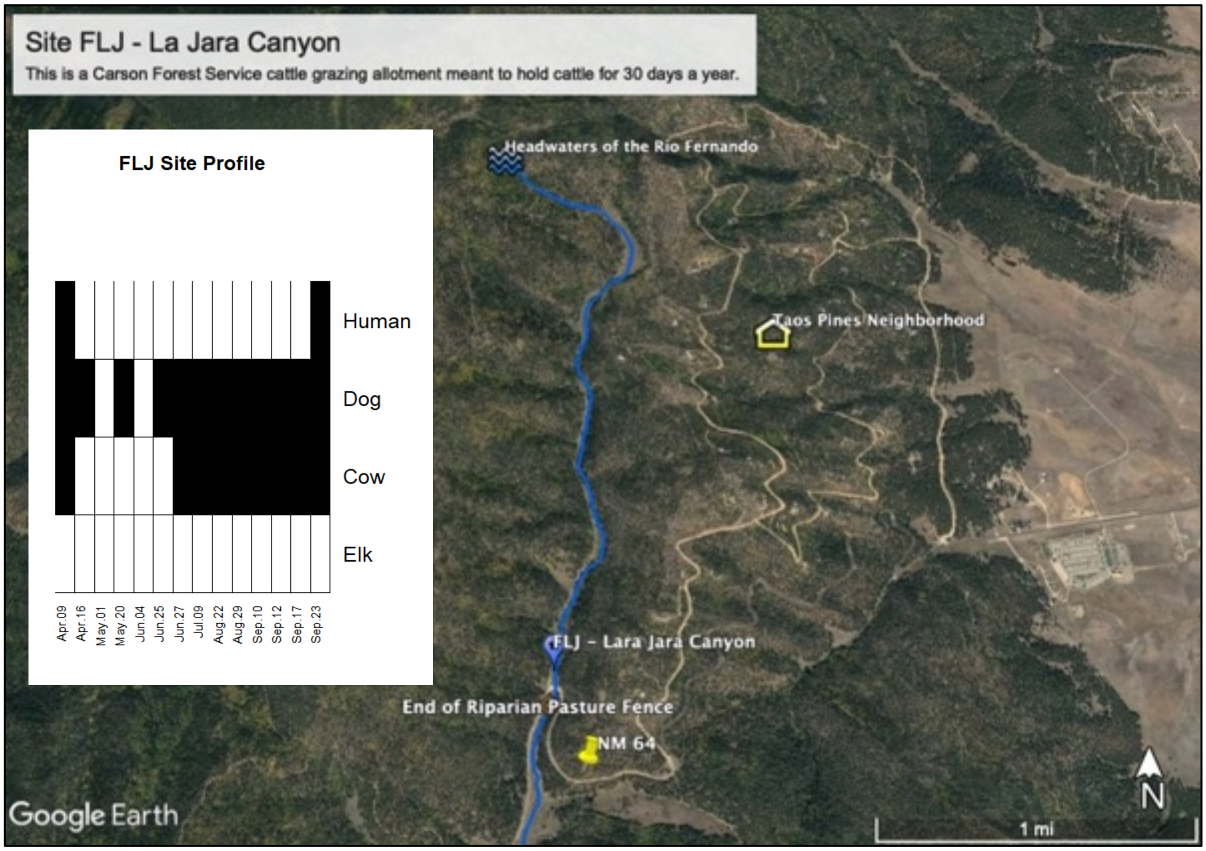
Site	Events	Human	Cow	Elk	Dog	Bird	Beaver
F16	7	2	X	X	5	7	X
F4	14	3	0	X	9	9	8
FLJ	14	2	9	0	12	X	X
FRE	14	0	5	0	3	13	X
PS3	12	8	0	X	1	11	X
F26	1	1	0	X	1	X	X

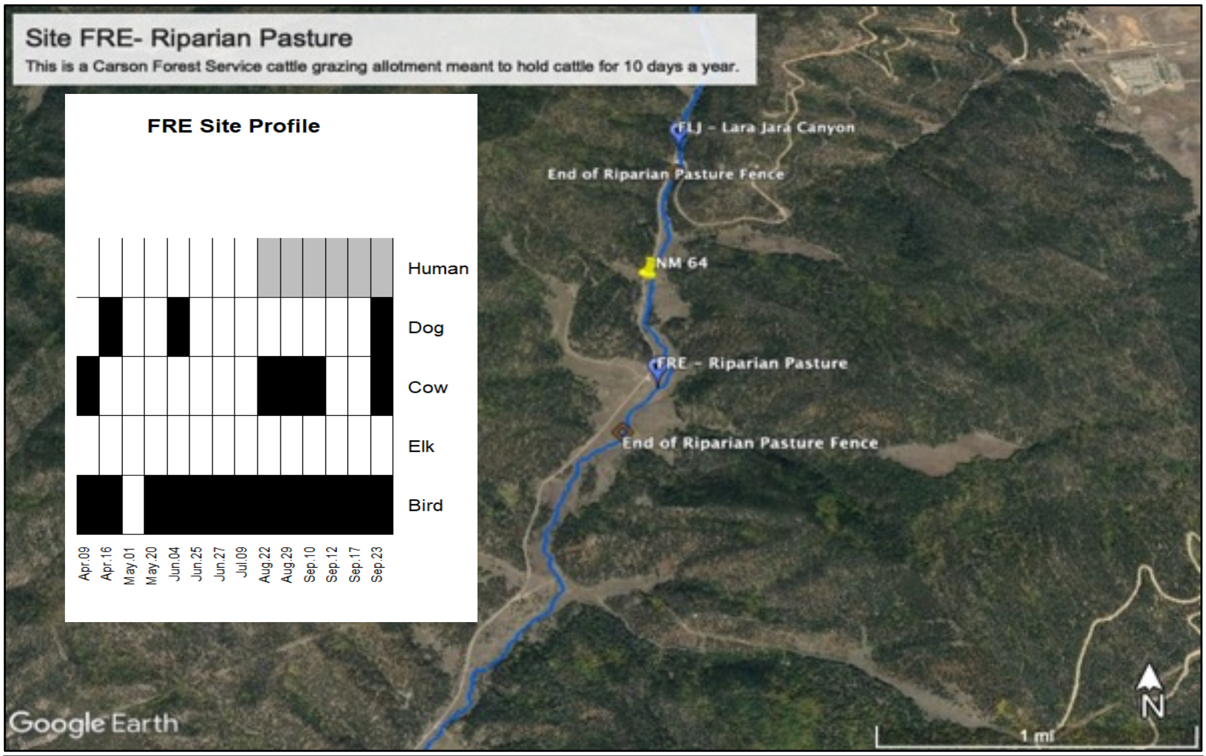
Figure 10: Frequency of Detection of species specific DNA markers at each site. X's indicate that the species was not tested for at that site.

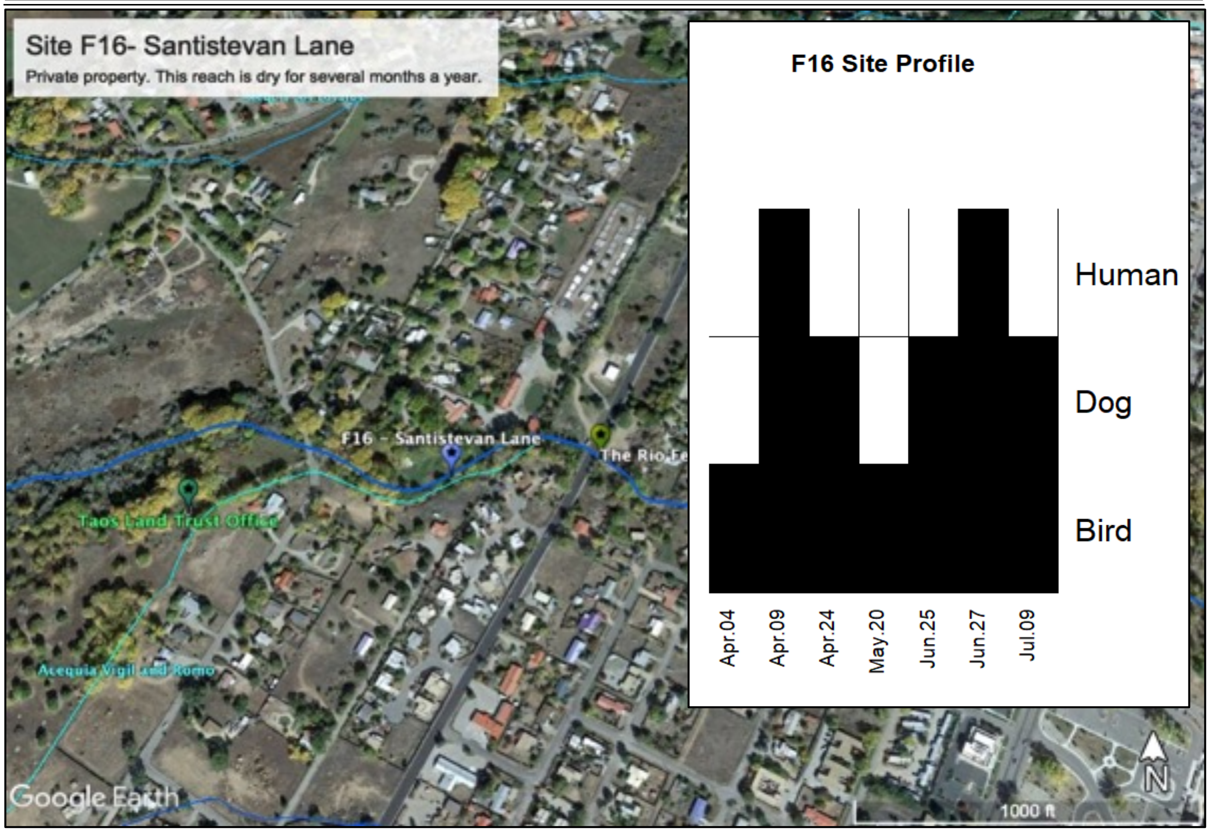
Site	Events	Human	Cow	Elk	Dog	Bird	Beaver
F16	7	0.29	X	X	0.71	1.00	X
F4	14	0.21	0.00	X	0.64	0.64	0.57
FLJ	14	0.14	0.64	0.00	0.86	X	X
FRE	14	0.00	0.36	0.00	0.21	0.93	X
PS3	12	0.67	0.00	X	0.08	0.92	X
F26	1	1.00	0.00	X	1.00	X	X

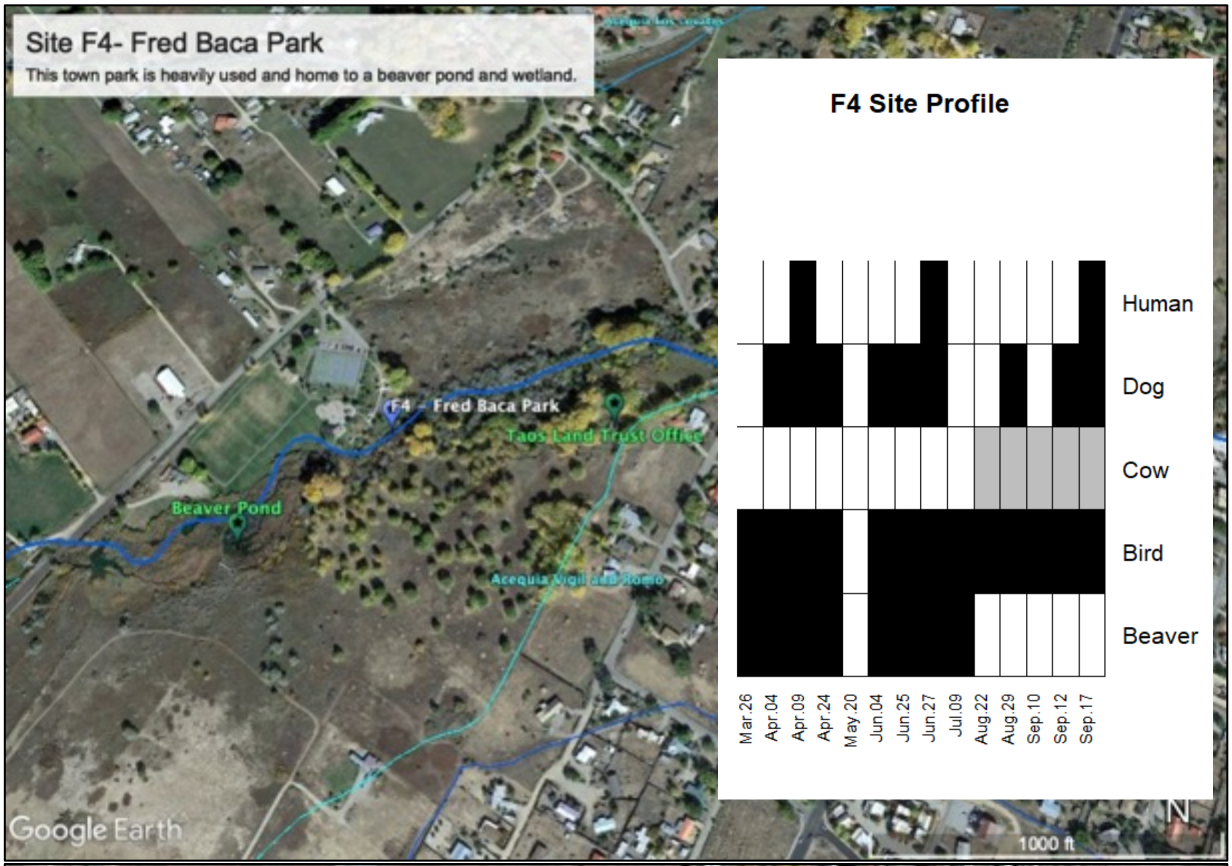
Figure 11: Proportional frequency of species specific DNA markers at each site. X's indicate that the species was not tested for at that site.

Figure 12 displays the sample site location map along with the species specific MST results for that site for all five sites. **The graphs use the following key: black = detected; white = not detected; gray = not tested for that source.**


Site FLJ - La Jara Canyon
This is a Carson Forest Service cattle grazing allotment meant to hold cattle for 30 days a year.
FLJ Site Profile
Human
Dog
Cow
Elk
Apr.09
Apr.16
May.01
May.20
Jun.04
Jun.25
Jun.27
Jul.09
Aug.22
Aug.29
Sep.10
Sep.12
Sep.17
Sep.23
Headwaters of the Rio Fernando
Taos Pines Neighborhood
FLJ – Lara Jara Canyon
End of Riparian Pasture Fence
NM 64
Google Earth
N
1 mi


Site FRE- Riparian Pasture
This is a Carson Forest Service cattle grazing allotment meant to hold cattle for 10 days a year.
FRE Site Profile
Human
Dog
Cow
Elk
Bird
Apr.09
Apr.16
May.01
May.20
Jun.04
Jun.25
Jun.27
Jul.09
Aug.22
Aug.29
Sep.10
Sep.12
Sep.17
Sep.23
FLJ – Lara Jara Canyon
End of Riparian Pasture Fence
NM 64
FRE – Riparian Pasture
End of Riparian Pasture Fence
Google Earth
1 mi
N


Site F16- Santistevan Lane
Private property. This reach is dry for several months a year.
F16 Site Profile
Human
Dog
Bird
Apr.04
Apr.09
Apr.24
May.20
Jun.25
Jun.27
Jul.09
F16 – Santistevan Lane
Taos Land Trust Office
Acequia Vigil and Romo
Google Earth
1000 ft
N


Site F4- Fred Baca Park
This town park is heavily used and home to a beaver pond and wetland.
F4 – Fred Baca Park
Taos Land Trust Office
Beaver Pond
Acequia Vigil and Romo
F4 Site Profile
Human
Dog
Cow
Bird
Beaver
Mar.26
Apr.04
Apr.09
Apr.24
May.20
Jun.04
Jun.25
Jun.27
Jul.09
Aug.22
Aug.29
Sep.10
Sep.12
Sep.17
Google Earth
1000 ft
N

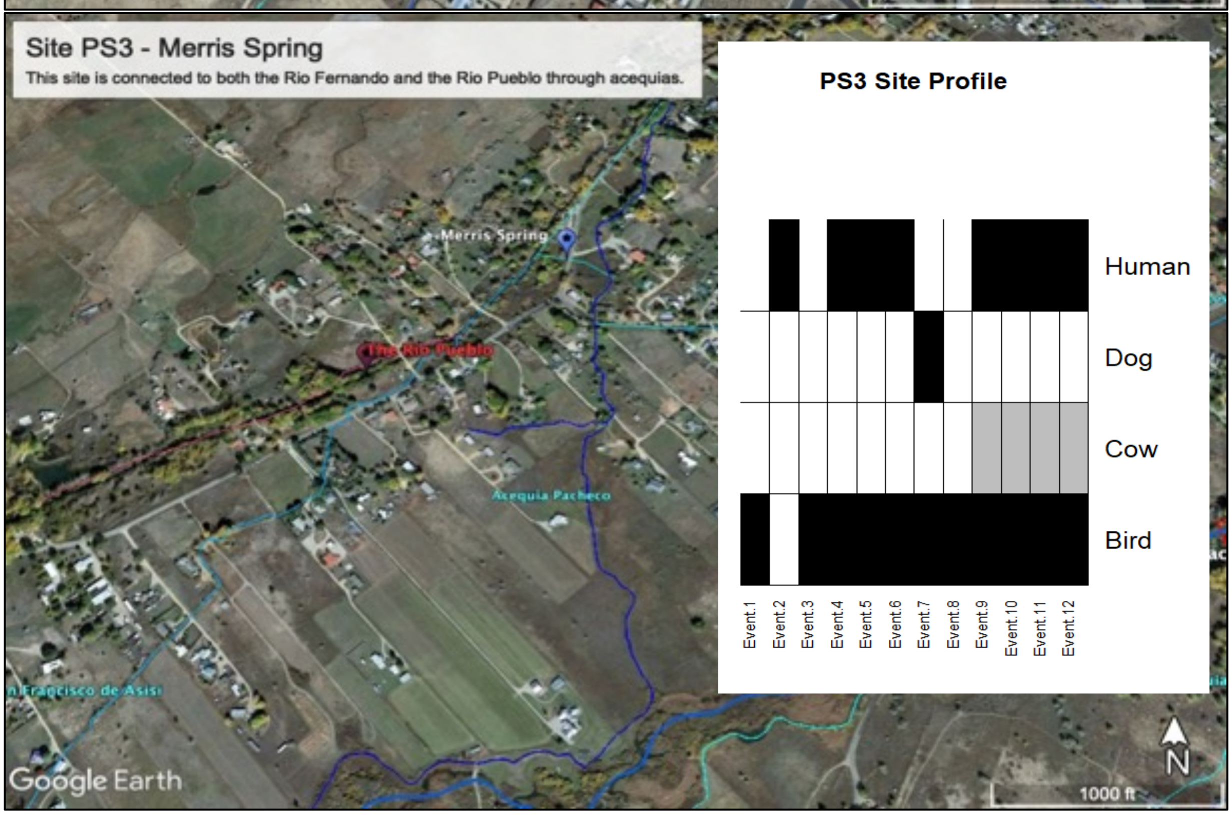

Site PS3 - Merris Spring
This site is connected to both the Rio Fernando and the Rio Pueblo through acequias.
Merris Spring
The Rio Pueblo
Acequia Pacheco
PS3 Site Profile
Human
Dog
Cow
Bird
Event.1
Event.2
Event.3
Event.4
Event.5
Event.6
Event.7
Event.8
Event.9
Event.10
Event.11
Event.12
Google Earth
1000 ft
N

Figure 12: Five Sample site location maps along with the MST results for that site. The graphs use the following key: black = detected; white = not detected; gray = not tested for that source.

4.5 MST Results Continued – Species Specific Pollution patterns

Water samples from all five sites were analyzed for human-associated fecal DNA. The site with the greatest frequency of detection was PS3. Four of the five sites detected human DNA markers. Site FRE did not detect human markers at all. To create the graphs below and site average concentrations we substituted values for the Non-detect and Detected-not-quantified results. Therefore, in the colored graphs below non-detected shows up as blue. This is why detection frequencies are provided in parentheses.

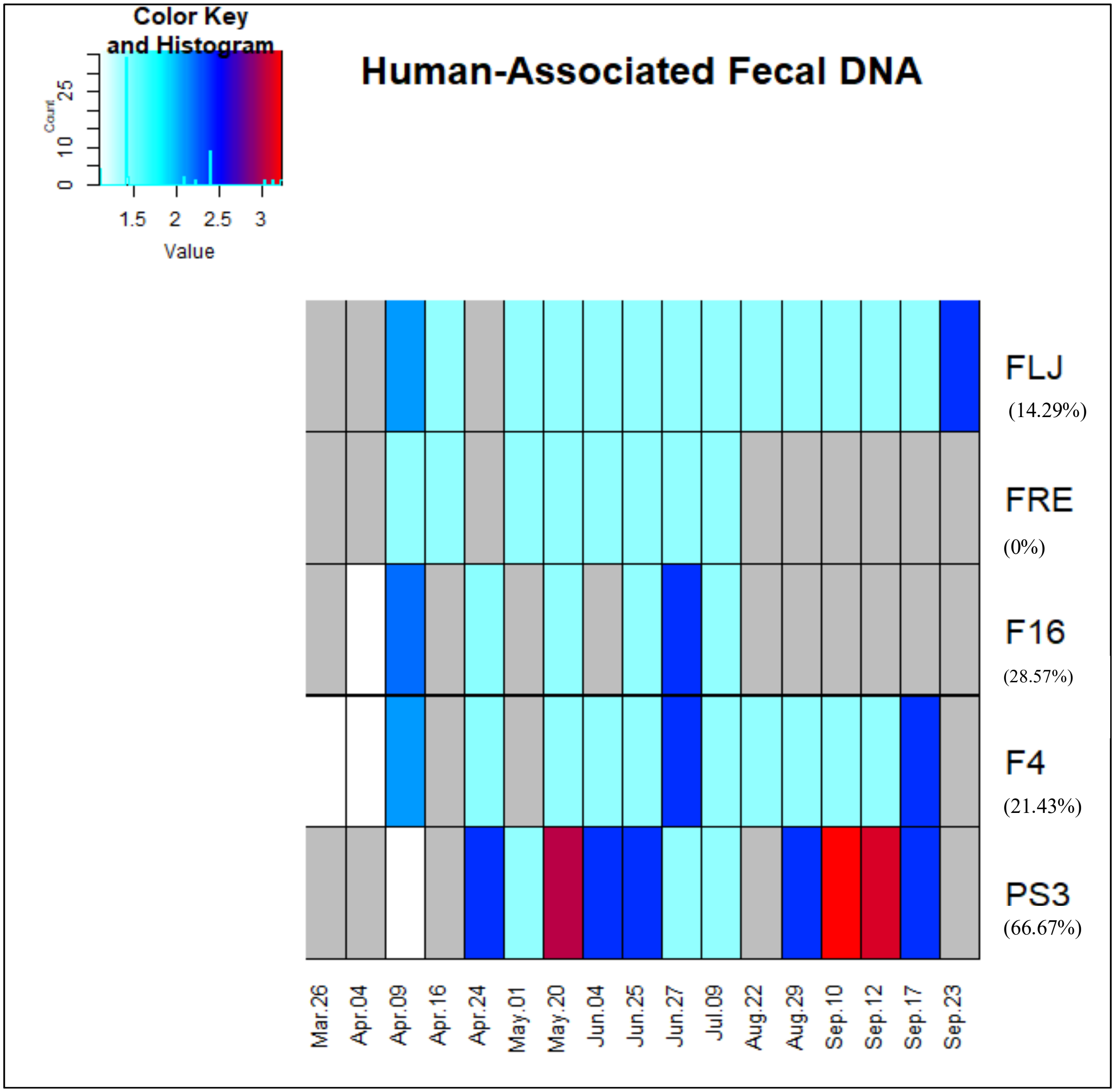


Figure 13. Gray boxes represent dates which no sample was collected at that site. Values in parentheses are frequencies of detection. Blue through red shows the level of markers found (histography provided).

Water samples from all five sites were analyzed for dog-associated fecal DNA. Sites FLJ and F4 had the highest levels of dog markers found (red). They were also the sites with the highest frequency of detection. Dog markers were detected at every site.

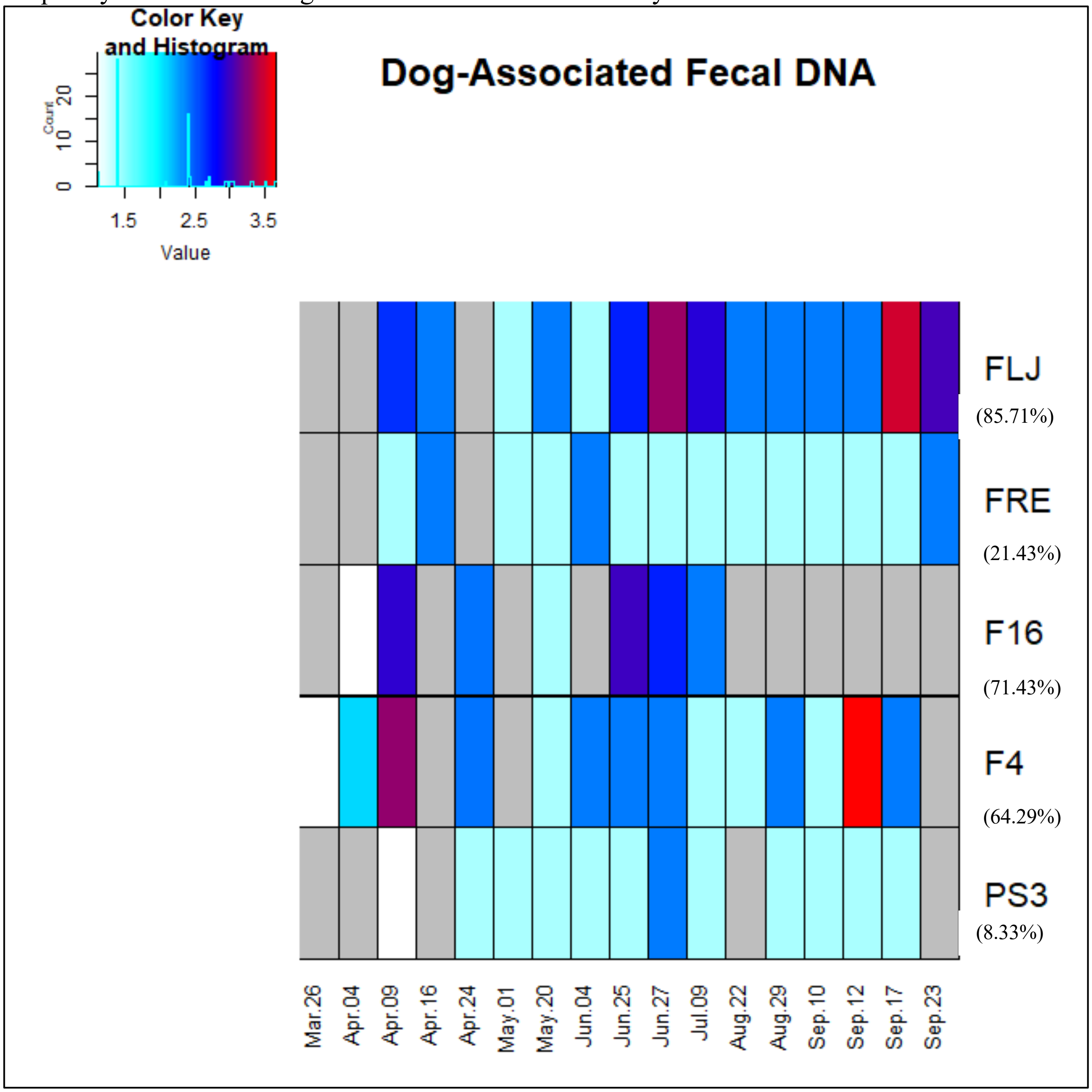


Figure 14. Gray boxes represent dates which no sample was collected at that site. Values in parentheses are frequencies of detection. Blue through red shows the level of markers found (histography provided).

Water samples from four of five sampling sites were analyzed for a cow-associated marker. Detections of the DNA were observed at two locations FLJ and FRE. The frequency of detection and concentrations of DNA were greater in samples at the later part of the sampling period, late June to September. The samples from FLJ and FRE were also analyzed for an elk-associated marker. The elk-associated marker was not detected in any water samples tested.

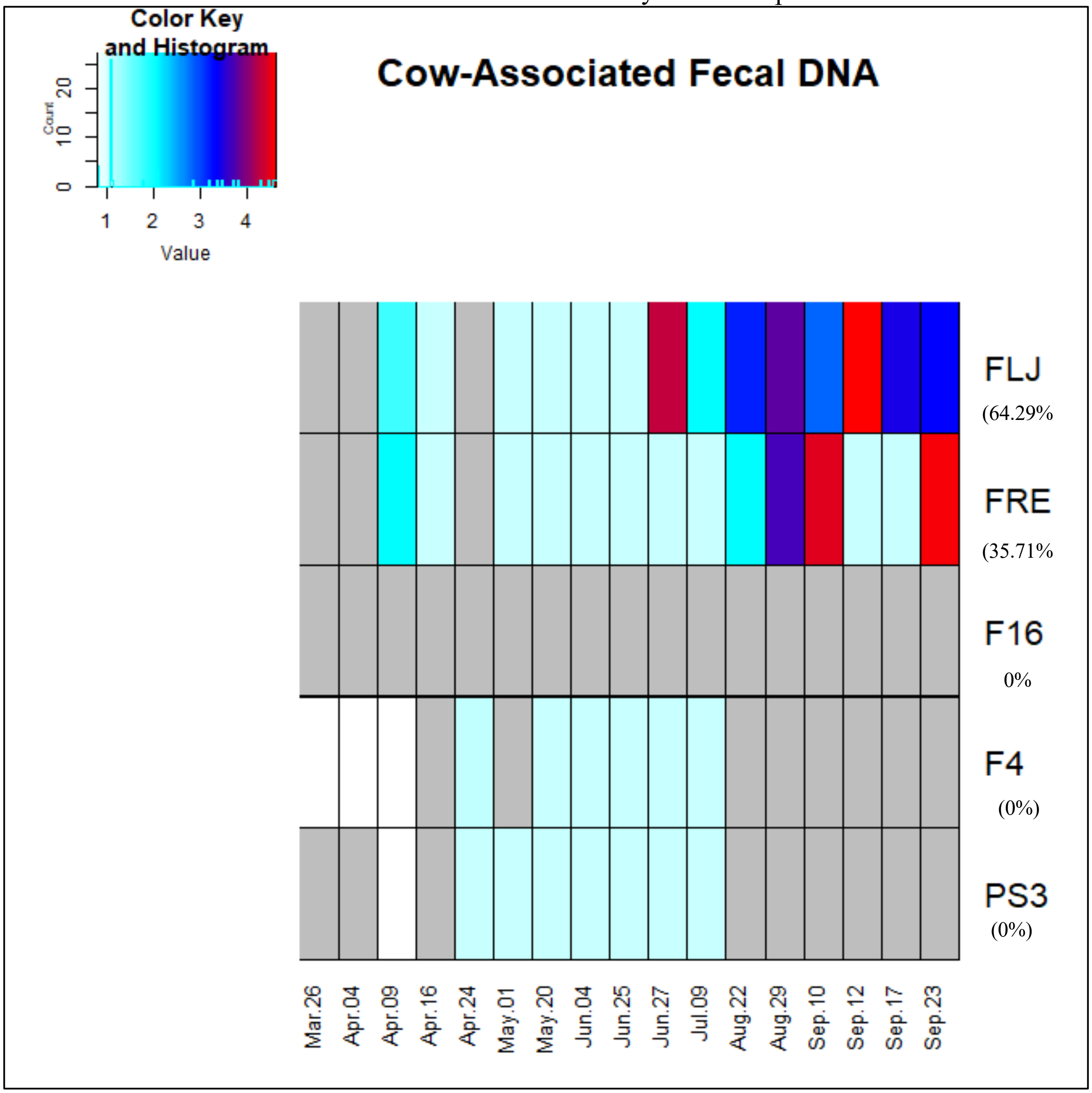


Figure 15. Gray boxes represent dates which no sample was collected at that site. Values in parentheses are frequencies of detection. Blue through red shows the level of markers found (histography provided).

Water samples were analyzed for bird-associated fecal DNA at all sites except FLJ. Bird-associated fecal DNA was detected at all sampling locations analyzed at frequencies of detection greater than 90%. During the sampling period evidence of avian fecal pollution was persistent at all sampling locations. Sites FRE and PS3 had the highest number of markers (red).

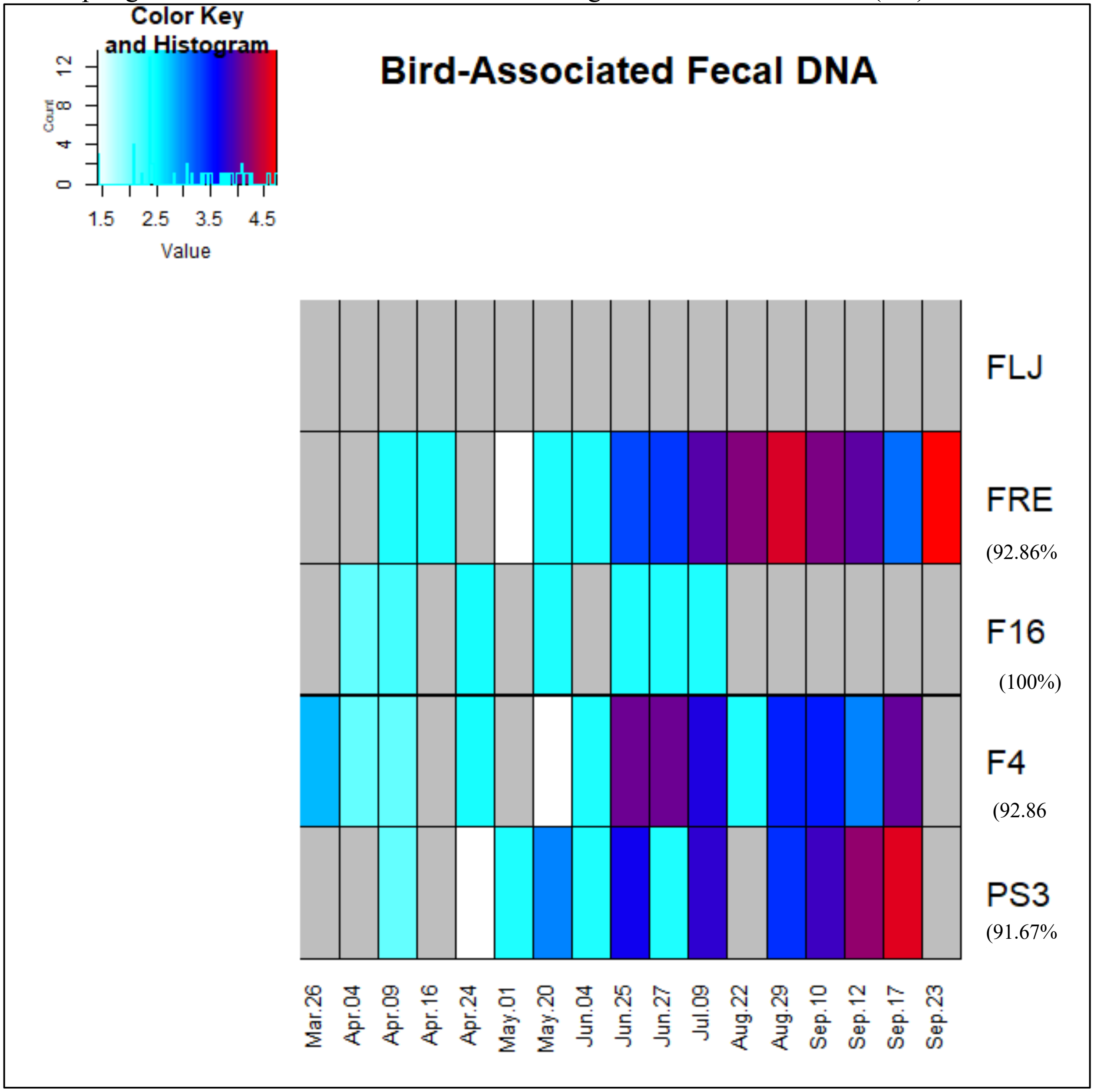


Figure 16. Gray boxes represent dates which no sample was collected at that site. Values in parentheses are frequencies of detection. Blue through red shows the level of markers found (histography provided).

Water samples from site F4 were analyzed for a beaver-associated marker. The frequency of detection was 57%. Detections were more common in the earlier sampling events from March to July.

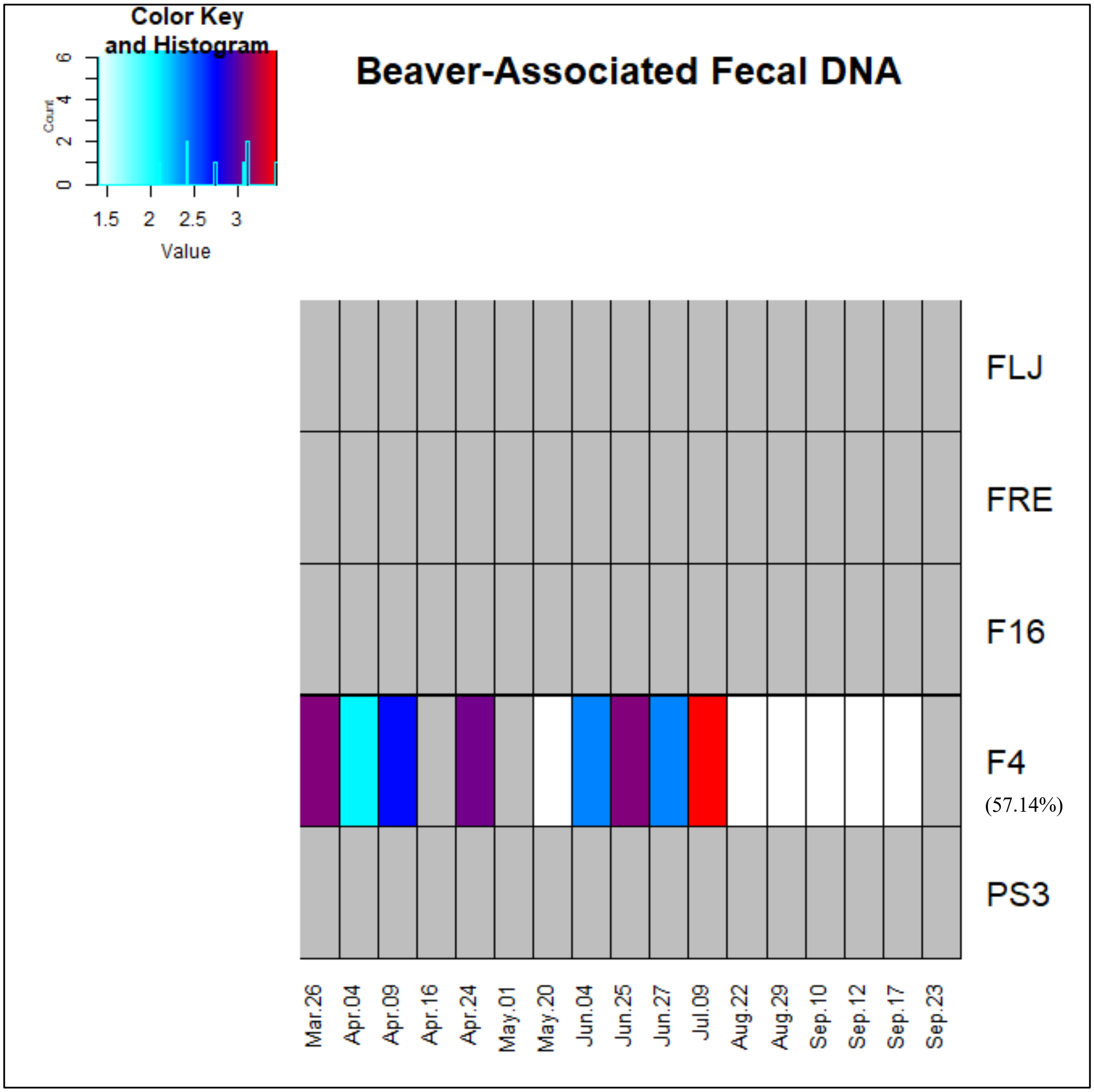


Figure 17. Gray boxes represent dates which no sample was collected at that site. Values in parentheses are frequencies of detection. Blue through red shows the level of markers found (histography provided).

5.0 DISCUSSION

The goal of this study was to determine the major sources of *E. coli* at five sites known to have *E. coli* concerns along the Rio Fernando de Taos. While sites cannot be compared to each other, each site can be examined by species DNA marker results, and over time.

5.1 Site FLJ, La Jara Pasture, Forest Road 5 (upper):

Dog markers were the most frequent, followed by cow. *E. coli* enumeration was highest during August and September (Figures 14 and 15). Dog and cow DNA markers were both highest in June and September (Figure 8). Rain events occurred on 4/24/19; 5/20/19; 9/12/19; 9/17/19 (Figure 9). There was no sample taken on 4/24, and on 5/1 and 5/20 the *E. coli* level detected were 8.4 and 25.3 CFU/100ml respectively. In the fall, both rain events coincided with high *E. coli* levels. It is important to know that in the spring, the grazing allotment is not in use by cattle. This indicates that fall runoff is contributing to *E. coli* levels and these markers are most often dog and cow. In September, the datasheets indicate heavy cattle trampling and very short grass at the sample site. The two human exceedances are likely due to camping activities in the area. If human was detected more regularly, we would suspect septic inputs from Taos Pines. But it is likely that any infiltration from this area would flow away from the Rio Fernando and that frequency of detection would have been higher (Figure 12).

This site was not sampled for birds due to cost limitations but it is likely that bird is a frequent input based on frequent observations at the site just downstream, FRE. It is also important to note that elk was tested for every time but never detected. This was a very unexpected result. Our fecal validations indicate that Elk would have been found if it was present. Further research is needed to understand if there are any other reasons we did not detect elk. They are known to be in the area year round.

Recommended management measures include implementing best management practices for livestock such as fencing the riparian area, resting this pasture, following the AOI, etc. Wetland restoration should be a major focus to give this site resilience in the face of campers, hikers, hunters, wildlife and cattle. Wetland restoration provides the ‘sponge’ that can filter out the *E. coli* and provide higher and more steady base flows to dilute the bacteria input. We also recommend providing waste bags and trash cans for pet trash at the entry way to this site (Forest Road 5).

5.2 Site FRE, Riparian Pasture (upper):

Bird markers were the most frequent, followed by cow. This is the only site where *E. coli* levels did not exceed water quality standards for the river on any of the sampling dates (Figure 9). Bird DNA markers were high from June – September and cow markers were highest in August and September (Figures 15 and 16). Cattle were scheduled to be grazed at this site from 9/21-9/30. However, the riparian pasture fence was down for the entire summer for replacement. We expected more frequent *E. coli* level exceedances this year due to this construction. Instead, this was one of the cleanest years (*E. coli levels*) for this site that we have seen in many years. It is possible that cattle and other wildlife behaved differently due to the construction and avoided the welding, trucks, and noise at the sample site. It is also possible that good snowpack this year helped the wetlands on the site to stay wet and better filter *E. coli* bacteria.

The pasture fence repairs are complete as of November 2019, and should limit the grazing of this pasture to the 10 allotted days. Rain events occurred on 4/24/19; 5/1/19; 5/20/19; 9/12/19; 9/17/19 (Figure 9). There was not any discernable pattern when comparing *E. coli* levels to these rain events. We sampled for human DNA markers seven times before cessation of testing because all the results were negative. Cessation of human sampling allowed us to reallocate funds for bird marker sampling. It is also important to note that elk was tested for every time but never detected (Figure 12).

Recommended management measures include anything that will increase flow of the river in this section. This will help dilute the natural bird input in the area. Methods to do this include wetland and stream restoration techniques that reconnect the river to its floodplain in this area. The pasture fence is a management measure that has been suspected to help with the cattle input. We are interested to see if our future results show a decrease in *E. coli* frequency and levels in this area. Monitoring by Water Sentinels- Rios de Taos will inform us of the success of this management. Pet waste signs, bags, and a trashcan are also recommended as this is a fairly popular location for hikers with pets, and dog DNA was the third most frequently detected source in the area (Figure 12).

5.3 *Site F16, Santistevan Lane (lower):*

Bird markers were the most frequent, followed by dog and then human. An *E. coli* level exceedance occurred once out of the sevens samples on July 9th, when the water was low and hot. This site was only sampled seven times due to dry conditions for the second half of the project (Figure 9). This section often dries up, while just downstream at F4, there is always at least some water, indicating springs in that area and a higher water table. At site F16, Bird DNA markers were at consistent but low levels, and were found in 100% of the samples (Figure 16). Dog markers were found five of the seven times and at slightly higher levels than the bird markers (Figure 14). Human markers were found two of the seven times, in April and June (Figure 13). The human marker levels were higher than any of the bird markers but only occurred twice. These are likely due to direct inputs into the river, however it is also possible that these are septic inputs. If there were on-going septic inputs at this site, we would expect more frequent human markers and higher *E. coli* levels. Neither sampling day that detected human (4/9/19; 6/27/19) corresponded with high *E. coli* levels (7/9/19) or with rain events.

E. coli levels were exceeded one time out of the seven sample days and occurred on July 9th when flows were low and temperatures were hot (Figure 8). There had also been rain on July 6th. This site had more frequent exceedances during the WBP study in 2017-2018 but dry periods decreased our sampling frequency during this study.

Recommended management measures include any practices that will increase flow in this area of the river. This includes erosion control projects and reconnecting the river to it's floodplain in this area. This will be difficult as the river is rip-rapped just above this sample site, helping the water to flow through quickly. Restoring the Taos Land Trust wetlands just downstream will also help this site. We also recommend increased capacity for homeless shelters as this is an urban area near the center of town, and homeless camps are frequently on the river in this section.

5.4 Site F4, Fred Baca Park (lower):

Bird and dog markers were the most frequent (64% frequency for each species), followed by beaver at 57% frequency. Dog markers were highest on April 9th and September 12th. This could indicate that spring snow runoff is carrying dog feces into the river, or that fresh pet waste just happened to be high in the area. The September 12th day corresponds with a large rain event and could also indicate that runoff with pet waste in it is the main conveyance of pet waste into the river. Bird markers were at the highest levels in June and September. June corresponds with high bird levels at this park and the high September 17th sample corresponds with a rain event and with fall bird migration.

E . coli levels were highest on 9/10/19 and 9/17/19 (Figure 8). The 9/17 sample corresponds with a rain event but the 9/10 does not. The dog marker was highest on 9/10/19, corresponding with that *E. coli* exceedance. The human marker was high on September 17th, also corresponding with the *E. coli* exceedance and a rain event. While there was not rain within 24 hours of the 9/10/19 sampling event, there was rain 48 hours before. This indicates that dog and human inputs may be entering this site during rain events.

Beaver markers were at the highest levels in April, June, and July. Beaver markers were not found on May 20th, or from August 22 – Sept 23 sample days. Baby beavers are usually born between May and June and begin swimming three to four weeks after that. This could explain the highest levels of beaver markers were found in July, but it does not explain why we stopped detecting beaver markers after that. Beaver pups generally disperse after 21-22 months after being born.

Human markers were found three times, on April 10, June 27, and Sept 17. There is a pit-toilet near the sample site that could be a source. There are also homeless in the area who camp near the sample site. Cow was sampled for 9 times as there is a small population of cattle just upstream of the sample site. Cow markers were not found during this time (April – July 9th). It is possible they would have been detected later in the sampling period when waters were lower but we chose to use resources to look for the bird marker that was being found frequently.

Recommended management measures include any practices that will increase flow in this area of the river. This is especially important because beaver and bird are natural inputs that cannot be controlled easily. This includes erosion control projects and reconnecting the river to its floodplain in this area. Any wetland restoration and invasive species plant/tree removal in the area will help to increase the functioning of the wetlands to filter the *E. coli* bacteria. We recommend increased capacity for homeless shelters as this is an urban area near the center of town, and homeless camps are frequently on the river in this section. We also recommend new pet waste signs and a campaign in the area to decrease input from dogs.

5.5 Site PS3, Merris Spring (Lower):

Bird and human markers were the most frequent at this site (92% and 67% respectively). This site had a much higher frequency of human input than any of the other sites (F16=0.29%, F4=0.21%, FLJ=0.14%, FRE=0). Levels of human DNA markers were highest on May 20th and September 10 and 12. Levels of bird markers were highest on September 12 and 17th.

E. coli levels were the highest on May 20th and September 12th, both corresponding with major rain events. There was no sample take on Sept 17th, and the April 24th sample did not show high levels even though it rained within 24 hours of that sample as well.

The fact that exceedances and DNA marker levels both correspond with rain events, indicates that runoff is contributing to the concern. However, there have been exceedances at this site many times without rain events and during cold weather in the past, indicating a septic issue in the area. It is probable that rain events in the wetland area are flooding leaky septic systems and causing them to input more bacteria into the system.

Dog markers were only detected once, and cow was not found at all. Recommended management measures should focus on two things in this area:

1) Install a public sewer system in this neighborhood. This area has had *E. coli* bacteria concerns for at least 20 years and is a known problem area by the NMED. They also recommend a sewer system be installed in this area based on their own experience.

2) Increasing flow and protecting the wetlands at this site however possible. Increasing flow, removing invasive plants, and generally protecting this wetland area is vital to filtering the natural bird input in the area.

If the septic systems cannot be replaced with a sewer system, other recommended management measures include mapping the septic tanks in the area and beginning to remove and replace the ones in the wetland and the floodplain. It is also recommended that the specific leaky septic is found and replaced immediately

It is also important to note that this location is at the intersection of Merris Spring and the Pacheco acequia. The water sampled goes into the Rio Fernando sometimes, when the water is delivered into the Pacheco, and into the Rio Pueblo de Taos other times, depending on local Mayordomo decisions (Figure 12).

5.6 Overall Final Discussion

Microbial source tracking is a fairly new method, with ever-emerging new information and accuracy. Our goal was to determine the primary sources of *E. coli* contamination at these five sites. We were able to achieve this goal thanks to the funding received. Without the frequent sampling, we would not be as confident in our results. Some limiting factors to this study were:

- Sites could only be sampled when water was present.
- Sites cannot always be compared to each other because they were not sampled at the same time and you cannot control the many differences in land-use at each site.
- Our sampling period was March 26-September 23rd -fall and winter information was not collected.
- We only know a species is present if we tested for it. This stressed the importance of knowing the potential inputs at a site before conducting the study. The 2017-2018 WBP sampling study was key to knowing what species to look for.

Important take-aways:

- Site PS3 has high levels of human input and needs to be addressed immediately. This input effects both the Rio Fernando and the Rio Pueblo.
- The upper Rio Fernando (site FLJ and FRE) are most impacted by bird, cow and dog. Stronger cattle grazing management (resting pastures, fences, etc) are necessary, along with wetland restoration at both sites to increase flows and better filter bird, dog, and cattle associated bacteria.
- Site F16, near the center of town is disconnected from the water table and dries up every year. We recommend erosion control structures to increase flow in this section of the river. This will be difficult as the river is rip-rapped just above this sample site, helping the water to flow through quickly. Restoring the Taos Land Trust wetlands just downstream will also help this site.
- Fred Baca Park, just downstream of the Taos Land Trust is impacted most by bird and dog. We recommend a pet waste campaign in the area and any restoration work that further restores the wetlands in this area and upstream at Taos Land Trust.

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