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2021 San Juan Human Bacteria Sampling and Investigation Study

Technical Report

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Table of Contents

Acronyms **2**

Introduction **3**

Sampling Design, Materials, and Methods **5**

Figure 1: San Juan River, Tributaries, and Sampling locations 5

Table 1: Sampling Dates, Transportation Method, and Locations Sampled 6

Results and Discussion **8**

Figure 2: *E.coli* Concentrations at all Surface Water Sampling Locations and Events 8

Figure 3: *B.dorei* HF183 Concentration Results of WWTP Outfall and Surface Water Sampling Locations 9

Figure 4: *B.dorei* HF183 Concentration Results for all Surface Water Sampling Locations and Events 10

Table 2: USGS Gauge 09365000 Flow, Turbidity, and Navajo Dam Release Data 11

in Comparison to Surface Water Results 11

Figure 5: *E.coli* and *B.dorei* HF183 Concentrations for all Sampling Locations during August 4-5th Sampling Events 12

Figure 6: *E.coli* and *B.dorei* HF183 Concentrations for all Sampling Locations during August 25-26th Sampling Events 13

Figure 7: *E.coli* and *B.dorei* HF183 Concentrations for all Sampling Locations 15

during September 29-30th Sampling Events 15

Figure 8: *E.coli* and *B.dorei* HF183 Concentrations for all Sampling Locations during October 27-28th Sampling Events 16

Conclusions **16**

References **18**

Appendix A. **20**

Acronyms

BMP	Best Management Practice
CFS	Cubic Feet per Second
CFU	Colony Forming Units
DNA	Deoxyribonucleic acid
DNQ	Detected Not Quantified
FIB	Fecal Indicator Bacteria
mL	Milliliter
MPN	Most Probable Number
MST	Microbial Source Tracking
ND	Non-Detect
NMED	New Mexico Environment Department
NPDES	National Pollution Discharge Elimination System
QA/QC	Quality Assurance Quality Control
qPCR	Qualitative Polymerase Chain Reaction
SJWG	San Juan Watershed Group
SWCD	Soil & Water Conservation District
SWQB	Surface Water Quality Bureau
TMDL	Total Maximum Daily Load
TSS	Total Suspended Solids
WWTP	Wastewater Treatment Plant

Introduction

Under the Clean Water Act (CWA), pathogenic bacteria contamination in fresh surface water is one of the main parameters monitored for water quality impairments in the United States. For recreational waters the presence and/or high quantities of pathogens increase human health risk for gastrointestinal, respiratory, eye, ear, nose, throat, and skin diseases (Tetra Tech & Herrera, 2011). Since pathogenic bacteria can be diverse and difficult to measure, *Escherichia coli* (*E.coli*) and enterococci is used as a fecal indicator bacteria (FIB) of other more harmful pathogens. *E.coli* is an aerobic bacteria that is naturally occurring in the environment and commonly found in the digestive system of all mammals (Rock & Rivera, 2014). In excess quantities it is an indicator of sewage and animal waste pollution that increases the probable presence of pathogens at elevated risk for water users.

While utilizing FIB's is a cost effective surrogate for determining human health risk, *E.coli* is plentiful in the feces of all mammals, as well as many cold blooded animals, and is impossible to differentiate between host organisms (Harwood et al., 2014). It is critical to have a firm understanding of the sources of pollution in a watershed to plan and implement best management practices that effectively mitigate this human health risk. Microbial source tracking (MST) was developed to determine the dominant sources of fecal contamination in environmental waters. Certain fecal microorganisms are strongly associated with specific hosts and using various methods, host-associated microorganisms can be quantified and used as an indicator of fecal pollution from their specific host (Harwood et al., 2014). While there are various techniques for MST, including speciation, biochemical reactions, and assemblages and ratios comparing two microorganisms in question, the DNA profile technique is most common for large scale watersheds (Simpson et al., 2002). The DNA profile MST technique uses qualitative polymerase chain reaction (qPCR) to pinpoint specific mRNA sections that correlate a specific bacteria species to their host. Through various comprehensive and multi-laboratory studies the DNA segment HF183 of the anerobic bacteria *Bacteroides dorei (B.dorei)* is the most common species and DNA marker used to quantify human source bacteria based on sensitivity and specificity in comparison to other tested bacterial species (Ahmed et al., 2016).

Bacteria pollution has been an ongoing concern throughout the San Juan Watershed. Within the state of New Mexico the stretch of the San Juan River from the Navajo Nation Boundary to the Animas River confluence has been listed on the New Mexico Environment Department Surface Water Quality Bureau's (NMED SWQB) CWA §303(d)/305(b) Integrated Report as impaired for *E.coli* since 2005. Going upriver, the stretch of the San Juan River from the confluence of the Animas River to the confluence of Cañon Largo has been listed as impaired for *E.coli* since 2006 (NMED SWQB, 2022). Both of these reaches have total maximum daily loads (TMDLs) for *E.coli* of 1.43×10^{12} cfu per day and 1.16×10^{12} cfu per day, respectively. Under §303(d) of the CWA, states, territories, and tribes are required to develop TMDLs for impaired waterbodies that calculate the maximum amount of pollutants, including FIB, that a waterbody can receive and still meet water quality standards. This information is critical to start the watershed planning process to best determine probable source(s) of the impairments and strategies to improve surface water quality.

In coordination with the New Mexico Environment Department (NMED) on a previous CWA §604(b) and privately funded project, the San Juan Watershed Group (SJWG) and the San Juan Soil & Water Conservation District (San Juan SWCD) conducted a microbial source tracking (MST) study in 2013-2014 to identify animal sources of bacteria along with *E.coli* and nutrient concentrations at three sampling sites on the Animas River and two sampling sites on San Juan River. The study found that 46% of San Juan River *E.coli* samples exceeded NMED's 410 cfu/100mL single sample standard for primary contact (ie: swimming). This study also tested for presence/absence of various source categories of bacteria and found that 94% of samples along the San Juan River were positive for human source bacteria, and 90% positive for ruminant source bacteria. The majority (79%) of all samples were quantifiable for human source bacteria and were analyzed for magnitude of concentrations, revealing a seven-fold increase in human source bacteria along the San Juan River between Farmington and the Hogback (jurisdictional boundary of the Navajo Nation). While ruminant source bacteria were expected due to livestock production in the river corridor (cattle, sheep) and the presence of wildlife (deer, elk), the near-constant presence of human sewage in the river was unexpected and alarming. Potential sources of human fecal bacteria include failing or improperly installed septic systems, illegal dumping of septage waste (by RVs and/or waste disposal companies), leaking sewer infrastructure, legal (permitted) discharges from wastewater treatment facilities, and/or outdoor defecation.

The 2013-2014 MST study provided a baseline for determining the presence of human fecal bacteria in the San Juan Watershed of New Mexico but specific nonpoint sources of human bacteria to the San Juan River were undetermined. A major assumption of this study was that treated wastewater from permitted point source discharges did not lead to detections of human source bacteria in surface water sampling downriver, but targeted MST sampling of point sources was not conducted to definitively rule out these contributions or lack thereof. While *E.coli* samples are culture dependent and detect only living bacteria, the cultureless qPCR method used in MST detects both non-viable and living bacteria DNA. To determine this dead vs viable ratio, a method using the photoreactive DNA binding dye Propidium Monoazide would need to be used on samples from WWTP outfalls and downstream from WWTPs to limit the detection of non-viable DNA and determine human health risk (Bae & Wuertz, 2009). Due to funding limitations, this method was not used during this study.

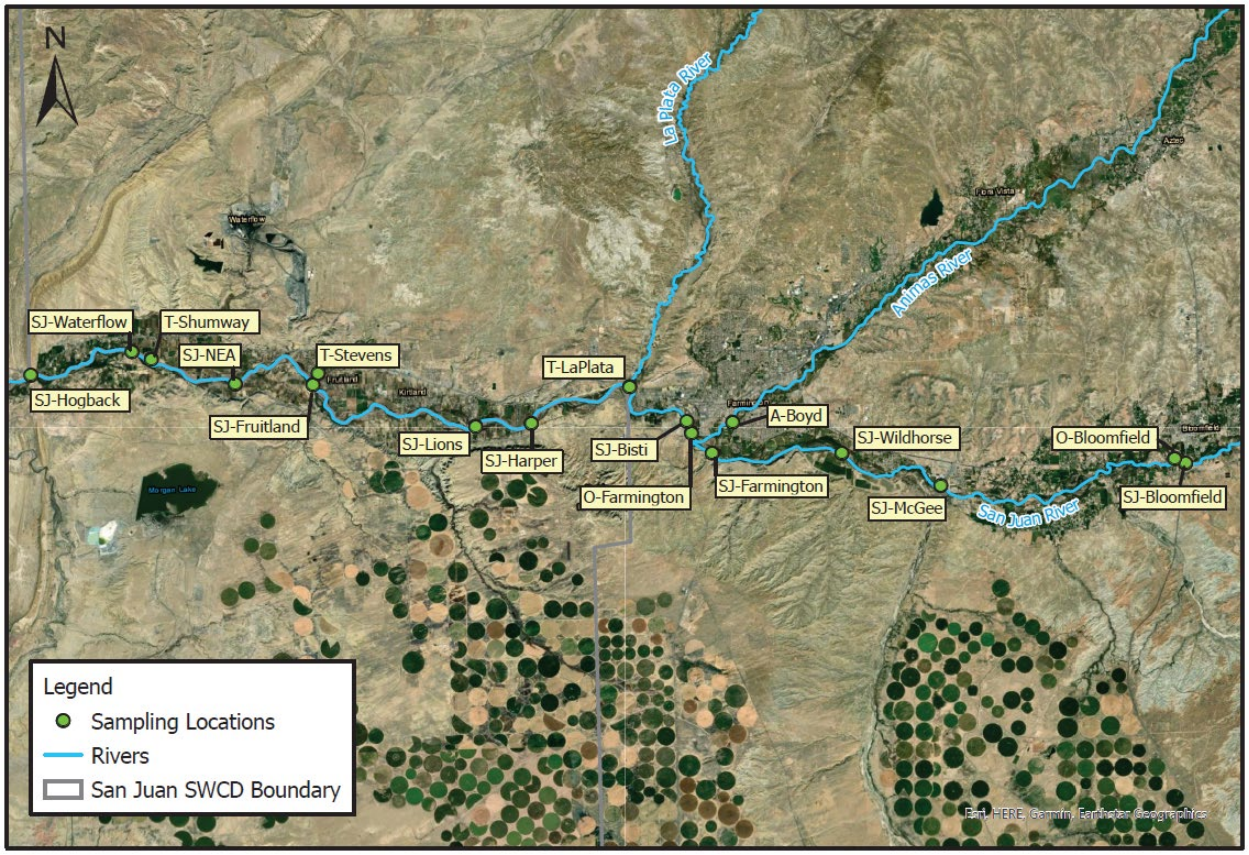
In continuation of San Juan Watershed planning efforts to address sources of bacteria pollution, the San Juan SWCD and SJWG conducted a 2021 follow up study of both *E.coli* and human source MST concentrations with a high sampling location density low sampling frequency. The goals of the study are fourfold, including (1) provide a surface water quality update on bacteria remediation efforts since the 2013-2014 MST Study, (2) further characterize spatial distribution of hotspot sources of human bacteria pollution, (3) investigate impacts of contributions from wastewater treatment plants (WWTP) on surface water quantities detected, and (4) provide data to inform concurrent investigations into opportunities to reduce human source bacteria pollution.

Sampling Design, Materials, and Methods

Bacteriological sampling for both *E.coli* culture and *B.dorei* HF183 qPCR quantification analysis were conducted at 17 sampling locations along the San Juan River, at two WWTP outfalls, and at the mouth of key tributaries, including the Animas River, La Plata River, Stevens Arroyo and Shumway Arroyo. Collecting data for both FIB and MST concentrations allowed for a comparison of human host specific data with host indifferent CWA water quality criteria.

In the effort of having data reflecting the geographic distribution of bacteria source hotspots, a sampling regime of a high density of sampling locations and low frequency of sampling events was prioritized. Sampling locations were determined through a local stakeholder effort lead by the SJWG using the following criteria: (1) legacy sampling locations from previous SJWG and San Juan SWCD water quality studies, (2) upstream and downstream of tributary confluences and suspected human bacteria sources (ie. housing divisions with known and/or suspected out-of-compliance onsite liquid waste systems), (3) tributaries into the San Juan River with legacy and/or existing §303(d)/305(b) *E.coli* impairment listings, and (4) permitted discharges from point human waste sources (ie. Farmington WWTP and Bloomfield WWTP).

Figure 1: San Juan River, Tributaries, and Sampling locations



The time of year for sampling was selected to capture the average time throughout the water year with an increased rate of primary recreation (ie. swimming) and probability of stormwater events. Various studies have shown a positive correlation between *E.coli* concentrations, total suspended solids (TSS), flow, and precipitation events, especially in watersheds with a disproportionate amount of impervious surface development (ie. concrete, asphalt, bare ground surfaces) (Petersen & Hubbart 2020, MCD 2018). Therefore, sampling was conducted on 4 sampling events from August to October of 2021.

Sampling for both *E.coli* concentrations and *B.dorei* HF183 MST was conducted on sampling days as described in the table below. Due to funding limitations, only select sampling locations on the San Juan River between Farmington and the Navajo Nation jurisdictional boundary were sampled for *B.dorei* HF183 MST during October sampling dates.

Table 1: Sampling Dates, Transportation Method, and Locations Sampled

Sampling Date	Method of Travel	Sampling Locations
8/4/2021	Raft	O-Bloomfield[1], SJ-Bloomfield[1], SJ-McGee, SJ-Wildhorse, SJ-Farmington, O-Farmington[2], SJ-Bisti, SJ-Harper, SJ-Lions
8/5/2021	Vehicle	A-Boyd, T-LaPlata, SJ-Fruitland, T-Stevens, SJ-NEA, T-Shumway, SJ-Waterflow, SJ-Hogback
8/25/2021	Raft	O-Bloomfield[1], SJ-Bloomfield[1], SJ-McGee, SJ-Wildhorse, SJ-Farmington, O-Farmington[2], SJ-Bisti, SJ-Harper, SJ-Lions
8/26/2021	Vehicle	A-Boyd, T-LaPlata, SJ-Fruitland, T-Stevens, SJ-NEA, T-Shumway, SJ-Waterflow, SJ-Hogback
9/29/2021	Vehicle	A-Boyd, T-LaPlata, SJ-Fruitland, T-Stevens, SJ-NEA, T-Shumway, SJ-Waterflow, SJ-Hogback
9/30/2021	Raft	O-Bloomfield[1], SJ-Bloomfield[1], SJ-McGee, SJ-Wildhorse, SJ-Farmington, O-Farmington[2], SJ-Bisti, SJ-Harper, SJ-Lions
10/27/2021	Raft	O-Bloomfield[3], SJ-Bloomfield[3], SJ-McGee[4], SJ-Wildhorse[4], SJ-Farmington[4], O-Farmington[5], SJ-Bisti, SJ-Harper, SJ-Lions
10/28/2021	Vehicle	A-Boyd[4], T-LaPlata[4], SJ-Fruitland, T-Stevens[4], SJ-NEA, T-Shumway, SJ-Waterflow, SJ-Hogback

[1]Sampled from outflow sluice or riverbank before rafting excursion
[2]Sampled by Farmington WWTP staff during routine NPDES permit water quality sampling
[3]Sampled from outflow sluice or riverbank for only *E.coli* before rafting excursion
[4]Sampled only for *E.coli*
[5]Sampled only for *E.coli* by Farmington WWTP staff during routine NPDES permit water quality sampling

E.coli and *B.dorei* HF183 marker samples were collected by San Juan SWCD staff and an a total of 16 volunteers for analysis at Jacobs Laboratory at the Farmington WWTP and LuminUltra Inc. laboratory in Miami, Florida, respectively. Each sampling event had a blank sample of deionized water for both *E.coli* and *B.dorei* HF183 marker analysis to ensure no cross contamination occurred between samples during sampling trips and shipping. Collection and sample bottles for lab analysis were provided by the above partners. Both raft and vehicle transportation were used to access sampling locations. Raft sample collection was conducted mid channel directly from the side of the raft using a site specific sterile 500

mL collection bottle. Samples collected via riverbank access was done using a 10 foot long fully extended sampling pole to ensure adequate flow and mixing of surface water into site specific sterile 500 mL collection bottle, unless not deemed possible during streams with low flow. This sample collection protocol was unable to be followed at the La-Plata sampling location, as low flows necessitated two scoops of water for enough volume for analysis.

Immediately after collection, samples were decanted into sterile 250 mL *E.coli* and *B.dorei* HF183 samples bottles, labeled, and stored in an ice cooler throughout the remainder of sampling trips. *E.coli* samples were delivered at the Jacobs Laboratory and analyzed within the quality assurance/quality check (QA/QC) 24 hour period. *B.dorei* HF183 samples were overnight shipped in the same ice cooler used during sample collection to LuminUlta Inc. Laboratory and were analyzed within the 24 hour QA/QC period. Due to shipping complications, *B.dorei* HF183 samples collected on August 5th, 2021 arrived at the laboratory three days after collection, diminishing sample quality. Heavy consideration was taken with this qualifier during data analysis.

At Jacob's Laboratory, *E.coli* concentrations were measured using the Idexx Quanti-Tray method with fluorescent Colilert reagent via the most probable number (MPN) per 100 mL sample (Jacobs 2021). The detection minimum and maximum were within the range of <1 and 24,196 MPN/100mL.

At LuminUltra Inc. Laboratory, *B.dorei* HF183 markers was analyzed for both presence and quantification using two repetitions of the library independent qPCR method with a unit of copies per 100 mL (LuminUltra 2021). The minimum limit of quantification varied between 150 and 500 copies/100mL depending on the volume filtered (range between 30-100 mL). Sample presence and quantification was bracketed into three categories: non-detect results (ND), detected not quantified (DNQ), and detected. Samples with ND results either had an absence of the *B.dorei* HF183 biomarker for both repetitions or only one repetition under 35 copies/100mL while the other repetition detected no presence. To qualify as DNQ, both repetitions detected quantities of the biomarker below the limit of quantification. For the sake of statistic and spatial analysis, samples with ND results were substituted for a 0.50 value and DNQ results were substituted with a value of 10.

Standard statistical analysis used included geometric mean, standard deviation, and correlation coefficients between *E.coli* and *B.dorei* HF183. *E.coli* concentrations were compared against the NMED CWA 410 cfu/100mL *E.coli* single grab exceedance limit. The monthly geometric mean exceedance limit of 126 cfu/100mL was not used during the analysis as five or more samples were not collected within a 30 day period at any sampling location. *B.dorei* HF183 quantification results were compared to the previously modeled 4,200 copies/100mL illness benchmark where 30 in 1,000 swimmers will experience a gastrointestinal illness (Boehm et al. 2015). Temporal trends with flows were analyzed comparing results per sampling event with USGS Gauge 09365000 flow data and Navajo Dam release measurements. Finally, results were georeferenced to analyze spatial trends in comparison to land use and local knowledge of potential pollutant sources and liquid waste projects recently completed since the 2013-14 MST Study.

Results and Discussion

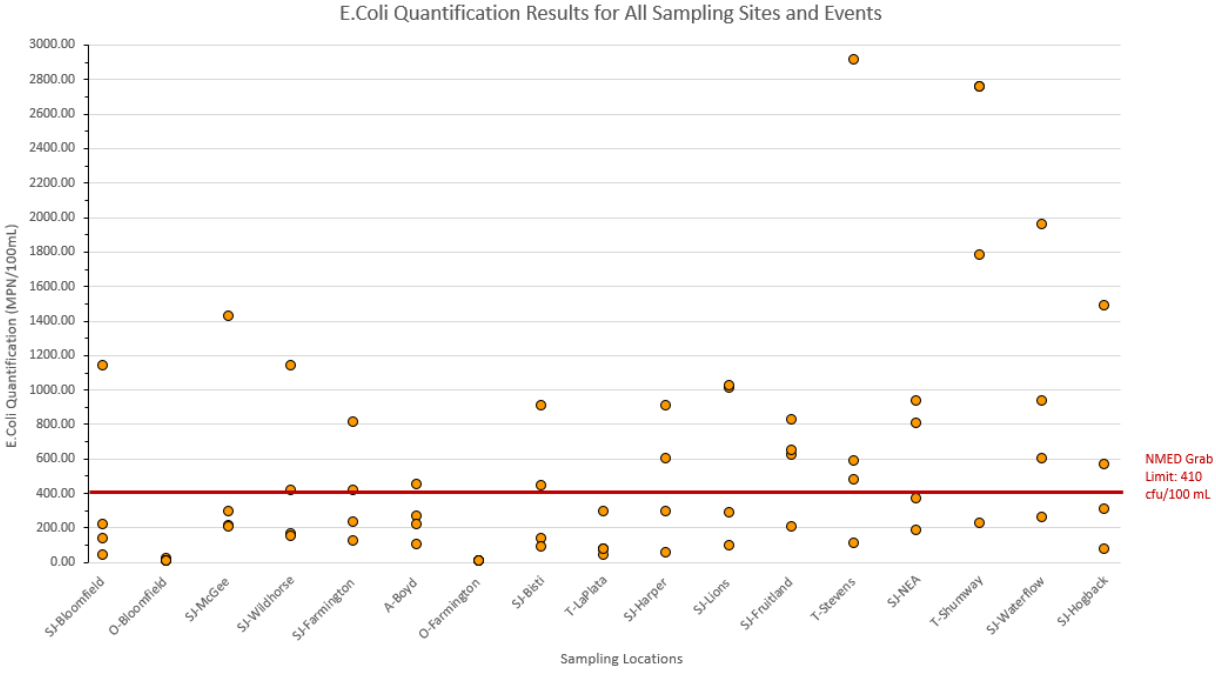
Other than the August 5th samples (sampling locations indicated in Table 1), all samples maintained time and temperature QA/QC requirements. All blanks had <1 MPN/100mL of *E.coli* and ND of *B.dorei* HF183 markers, indicating that no samples were contaminated before lab analysis.

General E.coli Result Distribution and Exceedances

Of the 60 *E.coli* surface water samples collected (WWTP outfalls are not under the jurisdiction of §303(d)/305(b) of the CWA), 48% were over the 410 cfu/100 mL single grab exceedance limit. Distribution of *E.coli* results for each sampling location were widely distributed, indicating high variability in concentration based on environmental factors. Sampling locations SJ-Fruitland, SJ-Waterflow, T-Stevens, and T-Shumway had the highest ratio of *E.coli* samples over the single grab exceedance limit (3/4 sampling events). Only one of the 17 samplings locations, T-La Plata had no single grab *E.coli* exceedances.

Number of Single Grab *E.coli* Exceedances *Over 410 cfu/100mL*
SJ-Bloomfield: 1/4
SJ-McGee: 1/4
SJ-Wildhorse: 2/4
SJ-Farmington: 2/4
A-Boyd: 1/4
SJ-Bisti: 2/4
T-LaPlata: 0/4
SJ-Harper: 2/4
SJ-Lions: 2/4
SJ-Fruitland: 3/4
T-Stevens: 3/4
SJ-NEA: 2/4
T-Shumway: 3/4
SJ-Waterflow: 3/4
SJ-Hogback: 2/4

Figure 2: *E.coli* Concentrations at all Surface Water Sampling Locations and Events

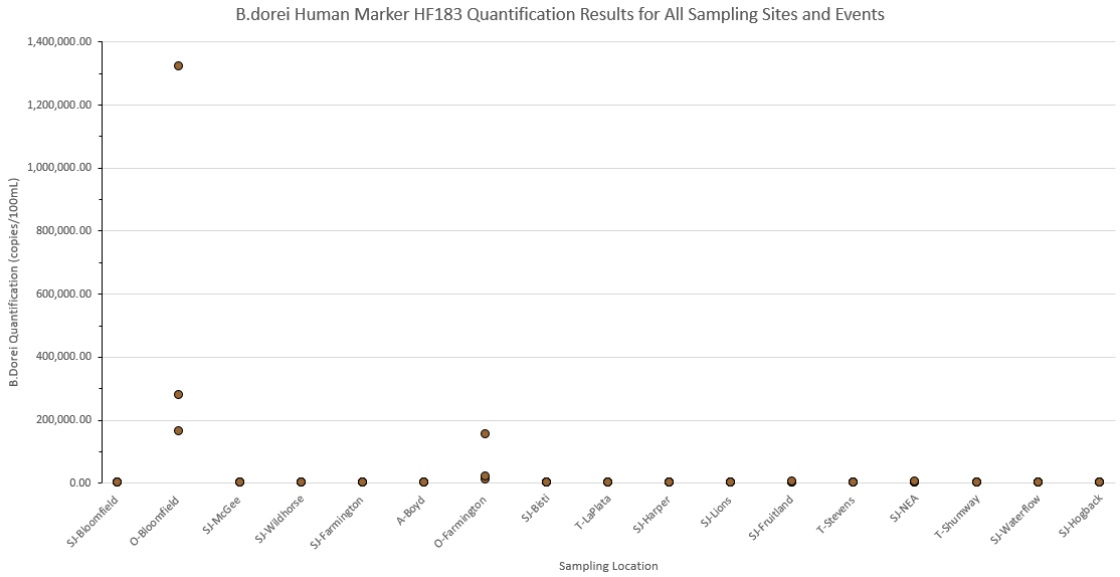


B.dorei HF183 WWTP Outfall Influence on Surface Water Concentrations

Based on the results of the Farmington and Bloomfield WWTP outfalls samples and the proceeding downriver sampling location, treated wastewater from these WWTPs do not appear

to be driving increases in downstream surface water *B.dorei* HF183 concentrations. The Farmington WWTP had *B.dorei* HF183 concentrations varying between 19,200 and 154,000 copies/100mL while having *E.coli* concentrations between 2 and 5.20 MPN/100mL. The Bloomfield WWTP had *B.dorei* HF183 concentrations between 164,000 and 1,320,000 copies/100mL while having *E.coli* concentrations between 5 and 10 MPN/100mL. Both facilities are well within their NPDES requirements for *E.coli*, indicating proper waste treatment. No correlating increases in *B.dorei* HF183 were seen in the downriver sampling locations of these significant concentrations from WWTP outfalls. Proceeding sampling events of sampling location SJ-McGee (approximately six miles from Bloomfield WWTP) and SJ-Bisti (approximately 0.25 mile from Farmington WWTP) displayed ND, DNQ, or *B.dorei* HF183 concentrations between 1,040 and 1,420 copies/100mL.

Figure 3: *B.dorei* HF183 Concentration Results of WWTP Outfall and Surface Water Sampling Locations



The high concentrations of *B.dorei* HF183 from WWTP outfalls is not necessarily an indicator of human health risk. As mentioned previously, qPCR methods detect both dead and viable DNA markers. Using the photoreactive DNA binding dye Propidium Monoazide during qPCR analysis is one of the most common methods to differentiate dead and viable MST DNA (Bae & Wuertz, 2009). Future sampling using this method is recommended to determine this dead vs alive ratio. Additionally, the decay rate of *B.dorei* HF183 introduced into a river water depends heavily on various factors, including temperature, turbidity, sunlight exposure, and predation of other macrophages (Dick et al. 2010). Decay rates in the San Juan Watershed for this fecal bacteria is yet to be seen and remains a data gap for the *B.dorei* HF183 contributions from WWTP outfalls detected in this study.

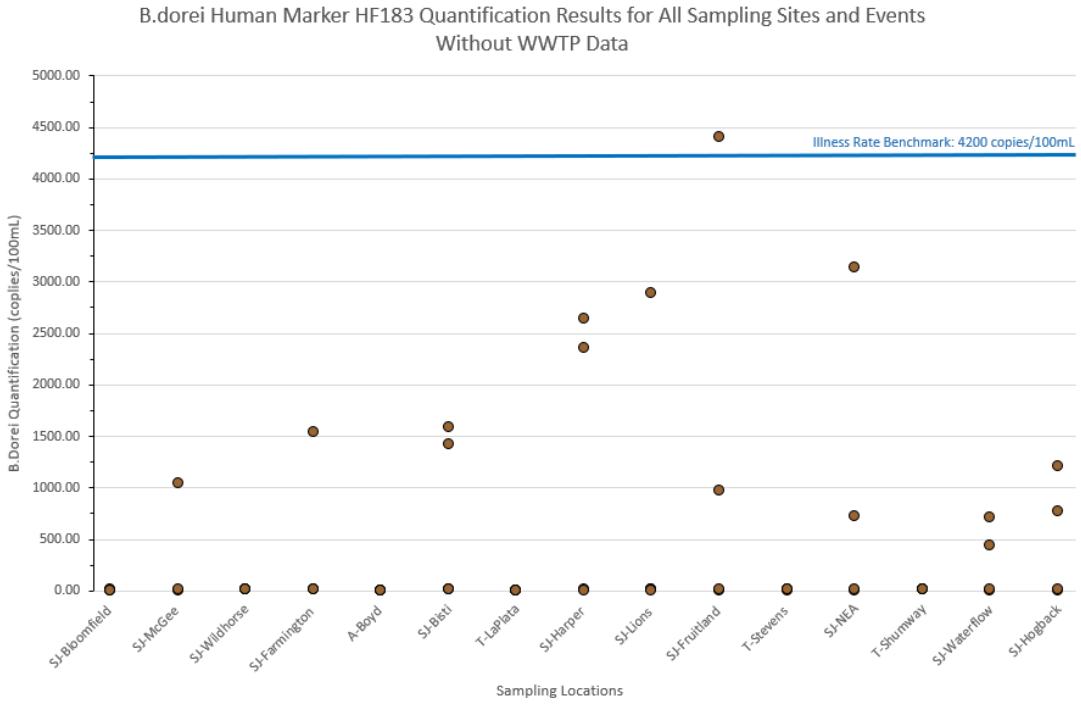
General B.dorei HF183 Result Distribution and Exceedances

Of the 52 *B.dorei* HF183 surface water samples collected, 29% were quantifiable - ranging between 437 to 4,400 copies/100mL. Only one of the samples, SJ-Fruitland during the October

28th sampling event, was over the 4,200 copies/100mL illness benchmark, with a concentration of 4,400 copies/100mL. Sampling locations SJ-Bloomfield, SJ-Wildhorse, A-Boyd, T-LaPlata, T-Stevens, and T-Shumway had ND or DNQ results for all sampling events. While all tributaries, and several San Juan River sampling locations, had no detectable human source bacteria via *B.dorei* HF183 (note that *B.dorei* HF183 from August 5th did not meet QA/QC requirements), all tributaries and various San Juan River sampling locations had one to three *E.coli* exceedances during these sampling events, indicating that bacteria contributions from these waterways are from non-human sources. Due to the design of this study the non-human source host organisms of fecal bacteria during these sampling events from the Animas River, La Plata River, Shumway Arroyo, and Stevens Arroyo are undetermined, but are suspected to be from wildlife and domestic ruminant sources from land use information (known livestock agriculture and feedlot use at varying locations along these tributaries). Future sampling for ruminant MST would supplement this data gap.

Number of Surface Samples with Quantifiable *B.dorei* HF183
SJ-Bloomfield: 0/4
SJ-McGee: 1/3
SJ-Wildhorse: 0/3
SJ-Farmington: 1/3
A-Boyd: 0/3
SJ-Bisti: 2/4
T-LaPlata: 0/3
SJ-Harper: 2/4
SJ-Lions: 1/4
SJ-Fruitland: 2/4
T-Stevens: 0/3
SJ-NEA: 2/4
T-Shumway: 0/3
SJ-Waterflow: 2/4
SJ-Hogback: 2/4

Figure 4: *B.dorei* HF183 Concentration Results for all Surface Water Sampling Locations and Events



This range of *B.dorei* HF183 results, in comparison to the results of the 2013 and 2014 MST study, indicate a significant improvement mitigating human source pollution in the San Juan Watershed. Over 90% of the 80 samples collected at SJ-Farmington (40 samples) and SJ-Hogback (40 samples) in 2013 and 2014 were quantifiable for *B.dorei* HF183. In 2013 and 2014, the average concentration for *B.dorei* HF183 at SJ-Farmington was approximately 3,000 copies/100mL, while SJ-Hogback was approximately at 24,000 copies/100mL. This drastic improvement over the past eight years could stem from a variety of reasons, including the

decommissioning of the Harper Valley WWTP in Kirtland, which on several occasions was not meeting their NPDES permit requirements, installation of the sewer extension from the Farmington WWTP to the Harper Valley Subdivision and surrounding community in 2016, and the decommissioning of the Central Consolidated School's Lagoon in Kirtland in 2016.

Correlation Between B.dorei HF183 and E.coli Concentrations

B.dorei and *E.coli* are two unrelated species of fecal bacteria in separate genuses, life patterns (anaerobic vs aerobic), and divergent decay rates. To investigate any correlation between *B.dorei* HF183 and *E.coli* concentrations, Pearson's Correlation Coefficients were calculated for each sampling event. August 4th and 5th sampling events had a -0.60 correlation, August 25th and 26th sampling events had a -0.16 correlation, September 29th and 30th sampling events had a -0.25 correlation, and October 27th and 28th sampling events had a 0.60 correlation. Therefore, during this study there was no consistent negative, positive, or strong correlation between *B.dorei* HF183 and *E.coli*.

Spatial Trends Per Sampling Event(s) for E.coli and B.dorei HF183 Surface Water Results

Spatial analysis of *E.coli* and *B.dorei* HF183 was conducted on a sampling event basis and is individually described below. The sampling frequency using to inform the goals of this study did not provide a sample size large enough to conduct a temporal analysis of both parameters investigated. However, to informally apprise the environmental conditions that could have influenced concentrations for each sampling event, flow and turbidity measurements form USGS Gauge 09365000 on the San Juan River downriver of the confluence of the Animas River and Navajo Dam release flows is provided in the Table 2. Spatial representation for both *E.coli* and *B.dorei* HF183 concentrations in connection to land use and distribution of sampling locations is available in Appendix A.

Table 2: USGS Gauge 09365000 Flow, Turbidity, and Navajo Dam Release Data in Comparison to Surface Water Results

Sampling Date	Flow (cfs)	Navajo Dam Release (cfs)	Turbidity (fnu)	% *E.coli* Results over Exceedance Limit[3]	% *B.dorei* HF183 Quantifiable Results
Aug 4-5	700-900[1]	400	500-1,500[5]	87%	13%
Aug 25-26	550-650	680	100-225	20%	27%
Sept 29-30	800[2]	760 to 680	500-4,000	80%	13%
Oct 27-28[4]	550-650	370	Not Available	0.07%	100%

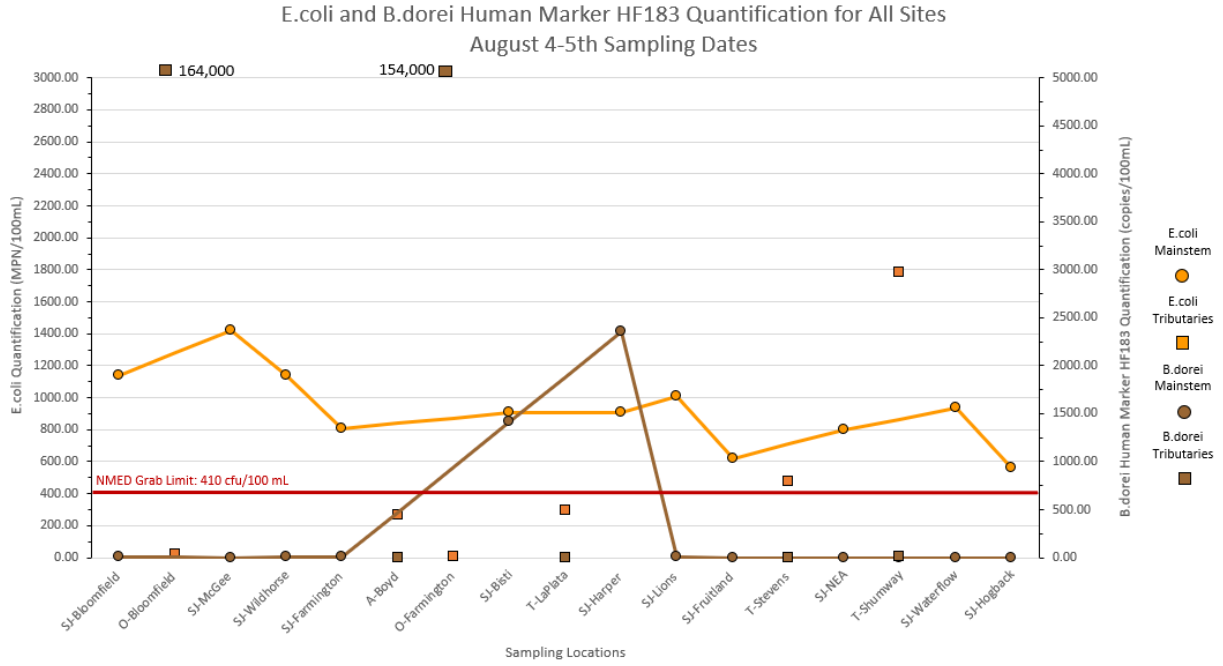
[1]Storm event on August 2nd leading to 3,000 cfs spike the evening of August 2nd
[2]Storm event on September 28th and 29th leading to 1,500 cfs spike afternoon of September 28th and 1,900 cfs spike on night of September 29th
[3]410 cfu/100mL Single Grab Exceedance Limit
[4]*E.coli* samples were collected at all 15 surface water sampling locations, *B.dorei* HF183 samples were collected at 7 surface water sampling locations
[5]Spike to 3,000 fnu in turbidity during the night of August 4th

For August 4th and 5th sampling events (Figure 5), surface water *E.coli* concentrations displayed the highest ratio of results, 87% over the 410 cfu/100mL single grab exceedance limit in comparison to all sampling events. This may have stemmed from a variety of conditions,

including the highest flow rate in comparison to all sampling events, 700-900 cfs according to USGS Gauge 09365000, from a storm system two days prior to sampling, and relatively low dilution from low Navajo Dam release flows (400 cfs) in response to this storm system.

The *E.coli* concentration at the SJ-Bloomfield sampling location was 1,140.50 MPN/100mL (the highest concentration measured at this site during the study). Since *B.dorei* HF183 was a DNQ at this location, this indicates that this high *E.coli* concentration was from non-human sources upriver. Generally, an increase in *E.coli* concentrations is evident between the city of Bloomfield and McGee Park, with a decrease and then plateau in concentrations between McGee Park and the town Fruitland, and finally a 400 MPN/100mL increase in concentration between Fruitland and the Waterflow community before decreasing back to 563 MPN/100mL. All *B.dorei* HF183 concentrations were ND or DNQ except for between the confluence of the Animas River and the Harper Valley Subdivision, which exhibited a linear increase to 1,400 copies/100mL and back to ND at the next downriver sampling location at Lions Park. Contributions of *B.dorei* HF183 from the Animas River and La Plata River between these locations is minimal at ND from the A-Boyd and T-LaPlata sampling locations. It is essential to emphasize that *B.dorei* HF183 quantification results from all tributary and downriver sampling locations from Lions Park did not meet QA/QC requirements, as described previously, and it is unknown whether the non-detects accurately represent human source bacteria at these locations during this sampling event.

Figure 5: *E.coli* and *B.dorei* HF183 Concentrations for all Sampling Locations during August 4-5th Sampling Events



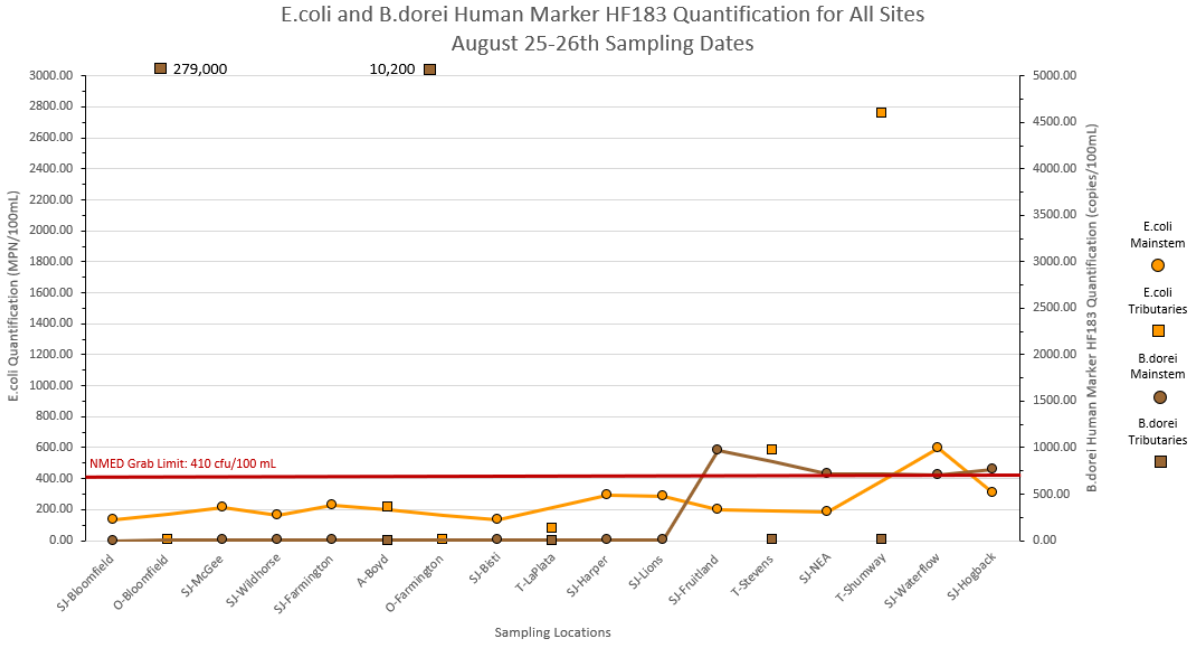
Unlike the *E.coli* results during the August 4th and 5th sampling events, the majority (80%) of *E.coli* concentrations from the August 25th and 26th sampling events (Figure 6) were below the 410 cfu/100mL single grab exceedance limit. This may be due to the lack of precipitation events mobilizing *E.coli* from the surrounding landscape to the San Juan River, as well as the increased Navajo Dam release flows of 680 cfs that sustained flows during these sampling events,

potentially diluting concentrations. *B.dorei* HF183 concentrations within the quantifiable range was approximately 27% for all sampling locations.

E.coli concentrations were over the single grab exceedance limit at T-Stevens and T-Shumway samplings locations (583 and 2,755 MPN/100mL); *B.dorei* HF183 concentrations at these sampling locations were qualified as DNQ, indicating that *E.coli* concentrations were from non-human sources. The *E.coli* concentration at the SJ-Waterflow sampling location was also over the single grab limit and exhibited a quantifiable range of *B.dorei* HF183 at 973 copies/100mL. *E.coli* concentrations were relatively stable between the city of Bloomfield and the community of Waterflow between 134 and 181 MPN/100mL and quickly increased to 598 MPN/100mL at the SJ-Waterflow sampling location (just downstream of Shumway arroyo confluence) and then decreased back to 305 MPN/100mL at the SJ-Hogback sampling locations. *B.dorei* HF183 concentrations remained either a ND and/or DNQ qualifier between Bloomfield and Lions Park, then increased to a steady range of 702 and 973 copies/100mL between Lions Park and the jurisdictional boundary of the Navajo Nation.

B.dorei HF183 concentrations indicate that human source contributions still occur throughout the Kirtland community and downriver through Fruitland and Waterflow. This area includes the western reach of the sewer lines feeding the Farmington WWTP (Harper Valley and town of Kirtland), but the south side of the river does not have sewer, and there are homes within the sewer service area which have yet to connect to it. Because decay rates in this watershed are undetermined, it is unclear whether the detected human source quantities are from residual contributions first detected along the river meandering through Kirtland, or additional sources entering at multiple points downstream.

Figure 6: *E.coli* and *B.dorei* HF183 Concentrations for all Sampling Locations during August 25-26th Sampling Events



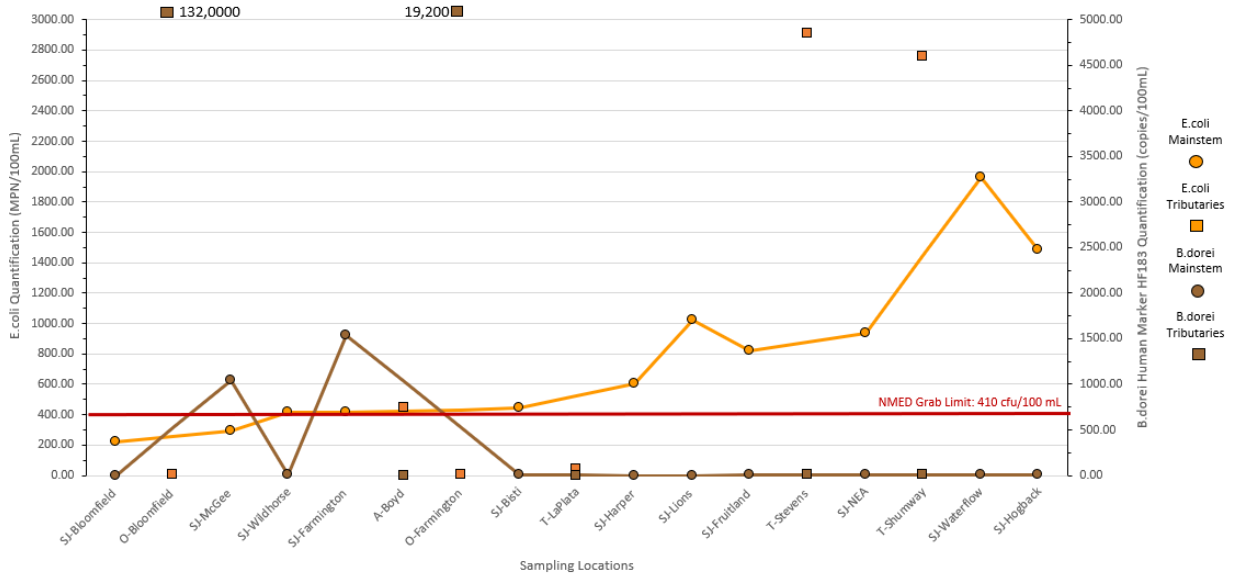
The September 28th and 29th sampling events had the second highest proportion of *E.coli* exceedances (Figure 7), with 80% having concentrations over the 410 cfu/100mL single grab

exceedance limit. This may have been influenced by the storm systems that passed through the watershed on September 28th and 29th, which increased flows drastically from 800 to 1,500 cfs the afternoon of September 28th and to 1,900 cfs on the night of September 29th (measured by USGS Gauge 09365000). Despite high E.coli, only 13% of *B.dorei* HF183 samples were within a quantifiable range.

E.coli concentrations were relatively stable between 217.60 and 445.70 MPN/100mL between the city of Bloomfield and the Bisti Bridge that crosses the San Juan River below the confluence of the Animas River. From there, *E.coli* concentrations increased to 1,020.40 MPN/100mL at Lions Park and significantly increased again at the SJ-Waterflow sampling location to 1,959 MPN/100mL before decreasing to 1,483.30 MPN/100mL at the jurisdictional boundary of the Navajo Nation. Inverse to the E.coli pattern and the *B.dorei* HF183 concentrations from the two August sampling events, concentrations at the Bisti Bridge and downriver to the jurisdictional boundary of the Navajo Nation were all in the ND and DNQ range, indicating that bacteria contributions in this downstream reach were from non-human sources. The only detectable human source bacteria on the September sampling was a spike between Bloomfield and McGee Park (ND to 1,040 copies/100mL) and between B Square Ranch (SJ-Wildhorse) and SJ-Farmington (ND to 1,540 copies/100mL) before dropping back to DNQ downriver of the Farmington WWTP.

The farthest East that the sewer lines extend from the Farmington WWTP is along the north side of the San Juan River to McGee Park. Sewer lines for the Bloomfield WWTP exist within city boundaries. The detected increase in human source bacteria between Bloomfield and McGee Park could have originated from either side of the San Juan River, as residential communities are concentrated along both sides of the river throughout the valley floor. At the same time, between B Square Ranch and the confluence of the Animas River, all the residential communities are on the north side of the San Juan River, indicating potential contributions of human source bacteria on this date from illegal dumping, unsewered communities like Totah subdivision, or other sources within the city of Farmington sewer service area.

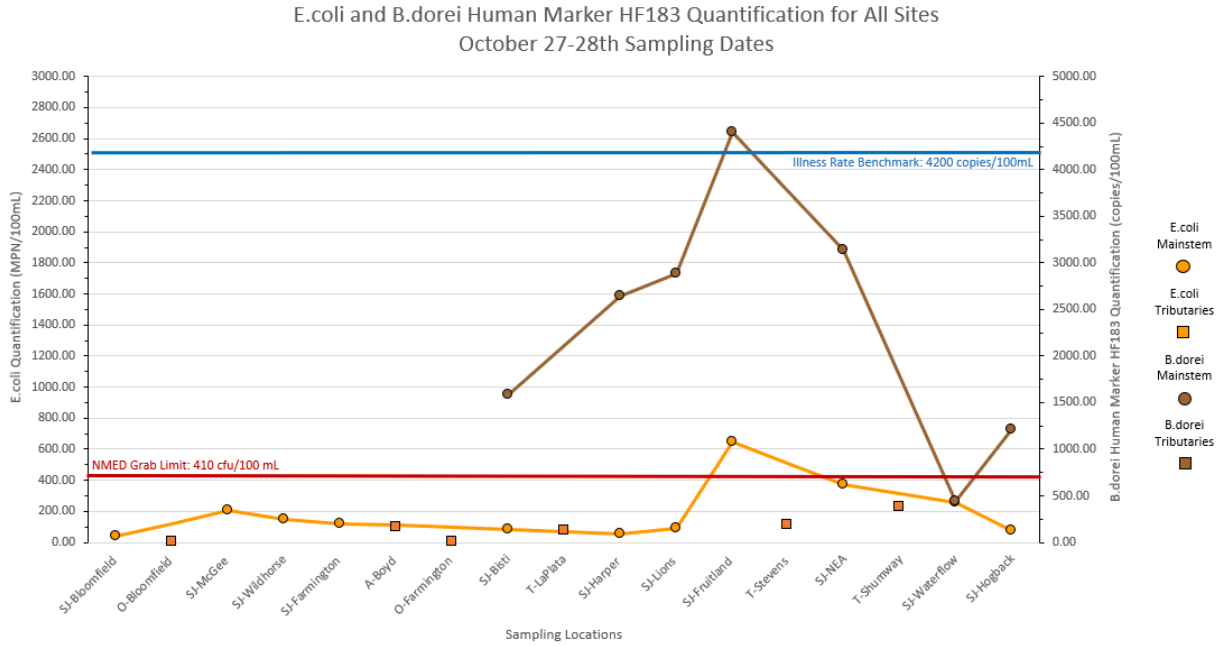
Figure 7: *E.coli* and *B.dorei* HF183 Concentrations for all Sampling Locations during September 29-30th Sampling Events



Finally, during the October 29th and 30th sampling events (Figure 8) *E.coli* samples were collected from all sampling locations while, due to funding constraints, *B.dorei* HF183 samples were only collected on San Juan River mainstem sampling locations between SJ-Bisti and SJ-Hogback. Only one sampling location (SJ-Fruitland) exhibited an *E.coli* concentration over the 410 cfu/100mL single grab exceedance limit. *B.dorei* HF183 concentrations were quantifiable for all sampling locations. This may have been influenced by the lowest flow conditions at 550-650 cfs as measured by USGS Gauge 09365000 that was maintained by the lowest Navajo Dam release rate during sampling events of 370 cfs.

E.coli concentrations remained low and fairly steady (41 to 92.50 MPN/100mL) between Bloomfield and Lions Park before a significant spike in Fruitland to 644 MPN/100mL at SJ-Fruitland and decreasing back to 75 MPN/100mL at the jurisdictional boundary of the Navajo Nation. *B.dorei* HF183 concentrations were detected at this sampling day's most upstream MST site at Bisti Bridge, increasing to 4,400 copies/100mL at SJ-Fruitland – the only illness benchmark exceedance across all sampling events – and then decreasing to 437 copies/100mL at Waterflow and increasing again to 1,210 copies/100mL at SJ-Hogback. This indicates that human source bacteria demonstrated a consistent presence throughout lower stretch of the San Juan River sampled as part of this study, with increases in concentration particularly between the Bisti Bridge and Waterflow and again between the confluence of Shumway Arroyo to the San Juan River and the jurisdictional boundary of the Navajo Nation. The spike of both HF-183 and E.coli at SJ-Fruitland was the only instance of human source bacteria potentially driving E.coli concentrations.

Figure 8: *E.coli* and *B.dorei* HF183 Concentrations for all Sampling Locations during October 27-28th Sampling Events



Conclusions

Overall, quantities of human source bacteria detected during this study reflect a drastic improvement in the reduction of human source bacteria contributions over the past eight years. Various projects conducted over this time-span are hypothesized to have contributed to this improvement, including the decommissioning of WWTP facilities not meeting discharge permits (Harper Valley WWTP), expansion of sewer infrastructure to the Farmington WWTP, and community outreach on septic care and illegal dumping. While this information is encouraging, the detected quantities still suggest that work addressing sources of human source bacteria is still needed to further improve water quality. This study did not find consistent geographical hotspots for human source bacteria (potentially due to higher prevalence of non-detects), which indicates that inputs of human source pollution may be episodic in nature. For this reason, watershed scale outreach efforts should continue, and specific remediation actions should be further developed through multijurisdictional watershed planning and continued water quality monitoring.

While these results indicate that human source bacteria concentrations have drastically improved, bacteria continues to be a water quality concern in the San Juan Watershed of New Mexico as exhibited through the 48% of samples that were over the 410 cfu/100mL single grab exceedance limit for *E.coli*. During the 2013-2014 San Juan Watershed MST study, ruminant source bacteria was more common than human source at 90-100% presence over all sampling events. Follow up MST is recommended for additional wild and domestic organisms, such as ruminants, for an update on this bacteria source and to further prioritize BMPs and outreach activities that will most effectively and efficiently address this water quality issue long term. In the interim, strategic public education on this water quality impairment, agriculture and soil health BMP implementation, and wetland and floodplain restoration initiatives throughout the study area is recommended to improve water infiltration and assimilative capacity of contaminants.

All tributaries investigated during this study, the Animas River, La Plata River, Shumway Arroyo, and Stevens Arroyo, did not have any quantifiable concentrations of human source bacteria, with the understanding that tributary samples on August 5th did not meet QA/QC requirements. However, these tributaries, excluding the La Plata and mouth of the Animas River, did display some of the highest *E.coli* concentrations seen throughout all sampling locations. Further watershed planning and water quality monitoring throughout the drainages of these tributaries is needed to characterize and address non-human bacteria contributions. Expansive water quality monitoring, MST sampling, and bacteria focused best management practices (BMPs) have been identified and conducted along the Animas River, as described in the Lower Animas Watershed Based Plan, which should be maintained to continue to reduce non-human source contributions in the Animas Watershed.

The high *B.dorei* HF183 concentrations detected from the Farmington and Bloomfield WWTP outflows did not drive subsequent increases in downstream surface water samples. This indicates that nonpoint sources of human source bacteria are the most likely contributors of human source pollution. However, further investigations into the differentiation between non-viable and alive *B.dorei* HF183 through the use of the Propidium Monoazide or other peer reviewed analytical methods from these WWTP outfalls is recommended to definitively determine whether any human health risk originates from these point sources.

Based on the NMED SWQB's *E.coli* water quality standard for waterways with the primary contact designated use, any assessment unit with more than one exceedance over the single grab exceedance limit is listed as impaired on the §303(d)/305(b) Integrated Report. With these conditions and the *E.coli* results from this study, the 2020-2022 E.coli impairment listing for the San Juan River below the confluence of the Animas River, Shumway Arroyo, and Stevens Arroyo is supported. In addition, the *E.coli* results on the San Juan River between the confluence of the Animas River and Cañon Largo would lead to this assessment unit being considered for relisting during the next integrated report cycle.

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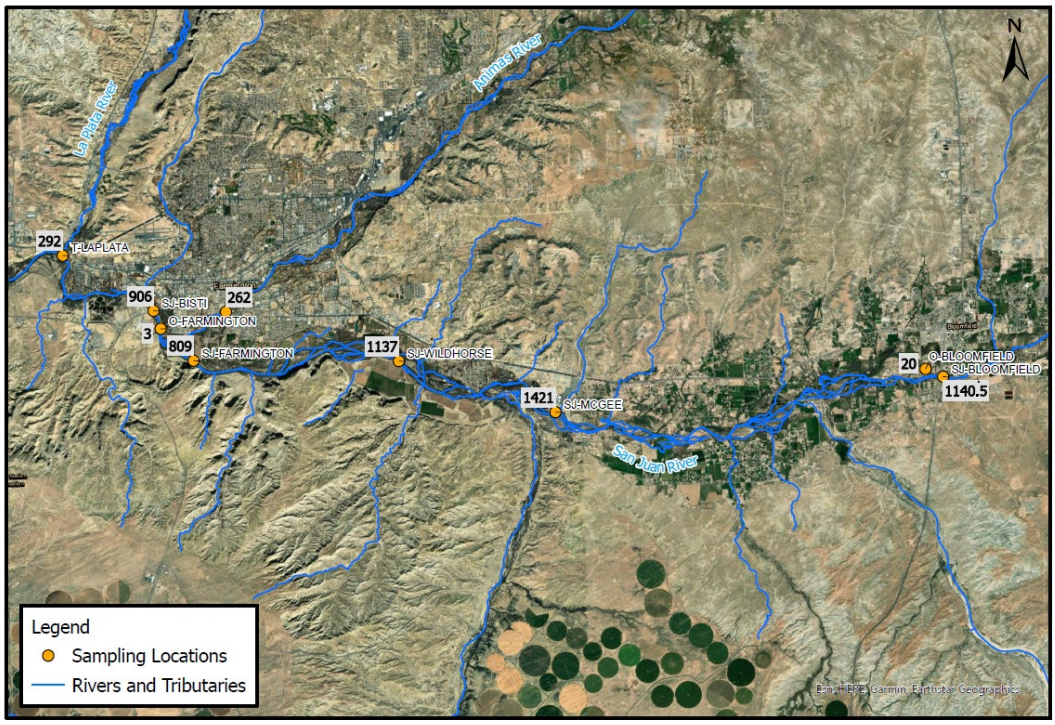
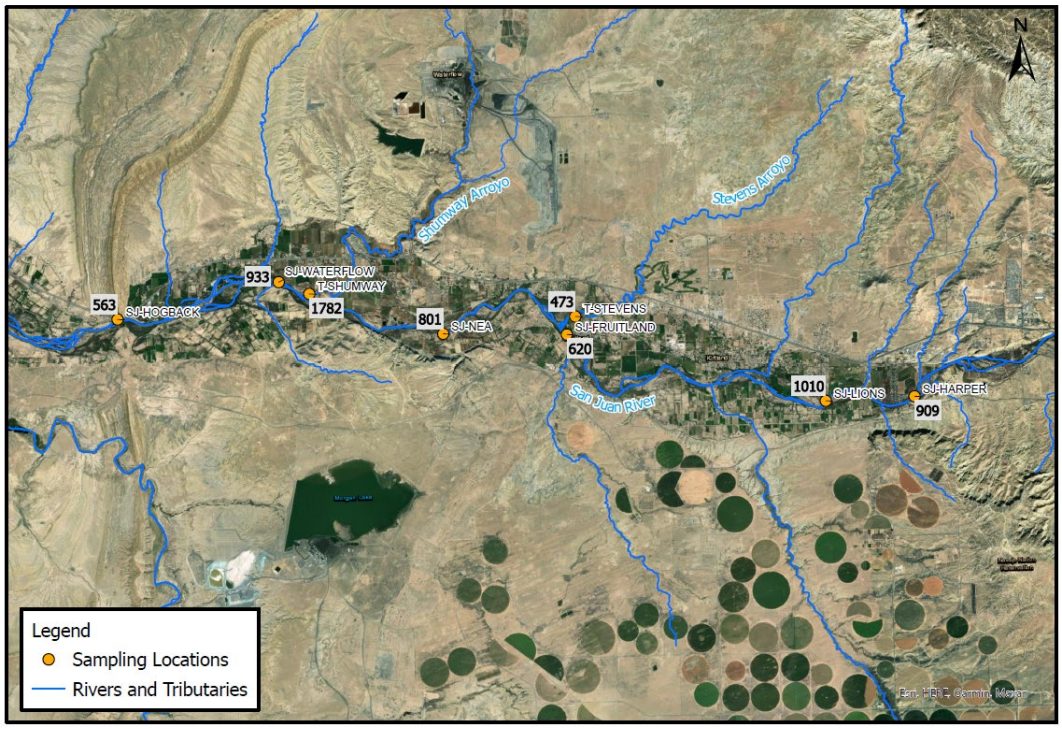
Appendix A.

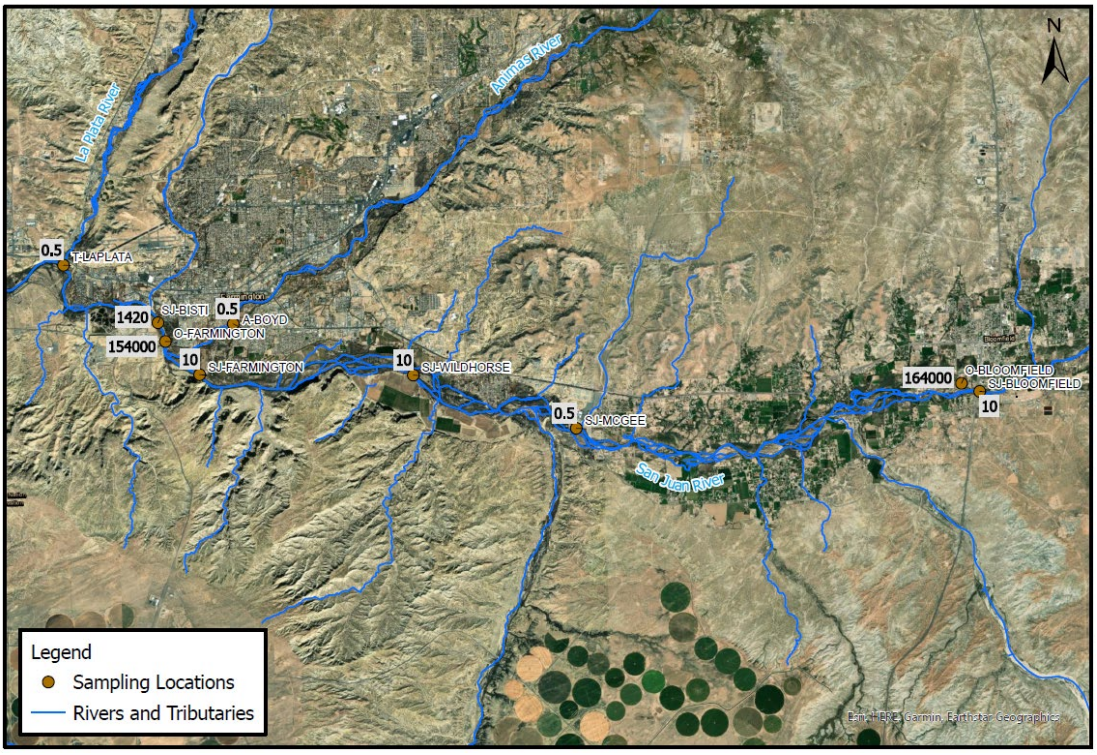
2021 San Juan Human Bacteria Sampling and Investigation Project
August 4th-5th E.coli Results



2021 San Juan Human Bacteria Sampling and Investigation Project
August 4th-5th E.coli Results



2021 San Juan Human Bacteria Sampling and Investigation Project
August 4th-5th B.dorei Human Marker Results



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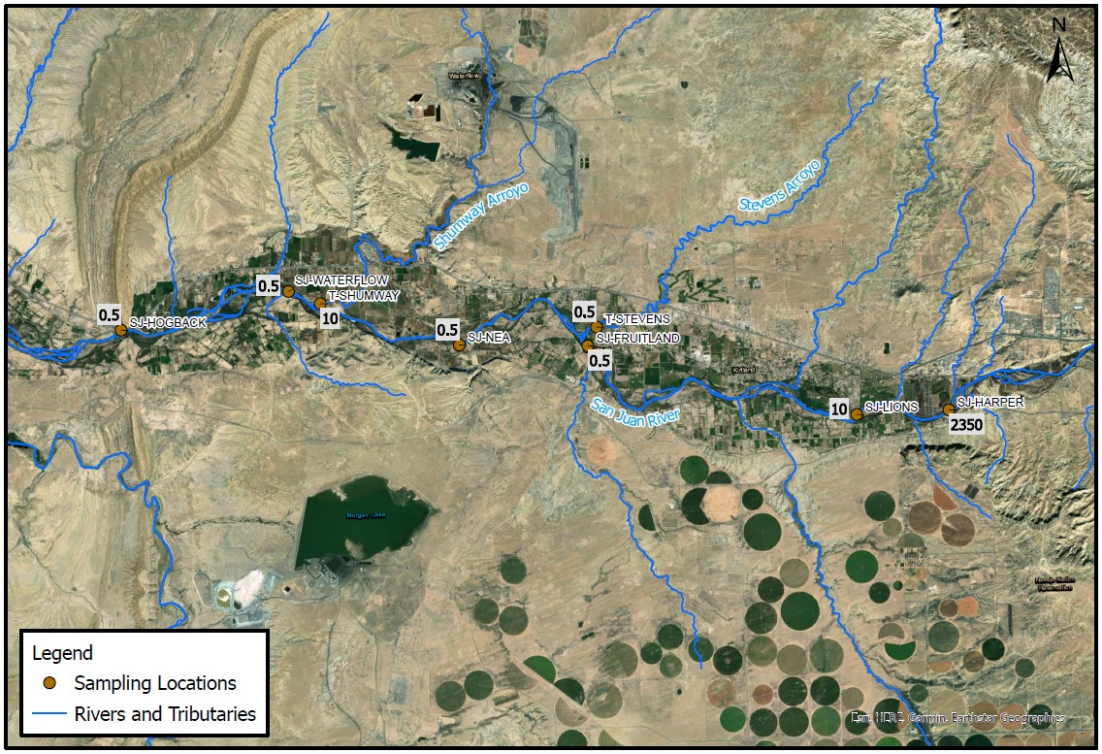
August 4th-5th B.dorei Human Marker Results



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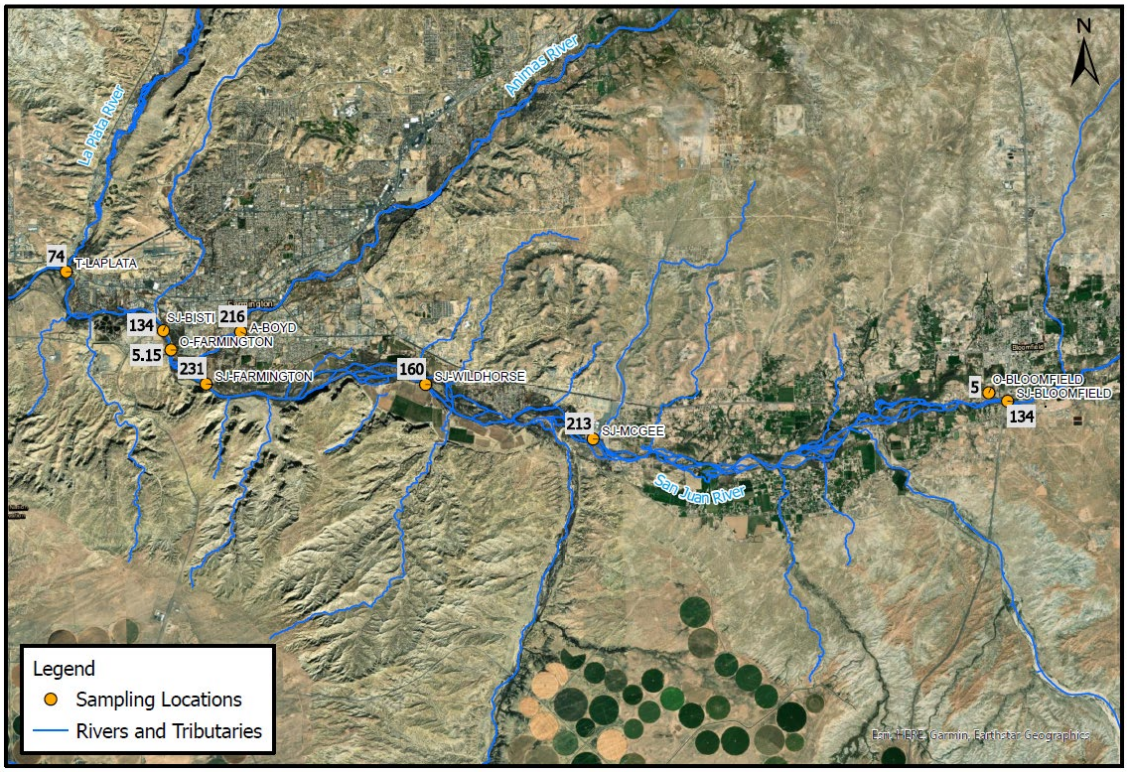
August 25th-26th E.coli Results



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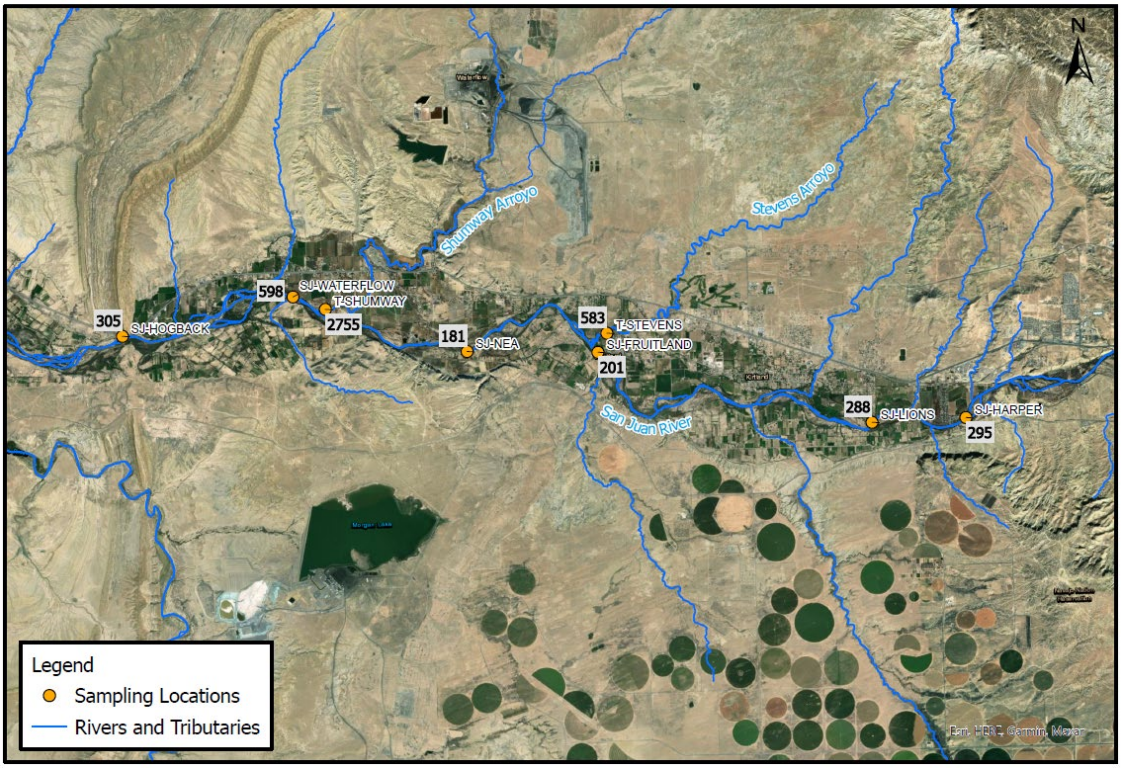

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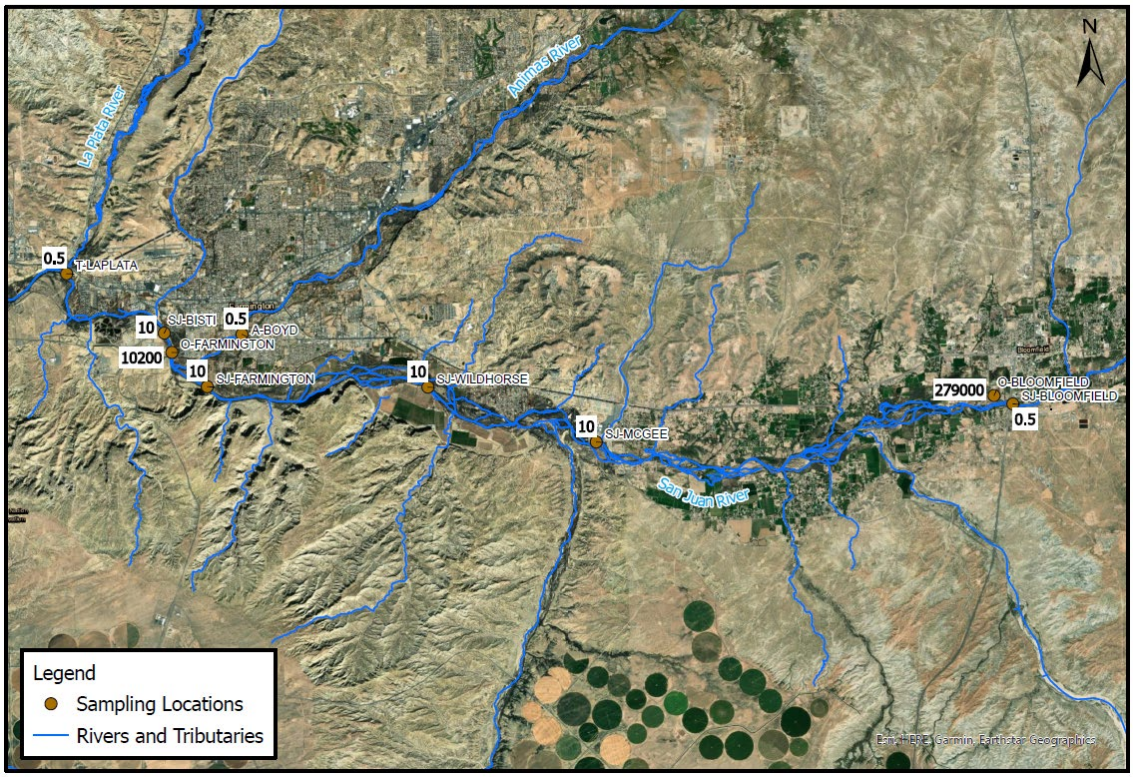
August 25th-26th B.dorei Human Marker Results



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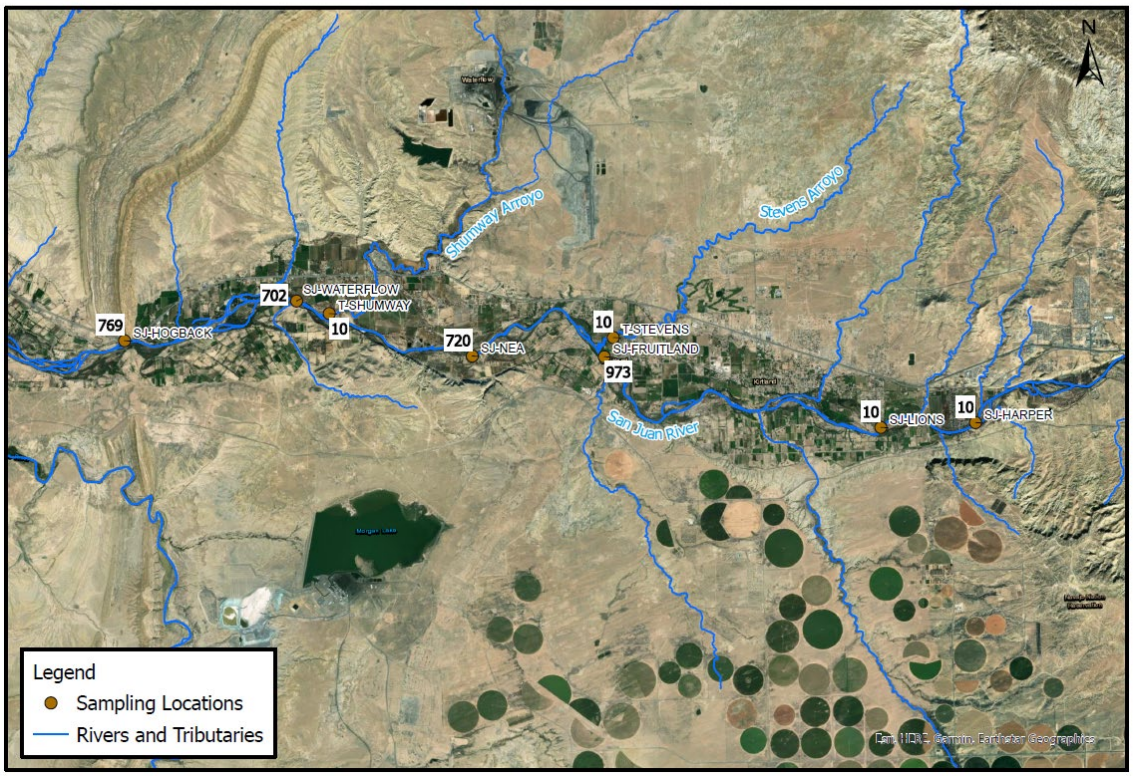
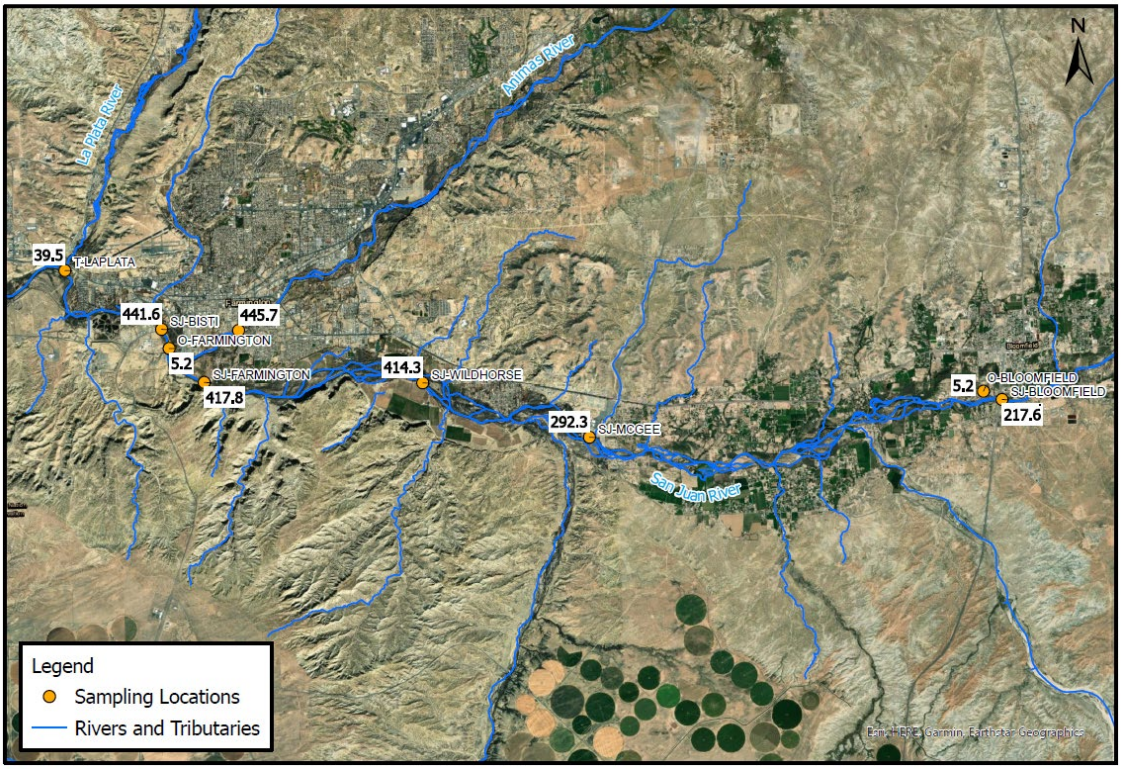
August 25th-26th B.dorei Human Marker Results



2021 San Juan Human Bacteria Sampling and Investigation Project
September 29th-30th E.coli Results



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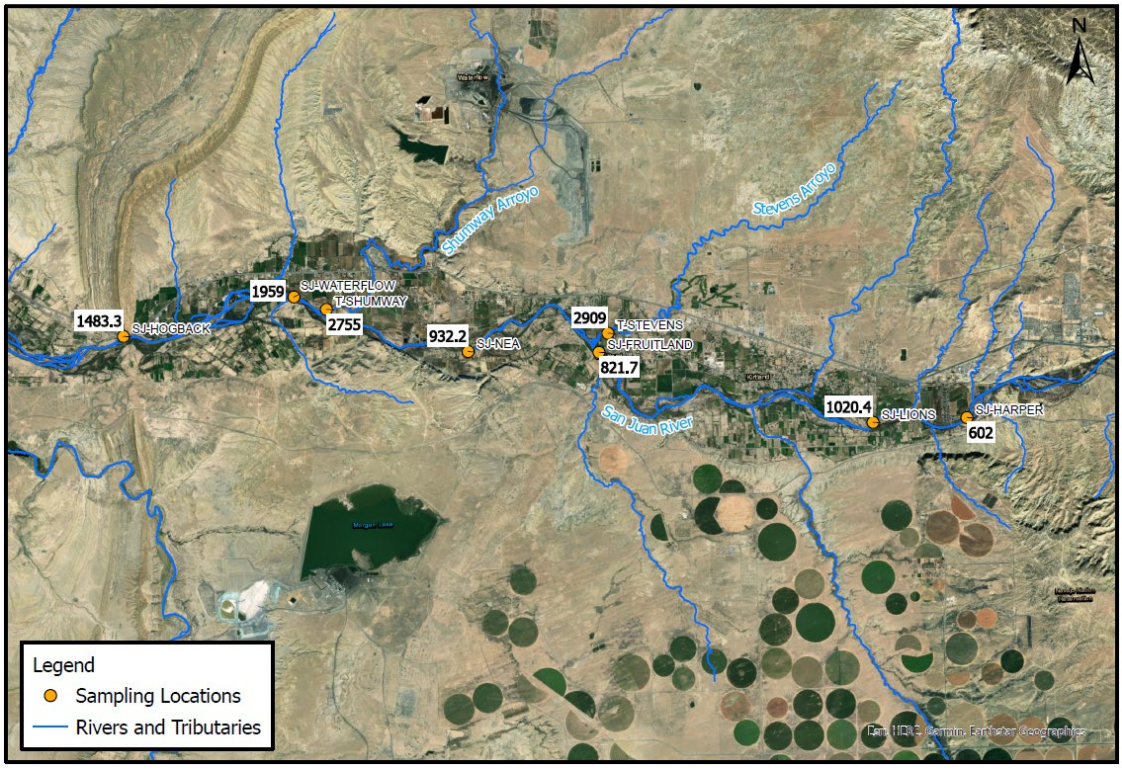
September 29th-30th E.coli Results



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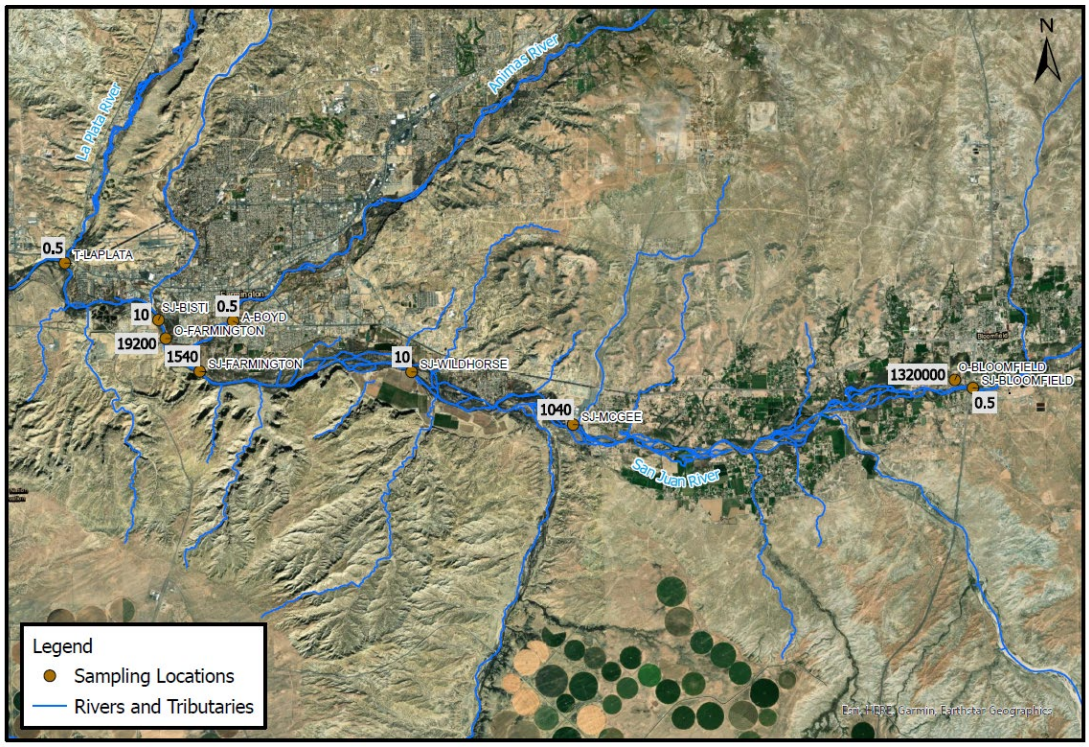
September 29th-30th B.dorei Human Marker Results



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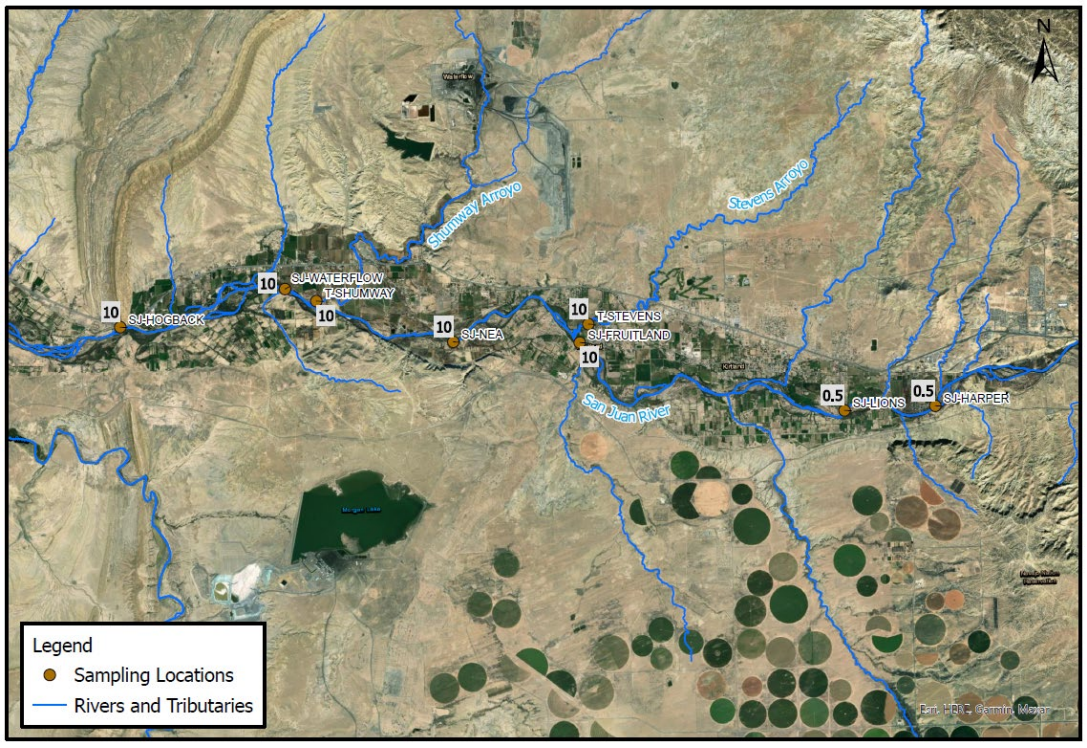
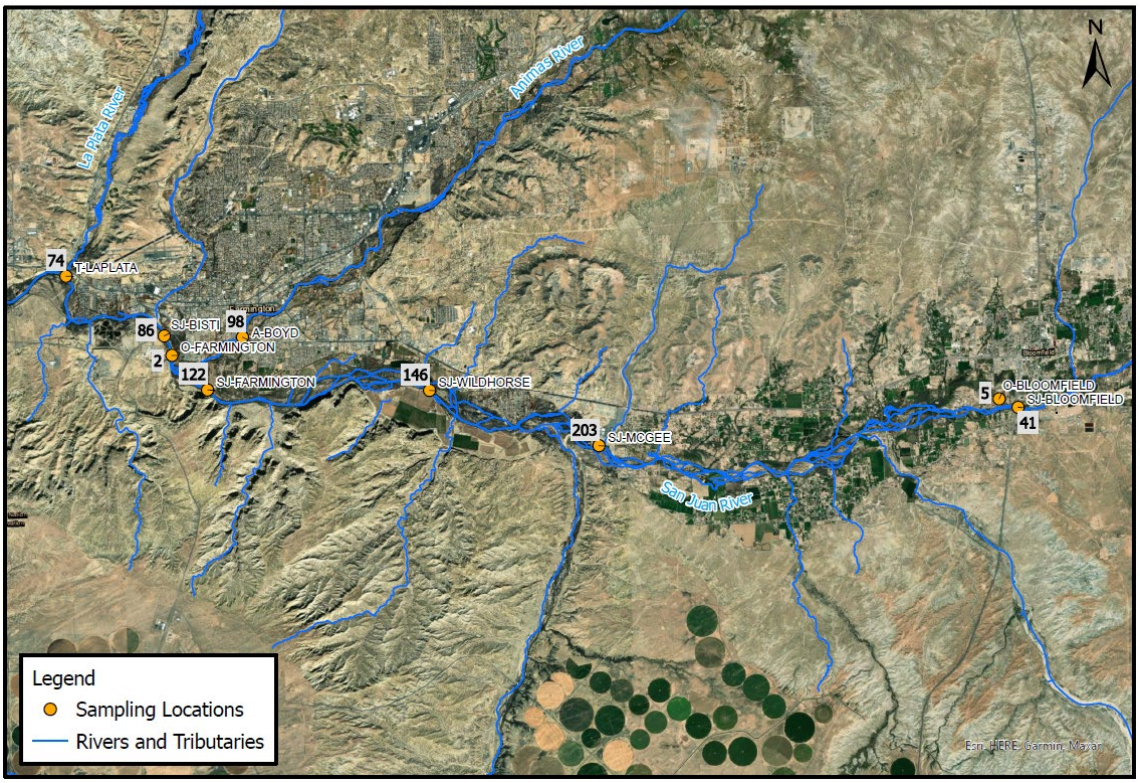
September 29th-30th B.dorei Human Marker Results



2021 San Juan Human Bacteria Sampling and Investigation Project
October 27th-28th E.coli Results



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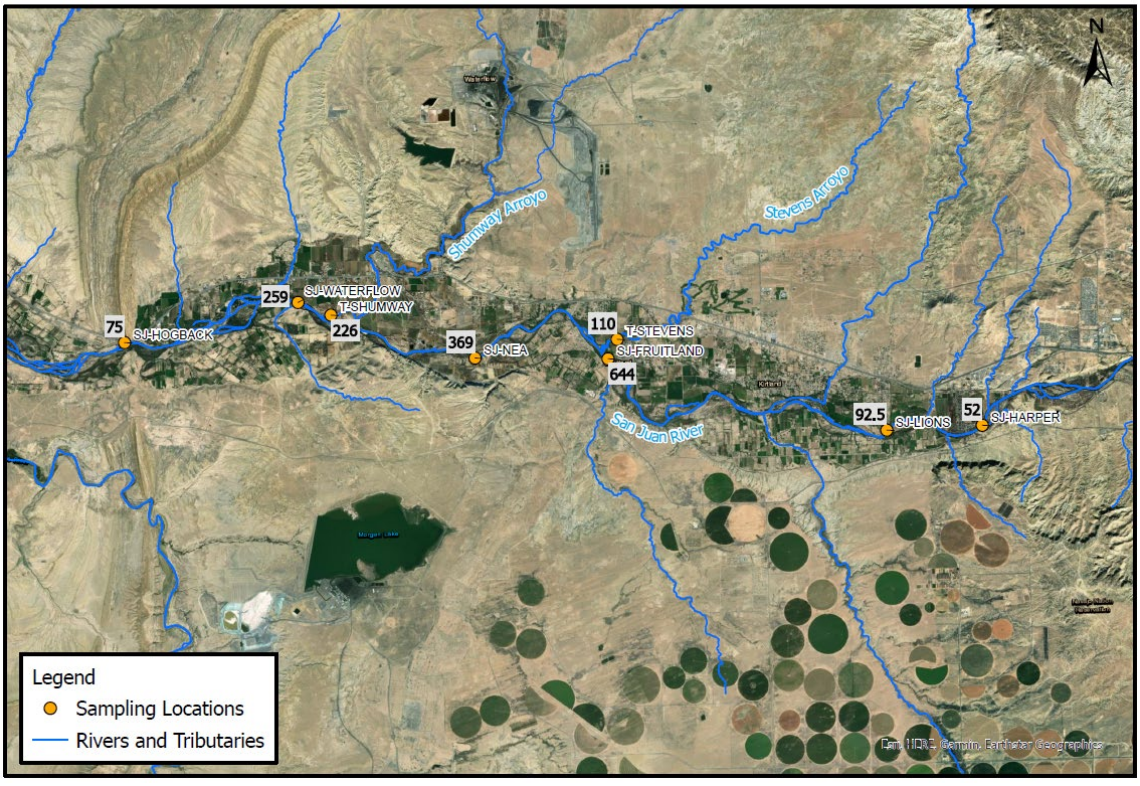
October 27th-28th E.coli Results



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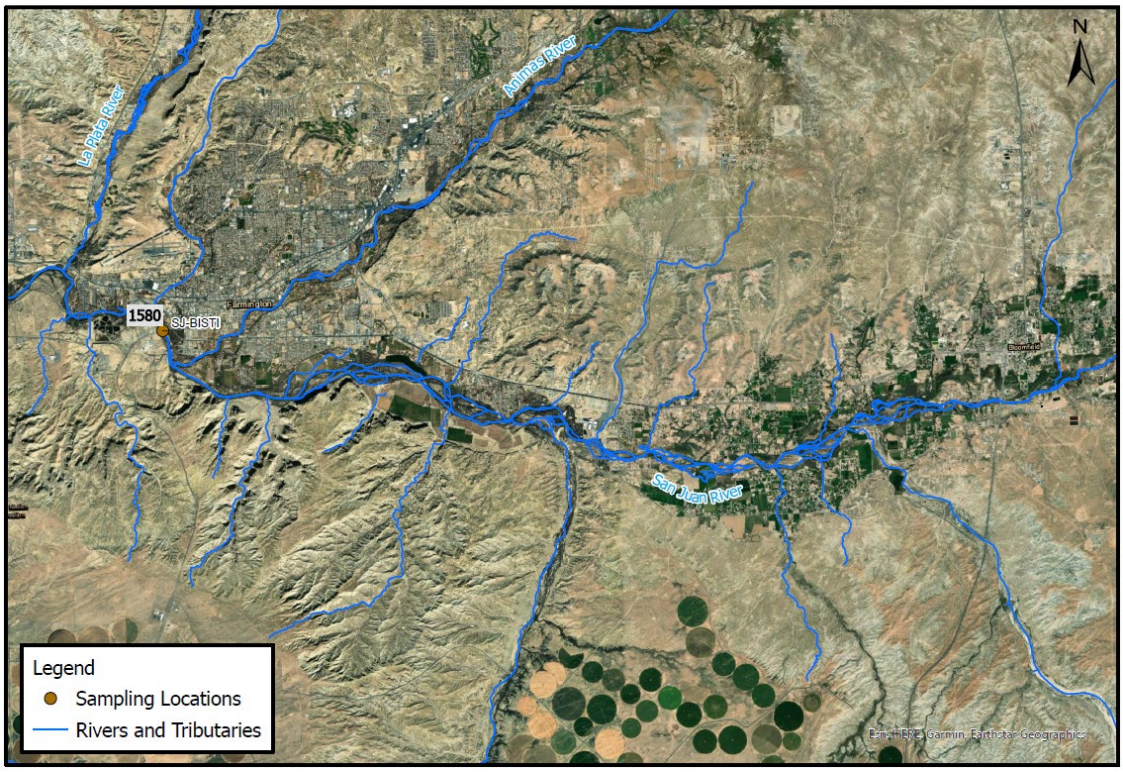
October 27th-28th B.dorei Human Marker Results



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October 27th-28th B.dorei Human Marker Results