Source Identification of Fecal Pollution in Rapid Creek, South Dakota

DNA Sampling Final Report

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Project Location: This project is located on the impaired segment of Rapid Creek beginning at Dark Canyon, running through the center of Rapid City, and ending below the Wastewater Treatment Plant (WWTP).

Project Abstract: Rapid Creek is a vital natural resource for wild brown trout, tourism and recreation in Rapid City, SD. Continuous parks and green spaces all along the creek creates and enhances a quality of life unparalleled in our region. Expanding urban development has and continues to impact Rapid Creek's water quality. Regular water testing by the South Dakota Department of Environment and Natural Resources (SDDENR) has resulted in listed total maximum daily load (TMDL) impairments for fecal coliform bacteria since 1998. A study by the SDDENR and the South Dakota School of Mines and Technology (SDSMT) in 2010 determined that the proposed study area would require a 95 percent reduction in the fecal coliform loading in high and moist flow zones in order to meet the TMDL standard. The specific host sources of fecal pollution have yet to be identified. Through diverse partnerships with local residents, H2E, Incorporated (H2E), Source Molecular Corporation, SDSMT, SDDENR, United States Geological Survey (USGS) and the City of Rapid City, the West Dakota Water Development District (WDWDD) funded the collection and analysis of deoxyribonucleic acid (DNA) samples from Rapid Creek to identify sources of the fecal pollution. By defining and understanding the host source(s) of fecal pollution in Rapid Creek and moving forward with corrective action, the WDWDD hopes to provide a healthy and accessible urban waterway for future generations to use and enjoy.

Key Definitions

*Definitions are from the Washington Department of Health, USGS and EPA.

Total Maximum Daily Load (TMDL) - A TMDL is a regulatory term in the U.S. Clean Water Act, describing a plan for restoring impaired waters that identifies the maximum amount of a pollutant that a body of water can receive while still meeting water quality standards.

Total Suspended Solids (TSS) - TSS are particles that are larger than 2 microns found in the water column. Anything smaller than 2 microns (average filter size) is considered a dissolved solid. Most suspended solids are made up of inorganic materials, though bacteria and algae can also contribute to the total solids concentration.

Total Coliform - The total coliform group is a large collection of different kinds of bacteria. Fecal coliforms are types of total coliform that mostly exist in feces. *E. coli* is a sub-group of fecal coliforms.

Fecal Coliform – Fecal Coliform is a sub-group of total coliform bacteria. They appear in great quantities in the intestines and feces of people and animals. The presence of fecal coliform in a water sample often indicates recent fecal contamination, meaning that there is a greater risk that pathogens are present than if only total coliform bacteria is detected.

Escherichia coli (E. coli) - *E. coli* is a sub-group of the fecal coliform group. Most *E. coli* bacteria are harmless and are found in great quantities in the intestines of people and warm-blooded animals. Some strains, however, can cause illness.

MPN/100 mL - Based on which dilutions showed positive for coliform and/or fecal coliform, a table of most probable numbers is used to estimate the coliform content of the sample.

Acknowledgements

This project was made possible by funding from the West Dakota Water Development District and through collaboration with the South Dakota Department of Environment and Natural Resources, the United States Geologic Survey, Source Molecular and the South Dakota School of Mines and Technology. A special thanks to Jaime Haueter (SDDENR), Galen Hoogestraat (USGS), Bill Eldridge (USGS) and Kelsey Murray (SDSMT) for coordinating with me on this project.

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Appendices

1. Background

Lower Rapid Creek has been listed as an impaired water body due to exceedances of fecal coliform bacteria criteria by the South Dakota Department of Environment and Natural Resources (SDDENR) in every listing cycle since 1998. Urban runoff pollution is a widespread and complex issue that was identified as a primary contributor to the overall bacterial load in Rapid Creek by the SDDENR in 2004. Within the proposed study area, Rapid Creek is listed for a variety of beneficial uses including cold water permanent fish life propagation, warm water permanent fish life propagation, immersion recreation, limited contact recreation, fish and wildlife propagation, recreation and stock watering, and irrigation. Rapid Creek is a nationally renowned wild brown trout fishery and a cornerstone of the fishing industry in the Black Hills. The creek also travels through the center of Rapid City and serves as an aesthetic feature for hikers, bicyclers, runners, and walkers. A big question mark has been placed on the fundamental value and the variety of beneficial uses of Rapid Creek due to the bacterial impairment designation. Recreational use of Rapid Creek also has the potential to make people ill, since the water concentrations of *Escherichia coli (E. coli)* exceeded the standard for over 1,000 samples. However, before effective remedial action can be taken, the sources of contamination must be identified. The West Dakota Water Development District (WDWDD) and its partners developed this project to characterize the fecal pollution in Rapid Creek.

2. Scope and Objectives

The objective of this project is to identify the host source(s) of fecal pollution in Rapid Creek through microbial source tracking genetic and molecular techniques. The project is located on the impaired segment of Rapid Creek beginning at Dark Canyon, running through the center of Rapid City, and ending below the Wastewater Treatment Plant (WWTP). Maps of each sampling location can be found in Appendix B. Sampling locations were strategically chosen as sites that would provide the most information to identify and isolate possible sources of contamination. Sampling locations also correspond with existing Unites States Geological Survey (USGS) and SDDENR monitoring sites in order to utilize flow, fecal coliform bacteria, Total Suspended Solids (TSS), and *E. coli* data that is already being recorded.

3. Collaboration

This project addresses the ongoing environmental concern in Rapid Creek through the collaborative efforts of community stakeholders and the WDWDD.

The WDWDD fostered partnerships with H2E, Incorporated (H2E), Source Molecular Corporation, South Dakota School of Mines and Technology (SDSMT), SDDENR, and the USGS. The City of Rapid City was notified of the project and assigned a staff member to assist with the study.

From planning to completion, each partner played an important role in the success of the project. H2E is a small local environmental consulting firm hired by the WDWDD to fill program management roles. H2E volunteered their time to research and develop a DNA Testing Plan for Rapid Creek. They were contracted by the WDWDD to complete the following tasks:

- Manage project budget.
- Oversee all aspects of the project to insure quality work.
- Establish and maintain working relationships with all project partners.
- Collaborate with the SDDENR, SDSMT and USGS for sample collection.
- Analyze and interpret the DNA testing results provided by Source Molecular Corporation.
- Write a summary report and present results to the WDWDD.

Source Molecular Corporation was contracted to complete laboratory tests on all collected samples. Tests identified and quantified the fecal bacteria from the following five host sources:

- Human – Test detects Human fecal bacteria
- Dog – Test detects Dog fecal bacteria
- Ruminant/Deer – Test detects Deer, Goat, Cattle, Chamois, and Sheep fecal bacteria
- Bird – Test detects Gull, Goose, Duck, Chicken, Sandpiper, Coot, Pigeon, Cormorant, Egrit, Pelican, Tern, Crow and Swan fecal bacteria
- Cattle/Cow – Test detects Cattle, Goat and Sheep fecal bacteria

Source Molecular provided a DNA quantification report that included all analysis results and a thorough analytical method explanation.

The USGS and the City of Rapid City conducted affiliated research that investigated the primary sources of bacteria in stormwater, which has been a persistent question for stormwater management in Rapid City. The two major objectives of this study were to quantify bacterial loads for various infrastructure elements along drainage flow paths and to identify the primary source species for the bacteria in the stormwater discharges.

The SDSMT conducted affiliated research that investigated the pathogenicity of the bacteria in Rapid Creek. Dr. Lisa Kunza, Dr. Linda DeVeaux, and Kelsey Murray applied a novel pathogenicity metric, which determined presence/absence as well as relative abundance of 30 genes, to water samples collected from Rapid Creek. Results showed that Rapid Creek has a presence of E. coli pathogenicity genes with significant gene transfer occurring. The presence of these pathogenicity genes may indicate a risk to human health.

The USGS, SDDENR, City of Rapid City, SDSMT and the WDWDD collaborated their project efforts to obtain as much data and information about Rapid Creek as possible.

4. Sampling Locations

Sampling locations were strategically chosen as sites that would provide the most information to identify and isolate possible sources of contamination. All sampling locations were tested for human, dog, cattle, ruminant and bird host sources. Maps of each of the sampling locations can be found in Appendix B.

1. DENR Site WQM 69 – Dark Canyon (460669)
    - Selected to coordinate with the DENR WQM 69 sites and to provide base information for Rapid Creek before the urban impacts of Rapid City.
2. USGS Station 6413200 – Below Canyon Lake
    - Selected to determine the effects of the bird population in Canyon Lake and the large concentration of septic systems in the Wonderland Drive drainage basin.
3. USGS Station 641600 and DENR Site WQM 173– East Saint Patrick Street
    - Selected to coordinate with both the USGS and SDDENR sampling sites and to help determine the effects of Rapid City's downtown area.
4. DENR Site WQM 92 – Below Wastewater Treatment Plant (WWTP) (460692)
    - Gives a good perspective of the possible impacts of the WWTP and the cattle feedlots in the area.

5. Sampling Procedure

Through collaboration with the SDDENR, SDSMT, USGS and Source Molecular, H2E collected monthly DNA samples for one year from November, 2016 through October, 2017. Samples were collected from four sites along the impaired segment of Rapid Creek, from Dark Canyon to below the WWTP. These samples were collected at established USGS gauging stations or SDDENR sampling locations and demonstrate seasonal variations on the water quality in Rapid Creek. Shipping kits, including sterile sample bottles were obtained from Source Molecular to help ensure quality assurance and quality control. Specific details regarding sample collection materials, methods, preservation and shipment can be found below.

5.1 List of Required Materials

- 500 ml leakproof sterile bottles
- Ice packs
- Zip lock bags
- Cooler
- Sharpie marker
- Pen
- Disposable gloves
- Paper towels
- Freezer

- Chain of Custody
- Waders

5.2 Sample Collection

The Water Grab Sample technique listed below was implemented to collect a 500-mL sample from each sampling location on a monthly basis. Real time flow data was recorded from USGS gaging station 06412810 each month. Below is the sampling protocol that was implemented.

1. Label each sample container with the date, time, and site number/name.
2. Record all applicable information on the Chain of Custody Sheet (see Appendix A).
3. Water samples will be collected before any other work is done at a site.
4. Collect water samples before stirring up the stream bottom, or collect samples upstream of agitated water. Samples will be collected by wading into the centroid of flow or mid-channel in the stream and reaching as far upstream as possible to avoid collecting stirred up water. If this is not possible due to high flow or other safety reasons, samples will be collected while standing on the edge of the water or on a rock.
5. Without touching the inside or lip of the sterile sample battles or caps, hold the uncapped bottle upside down and submerse it about 1 foot below the surface mid-stream until filled. Tip the bottle upright and allow water to fill the bottle. Remove the bottle from the water and screw on the cap.
6. Store and transport samples using the Sample Shipment Directions below.
7. Complete the Chain of Custody Sheet (see Appendix A) and include in sample shipment.

5.3 Sample Collection QA/QC

In addition to Source Molecular's lab analysis QA/QC, sample collection QA/QC was implemented. The key aspects of quality control associated with sample collection are as follows:

1. Field personnel will be thoroughly trained in proper sample collection protocol.
2. Field personnel will be thoroughly trained to recognize and avoid potential sources of sample contamination.
3. Sample gear or equipment that comes in direct contact with the water sample will be made of non-contaminating materials and will be cleaned between sampling events.
4. Sample containers will be sterile and the recommended type of material.
5. Conditions for sample collection, preservation and holding times will be followed.

Additional QA/QC samples were shipped to Source Molecular for analysis. One blank per sampling trip was analyzed. Duplicate samples were also sporadically taken and sent to the lab to help confirm accurate results. Human bacteria are most harmful to other humans compared to other species and it is generally easier to control if it is identified. Therefore, human bacteria identification and validation was prioritized first and all duplicate samples were tested for human markers.

5.4 Sample Shipment

After collection, samples were immediately shipped to Source Molecular via an overnight courier using the recommended packing instructions below:

- Wrap leakproof sterile bottles with abundant paper towels and put them individually in zip lock bags.
- Place ice packs in zip lock bags and pack along with sample(s).
- Wrap the zip lock bag with abundant paper towels and insert everything in another zip lock bag.
- Do not let ice packs directly touch the samples (adding additional packing material will prevent this).
- As an added precaution, put zip lock bags and packing material in two overlapping garbage bags.
- Wrap the garbage bags tightly and put everything in a sturdy cooler.

Ship the sample(s) via overnight courier to:

Source Molecular Corporation
4985 SW 74th Court
Miami, Florida 33155 USA

6. Sample Analysis

Source Molecular is a private commercial laboratory who was contracted to provide sample analysis services. Source Molecular maintains quality control for sample analysis with the latest scientific laboratory equipment and highly trained, degreed personnel. Source Molecular's QA/QC summary can be found in Appendix D. Source Molecular uses scientifically rigorous molecular analytical methods that enable it to identify with high confidence any contamination, to avoid false positives. Their stringent methodologies and expertise in understanding fecal contamination helped ensure accurate results.

Source Molecular performed quantification fecal contamination testing from Human, Ruminant, Bird, Dog and Cattle host sources for each sample. The test results were delivered back to the WDWDD within 5-10 business days. Source Molecular also provided a DNA quantification report that will include all analysis results and a thorough analytical method explanation for each set of tests.

7. Project Budgets

The WDWDD obligated $53,014.00 for DNA sample collection, testing and analysis. The detailed DNA testing plan budget can be found in Appendix C.

8. Results

The DNA testing results are graphically illustrated in Figures 1, 3, 5, 7 and 8. Each figure shows one sampling site for the 12 months from November, 2016 through October, 2017 with all five possible host sources (human, dog, ruminant/deer, bird, and cattle/cow). Source Molecular DNA markers that returned positive values below the quantification level were given a "low" value and were not assigned a numerical value by Source Molecular. To graphically illustrate these results, a value of 25 copies/100 mL were given to those samples. A value of 0 copies/100 mL was assigned to absent DNA markers.

Flow data portrayed in the Figures 2, 4, 6 and 9 was obtained from the USGS Station (064125000) Rapid City Above Canyon Lake. Average daily discharge in cubic feet per second was implemented for the day that each sample was collected.

Numeric surface water quality standards are defined in the fecal coliform and *E. coli* bacteria TMDL for lower Rapid Creek. These standards were implemented for comparison in the analysis of the collected data. The TSS daily maximum standard is 53 mg/L. The *E. coli* standard for a single sample is 235 cfu/100 mL. Throughout the Results section of this report these standards are referred to as the TSS standard and *E. coli* standard.

8.1 Dark Canyon Figures

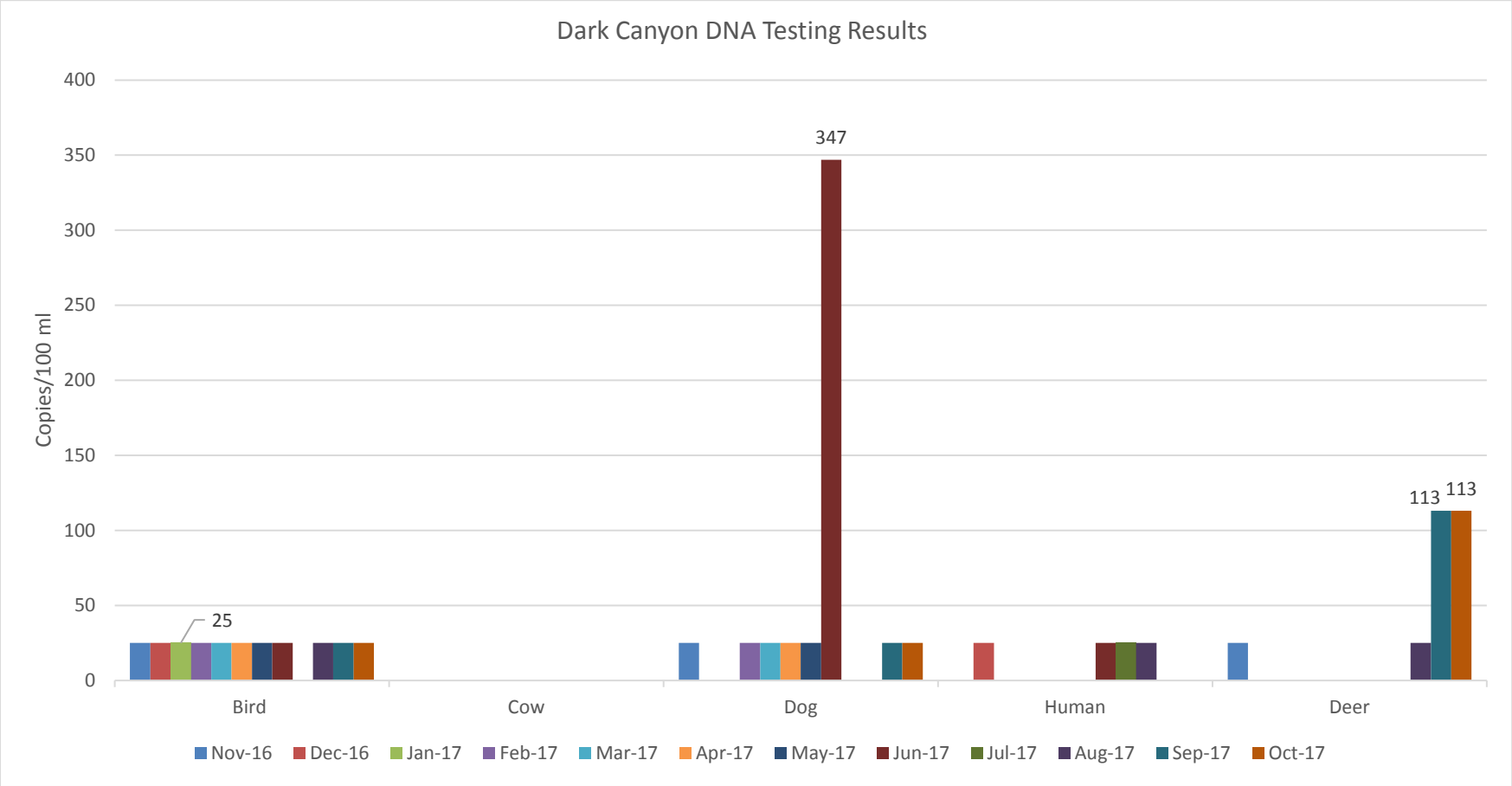
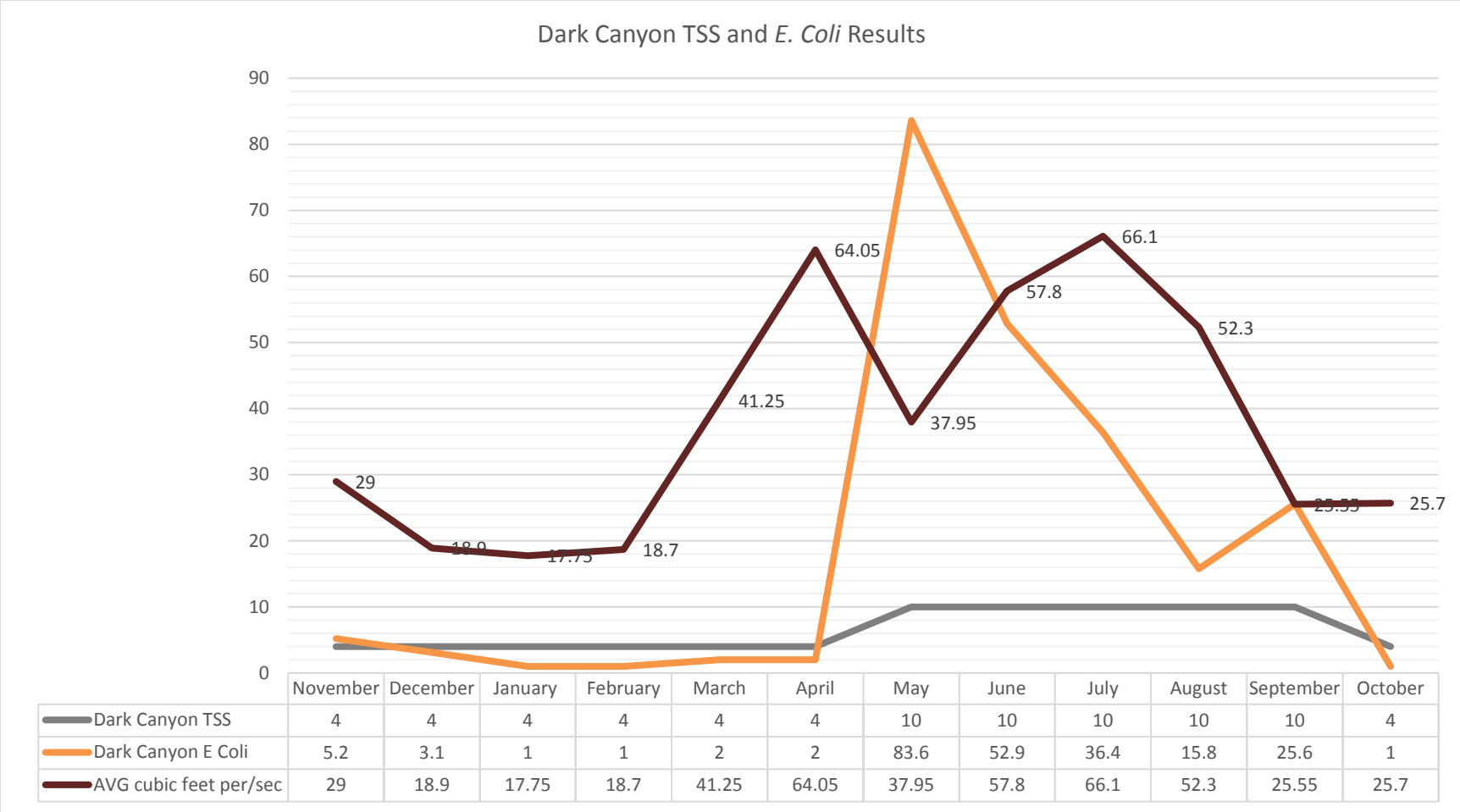


Figure 1: This Figure shows the quantified DNA testing results in copies/100 mL for the five tested host sources at the Dark Canyon site from November, 2016 through October, 2017. The general trend of data from Dark Canyon follows a clean creek with very few quantifiable concentrations of any of the tested host sources.



	November	December	January	February	March	April	May	June	July	August	September	October
Dark Canyon TSS	4	4	4	4	4	4	10	10	10	10	10	4
Dark Canyon E Coli	5.2	3.1	1	1	2	2	83.6	52.9	36.4	15.8	25.6	1
AVG cubic feet per/sec	29	18.9	17.75	18.7	41.25	64.05	37.95	57.8	66.1	52.3	25.55	25.7

Figure 2: This Figure shows the TSS and *E. coli* data at Dark Canyon as well as the Rapid Creek flow data from November, 2016 through October, 2017. TSS concentrations ranged from 4 mg/L to 10 mg/L which is well below the TSS standard of 53 mg/L. *E. coli* data during the winter months showed very low concentrations ranging from 1 to approximately 5 mpn/100 mL. An increase in *E. coli* concentrations was observed during the summer months with a peak of 84 mpn/100 mL which is still below the *E. coli* standard.

8.2 Below Canyon Lake Figures

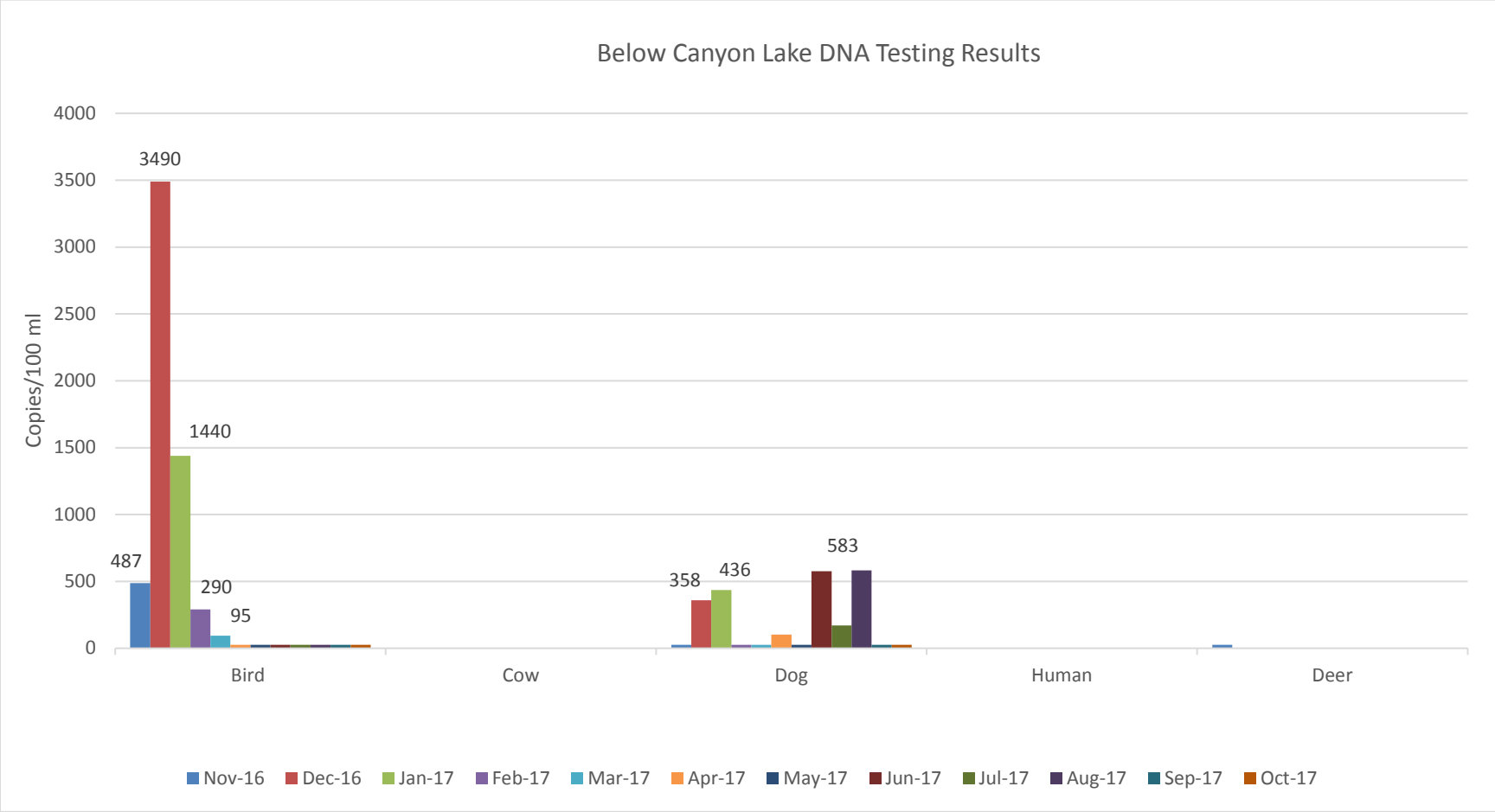
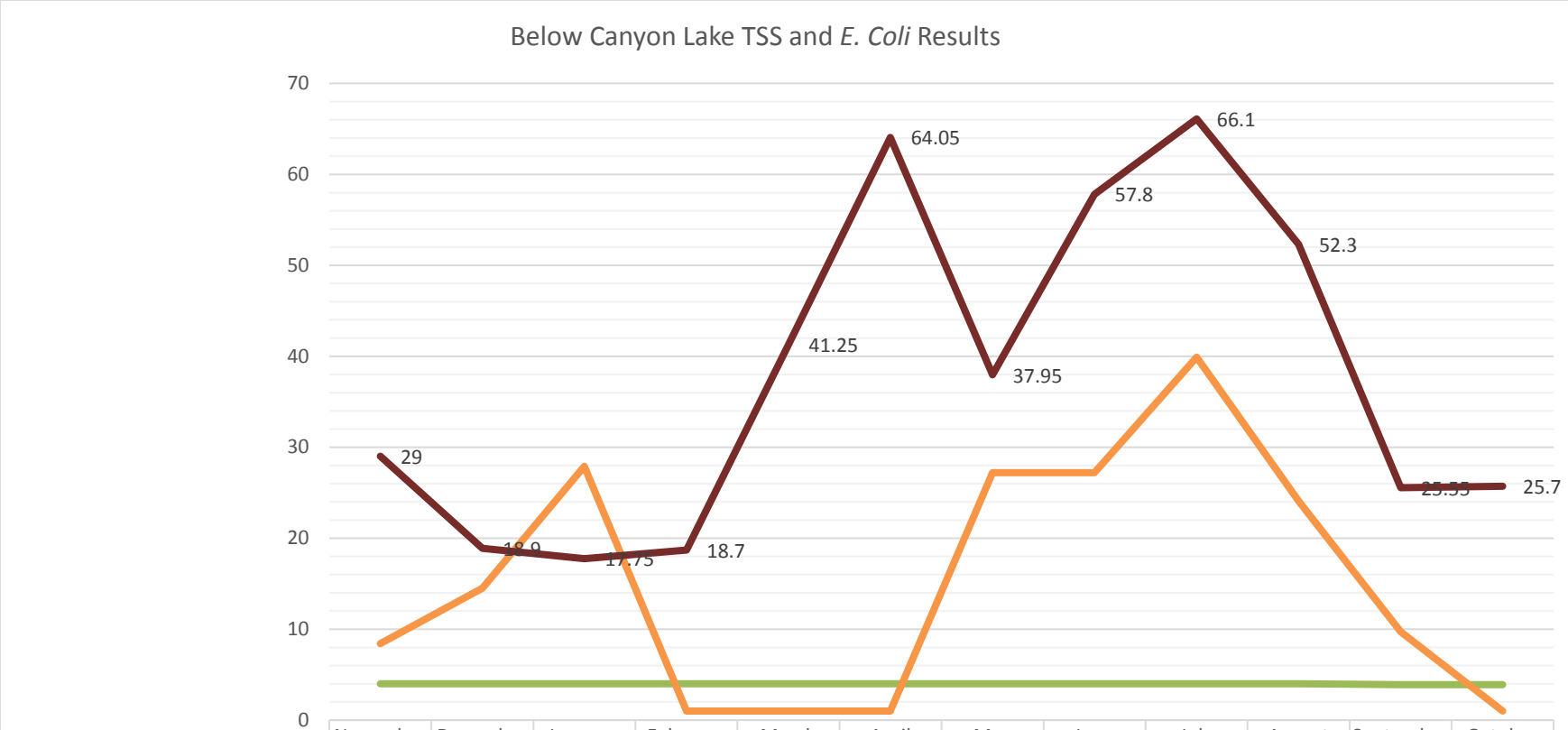


Figure 3: This Figure shows the quantified DNA testing results in copies/100 mL for the five tested host sources Below Canyon Lake from November, 2016 through October, 2017. Data shows a noticeable impact of the bird populations in Canyon Lake, especially during the winter months. Impacts from dog was also evident throughout the year which is likely related to the dog park located upstream from Canyon Lake.



	November	December	January	February	March	April	May	June	July	August	September	October
Below Canyon Lake TSS	4	4	4	4	4	4	4	4	4	4	3.9	3.9
Below Canyon Lake E Coli	8.4	14.5	27.9	1	1	1	27.2	27.2	39.9	24.1	9.7	1
AVG cubic feet per/sec	29	18.9	17.75	18.7	41.25	64.05	37.95	57.8	66.1	52.3	25.55	25.7

Figure 4: This Figure shows the TSS and *E. coli* data below Canyon Lake as well as the Rapid Creek flow data from November, 2016 through October, 2017. TSS concentrations were low year-round which is likely due to sediment dropping out into Canyon Lake. Lower concentrations of *E. coli* were observed during the winter months with an increase observed during the summer months. *E. coli* concentrations peaked at 66.1 mpn/100 mL, which is still below the *E. coli* standard.

8.3 East Saint Patrick Street Figures

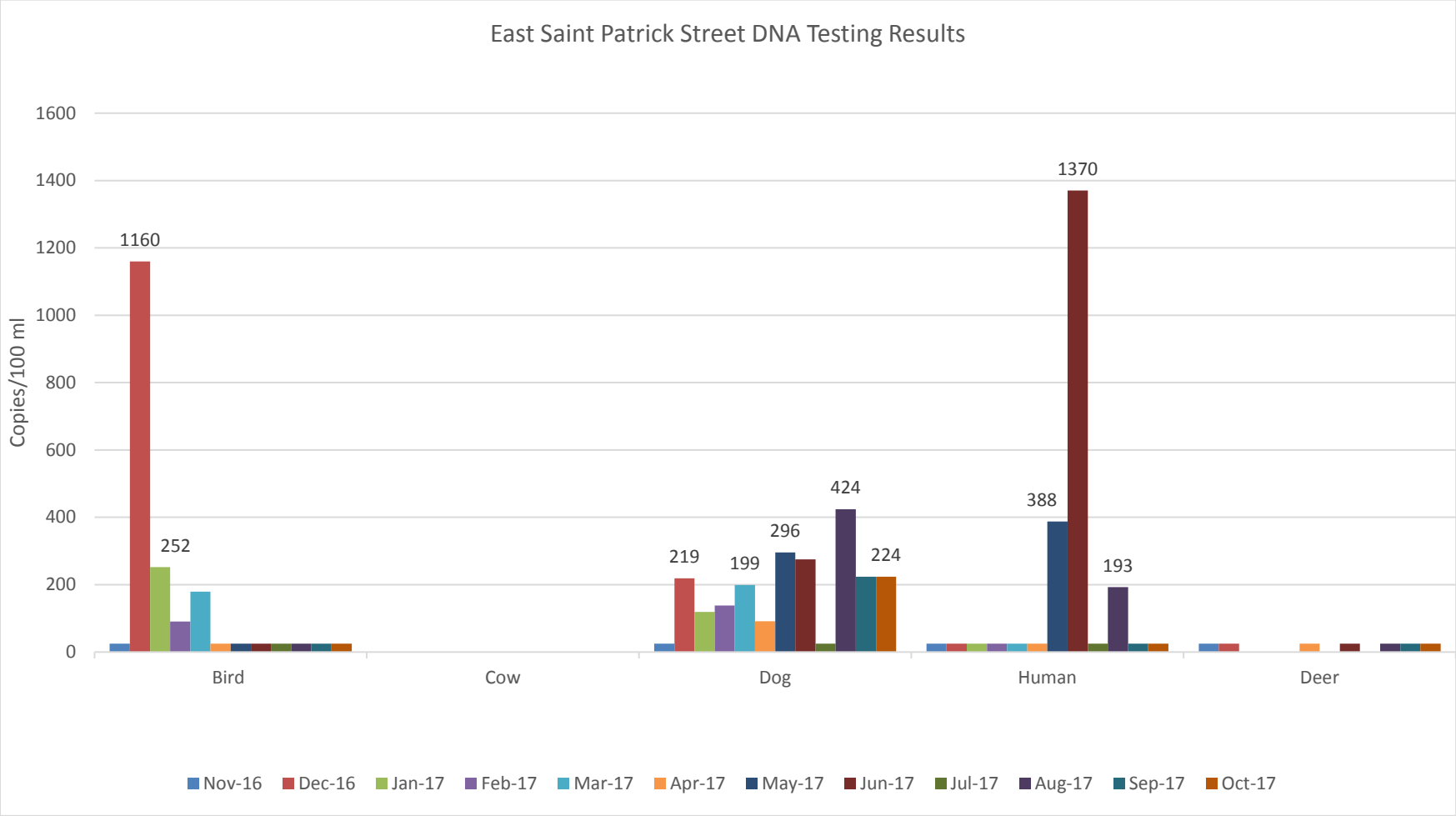
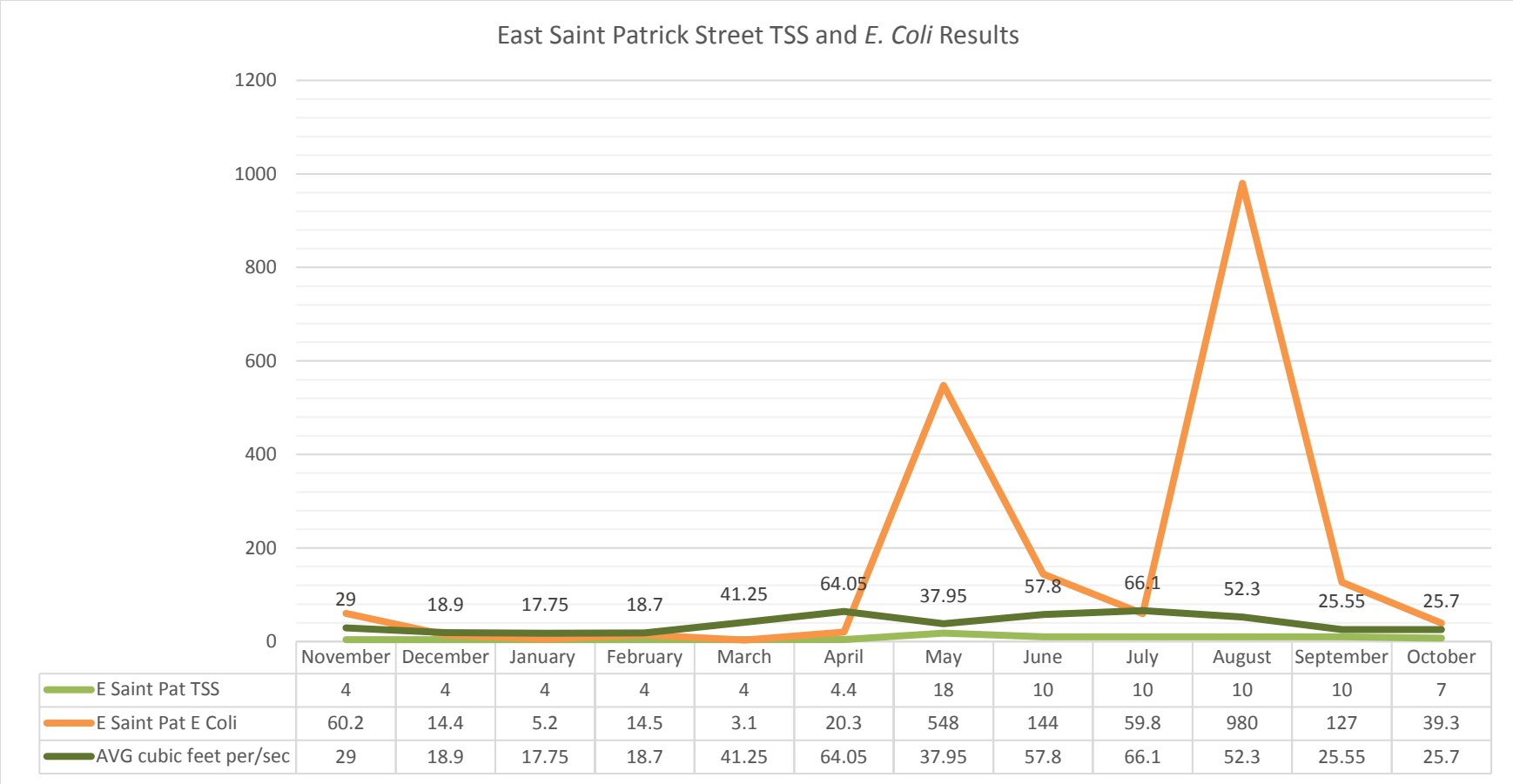


Figure 5: This Figure shows the quantified DNA testing results in copies/100 mL for the five tested host sources at East Saint Patrick Street from November, 2016 through October, 2017. Data shows a noticeable impact of the bird populations during the winter months. Impacts from dog was also evident throughout the year which is likely related to the bike path and green way along Rapid Creek through Rapid City. Impacts from human can be seen in the summer months which could be due to immersion recreation, the transient population, the septic systems located in the industrial park as well as possible leaks in the city's sewer system.



	November	December	January	February	March	April	May	June	July	August	September	October
E Saint Pat TSS	4	4	4	4	4	4.4	18	10	10	10	10	7
E Saint Pat E Coli	60.2	14.4	5.2	14.5	3.1	20.3	548	144	59.8	980	127	39.3
AVG cubic feet per/sec	29	18.9	17.75	18.7	41.25	64.05	37.95	57.8	66.1	52.3	25.55	25.7

Figure 6: This Figure shows the TSS and *E. coli* data at East Saint Patrick Street as well as the Rapid Creek flow data from November, 2016 through October, 2017. TSS concentrations were low year-round with a peak of 18 mg/L which is well below the TSS standard. An increase in *E. coli* concentrations were observed at this site compared to the two upstream locations. However, a similar trend of lower concentrations of *E. coli* were observed during the winter months with an increase observed during the summer months. *E. coli* concentrations peaked at 980 mpn/100 mL, which exceeds the *E. coli* standard by over 4 times.

8.4 Below WWTP Figures

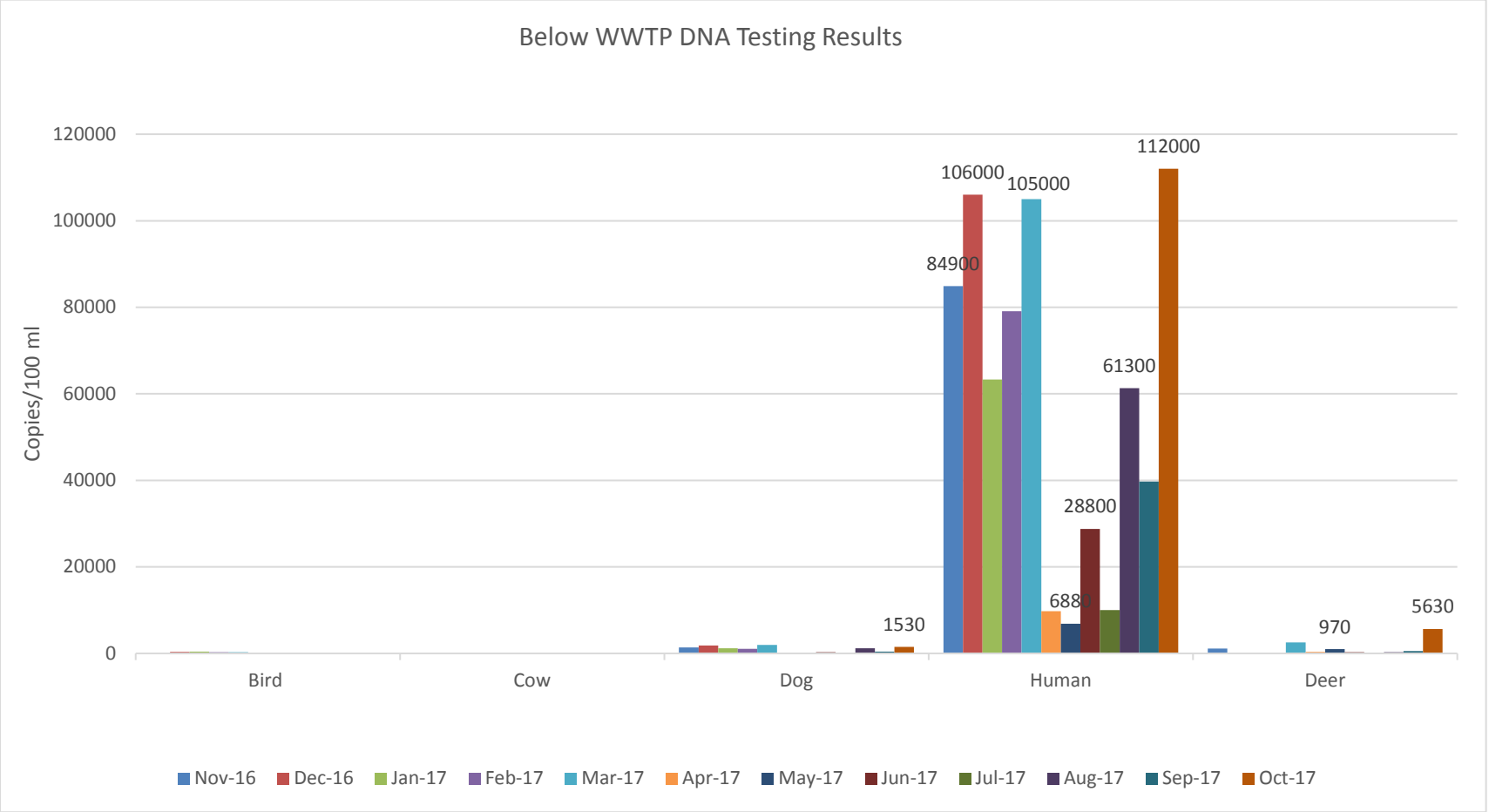


Figure 7: This Figure shows the quantified DNA testing results in copies/100 mL for the five tested host sources below the WWTP from November, 2016 through October, 2017. Much higher counts of contamination were observed at this site compared to the three upstream locations. Data shows that Human impacts are by far the main contributor to the contamination in Rapid Creek below the WWTP. This was expected because this sampling site is right below the WWTP outfall. Higher counts of human DNA markers were observed during the winter months with lower counts observed during the summer. The WWTP must meet more strict immersion recreation standards during the summer which contributes to this pattern. No quantifiable concentrations from cattle was observed year-round.

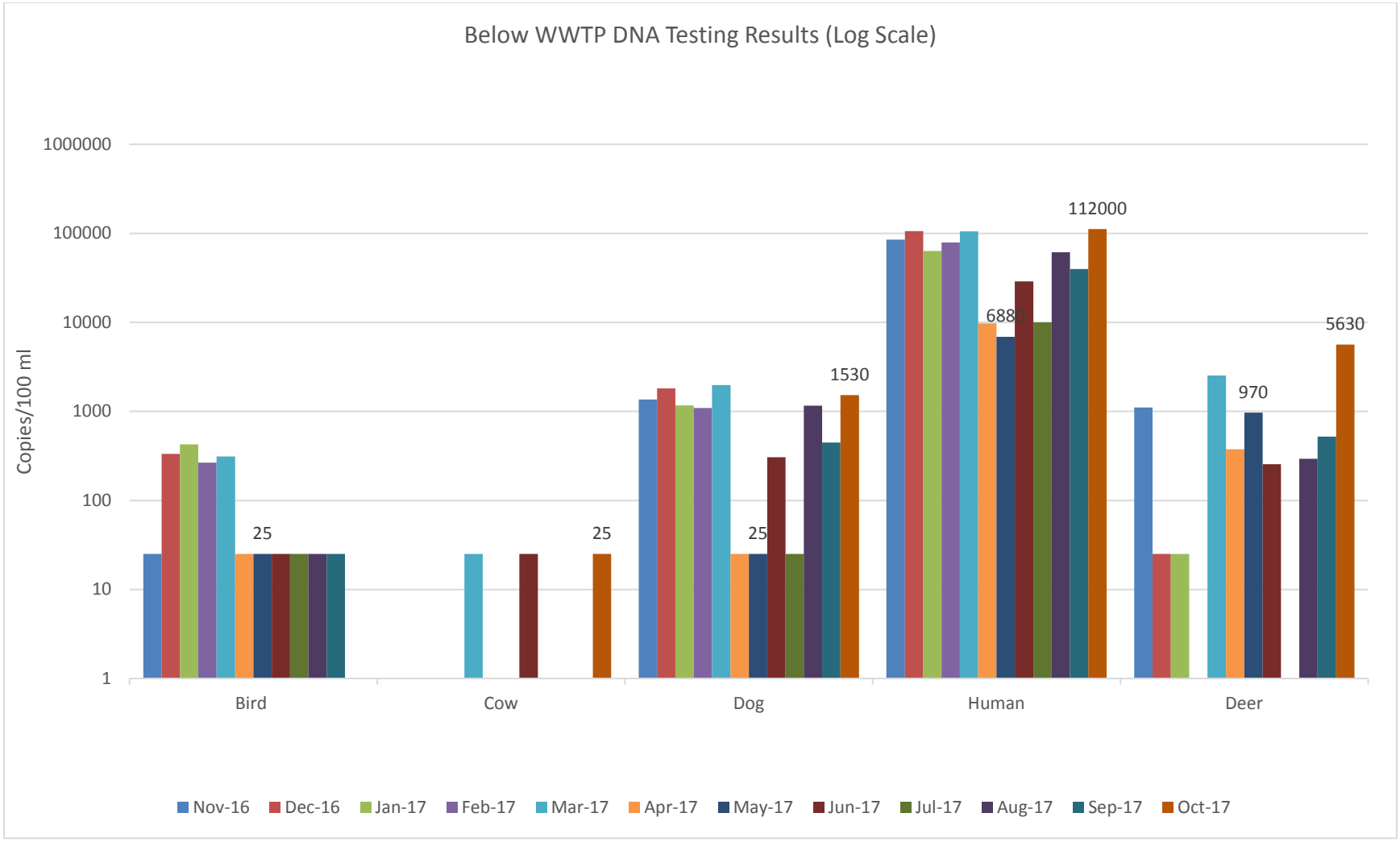
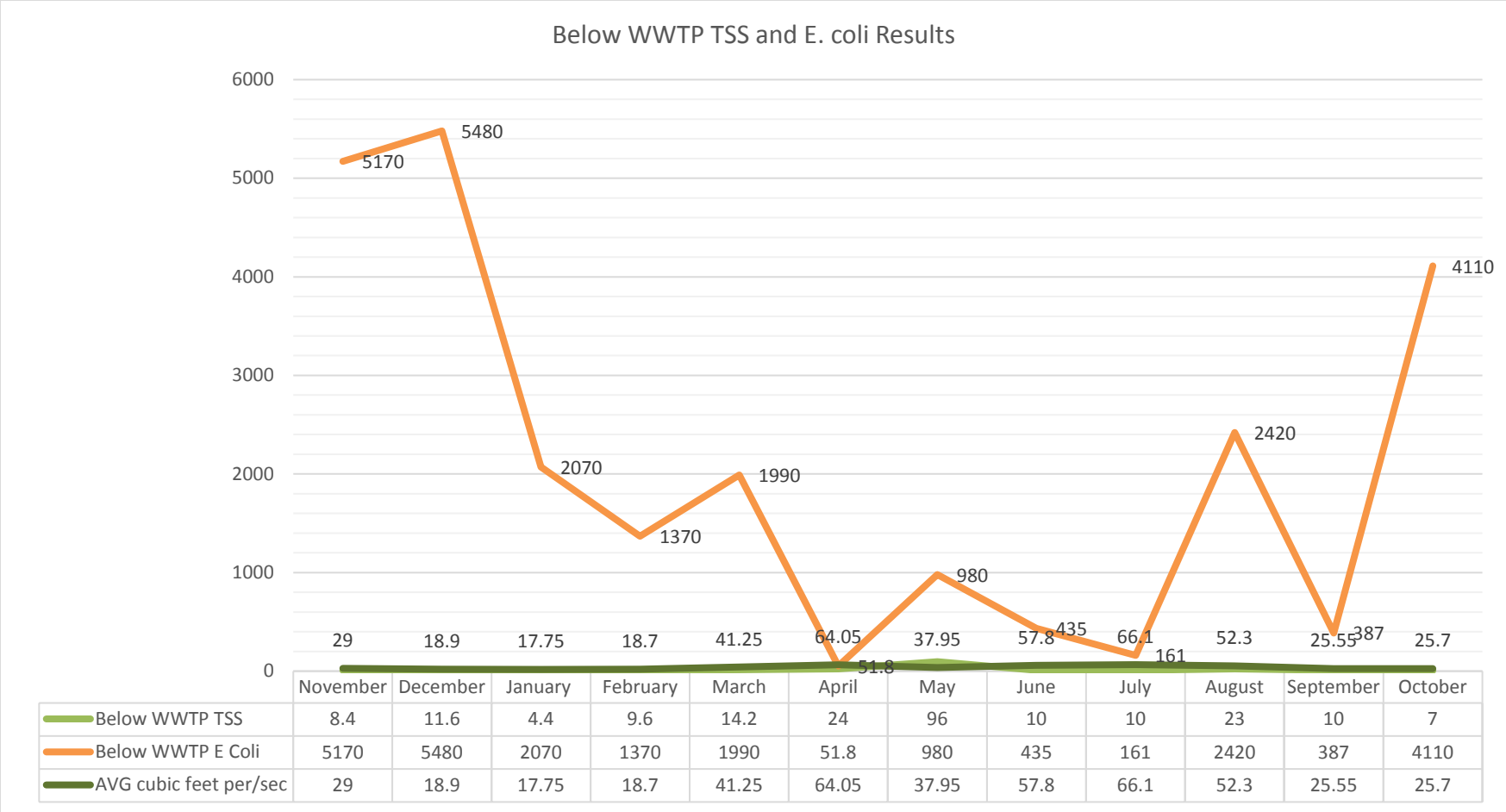


Figure 8: Unlike the other DNA testing results figures, this Figure has a log scale to better show impacts from the host species that are not Human. Data shows that Human impacts are by far the main contributor to the contamination in Rapid Creek below the WWTP. Data shows a noticeable impact of the bird populations during the winter months. Impacts from the dog and ruminant host sources was also evident throughout the year. No quantifiable concentrations from cattle was observed year-round.



	November	December	January	February	March	April	May	June	July	August	September	October
Below WWTP TSS	8.4	11.6	4.4	9.6	14.2	24	96	10	10	23	10	7
Below WWTP E Coli	5170	5480	2070	1370	1990	51.8	980	435	161	2420	387	4110
AVG cubic feet per/sec	29	18.9	17.75	18.7	41.25	64.05	37.95	57.8	66.1	52.3	25.55	25.7

Figure 9: This Figure shows the TSS and *E. coli* data below the WWTP as well as the Rapid Creek flow data from November, 2016 through October, 2017. TSS concentrations were slightly higher at this site compared to the three upstream locations but remained relatively low year-round. May, 2017 had a peak of 96 mg/L which is the only month that exceeded the TSS standard. A large increase in *E. coli* concentrations were observed at this site compared to the three upstream locations. An opposite trend of higher concentrations of *E. coli* were observed during the winter months with a decrease observed during the summer months. The WWTP must meet more strict standards during the summer, which contributes to this pattern. *E. coli* concentrations peaked at 5,480 mpn/100 mL, which exceeds the *E. coli* standard by over 23 times.

8.5 Overview Figures

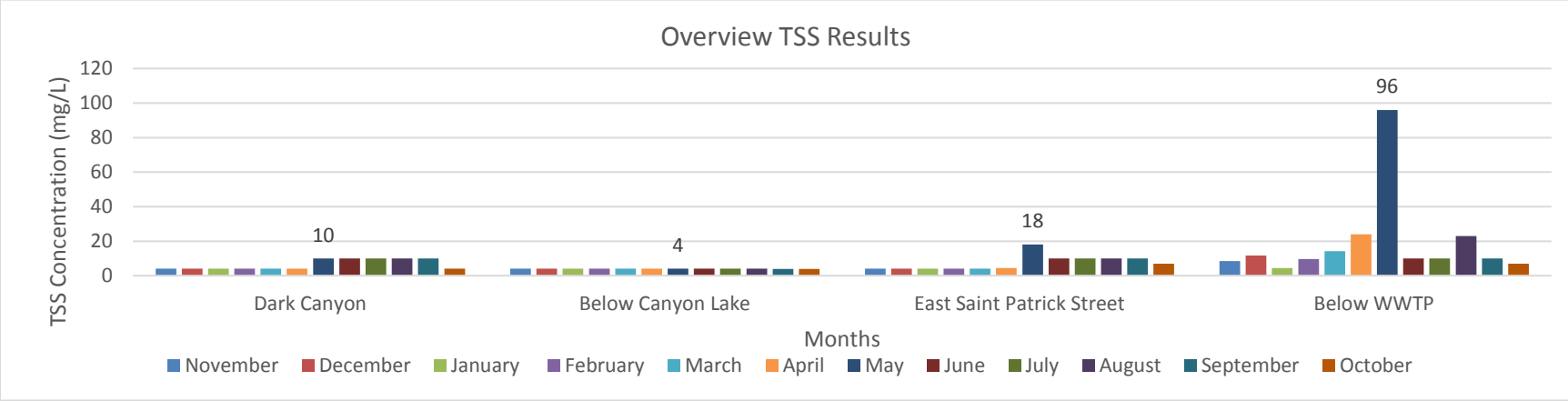


Figure 10: This Figure shows the TSS data for all four sampling locations from November, 2016 through October, 2017. TSS data remained below the TSS standard at all four sampling locations all year with the exception of one month below the WWTP.

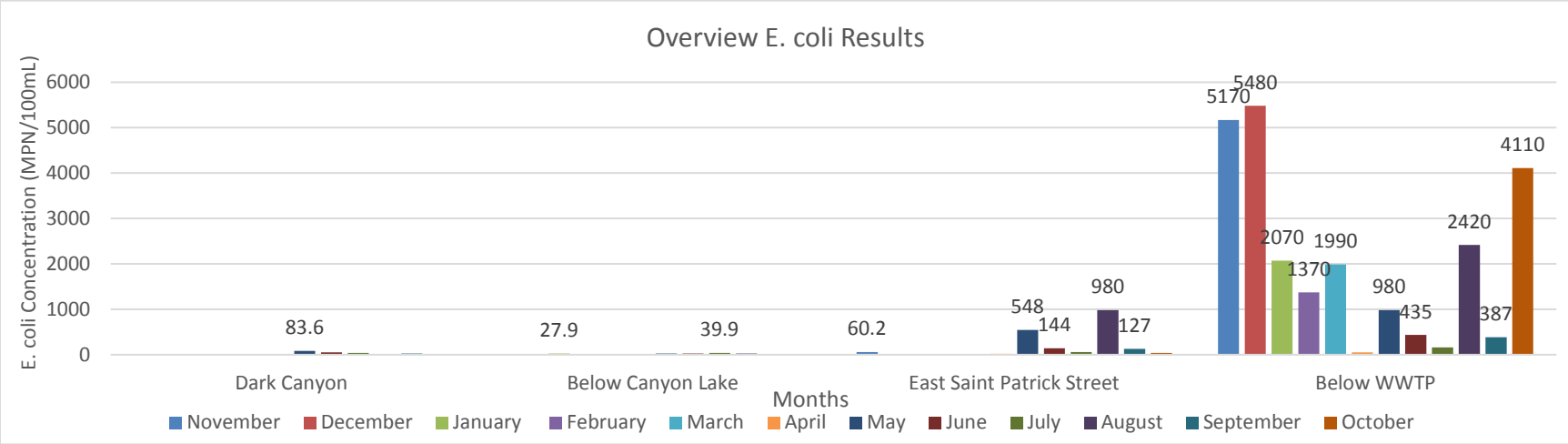


Figure 11: This Figure shows the *E. coli* data for all four sampling locations from November, 2016 through October, 2017. Results show increasing concentrations of *E. coli* as you proceed downstream with exceedances in the *E. coli* standard at East Saint Patrick Street and below the WWTP.

8.6 Conclusions

The Dark Canyon site was selected to coordinate with the DENR WQM 69 site and to provide a base of information for Rapid Creek prior to any possible urban impacts associated with Rapid City. The general trend of data shows a clean and clear creek at Dark Canyon year-round. TSS concentrations ranged from 4 mg/L to 10 mg/L which is well below the TSS standard of 53 mg/L. *E. coli* data during the winter months showed very low concentrations ranging from 1 to approximately 5 mpn/100 mL. An increase in *E. coli* concentrations was observed during the summer months with a peak of 84 mpn/100 mL. Throughout the entire year of testing, Dark Canyon *E. coli* concentrations were below the *E. coli* criterion of 235 mpn/100 mL.

The DNA testing data at Dark Canyon revealed very few quantifiable concentrations of any of the tested host species throughout the year of testing. No quantifiable concentrations of human, cattle or bird host species were detected during this time. This shows that there is very little impact to the fecal pollution in Rapid Creek from the septic systems and cattle grazing above Dark Canyon. Some impact from ruminant and dog host sources was noted, but at low concentrations compared to the downstream locations.

The second sampling site is located downstream from Dark Canyon and below the Canyon Lake spill way. This site was selected to determine the possible effects of the bird population in Canyon Lake and the large concentration of septic systems within the Wonderland Drive drainage basin on Rapid Creek. Throughout the year of testing, there were no exceedances of the TSS or *E. coli* standards. TSS concentrations were low year-round which is likely due to sediment dropping out into Canyon Lake. Lower concentrations of *E. coli* were observed during the winter months with an increase observed during the summer months. *E. coli* concentrations peaked at 66.1 mpn/100 mL, which was still below the *E. coli* standard.

The DNA testing data below Canyon Lake shows a noticeable impact of the bird populations in Canyon Lake, especially during the winter months. Dog DNA markers were also evident throughout the year which is likely related to the Dog Park located upstream from Canyon Lake. Data showed that human, cattle and ruminant host sources were not contributing sources. Therefore, there was no impact to the fecal pollution from the septic systems on the Wonderland Drive drainage basin or from grazing upstream on Rapid Creek.

The third sampling site is located off of East Saint Patrick Street and was selected to coordinate with USGS Station 641600 and DENR Site WQM 173 and to help determine the effects of Rapid City's downtown area. TSS concentrations were low year-round with a peak of 18 mg/L which is well below the TSS standard. Overall, an increase in *E. coli* concentrations were observed at this site compared to the two upstream locations. However, a similar trend of lower concentrations of *E. coli* were observed during the winter months with an increase observed during the summer months. *E. coli* concentrations peaked at 980 mpn/100 mL, which exceeds the *E. coli* standard by over 4 times.

The DNA testing data at East Saint Patrick Street shows a noticeable impact of the bird populations during the winter months. Impacts from dog was also evident throughout the year which is likely related to the bike path and green way along Rapid Creek through Rapid City. Impacts from

Human can be seen in the summer months which could be due to immersion recreation, the transient population, possible leaks in the city's sewer system, as well as the septic systems located in the industrial park.

The last site is farthest downstream and gives a good perspective of the possible impacts that the WWTP and the cattle feedlots have to the surrounding area. TSS concentrations below the WWTP were slightly higher at this site compared to the three upstream locations but remained relatively low year-round. May, 2017 had a TSS peak of 96 mg/L which is the only month that exceeded the TSS standard. A large increase in *E. coli* concentrations were observed at this site compared to the three upstream locations. An opposite trend of higher concentrations of *E. coli* during the winter months with a decrease observed during the summer months was observed. The WWTP must meet more strict standards during the summer, which contributes to this pattern. The *E. coli* concentrations peaked at 5,480 mpn/100 mL, which exceeds the *E. coli* standard by over 23 times.

The DNA testing data below the WWTP showed very elevated levels of human every month with the lowest readings at 6,880 copies/100 mL in May, 2017. In comparison, the highest reading from any of the other three locations was 3,490 copies/100 mL (Bird in December, 2016 below Canyon Lake) which is approximately half of the smallest reading at below the WWTP.

Much higher counts of fecal pollution were observed at this site compared to the three upstream locations. Data shows that Human impacts are by far the main contributor to the fecal pollution in Rapid Creek below the WWTP. This was expected because this sampling site is right below the WWTP outfall. Higher counts of Human DNA were observed during the winter months with lower counts observed during the summer. The WWTP must meet immersion recreation standards during the summer months which contributes to this pattern. No quantifiable concentrations from cattle was observed year-round indicating that the cattle feedlots located in the area do not contribute to the fecal pollution in Rapid Creek.

This study attributes little to no impact to fecal pollution in the study area from the non-point sources of septic systems and cattle grazing. Cattle host sources were absent or below quantifiable levels at all four locations throughout the 12 months of testing. Human sources of fecal pollution are introduced at East Saint Patrick Street which is before most of the possible septic system influences. Influence from septic systems located northeast of the WWTP could be isolated by collecting DNA samples above and below the WWTP. However, based on no impact from the septic systems along Dark Canyon and from the Wonderland Drive drainage basin it is unlikely that they are a major contributor. The large spike of E. coli and human DNA markers below the WWTP can be contributed to the WWTP outfall being located just above the sampling site. Discharge from the WWTP is required to meet their National Pollution Discharge Elimination System permit limits that are issued by the State of SD. The WWTP continues to upgrade their facility to continue to comply with the standards.

Corrective action would be most effective if it was geared towards reducing the impacts of the bird populations throughout the winter, specifically the months of December, January, February and March, and reducing the impacts from dogs year-round. The influence from dogs increases as you

move downstream which is likely due to owners not cleaning up after their pets in the various parks and greenways located along Rapid Creek.

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Appendices

Appendix A: Chain of Custody

Chain Of Custody Record

Source Molecular Corporation
Leader in Genetic Microbial Source Tracking

SHIPPING ADDRESS:
4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

Company Name:	
Contact Name(s):	
E-mail Address(es):	
Phone:	
Address:	
City/State/Zip:	
Country:	

Sample ID	Media	Sample Date	Sample Time	# of Containers	Analysis Requested (see pg. 2) Mark boxes with "X"						Quantification (Yes, No, If Positive)	Comments

Completed by Client:

SAMPLES DELIVERED BY _____
SAMPLER NAME _____
SAMPLE SITE _____
SIGNATURE _____

Completed by Source Molecular:

SAMPLES RECEIVED BY _____
RECEIVED DATE/TIME _____
TEMPERATURE _____
CONDITION _____
SIGNATURE _____

Explanation of Terms Used in Chain-of-Custody Record

Sample ID:	The specific sample identification number, unique to each sample set.
Media:	The sample media (e.g. water, soil).
Sample Date:	The date that the sample was taken, including month, day, and year.
Sample Time:	The time the sample was taken.
# of Containers:	The number of containers for that particular sample set.
Analysis:	The type(s) of analysis required for that particular sample or sample set.
Quantification:	The type of testing to be performed. Select "Yes" for quantification analysis. Select "No" for only presence or absence analysis. Select "If Positive" for presence or absence with quantification of the host biomarkers that are positive.
Samples Delivered By:	The signature of the individual who is delivering the samples to the laboratory. Under most circumstances, this will be one of the individuals who performed the sampling.
Sampler Name:	The name of the individual(s) performing the sampling.
Sample Site:	The name of the site being sampled.
Samples Received By:	The name of the individual who receives the samples for the laboratory.
Received Date/Time:	The date and time the samples are delivered to the lab, including hour, month, day, and year.
Temperature:	The temperature of the samples upon arrival, in degrees Celsius.
Condition:	The condition of the samples upon arrival.
Signature:	The signature of the individual who receives the samples for the laboratory.

Recommended Quantities

Water samples:
500mL of water in leak proof bottles per sample.

Filters, sediment, shellfish or other sample types:
Contact us for details.

Available Analysis

Human
Human Bacteroidetes ID: Dorei *(Default)*
Human Bacteroidetes ID: EPA
Human Bacteroidetes ID: Steri
Human Bacteroidetes ID: Spp.
Human Bacteroidetes ID: Fragilis

Cattle
Cow Bacteroidetes ID: EPA 1 *(Default)*
Cow Bacteroidetes ID: EPA 2

Pig
Pig Bacteroidetes ID

Bird
Bird Fecal ID

Gull
Gull Fecal ID

Goose
Goose Bacteroidetes ID

Chicken
Chicken Bacteroidetes ID

Dog
Dog Bacteroidetes ID: Target 1 *(Default)*
Dog Bacteroidetes ID: Target 2

Deer/Elk
Ruminant Fecal ID
Elk Bacteroidetes ID

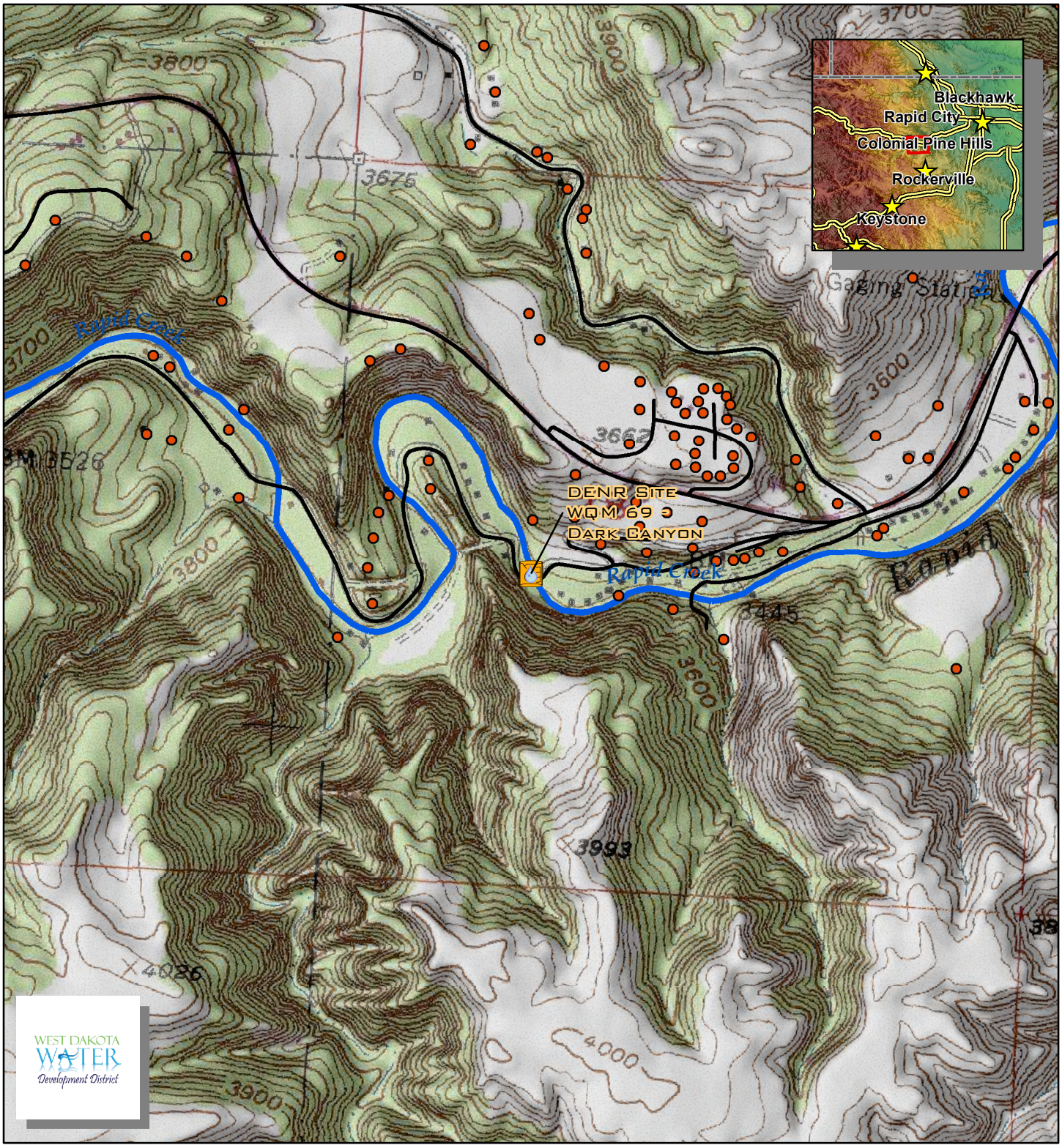
Ruminant
Ruminant Fecal ID: Target 1 *(Default)*
Ruminant Fecal ID: Target 2

Horse
Horse Bacteroidetes ID

Beaver
Beaver Fecal ID

General
General Bacteroidetes ID
General Enteroccocus ID
EPA Method 1611
EPA Method B

Appendix B: Sample Location Maps



0 0.25 0.5 Miles

Scale 1:12,500

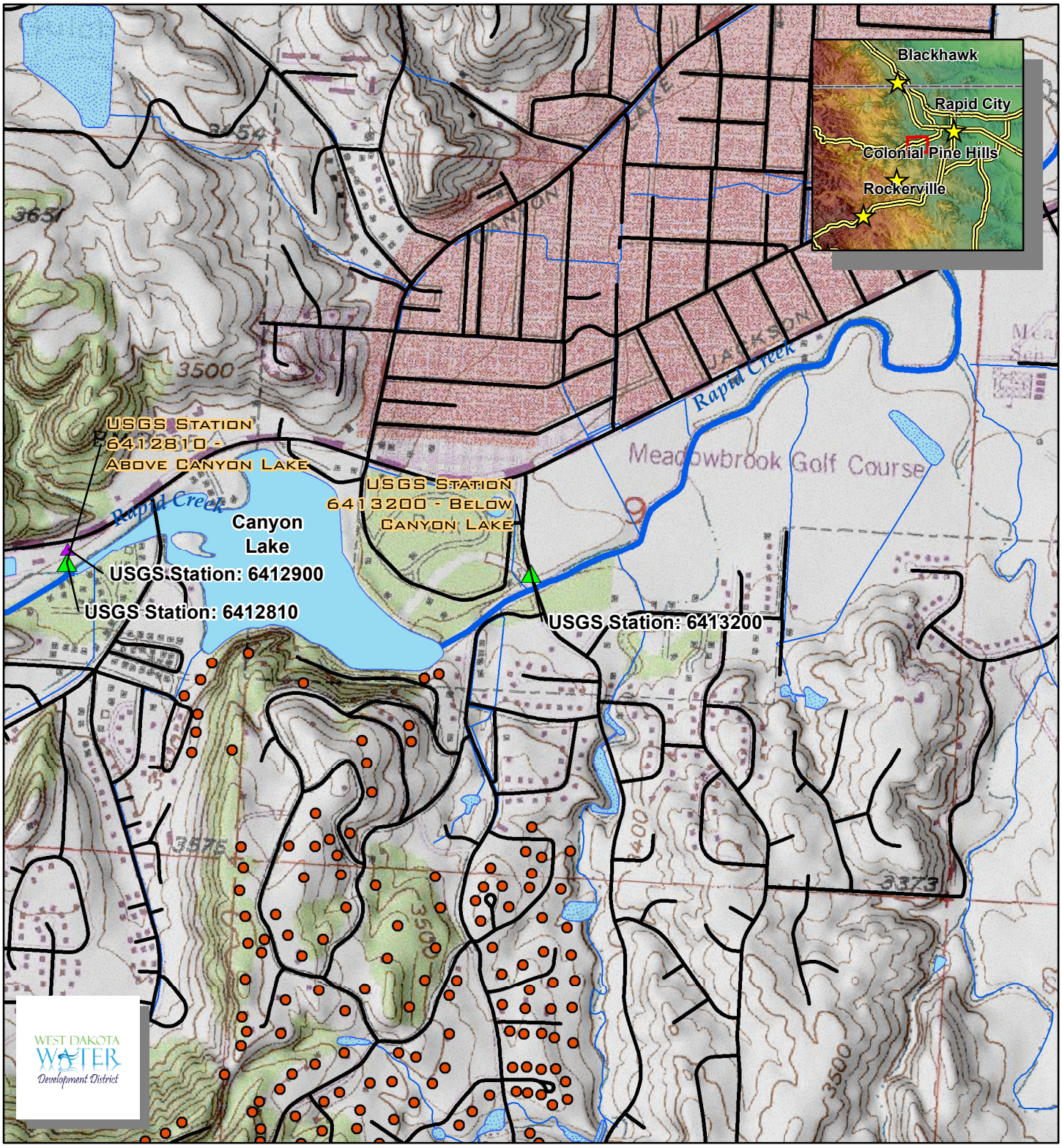
Rapid Creek Proposed Sampling Location 1:

DENR Site WQM 69 - Dark Canyon

- Local Roads
- DENR Existing Sampling Location
- Septic System Locations (2007)

Prepared by:

Date: 2/17/2016
User Name: eenglund
H:\GIS\Clients\West Dakota Watershed District\Rapid_Creek_Sampling_Locations.mxd



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Scale 1:12,500

Rapid Creek Proposed Sampling Location 3:

USGS Station 6413200 - Below Canyon Lake

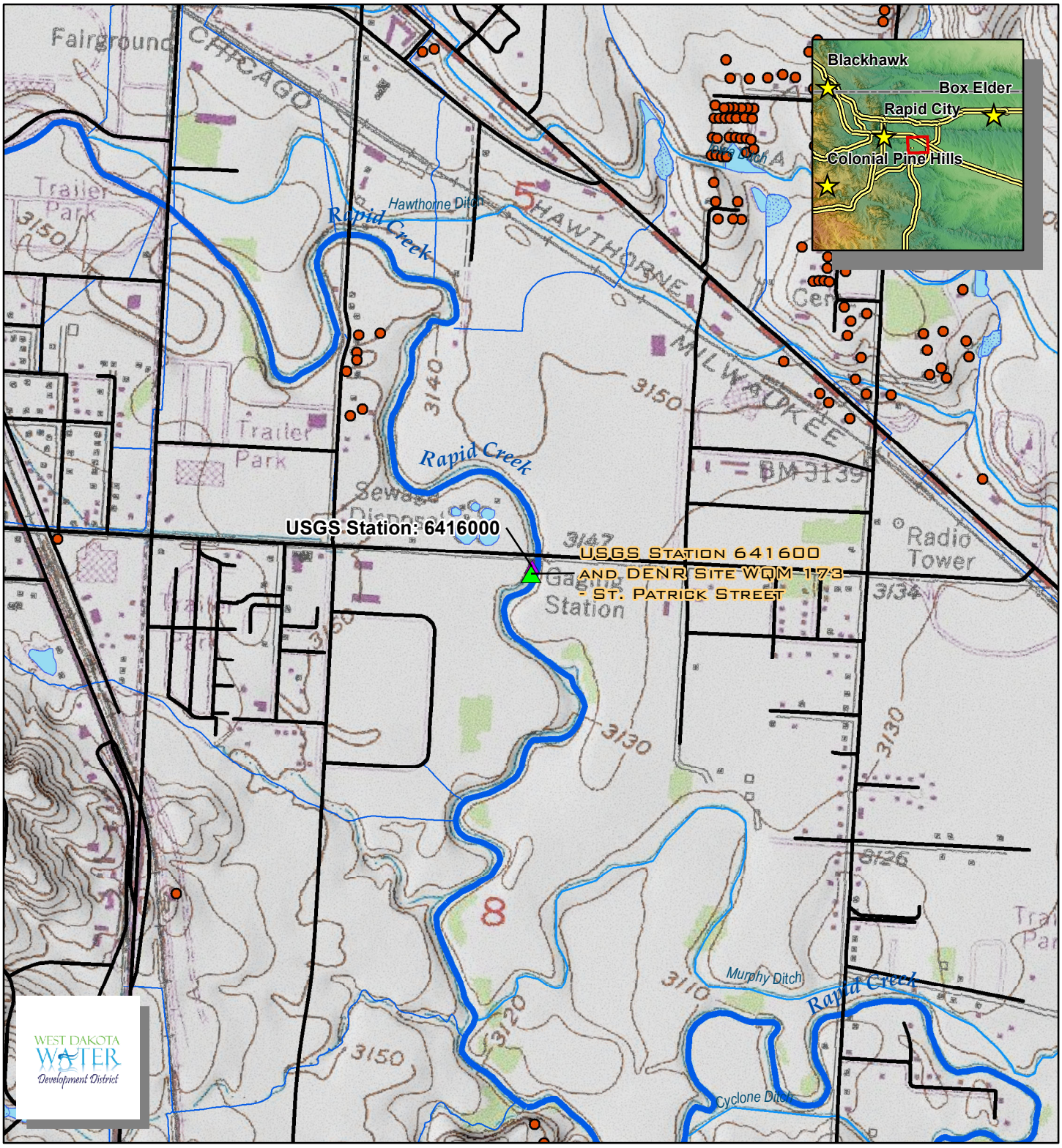
Local Roads

USGS Guaging Station Sampling Location

Septic System Locations (2007)

Prepared by:

Date: 2/17/2016
User Name: eenglund
H:\GIS\Clients\West Dakota Watershed District\Rapid_Creek_Sampling_Locations.mxd



0 0.25 0.5 Miles

Scale 1:12,500

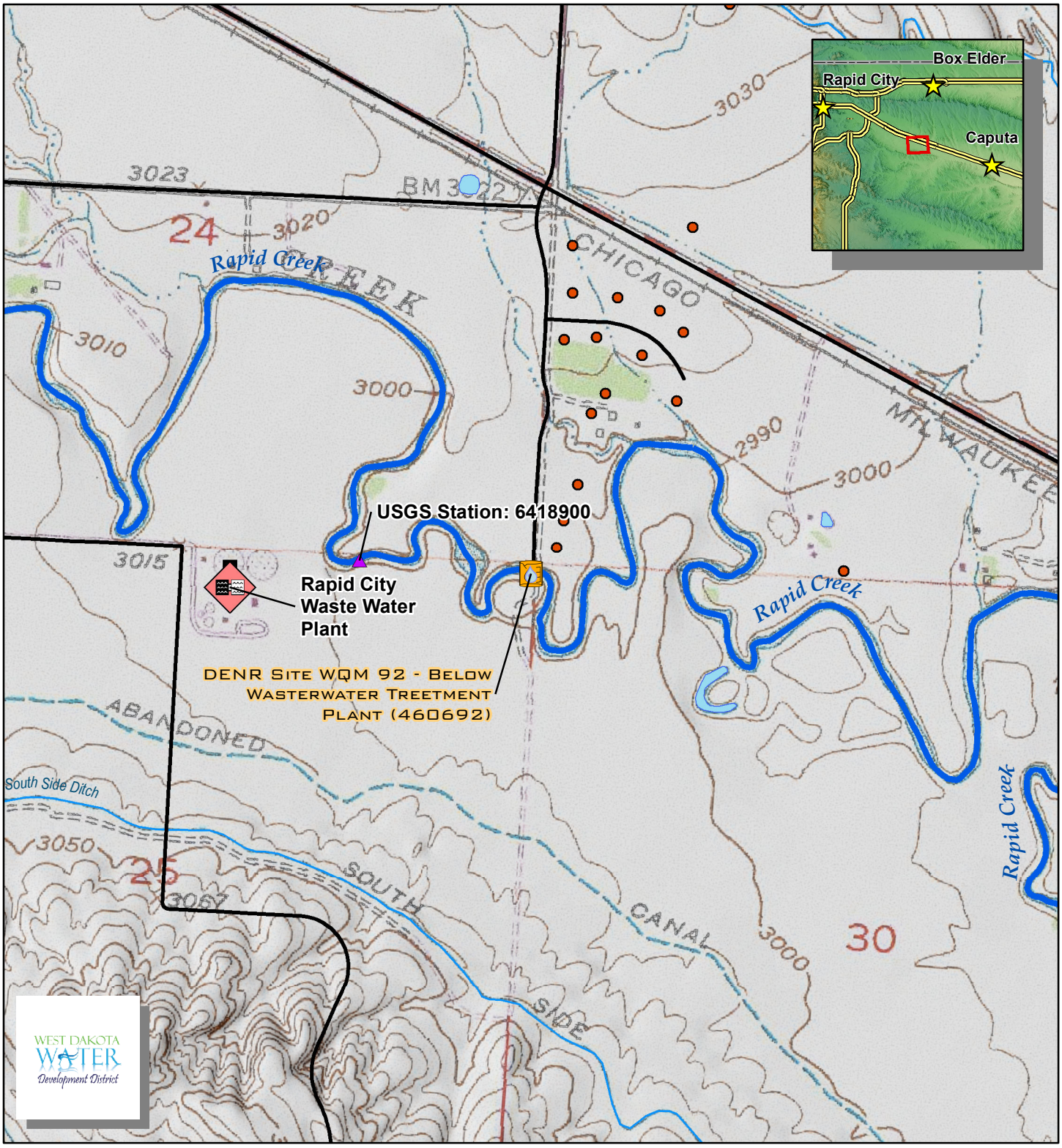
Rapid Creek Proposed Sampling Location 4:

USGS Station 641600 and DENR Site WQM 173 - St. Patrick Street

- Local Roads
- USGS Guaging Station Sampling Location
- Septic System Locations (2007)

Prepared by:

Date: 2/17/2016
User Name: eenglund
H:\GIS\Clients\West Dakota Watershed District\Rapid_Creek_Sampling_Locations.mxd



0 0.25 0.5 Miles

Scale 1:12,500

Rapid Creek Proposed Sampling Location 6:

DENR Site WQM 92 - Below Wasterwater Treetment Plant (460692)

- Local Roads
- DENR Existing Sampling Location
- Septic System Locations (2007)

Prepared by:

Date: 2/17/2016
User Name: eenglund
H:\GIS\Clients\West Dakota Watershed District\Rapid_Creek_Sampling_Locations.mxd

Appendix C: Detailed Project Budget

Detailed Budget

Project Funds		
	Approved Expenditure	**Actual Cost**
WDWDD Committed Funds	$ 40,000.00	
Additional Requested Funds from WDWDD	$ 13,014.00	
TOTAL	**$ 53,014.00**	**$ 50,240.46**

Project Costs		
	Approved Budget	**Actual Cost**
A. Personnel		
Total Personnel	$ -	$ -
B. Fringe Benefits		
Total Fringe Benefits	$ -	$ -
C. Travel		
Travel for Staff: 50 mi/mo x $0.55/mi x 12 mo	$ 330.00	$ 360.00
Total Travel	$ 330.00	$ 360.00
D. Equipment		
Total Equipment	$ -	$ -
E. Supplies		
Disposable coolers: 12 sampling trips x 1 cooler/trip x $16.00/cooler	$ 192.00	$ -
Total Supplies	$ 192.00	$ -
F. Contractual		
H2E, Incorporated		
(1) DNA Sampling Plan: $74.50/hr x 60 hours	$ -	$ -
(2) Sample Collection		
*Monthly Sampling: 1 FTE x $74.50/hr x 6 hrs/mo x 12 mo	$ 5,364.00	$ 5,400.00
(3) Developing Outputs		
*Final Report: $74.50/hr x 8 hrs/week x 8 weeks	$ 4,768.00	$ 3,110.00
Source Molecular: Sample Testing		
(1) 4 samples/mo x 12 mo x 5 tests/sample x $215/test x 0.65 (30% discount)	$ 36,120.00	$ 36,120.00
(2) Sample Collection QA/QC: 2 samples/mo x 12 mo x $175/sample	$ 4,200.00	$ 4,200.00
Midcontinent Testing: Winter TSS and E. coli		
(1) 4 months of E. coli and TSS: $166.14/month	$ 664.56	$ 664.56
Total Contractual	$ 50,452.00	$ 49,494.56
G. Construction		
Total Construction	$ -	$ -
H. Other		
Overnight sample shipments: 12 coolers x $170/cooler	$ 2,040.00	$ 385.90
Total Other	$ 2,040.00	$ 385.90
K. TOTALS	**$ 53,014.00**	**$ 50,240.46**

Appendix D: Source Molecular QA/QC Summary

Source Molecular QA/QC Summary

Special Training/Certification

Individuals appointed to MST projects hold at minimum a Bachelor's degree and have a sound knowledge in genetics and molecular biology. Individuals must have had 1 year of previous hands-on qPCR experience at another laboratory. Trainees undergo supervised hands-on training by the Laboratory Manager, which typically lasts 1-4 months depending on experience. An initial demonstration of technical capability is required before personnel are permitted to work independently on client projects. This involves:

- Successfully preparing five, 5-point standard curves that satisfy accuracy and precision criteria; and
- A side-by-side comparison test in which the trainee's results are compared to a qualified individual's results after both independently prepare and analyze the same randomly selected client samples (5 batches up to 100 samples).

Training records are documented by the Laboratory Manager and hard copies are kept on file in the company's office.

Quality Objectives and Criteria

Quality control procedures are utilized to monitor the validity of test results. Source Molecular ensures that only valid results are reported to the client by continuously monitoring and reviewing the performance of tests. Key performance acceptance criteria and Data Quality Indicators are described below.

Data Quality Indicators and QC Requirements for MST Tests

Data Quality Indicators	**QC Item/Activity Used to Assess Measurement Performance**	**Purpose**	**Frequency**	**Measurement Performance Criteria**
Accuracy/Bias	Extraction blank	Evaluates contamination during DNA extraction/purification	Once every week samples are extracted	No detection or detection at least 3 C_T units above sample C_T values
Accuracy/bias	Diluted sample	Monitors for sample matrix inhibition affects	Every sample analyzed	C_T value must be greater than that of unknown sample
Accuracy/bias	Positive control	Monitors for false negatives	One reaction for every sample analyzed	C_T value below 35. No false negatives

Accuracy/bias	Negative control	Monitors for false positives	Three reactions for every sample analyzed	No detection or detection at least 3 C_T units above sample C_T values
Accuracy/bias	Standard Curve	Monitors overall reaction performance and efficiency -Ensures confidence and comparability between sample data -Sets linear dynamic range to accurately quantify samples	One curve in duplicate for every sample analyzed and requiring quantification	R^2: ≥0.98 Efficiency: 80-110% Slope: -3.0 - -4.0 Analytical Limit of Quantification (copies). Sample unknown within the linear dynamic range limits
Precision/ Comparability	qPCR duplicates	Ensures precision and confidence in data	Every sample analyzed	± 1 standard deviation unless CT value ≥33

Analytical Methods

Test methods meet the needs of the project and are appropriate for the tests undertaken. The microbial source tracking tests aim to identify potential animal host sources of fecal contamination in water samples. Currently, no standard methods exist for microbial source tracking. Genetic markers used for microbial source tracking tests are adopted by the Source Molecular laboratory from published, peer-reviewed scientific texts or journals whenever possible. Tests have been validated internally and/or externally in the microbial source tracking research community. If possible, reference methods published as international, national or regional standards are used. The laboratory ensures that the latest edition of a standard is used unless it is not appropriate or possible to do so.

Quality Control

Quality control procedures are utilized to monitor the validity of test results. Source Molecular ensures that only valid results are reported to the client by continuously monitoring and reviewing the performance of tests. All QC criteria must be met for the results to be considered valid and reported to client.

Statistical calculations are calculated automatically by the qPCR software. These include qPCR replicate standard deviations, replicate means and standard curve efficiency, slope, y-intercept and coefficient of linear regression (R^2).

QC Item/Activity	Data Quality Indicator	Frequency
Extraction blank	Accuracy/Bias - Evaluates contamination during DNA extraction/purification	Once every week samples are extracted
qPCR duplicates	Precision, Comparability -ensures precision and confidence in data	Every sample analyzed
Diluted sample	Accuracy/Bias -Monitors for sample matrix inhibition affects	Every sample analyzed
Positive control	Accuracy/Bias -Monitors for false negatives	One reaction for every sample analyzed
Negative control	Accuracy/Bias -Monitors for false positives	Three reactions for every sample analyzed
Standard Curve	Accuracy/Bias, Comparability, Sensitivity -Monitors overall reaction performance and efficiency -Ensures confidence and comparability between sample data -Sets linear dynamic range to accurately quantify samples	One curve in duplicate for every sample analyzed and requiring quantification

Instrument/Equipment Testing, Inspection, and Maintenance

Access to laboratory equipment is controlled to ensure that only authorized personnel use the equipment. Instructions on the use and maintenance of equipment are readily accessible by authorized personnel.

Generally, the handling, transport, storage, use and maintenance of equipment are outlined in the manufacturer's manual. Manuals are located in the laboratory at all times. Specific requirements, if any, are outlined in the test method standard operating procedures.

The manufacturer's manual is critical in describing the safe handling requirements of the equipment, to avoid any damage, alteration, contamination, deterioration, change of integrity or reliability and condition of the equipment (or samples). The manufacturer's manual also provides guidance for suitable environmental conditions for the calibrations, inspections, measurements and tests performed. These guidelines should be followed at all times unless specified otherwise in standard operating procedures.

Routine test work is completely discontinued on equipment that shows minor non-conformances. Not only do we do this for ethical reasons in support of our customer, but minor non-conformances are often indicative of major breakdowns in expensive equipment. These breakdowns need to be avoided wherever possible. Out of service equipment is clearly marked with an "out of service" label.

General Equipment

General service equipment is maintained by cleaning and performing safety checks as necessary. Calibrations or performance checks will be necessary where the setting can significantly affect the test or analytical result (e.g., the temperature of a water bath). Instructions on the use and maintenance of general equipment are located in the laboratory at all times.

Volumetric Equipment

The correct use of volumetric equipment is critical to analytical measurements. Volumetric equipment are suitably maintained and calibrated as specified in the Equipment Records and Inventory datasheet located in the web-based storage system.

Attention is paid to the possibility of contamination arising from the equipment or cross-contamination from previous use. The type used, cleaning, storage and segregation of volumetric equipment are critical. Volumetric equipment should be sterilized with 10% bleach solution and 70% ethanol, DNA Away, or autoclaved as appropriate. Instructions on the use and maintenance of volumetric equipment are located in the laboratory at all times.

Measuring Equipment

Measuring equipment, which include the real-time qPCR instrument, must be used correctly, with care and requires stringent calibration and maintenance plans to ensure adequate performance. Such equipment shall not be used for measurement of customer test items if they go out of calibration. If this occurs, items must be re-measurement once the equipment has been re-calibrated. Operating instructions for the Applied Biosystems StepOnePlus Real-Time qPCR System are located in the laboratory office and also in the StepOnePlus Software Instrument Maintenance Manager.

Instrument/Equipment Calibration and Frequency

All measurement and test equipment having an effect on the accuracy or validity of tests are calibrated and/or verified before being put into service. Calibration records for these equipment, including calibration dates and due dates, are maintained in the Source Molecular web-based storage system. Equipment may be calibrated internally or externally. External calibration

services must be conducted by a calibration laboratory that demonstrates competence by being accredited and demonstrating measurement capability and traceability. The frequency of calibration depends on the accuracy requirements of the test, the stability of the instrument and manufacturer recommendations. It is crucial that calibration measurements are traceable to the International System of Units (SI) whenever possible. Calibration reports, traceability certificates and certificates of analysis are maintained in the Calibration Certificates binder that is kept in the laboratory office. Records are retained for 5 years or more at the discretion of the laboratory.

The procedures for checking newly received equipment are as determined by manufacturers' specification and/or those determined by the laboratory during procurement.

After repair, equipment must be calibrated, when appropriate, and verified to perform correctly by following procedures in the manufacturer's manual and/or by comparing pre-nonconformance and post-repair tests.

Anytime the equipment goes outside the direct control of the laboratory, the function and calibration status must be verified before the equipment can be returned to service. This is done by ensuring that calibration stickers and calibration reports are correct, calibration values are within a specified range (if applicable) and that all components of the instrument are functioning properly. This, along with other key information, is recorded and documentation is stored for 5 years or more at the discretion of the laboratory. When verification of the calibration status and functionality of the equipment is not possible, the equipment must be re-calibrated and serviced, respectively.

Generally, spare parts do not have to be kept on hand in the laboratory. Any parts that are needed as part of equipment servicing are provided and installed by the manufacturer or service contractor.

Inspection/Acceptance of Supplies and Consumables

For all test methods, only services and supplies of the required quality and grade are used. If the specified reagent or material is discontinued by the manufacturer, an alternative from a different manufacturer may be purchased as long as the grade and specifications are identical to the discontinued item. The Laboratory Manager verifies and approves the alternate items and the change is made in the appropriate SOP. Supplies, materials and consumables to be purchased are determined by the Laboratory Manager and entered into an electronic "order list" that includes a description of the item, the name of the vendor, the item catalogue number, the quantity and the cost and is stored for a minimum of 5 years.

Shipments are received at the receiving area and brought to the laboratory. The Laboratory Manager or other authorized personnel is responsible for checking shipments for accuracy. Packing slips are checked against package content labels and matched with the electronic order list. Certificates of analysis (COA) are verified (when applicable) to ensure the received item

meets minimum specifications. All standards, reagents, filters, and other consumable supplies are purchased from manufacturers with performance guarantees and industry recognition, and are inspected upon receipt for damage, missing parts, expiration date, and storage and handling requirements. Labels on reagents, chemicals, and standards are examined to ensure they are of appropriate quality. Reagents are marked with the "date received". Primers and plasmid DNA standards are quantitated and aliquoted for storage at -80˚C.

Once the materials are verified, the appropriate box is checked next to the item in the order list and an electronic signature is created. If a discrepancy is found that could affect the quality of laboratory output, the supplier is contacted and the material is replaced.

All supplies will be stored as per manufacturer labeling and discarded past expiration date. Long term storage of nucleic acids is in a -80˚C feezer. Whenever possible, consumables and reagents that come into contact with test samples are received pre-sterilized and disposable (e.g. filtering funnels). They are used once and not re-used. Specific information of supply and consumable vendors are specified in individual Test Method SOPs' materials list.