Ozarks Environmental and Water Resources Institute (OEWRI)
Missouri State University (MSU)

Bacteria Source Tracking to Support Watershed Planning, Pearson Creek, Greene County, Missouri.

FINAL REPORT

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SCOPE AND OBJECTIVES

Pearson Creek, a tributary of the James River, in Greene County Missouri is on the state's 303d list of impaired waters due to high concentrations of *E. coli* bacteria. Pearson Creek consistently exceeds the Missouri Department of Natural Resources (MDNR) water quality standards for Whole Body Contact Recreation (WBCR) Class-A designation of 126 MPN/100 mL from both urban and rural nonpoint pollution sources (Richards and Johnson 2002, Owen and Pavlowsky 2014, MDNR 2014, MDNR 2018). The City of Springfield and Greene County have been working to identify bacteria sources that will ultimately reduce *E. Coli* concentrations in the stream. In 2017, Greene County started monitoring bimonthly water quality at the United States Geological Survey (USGS) gaging station at Farm Road (FR) 148. That same year, the City of Springfield provided funding for a wastewater exfiltration study aimed at pinpointing specific bacteria (and other pollutants) source areas along Pearson Creek. Results of these efforts have identified the site at the FR 148 Bridge and State Highway YY as having high and sustained *E. Coli* concentrations (Owen et al. 2018). However, *E. Coli* concentrations are only an indicator of fecal contamination, but does not specifically identify the source of the pollution. Therefore, Greene County is contracting the Ozarks Environmental and Water Resources Institute (OEWRI) at Missouri State University to begin to isolate the source by performing a bacteria source tracking study on two hotspots identified along Pearson Creek. The objectives of this study are: (i) collect water samples at four sites, approximately two weeks apart to assess variability (ii) perform sample analysis that includes both IDEXX *E. Coli* counts and identification of human and bovine markers, and (iii) reporting of results to Greene County.

STUDY AREA

The Pearson Creek Watershed (12-digit Hydrologic Unit Code (HUC) 110100020107) is located in east Springfield in Greene County, Missouri. The watershed is approximately 59.2 km^2 (22.9 mi^2) and drains the eastern edges of Springfield and flows south to its confluence with the James River (Figure 1). The underlying geology of the watershed is Mississippian age limestone within which a karst landscape has formed where sinkholes, losing streams, and springs are common (Bullard et al. 2001). There are 23 mapped springs within the basin with the largest being Jones Spring in the southwest portion of the watershed. Land use of the watershed ranges from high-low density urban in the western half of watershed to residential, livestock grazing, and forage crop production outside the city limits to the east (Hutchison 2010). Samples were collected at four sites located along the main stem of Pearson Creek. These sites were located at bridges over the stream at Farm Road 193, Farm Road 148, Farm Road 144 (E. Catalpa St.), and State Highway YY (Division St.) (Table 1, Figure 1, Photos 1-4). Drainage areas ranged from 26.5 km^2 at PC_SHYY to 56.2 km^2 at PC_193 (Table 2).

METHODS

Water Sampling

Samples were collected on August 7th and August 16th**.** During the first sampling day a duplicate sample was collected from two randomly selected sites and during the second sampling day duplicate samples were collected from the other two sites. Water samples were collected in 8 L sterilized polypropylene carboy containers. All samples were placed on ice after collection and transported to the Microbiology Laboratory at MSU within two hours of sampling. During the bacteria source tracking sampling, additional samples were collected for quantifying bacteria using the IDEXX method to compare with the source tracking results. These samples were collected in sterile 125 mL plastic bottles and processed in the OEWRI laboratory at MSU within two hours of sample collection. Duplicate samples were also collected at the same locations as the bacteria source tracking duplicates.

Laboratory Methods

IDEXX

The IDEXX Quanti-Tray/2000 system is used to analyze water samples for the presence of total coliform and *E. coli* following manufacturers recommendations and laboratory SOPs (OEWRI, 2013).

DNA Extraction

From all water samples, one liter of water per sample was filtered through 0.22 μm Sterivex filters (Millipore Corporation, MA) using a peristaltic pump (Masterflex, Cole–Pamer Co, Vernon Hills, IL, USA). Filters were broken and membranes were removed and cut into small pieces using sterile scissors and then placed in 1.5 mL micro-centrifuge tubes that were used for DNA extraction. Genomic DNA from the 12 water samples was extracted using PowerSoil DNA isolation kit (Mo Bio, Carlsbad, CA). All extraction steps were followed according to the manufacturer's instructions, and DNA samples were stored at -20°C until analyzed.

Real-time PCR for specific marker genes

Bacteroidetes specific to human and bovine fecal bacteria were determined using qPCR. Detection of bacterial contamination of human and bovine fecal material was performed using the group-specific primers (Table 3). These assays were carried out using the same master mix concentrations and qPCR-cycling conditions as described previously (Mirza et al., 2017). Briefly, qPCR was carried out in 25 μL volumes containing 12.50 μL of iTaq Fast SYBR green supermix with ROX (Bio–Rad, Inc., Hercules, CA), 100 nM primers, and 10 ng of template DNA. PCR conditions were as follows: 94°C for 2 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 1 min, and extension at 72°C for 30 s. PCR grade water was used as a negative control. The specificity of the qPCR products was confirmed by melting curve

analysis. A standard curve was generated from serial dilutions (100 to 10–9) of plasmid DNA or serially diluted PCR product of the specific marker gene. The qPCR efficiency (E) was calculated according to the equation $E = 10^{[-1/slope]}$. Detection limits for this analysis range from 10-50 copies per 1,000 mL.

QA/QC

The PCR primer combination used in this study has been previously well tested and optimized for the specific amplification of bacterial marker genes from human (Green et al., 2014a; Ahmed et al., 2015) and bovine (Ravaliya et al 2014; Shanks et al., 2010) fecal materials. The positive standard DNA material (plasmid or gene amplicon) that was amplified from the fecal material of different source animals (human and cattle) showed consistent PCR amplification. This was used as a reference material for our unknown water samples. The negative samples (sterile water) did not show any amplification. The regression line of the standard curve generated through serial dilutions of specific marker genes showed a coefficient of determination of >0.99 and a PCR amplification efficiency of 100% (+/- 10%). The specificity of the amplicon was confirmed by the melting curve analysis, which indicated the presence of a single peak for each marker gene (Figure 4).

RESULTS AND DISCUSSION

Hydrology

Discharge during both sampling dates occurred during low flow conditions where stage was similar, but flow exceedance was lower during the second sampling date suggesting low discharge variability in this watershed. For samples collected on August 7th the stage was 1.96 ft and the discharge was 3.12 ft^3/s (Table 4). On August 16th, the stage was 2.11 ft and the discharge was 5.70 ft^3/s. The stage was only 0.15 ft higher on August 16th, the discharge was about 2.5 ft^3/s higher compared to August 7th. While the stage increased only 8% between the two sampling days, the discharge increased 83%. The August 7th sampling date was at the end of a very low flow period that lasted over a week (Figure 2). In contrast, the August 16th sampling occurred after a series of small runoff events that raised the base flow level compared to the August 7th event. However, a flow duration curve created from the 2018 water year data from the gage shows that the discharge during the August 7th had a flow exceedance of 96% while the discharge during the August 16th event had a flow exceedance of 60% (Figure 3). This is an important point hydrologically as discharge went from a fairly low base flow on August 7th to a fairly high base flow on August 16th with a little increase in stage. This suggests that small differences in stage can be significant in this watershed that has relatively low variability throughout the year.

IDEXX Results

Results of the bacteria analysis using the IDEXX method showed higher concentrations at three of the four sites during the low flow sampling event on August 7th compared to high flow event on August 16th suggesting there may be a dilution effect during the second sampling day. On August 7th, *E. Coli* concentrations ranged from 290.9 MPN/100 mL at PC_SHYY to 1,732.9 MPN/100 mL at PC_148 (Table 5). The second highest concentration was at PC_193 just downstream of PC_148 at 613.1 MPN/100 mL. On August 16th, *E. Coli* concentrations ranged from 78.5 MPN/100 mL at PC_193 to 770.1 MPN/100 mL at PC_SHYY (Table 6). The second highest concentration during this sampling day was at PC_148 at 325.5 MPN/100 mL. Overall, concentrations were higher during the August 7th sampling that was during a relatively low base flow period for all sites but PC_SHYY suggesting dilution from storm runoff may have lowered the concentration at three of the four sites that may be influenced by a local pollution source. However, the site at PC_SHYY was 2.5 times higher on August 16th compared to August 7th suggesting the pollution source at this site is relatively inconsistent. Duplicate analysis shows relative percent difference (RPD) between samples ranged from 5.9% to 45.4% and averaged about 23% for this study. Additionally, total coliform concentrations for all samples exceeded the upper limit of the IDEXX analysis, which is 2,419.6 MPN/100 mL.

qPCR Results

The results of the qPCR results show positive human and bovine specific markers were found during sampling, but the results were not consistent. On August 7th, site PC_148 was positive for a bovine specific marker at 586 copies/1,000 mL (Table 8). However, no other sites were positive for human or bovine specific markers despite having relatively high *E. Coli* concentrations from the IDEXX test method. On August 16th, the PC_SHYY was positive for both bovine (1,920 copies/1,000 mL) and human (561 copies/1,000 mL) markers while the remaining sites were non-detect. During the August 16th sample day PC_SHYY had the highest *E. Coli* concentrations measured from the IDEXX method and it was the only site that had an increase in *E. Coli* concentration during this sample day. Again this suggests there is an inconsistent cattle and human source at this site. Furthermore, there are no sanitary sewers located upstream of PC_SHYY suggesting onsite wastewater systems are the source of the human specific markers. Field duplicates were all non-detect for this project. While these results are somewhat inconclusive, these data provide important information on bacteria pollution source variability over a short timeframe.

CONCLUSIONS

There are five main conclusions from this project:

1. Sampling occurred during base flow conditions in August 2018, but the August 7th sampling was a low base flow event and the August 16th was a relatively high base flow even though the stage was only 0.15 ft higher, but the discharge nearly doubled. Flow exceedance for August 7th sample discharge was 96% and flow exceedance was 60% for the August 16th sample discharge. This shows low hydrologic variability in the flow duration curve at this gaging station when such a small change in stage can represent such a large difference in flow exceedance compared to the 2018 water year flow duration curve.

2. Results of the IDEXX based analysis show total *E. Coli* concentrations varied by event suggesting pollution sources may differ among sites. During the low base flow sampling on August 7th, *E. Coli* concentrations were much higher at PC_193, PC_148, and PC_CAT compared to the August 16th sampling at a slightly higher stage. This indicates the higher flow is diluting the *E. Coli* concentrations at these sites suggesting a localized source. In contrast, the site at PC_SHYY had higher *E. Coli* concentrations during the higher flow sampling suggesting an inconsistent source at this site.

3. There were no positive human specific markers found at PC_148. Past studies along Pearson Creek show increased concentrations of *E. Coli* were found around sanitary sewer infrastructure that crossed or that was in close proximity to the stream. However, these data indicate that no human specific markers were found during the two sampling dates and provides evidence that bacteria pollution in the stream are from another source. More sampling may be needed to replicate this finding since high bacteria and nutrient inputs have been routinely associated with this site in the past.

4. There were positive bovine specific markers found at PC_148 and PC_SHYY, but not during both sampling days. Bovine specific markers were found on August 7th at PC_148 and at PC_SHYY on August 16th. IDEXX results indicate that PC_148 has a localized bacteria source and the positive bovine specific markers suggest it is from cattle. At PC_SHYY, no positive bovine specific markers were found during the August 7th sampling, but were found during August 16th sampling. *E. Coli* concentrations at this site were also higher during the August 16th sampling indicating an inconsistent source following a series of small storms may be contributing to the bacteria pollution at this site and the positive bovine specific markers suggest it is from cattle operations.

5. Human specific markers were found at PC_SHYY on August 16th, but there are no sanitary sewers upstream of this site indicating onsite wastewater sources during the slightly wetter

conditions. During the low base flow event on August 7th no human specific markers were found at PC_SHYY. During the higher base flow event on August 16th human specific markers were identified indicting a domestic wastewater pollution source. However, no sanitary sewers are located upstream of this site indicating the pollution source is from onsite wastewater systems that are not functioning properly that may be connected to the stream during wetter conditions and not connected during dryer conditions of August 7th.

REFERENCES

Bullard, L., K.C. Thomson, and J.E. Vandike, 2001. The Springs of Greene County Missouri. Missouri Department of Natural Resources Geological Survey and Resource Assessment Division. Water Resources Report No. 68.

Hutchison, E., C., D., 2010. Mass Transport of Suspended Sediment, Dissolved Solids, Nutrients and Anions in the James River, SW Missouri. Unpublished Masters Thesis, Missouri State University.

Layton, A., L. Mckay, D. Williams, V. Garrett, R. Gentry, and G. Sayler (2006) Development of Bacteroides16S rRNA geneTaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. Appl. Environ. Microbiol. 72:4214–4224.

Mirza, B.S., D.L. Sorensen, R.R. Dupont, and J.E. McLean (2017). New arsenate reductase gene (arrA) PCR primers for diversity assessment and quantification in environmental samples. Appl. Environ. Microbiol 83: eAEM2725-16.

Missouri Department of Natural Resources (MDNR) (1998) Section 303(d) Waters State of Missouri. Prepared by the Missouri Clean Water Commission.

Missouri Department of Natural Resources (MDNR) (2014). The Code of State Regulations (CSR); Division 20, Clean Water Commission. Chapter 7-Water Quality, pp 1-47.

Missouri Department of Natural Resources (MDNR) (2018). 2018 EPA Approved Section 303(d) Listed Waters. https://dnr.mo.gov/env/wpp/waterquality/303d/docs/2018-303d-list-cwc-approved-1-4-2018.pdf

Owen, M.R. and R.T. Pavlowsky (2014). Water Quality Assessment and Load Reductions for Pearson Creek, Springfield, Missouri. Final Report to the James River Basin Partnership. Ozarks Environmental and Water Resources Institute EDR-14-001.

Owen, M.R., R.T. Pavlowsky, and G. Roman (2018). Wastewater Exfiltration Sources

in Pearson Creek, Springfield, Missouri. Ozarks Environmental and Water Resources Institute EDR-18-001.

Ozarks Environmental and Water Resources Institute (OEWRI) (2013) Standard Operating Procedure for: Escherichia coli and Total Coliform using the IDEXX Quanti-Tray/2000 System with Colilert Reagent. Ozarks Environmental and Water Resources Institute, Missouri State University.

Richards, J. M. and B. T. Johnson (2002). Water Quality, Selected Chemical Characteristics, and Toxicity of Base Flow and Urban Stormwater in the Pearson Creek and Wilsons Creek Basins, Greene County, Missouri, August 1999 to August 2000. Water-Resources Investigations Report 02-4124, United States Geological Survey.

United States Environmental Protection Agency (2011). Region 7 Total Maximum Daily Load Pearson Creek Greene County, Misssouri. https://dnr.mo.gov/env/wpp/tmdl/docs/2373-pearson-ck-tmdl.pdf

TABLES

Table 1. Sample site locations

Site	North_m	East_m	Location
PC_SHYY	4,119,561.62	484,841.75	E State Highway YY (E Division st.) bridge
PC_Cat	4,115,668.01	482,249.92	E FR 144 (E Catalpa st.) bridge
PC_148	4,114,632.82	482,382.21	FR 148 bridge south of State Highway D
PC_193	4,114,034.20	482,560.81	FR 193 bridge near railroad tracks

Table 2. Drainage area characteristics

Site	Ad (km^2)	% Urban	% Agriculture	% Forest	% Water	% Other
PC_SHYY	26.5	11.8	72.1	14.4	0.4	1.2
PC_Cat	49.3	17.7	64.6	16.4	0.4	0.9
PC_148	55.4	22.7	58.9	17.1	0.5	0.9
PC_193	56.2	22.8	58.5	17.3	0.5	0.9

Table 3. PCR Primers used in qPCR

Primer	Sequence	Marker	Reference
F Primer HF183	5'- ATCATGAGTTCACATGTCCG	Human	Layton et al., 2006
R Primer SSHBacR	5'- TACCCCGCCTACTATCTAATG		
BoBac367f	5′- GAAG(G/A)CTGAACCAGCCAAGTA	Bovine	Layton et al., 2006
BoBac467r	5′- GCTTATTCATACGGTACATACAAG		

Table 4. Hydrology at USGS gaging station at Farm Road 148 during sampling

Date	Time	Stage (ft)	Q (ft^3/s)	% Exceedance
8-7-2018	9:30 am	1.96	3.12	96%
8-16-2018	2:45 pm	2.11	5.70	60%

Table 5. IDEXX sample results from August 7th

Site	Time	Total Coliform (MPN/100 mL)	*E. coli* (MPN/100 mL)
PC_SHYY	10:00 AM	>2419.6	290.9
PC_Cat	9:40 AM	>2419.6	488.4
PC_148	9:30 AM	>2419.6	1,732.9
PC_193	9:20 AM	>2419.6	613.1

Table 6. IDEXX sample results from August 16th

Site	Time	Total Coliform (MPN/100 mL)	*E. coli* (MPN/100 mL)
PC_SHYY	3:15 PM	>2419.6	770.1
PC_Cat	3:00 PM	>2419.6	167.0
PC_148	2:45 PM	>2419.6	325.5
PC_193	2:35 PM	>2419.6	78.5

Table 7. Duplicate analysis of IDEXX samples

Site	Date	Total Coliform (MPN/100 mL)	*E. coli* (MPN/100 mL)
PC_SHYY	8/7/2018	>2,419.6	290.9
PC_SHYY	8/7/2018	>2,419.6	344.8
	RPD		-17.0
PC_Cat	8/7/2018	>2,419.6	488.4
PC_Cat	8/7/2018	>2,419.6	307.6
	RPD		45.4
PC_193	8/16/2018	>2,419.6	78.5
PC_193	8/16/2018	>2,419.6	83.3
	RPD		-5.9
	Avg. RPD		**22.8**

Table 8. Group Specific Bacterial Contamination

Copies per 1,000 mL of water. Non-detect (-). Duplicate samples in yellow.

Sample	Human	Bovine
August 7th		
PC_SHYY	-	-
PC_SHYY	-	-
PC_Cat	-	-
PC_Cat	-	-
PC_148	-	586
PC_193	-	-
August 16th		
PC_SHYY	561	1,920
PC_Cat	-	-
PC_148	-	-
PC_193	-	-
PC_193	-	-

FIGURES

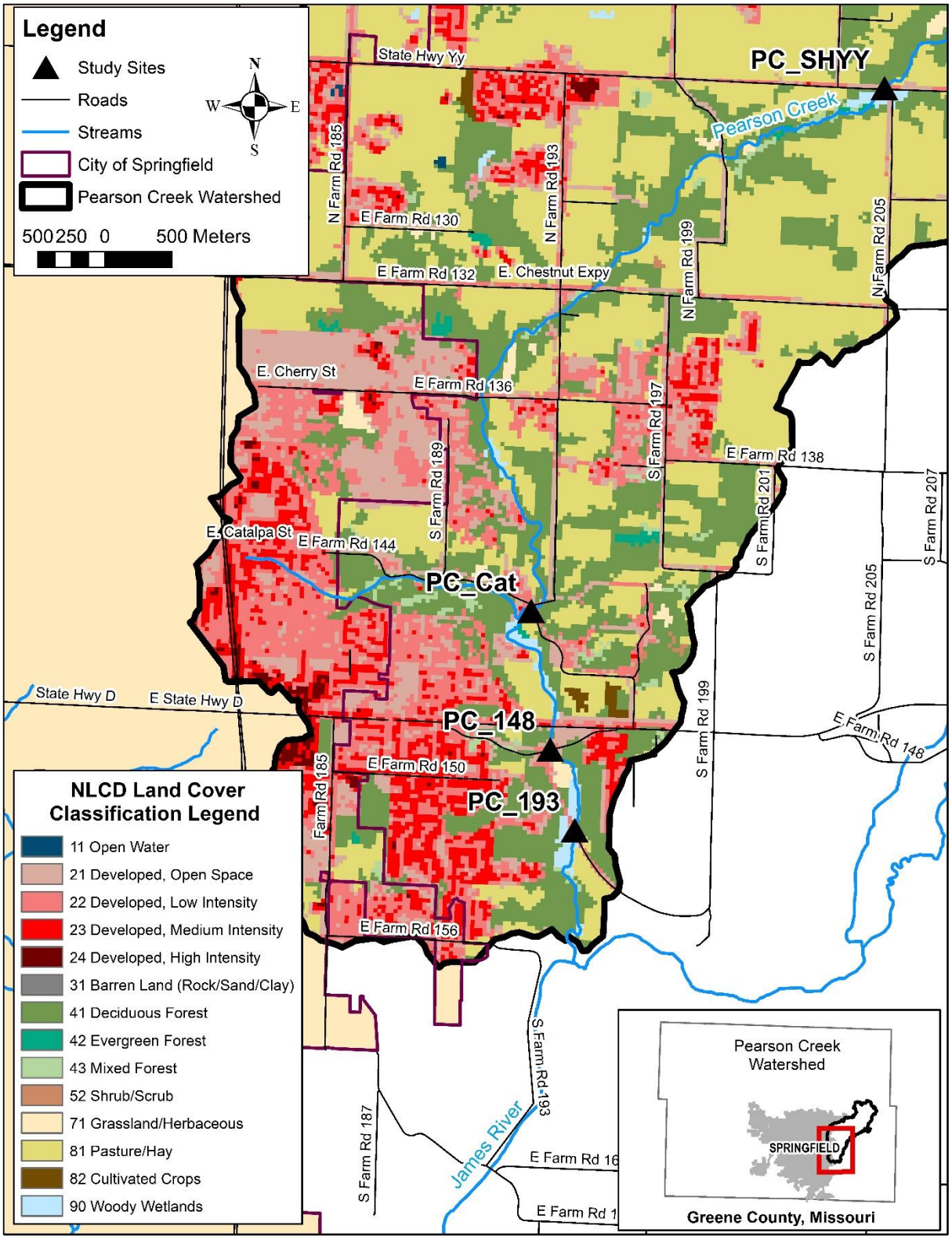

Figure 1. Land use map and sample locations.

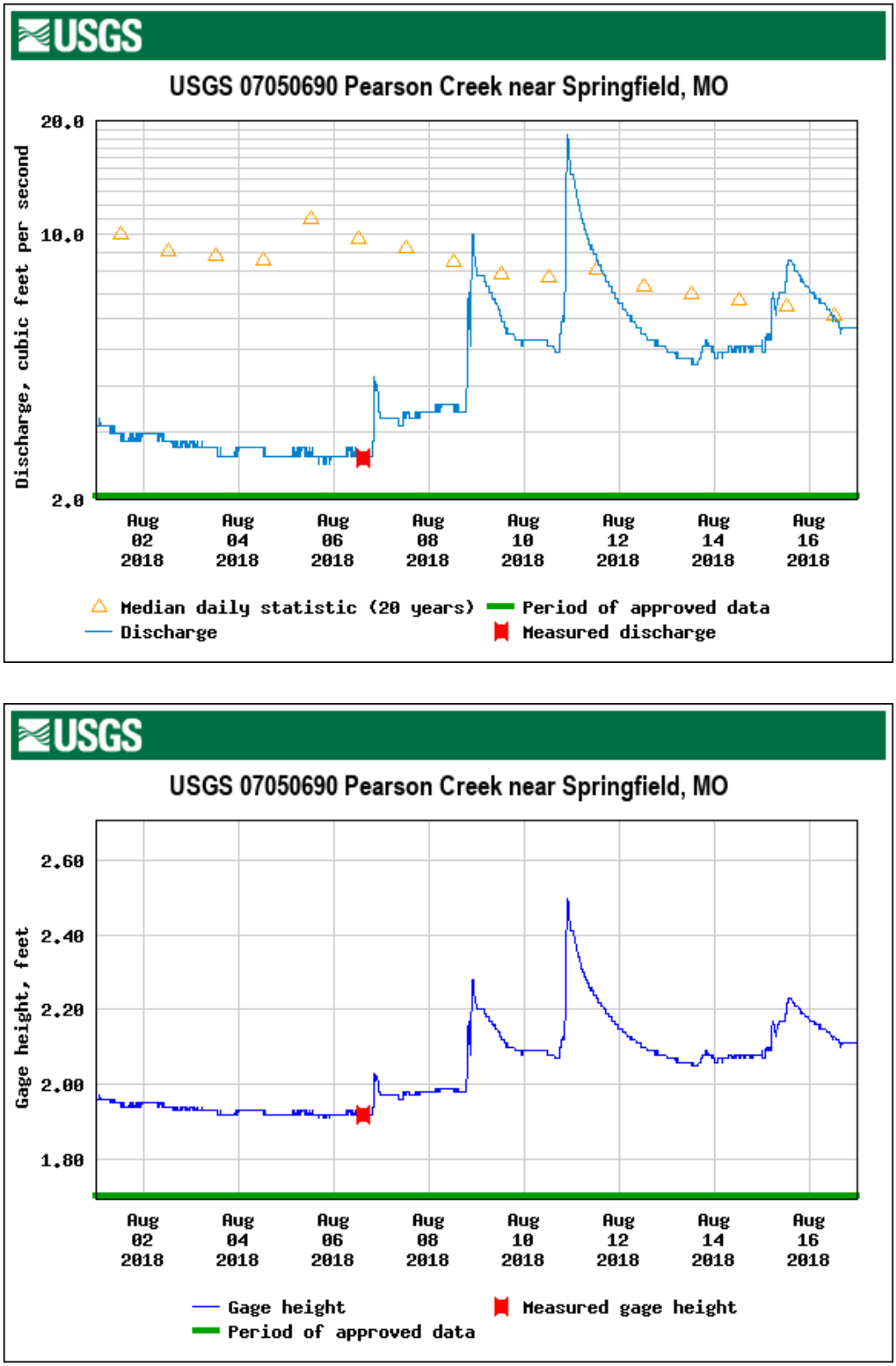

Figure 2. Hydrograph (A) and stage (B) from the USGS gaging station on Pearson Creek near Springfield over the study period.

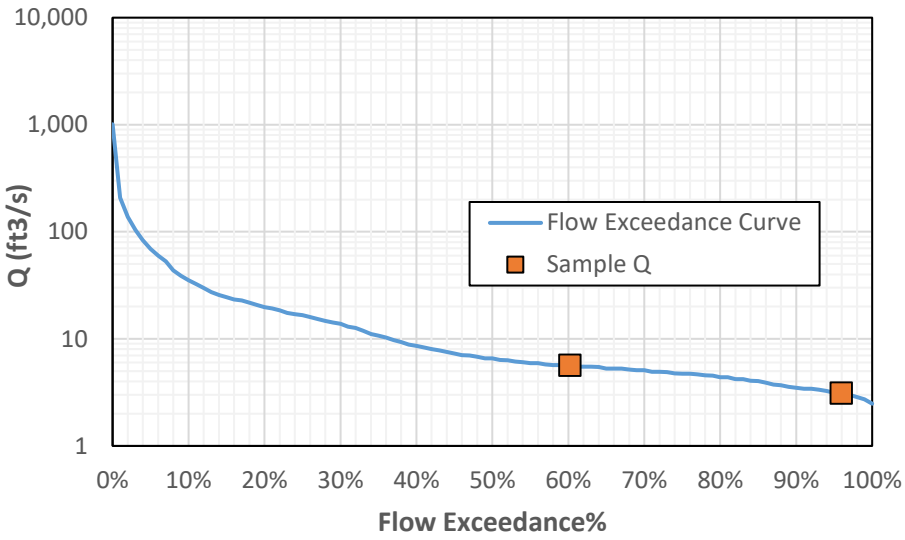

Figure 3. Flow exceedance curve for water year 2018 at the USGS gaging station on Pearson Creek near Springfield with sample discharge at the FR_148 site.

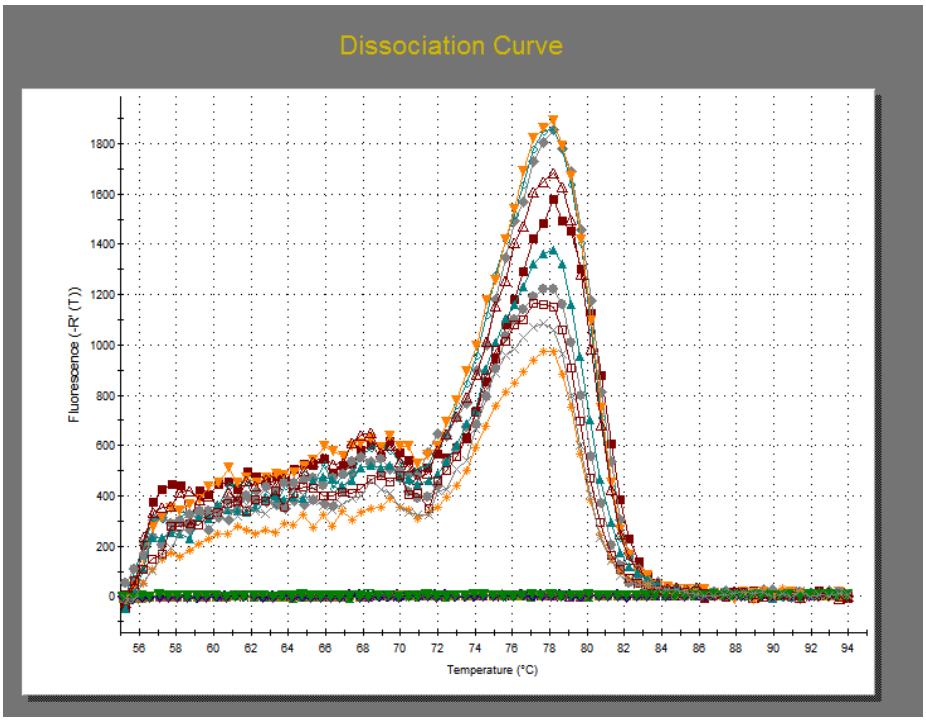

Figure 4. A dissociation curve of bovine specific primers indicating a specific amplification of single DNA fragment.

PHOTOS

Photo 1. Looking upstream at the FR 193 Bridge. Site PC_193 (8-7-18)

Photo 2. Looking upstream at the FR 148 Bridge. Site PC_148 (8-7-18)

Photo 3. Looking upstream at the FR 144 (Catalpa) Bridge. PC_Cat (8-7-18)

Photo 4. Looking upstream at the State Highway YY (Division St.) Bridge. PC_SHYY (8-7-18)