CWA Section 319 Nonpoint Source Pollution Control Program Assessment Project

Final Report

February 17, 2016

CROOK COUNTY NATURAL RESOURCE DISTRICT

BELLE FOURCHE RIVER WATERSHED PROJECT IMPLEMENTATION: PHASE III

PROJECT NUMBER: NPS2011D/2012B GRANT NUMBER: C9008630-11, 12

This Project was conducted in cooperation with the State of Wyoming and the United States Environmental Protection Agency, Region 8.

SECTION 1.0 EXECUTIVE SUMMARY

Project Title: Belle Fourche River Watershed Plan-Phase III

Project Start Date: May 1, 2012

Project Completion Date: December 31, 2015

Budget Summary

In Accordance with Final Agreement (Amendment #3)

Total 319 Funds Awarded: $382,130.00

Total 319 Funds Expended: $382,130.00

Total Non-federal Match Commitment: $220,080.00

Total Non-federal Match Expended: $222,376.90

Total Project Budget: $604,210.00

Total Project Expenditures: $604,506.90

Accomplishments Summary

The Crook County Natural Resource District's (CCNRD), Belle Fourche River Watershed Plan Implementation Project-Phase III built upon past accomplishments from Phases I-II and was the third of six planned implementation phases to address TMDLs in the Belle Fourche River Watershed (Watershed). The purpose of the Phase III workplan was to direct the District's efforts at addressing fecal coliform and *Escherichia coli* (*E. coli*) impairments in accordance with actions outlined in *Belle Fourche River Watershed TMDLs for Pathogens, Ammonia, and Chloride*.

The CCNRD's Nonpoint Source Program is focused on four watershed improvement approaches: water quality monitoring, stakeholder participation, community education and outreach, and implementation of Best Management Practices (BMPs) to reduce total coliform loading and *E. coli* concentrations in the Watershed. Funding received to complete Phase III allowed for: the collection of 538 water quality samples in the Belle Fourche River Watershed, the continuation of information and education outreach programs via tours, presentations, and 16 school programs, and the coordination of a landowner advisory committee (now made-up of the Board of Supervisors) for the purpose of seeking local citizen leadership and guidance. Under Phase III, the District was also able to secure 14 water quality improvement agreements with landowners and complete four septic remediation projects.

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SECTION 2.0 BACKGROUND

The 2010 Wyoming Department of Environmental Quality's 303(d) list included five impaired segments on two waterbodies within the Upper Belle Fourche River subbasin. A significant portion of the two impaired waterbodies, Donkey Creek and the Belle Fourche River, are located within Crook County, Wyoming. Donkey Creek, a class 3B stream, is categorized as impaired for recreational designated uses due to levels of fecal coliform from the Belle Fourche River upstream to an undetermined distance above Antelope Butte Creek, (approximately 56-miles). The Belle Fourche River is a class 2ABww stream with four impaired segments within Crook County: a segment categorized as impaired for recreational designated uses due to levels of fecal coliform from Arch Creek downstream to Sourdough Creek (approximately 58.5-miles); a segment from Keyhole Reservoir upstream to Donkey Creek is categorized as impaired for recreational designated uses due to elevated *Escherichia coli* (*E. coli)* levels, including another segment directly upstream of the confluence with Donkey Creek to an undetermined distance above Rush Creek (combined total of 26.6-miles); the fourth impaired segment of the Belle Fourche River is impaired for warm water aquatic life designated uses due to levels of ammonia and chloride from Keyhole Reservoir upstream to Donkey Creek (approximately 21.2-miles).

Streamflow throughout the Watershed varies over time and is influenced by a number of natural and anthropogenic factors. For example, a variety of the intermittent streams located throughout the western portion of the Watershed develop into perennial streams when influenced by discharges from coal mines and wastewater treatment facilities. Conversely, the eastern portion of the Watershed is dominated by perennial streams draining from the Black Hills. Peak flows are typically between March and June and the low flows occur in the months of November and December. In addition to habitat provided for various aquatic life, the Belle Fourche River provides numerous recreational opportunities, particularly where it forms Keyhole Reservoir. The Belle Fourche River also is an important irrigation source for cropland in northeastern Wyoming.

When the Wyoming Department of Environmental Quality (DEQ) last evaluated the water quality of the Belle Fourche River, the results indicated that most waterbody segments were in attainment of their designated uses. However, within Crook County, the aforementioned segments on Donkey Creek and the Belle Fourche River did not support their designated recreation uses. Multiple agencies and organizations throughout the Belle Fourche River Watershed collected data and information that were used to develop a Total Maximum Daily Load (TMDL) document and implementation plan. The DEQ contracted development of the TMDLs to Tetra Tech who submitted a total of seven TMDLs in a single document during 2011. The revised TMDLs for five stream segments were finalized for DEQ in August 2013 and approved later that year by the Environmental Protection Agency (EPA). The *Belle Fourche River Watershed TMDLs for Pathogens, Ammonia, and Chloride* document and implementation plan were used as guiding documents for the scope of this Project. The WDEQ has reviewed the implementation plan associated with the TMDLs and determined that it addresses the nine key elements of watershed-based planning established by EPA. The scope of this Project parallels the recommended implementation activities used to reduce fecal coliform and *E. coli* loads to achieve reductions identified in the TMDL. In addition to the implementation plan for *Belle Fourche River Watershed TMDLs for Pathogens, Ammonia, and Chloride*, this project was also consistent with the CCNRD's long-range plan and the *Wyoming Nonpoint Source Management Plan*.

While the sources of the pollutants of concern are mostly listed as "unknown" in the TMDL, there are a number of potential sources that exist within the Belle Fourche River Watershed. Potential *E. coli* sources include, but are not limited to: septic systems, wastewater treatment plants (WWTPs) and municipal WWTPs, corrals, feedlots, and pastures adjacent to or near surface waters, pet waste, recreational vehicle waste, and wildlife sources. Activities under Phase III were directed at addressing bacteria impairments for streams in the Belle Fourche River Watershed (within Crook County) via public outreach and adaptive techniques to implement effective Best Management Practices (BMPs) and reduce pollutant loads. Water quality data collected will be used to determine the effectiveness of BMPs over the life of the TMDLs to ensure that project goals are being achieved. Monitoring

under this project also enabled the District to better identify long-term trends in pollutant sources causing the impairments.

The *Belle Fourche River Watershed TMDLs for Pathogens, Ammonia, and Chloride* (TMDL document), identified the following load reduction recommendations for *E. coli*:

- In the upper Belle Fourche River, an *E. coli* load reduction of 82% during high flow conditions needs to be achieved throughout the primary contact recreation season (May through September).
- In the lower Belle Fourche River, an *E. coli* load reduction of 85% during high flow conditions needs to be achieved throughout the primary contact recreation season (May through September).
- In Donkey Creek, an *E. coli* load reduction of 89% during high flow conditions, 57% during moist flow conditions, 20% during mid-range flow conditions, 54% during dry flow conditions, and 50% during low flow conditions need to be achieved during the primary contact recreation season (May through September).

Data summarized in the TMDL document indicated that in the Belle Fourche River above Keyhole Reservoir, *E. coli* samples were consistently greater than water quality standards across all flow zones except the low flow zone during the summer. The TMDL targets were not exceeded during the winter according to the document. In the Belle Fourche River below Keyhole Reservoir, *E. coli* samples were less frequently above water quality standards than in Donkey Creek or the Belle Fourche River upstream. This is likely due to factors such as increased UV exposure and less aerobic conditions common in a reservoir setting. In Donkey Creek, *E. coli* samples routinely failed to meet water quality standards during the summer during all flow conditions. The Crook County Natural Resource District (CCNRD) has not had the resources thus far to monitor in the winter, therefore data during the winter have not been available to evaluate.

SECTION 3: GOALS AND OUTCOMES

The CCNRD's overarching goal under the Phase III project was to reduce fecal coliform and bacteria loading to waterbodies within the Belle Fourche River Watershed (within Crook County), that are listed as impaired for recreation use due to high levels of *E. coli*/fecal coliform. To accomplish this, the District: 1) Worked with the District Board of Supervisors (BoS) and landowners to implement Best Management Practices (BMPs) in alignment with the recommendations from the Belle Fourche River Watershed TMDLs, 2) Conducted public education and outreach to raise the community's awareness of fecal coliform and *E. coli* impairments in the Belle Fourche River Watershed, and 3) Continued its water quality monitoring to further identify pollutant sources and evaluate project effectiveness. Specific goals identified for each task under the Phase III Project Implementation Plan included:

- *Conduct Efficient and Effective Project Management and Administration*
- *Coordinate with Landowner Advisory Committee (LAC) to Ensure Water Quality Remediation Efforts are Locally Led*
- *Increase TMDL Awareness, BMP Implementation and Public Outreach*
- *Continue Water Quality Sampling & Analysis in Addition to Monitoring for BMP Effectiveness*
- *Utilize Microbial Source Tracking (MST) as a Tool to Assist in Determining Bacteria Sources in Impaired Streams*
- *Implement BMP Projects for Septic, Off-Stream Watering & AFO/Wintering*
- *Generate Final Report to WDEQ with Outcomes from Project*

To reduce fecal coliform and *E. coli* levels in the Belle Fourche River Watershed within Crook County under Phase III, the District initially planned to concentrate on the following BMP targets: 1) Animal Feeding Operation (AFO), Improvements (including practices such as livestock management and grazing planning, as well as wintering

BMPs), 2) Off-Stream Watering Projects (including alternative watering techniques and riparian corridor BMPs), and lastly, 3) Septic System Remediation Projects.

Based on landowner site needs and interest in voluntary cost-share assistance, the BMPs implemented under Phase III consisted mostly of off-stream watering projects that were approved by the BoS. It could be argued that many of these projects also met objectives considered under AFO practices as the projects implemented resulted in improved plant health and productivity and less impact to riparian areas. Practices such as fencing and watering tanks were installed to enable ranchers to split up pastures to allow for intensive, rotational grazing patterns. Tanks installed provided more seasonal watering sources for livestock, thus creating a more uniform grazing plan on rangeland and less reliance on the Belle Fourche River and the tributaries that drain into it. The aforementioned rangeland practices also reduced the frequency of livestock directly defecating in surface waters, thereby reducing the concentration of pathogens and other pollutants entering waterbodies.

Besides improving in-stream water quality and aquatic habitat conditions, the use of off-stream watering systems reduced grazing impacts to the riparian zone of the Belle Fourche River and its tributaries. Riparian buffers are critical to improving and protecting water quality by filtering pollutants from runoff from adjacent lands before the runoff reaches surface waterbodies. Lastly, the CCNRD continued to provide cost-sharing assistance to remediate failing or failed septic systems for the purpose of reducing fecal coliform and *E. coli* concentrations. Reducing pathogen effluent from failed septic systems was achieved by working with the District Board of Supervisors and County officials to identify high priority areas to provide assistance to landowners who met specific eligibility. Septic system remediation projects funded under Phase III comply with the WDEQ Nonpoint Source Program Septic System Eligibility Criteria.

Beyond project implementation, the District achieved its goal of raising the awareness of water quality issues within the County. In addition to classroom lessons and field tours of projects completed, the District met regularly with County leaders as well as resource managers from other agencies to discuss monitoring results and projects completed under Phase III. Although it is hoped that even more outreach activities will be accomplished under Phase IV, the District made great strides in building relationships with landowners and working cooperatively to install practices that benefit the landowners as well as water quality.

Through the assistance of the state and cooperating landowners, the monitoring program continued to operate successfully and contributed to the development of a long-term dataset for use in evaluating water quality trends. For numerous reasons including landowner support, logistics, and budget constraints, the District focused its water quality monitoring efforts on the segment of the Belle Fourche River and Donkey Creek for which TMDLs were prepared. This enabled the District to collect data that can be used to help assess whether TMDL reduction goals are being met. Monitoring locations were determined by landowner cooperation and proximity to potential pollutant sources identified in the TMDL report. The District also prioritized having monitoring sites above and below the confluence of the Belle Fourche River and Donkey Creek to assess the impact Donkey Creek has on water quality along the Belle Fourche River. Therefore, monitoring data collected under Phase III can be used to evaluate the cumulative effectiveness of BMP projects implemented in the long-term. At present, the monitoring data does not indicate any considerable changes in water quality for the listed segment of the Belle Fourche River and Donkey Creek. This is not surprising given the scale of pollutant loading in the watershed, the remaining opportunities for additional BMP implementation, and the time it can take to observe positive water quality trends after practices are installed. Whereas the monitoring data provides a long-term view of water quality conditions in the impaired section of the Watershed, more immediate, quantifiable results on BMP effectiveness are also included in this report. Calculated using State-derived or accepted methods, pollutant load reductions provided in Section 5 reflect the benefits of BMPs and septic remediation projects completed.

SECTION 4.0 TASK ACTIVITIES

The following table provides a breakdown of accomplishments under the Phase III workplan by task:

Table 1. Task Summary

Task #1: Administration	
Task Description	Administer Duties as Required by Section 319; Manage the Project; Generate and File Reports, Contracts, and Reimbursement Requests.
Actual Deliverables	• Submittal of 12 Quarterly Reimbursement Requests; 6 Semi-Annual and 3 Annual Progress Reports; Annual MBE/WBE reports. • Administration of Phase III Contract (fiscally and task implementation-wise).
Task #2: Landowner Advisory Committee	
Task Description	Coordinate Project Guidance and BMP Implementation with the Belle Fourche River Watershed "Landowner Advisory Committee" (LAC).
Actual Deliverables	• Conducted 43 Board of Supervisor Meetings (BoS); Provided 41 monthly updates at County Commissioners' meetings; Provided correspondence and minutes of BoS meetings to non-attendees.
Task #3: Information and Education	
Task Description	Conduct 30 Information and Education Activities to Increase TMDL Awareness; Promote Voluntary BMP Implementation; Ensure that Community Leaders are Involved in Directing Educational Efforts; Inform and Educate Watershed Stakeholders and General Public about Nonpoint Source Pollution; Encourage the Public to Communicate with Municipal/County Elected Officials; Initiate Conversation at Meetings with Crook County Board of Commissioners; Encourage Neighboring Landowners to Conduct Successful BMP Projects; Coordinate a Pet Waste Awareness Campaign.
Actual Deliverables	• Organized two CCNRD watershed/BMP tours; website postings; 16 Classroom Visits and/or Water Quality Demonstrations using Enviroscape Models; two presentations, distribution of flyers and other outreach materials; posted monthly Board meeting outcomes and informational articles in the local newspapers.
Task #4: Water Quality Monitoring	
Task Description	Continue Water Quality Monitoring on Belle Fourche River and Donkey Creek to Measure TMDL Pollutant Load Reductions and Evaluate BMP Implementation Effectiveness.
Actual Deliverables	• During the period 2013-2015, 538 water quality samples were collected. This number is based on *E. coli*, ammonia, chloride, nitrate-nitrogen, and nitrite-nitrogen samples obtained; it does not include flow measurements, duplicates and field blanks or basic physical or chemical parameters also collected each sampling event. Basic water quality parameters recorded include temperature, pH, conductivity, total dissolved solids (TDS) and turbidity. • Maintained up-to date Sampling and Analysis Plan. • Memorandum of Understanding with Campbell County Conservation District for monitoring assistance as needed. In 2014, both District's monitoring staff assisted each other in data collection.
Task #5: Microbial Source Tracking (MST)	
Task Description	• Utilize Microbial Source Tracking (MST) as Recommended by the Belle Fourche River Watershed TMDLs to Assist in Determining Bacteria Pollutant Sources in Impaired Streams.
Actual Deliverables	• Tested for 3 Host Sources (Canine, Cow or Human) ; Conducted 42 quantification tests for each host over the 2013-2014 primary contact seasons at three sites using pyrosequencing analysis. *Results were inconclusive.* • Tested for 3 Host Sources (Canine, Cow or Human) ; Conducted 20 quantification tests for each host over the 2015 primary contact season at three sites using quantitative

	polymerase chain reaction (qPCR) analysis. Tests for which sources were identified indicated low concentrations of ruminant markers (in sampling area, ruminant sources include antelope, deer, and cow). The only moderate concentration of markers observed was for one sampling event on Donkey Creek; the tests indicated humans as the source.
Task #6: BMP Implementation	
Task Description:	Employ BMP Implementation as Recommended by the Belle Fourche River Watershed TMDLs to Reduce *E. Coli* Loading to Impaired Streams.
Actual Deliverables	• Implemented 4 septic system replacement projects and 14 off-stream watering BMP contracts. Totals for certain practices are as follows: pipeline installed (61,242 feet), fencing installed (4,215 feet), upland storage and stock tanks installed (33). Acreage Treated: 16,997 acres; Cattle affected: 4,705. *Other metrics provided in Section 5.0 below.*
Task #7: Final Report	
Task Description:	Generate Final Report
Actual Deliverables	• Submittal of Draft Final Report to WDEQ nearly 60 days prior to project expiration date. Final Report will be submitted within 60 days of close of project.

SECTION 5.0 BMP IMPLEMENTATION

Table 2. *BMP Summary* and load reduction estimates in Table 3 provide detailed summaries of what the CCNRD Board of Supervisors (BoS), and District staff were able to accomplish with the budget allocated for implementing practices intended to address fecal coliform and *E. coli* concentrations in the Belle Fourche River drainage. Although the DEQ and BoS agreed to use funds to implement projects outside of the listed reaches, the District specifically encouraged landowners near the confluence of Donkey Creek and the Belle Fourche River (and other listed segments) to engage in BMP opportunities. Within the impaired segment of the Belle Fourche River, elevated concentrations of fecal coliform and *E. coli* are routine; there had also been a lack of projects implemented at this location in previous years. Concentrating efforts on the listed segment as well as implementing projects elsewhere on or near the Belle Fourche River is not only improving water quality; it also appears to be generating increased landowner interest in working with the District to implement BMPs. The DEQ and CCNRD agreed that implementing projects outside of the impaired segment was advantageous where there were significant opportunities to improve water quality or to encourage key landowners to become involved in the District's efforts. Additional landowner interest is critical to the CCNRD's efforts to ultimately have the Belle Fourche River meet water quality standards throughout the County. As noted in Section 4.0, the District was able to complete 18 projects, which includes the remediation of four failing septic systems.

With the assistance of the DEQ, load reduction estimates have been provided for BMPs implemented under this project. *E. coli* load reduction estimates for off-stream watering projects were calculated using a spreadsheet tool developed by DEQ. Nutrient and sediment load reduction estimates for off-stream watering projects were calculated using the *Spreadsheet Tool for the Estimation of Pollutant Loads (STEPL)*, developed by Tetra Tech, Inc. for the USEPA. *E. coli* and nutrient reduction estimates for septic system projects were calculated using the DEQ *NPS Septic System Load Reduction Model*. These load reduction estimates are provided for reporting purposes only. Due to the complexity of nonpoint source pollution, these are only simple estimates of the short-term benefits of BMPs implemented under this project. The CCNRD will continue to use water quality monitoring to evaluate long-term changes in water quality.

Table 2. BMP Summary

BMP Name/ Description	Pipeline Installed (ft.)	Fencing Installed (ft.)	Storage/Stock Tanks Installed (ea.)	Wells Installed (ea.)	Spring Development (ea.)	Septic Rehab (ea.)	Heavy Use Protection (ft.)	Date Completed
Oak Creek Off-Stream Watering	6103	317	1	0	0	0	0	8/30/2013
Black Tail Creek Off-Stream Watering	3436	0	2	0	0	0	0	10/1/2013
Arch Creek Off-Stream Watering	5098	0	4	1 (w/pump)	0	0	0	10/1/2013
Lytle Creek Off-Stream Watering	3606	3898	3	0	2	0	0	3/20/2014
Spring Creek Off-Stream Watering	8863	0	4	1 solar pump	1	0	0	7/6/2014
Septic Remediation(straightpipe)-Spring Creek & N. Redwater Creek confluence	0	0	0	0	0	1	0	5/23/2014
Lame Jones Creek Off-Stream Watering	6608	0	2	0	0	0	0	12/30/2013
Buck Creek Off-Stream Watering	5571	0	3	0	0	0	0	7/13/2014
Trail Creek Off-Stream Watering	2500	0	1	1 (w/pump)	0	0	0	7/17/2014
Well Creek & Miller Creek Off-Stream Watering	0	0	0	2 solar pumps	0	0	192	7/31/2014
Spring Creek Off-Stream Watering	13757	0	4	1 solar pump	0	0	0	9/30/2014
Rush Creek Off-Stream Watering	4500	0	5	2 solar pumps	0	0	0	8/6/2014
N. Fork Hay Creek Off-Stream Watering	600	0	2	1 solar pump	0	0	0	9/2/2014
Oak Creek Off-Stream Watering	600	0	1	1 (w/pump)	0	0	0	9/2/2014
Septic Remediation (tank without leachfield)-Tributary to Belle Fourche River	0	0	0	0	0	1	0	10/26/2014
Septic Remediation (tank & overland flow)-S. Redwater Creek	0	0	0	0	0	1	0	7/22/2014
Septic Remediation (tank and overland flow)-Tributary to Belle Fourche River	0	0	0	0	0	1	0	5/10/2015
Trail Creek and Rush Creek Off-Stream Watering	0	0	1	2 solar pumps /windmill conversion	0	0	0	10/30/2015
Totals	**61,242**	**4,215**	**33**	**3 wells, 11 pumps**	**3**	**4**	**192**	

Table 3. Load Reduction Target Estimates from BMP Projects: E. coli and Fecal Coliform

BMP Name/ Description	HUC12	Acreage Treated	Stream Distance (ft)	Cattle Affected	Annual Load Reduction (Fecal coliform organisms per year)	Annual Load Reduction (Most Probable Number/ Year)	Annual Total Nitrogen Load Reduction (lbs./ year)	Annual Total Phosphorus Load Reduction (lbs./year)	Annual Sediment Load Reduction (tons/ year)
Oak Cr. Off-Stream Watering	101202020108	1510	52597	150	N/A	2.27E+13	513.6	61.3	23
Black Tail Cr. Off-Stream Watering	101202010904	630	3472	50	N/A	7.59E+12	220.1	27.8	11.4
Arch Cr. Off-Stream Watering	101202010702	2572	0	200	N/A	3.04E+13	861.6	99.3	35.0
Lytle Cr. Off-Stream Watering	101202010706	2349	0	100	N/A	1.53E+13	788.9	91.5	32.6
Spring Cr. Off-Stream Watering	101202010706	727	1200	175	N/A	2.64E+13	252.8	31.7	12.8
Septic Remediation (straightpipe)-Spring Cr. & N. Redwater Cr. confluence	101202030101	0	200	0	1.523E+13	N/A	18.66	5.33	N/A
Lame Jones Cr. Off-Stream Watering	101202020102	4571	7416	90	N/A	1.40E+13	1507.8	167.5	54.8
Buck Cr. Off-Stream Watering	101202020102	730	3000	400	N/A	6.03E+13	253.8	31.8	12.8
Trail Cr. Off-Stream Watering	101202010501	200	1000	100	N/A	1.51E+13	77.6	11.8	6.0
Well Cr. and Miller Cr. Off-Stream Watering	101202010501, 101202010503	250	150	800	N/A	1.20E+14	90.1	12.1	5.4
Spring Cr. Off-Stream Watering	101202010106	1200	500	900	N/A	1.36E+14	410.9	49.8	19.1
Rush Cr. Off-Stream Watering	101202010504	800	500	600	N/A	9.04E+13	277.4	34.5	13.8
N. Fork Hay Cr. Off-Stream Watering	101202030407	290	800	240	N/A	3.62E+13	104.0	13.8	6.1
Oak Cr. Off-Stream Watering	101202020109	730	3000	400	N/A	6.03E+13	253.8	31.8	12.8
Septic Remediation (tank without leachfield)-Tributary to Belle Fourche River	101102010301	0	0	0	2.418E+12	N/A	18.66	5.33	N/A
Septic Remediation (tank & overland flow)-S. Redwater Cr.	101202030102	0	500	0	9.22E+08	N/A	1.87	0.53	N/A
Septic Remediation (tank and overland flow)-Tributary to Belle Fourche River	101202010806	0	300	0	1.996E+11	N/A	8.58	2.45	N/A
Trail Cr. and Rush Cr. Off-Stream Watering	101202010502	438	834; 400	500	N/A	7.53E+13	154.9	20.0	8.5
Totals		**16,997**		**4,705**	**1.78E+13**	**7.10E+14**	**5767.2**	**684.6**	**254.1**

*Stream distance for off-stream watering projects determined by measuring distance between nearest waterbody and closest watering source implemented.

SECTION 6.0 MONITORING RESULTS

Under Phase III, the goal of producing defensible data for use in evaluating load reductions and BMP effectiveness was continued. The District was successful in supporting this goal by: 1) Collecting credible data under a DEQ-approved Sampling and Analysis Plan, 2) Developing monitoring strategies based on long-term trends in data previously collected, 3) Furthering our understanding of microbial source tracking methods and the data results obtained from this type of analysis during the project period, and 4) Acquiring the permission of landowners to sample at strategic locations within the impaired segment of the Belle Fourche River and Donkey Creek.

Monitoring over the years has helped the CCNRD hone in on high priority areas deemed necessary for fecal coliform or *E. coli* load reductions. In recent years, the District has been able to collect data on the Belle Fourche River above and below the confluence with Donkey Creek, as well as a site directly on Donkey Creek near the County line. In 2015, the District was able to resume monitoring at a site directly below the confluence-a prime location for assessing the influence of Donkey Creek on water quality conditions of the Belle Fourche River. The site had been sampled in years past, but the landowner had, for some time, not expressed an interest in allowing samples to be collected. However, as the District has continued working with the landowner to implement off-stream water improvement projects and talked about the purpose of the monitoring program, the landowner became more receptive to the idea of allowing monitoring to resume.

Besides landowner cooperation, adjustments in sampling plans were influenced by data trends, and prioritization of sampling sites (in this case, the impaired segment of the Belle Fourche River above and below the confluence of Donkey Creek and Donkey Creek itself). Figures 1 and 2 depict sampling locations. Table 4 provides general information about the sites that were monitored during Phase III.

Figure 1. Water Quality Sampling Locations: 2012-2014

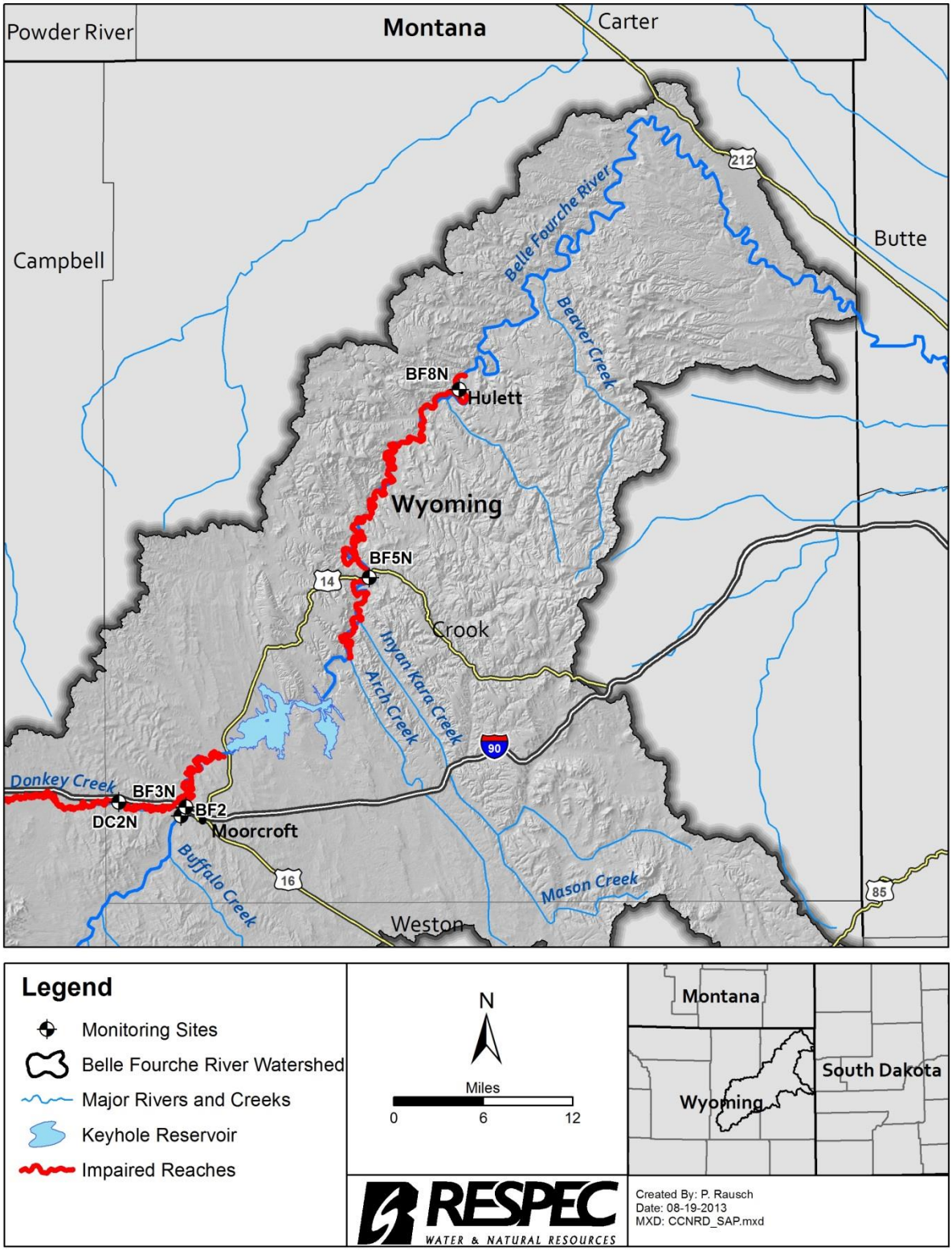

Figure 2. Water Quality Sampling Locations: 2015

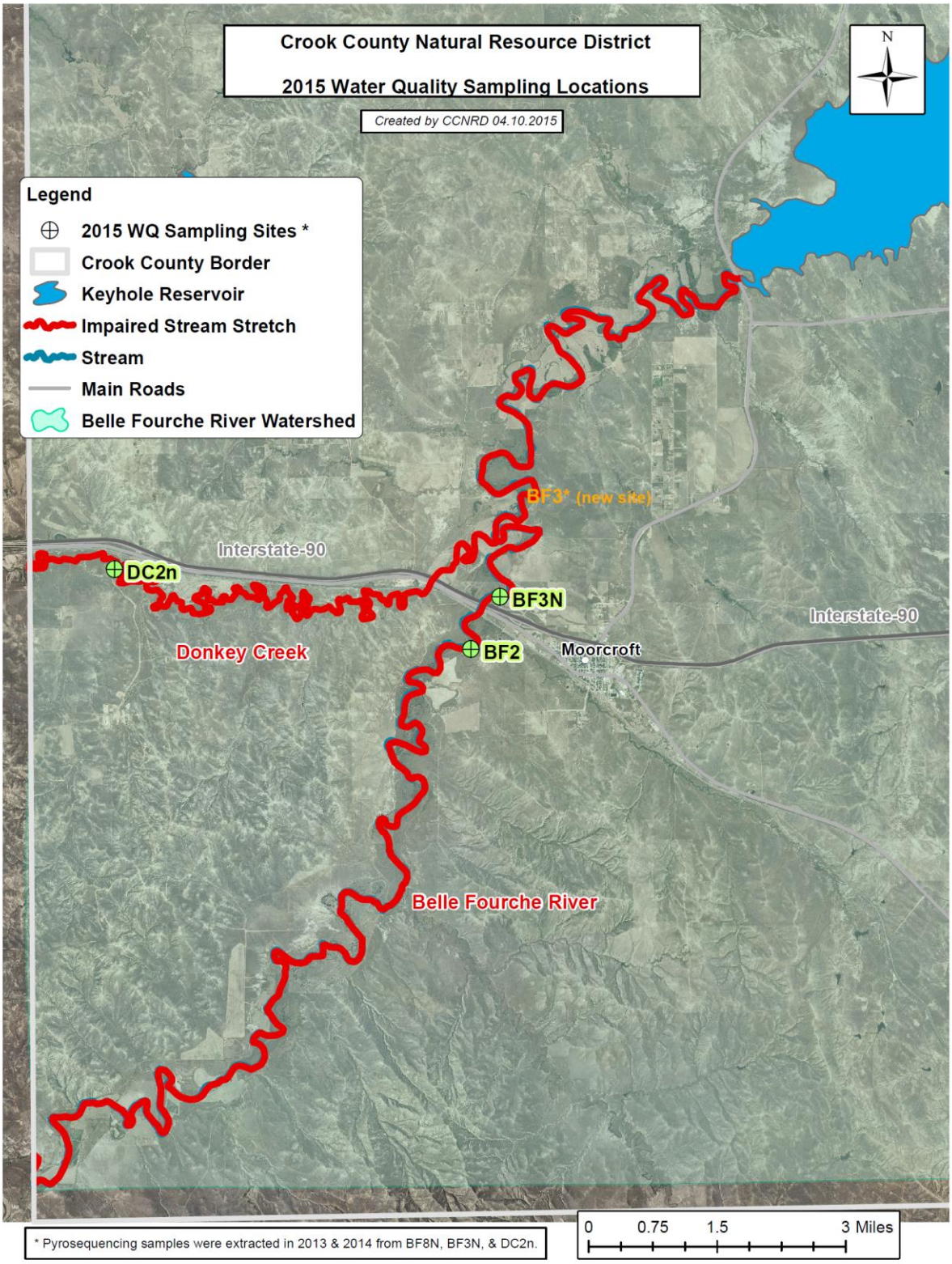

Table 4. Water Quality Sampling Locations

Sampling Site	General Site Location	Site Coordinates (Latitude/Longitude)	Land Ownership	Elevation (ft)	Years Monitored
BF2	Belle Fourche River, on state land above the Town of Moorcroft & downstream of the confluence of Buffalo Creek. On Belle Fourche River Road (about a mile off of highway) above the Moorcroft wastewater treatment plant.	44°16.067N, –104°58.788W	State Property with Leasee ~ *Consent Form Filed*	4,155	2010 2011 2012 ***2013*** ***2014*** ***2015***
BF5n	Belle Fourche River, below Key Hole Reservoir, approximately 150 meters upstream of the Highway 14 bridge.	44°29.923N, –104°43.412W	Private Landowner ~ *Consent Form Filed*	3,921	***2013***
BF8N	Belle Fourche River, above the Town of Hulett and downstream of the confluence of Black Tail Creek. **Downstream 100-yards from original BF3N*	44°40.401N, –104°36.100W	Private Landowner ~ *Consent Form Filed*	3,744	2010 2011 ****2013*** ***2014***
BF3N	Belle Fourche River, 300 meters downstream of I-90 below Moorcroft & upstream of the confluence of Donkey Creek.	44°16.601N, –104°58.377W	State Property with Leasee ~ *Consent Form Filed*	4,181	2011 2012 ***2013*** ***2014*** ***2015***
BF3	Belle Fourche River, north of I-90 and approximately 624 meters downstream of confluence with Donkey Creek.	44°17.431N, –104°58.288W	Private Landowner ~ *Consent Form Filed*	4,136	***2015***
DC2n	Donkey Creek, upstream of the confluence with the Belle Fourche River; 5 miles west of the Town of Moorcroft on Hwy 51, onto Wessex Road/Donkey Creek Road; across railroad tracks and bridge; ¼ mile upstream on East side of road.	44°16.878N, –105°03.821W	Private Landowner ~ *Consent Form Filed*	4,204	2012 ***2013*** ***2014*** ***2015***

SAP Methods Utilized Under Phase III

During the period 2013-2015, 538 water quality samples were collected. Parameters monitored include total coliform, *E. coli*, ammonia, chloride, nitrate-nitrogen, and nitrite-nitrogen (nitrate and nitrite not collected in 2015 due to a lack of exceedances in last couple years). Field chemistry parameters collected each sampling event included temperature, pH, conductivity, total dissolved solids (TDS) and turbidity. Flow measurements and site photographs were also taken as were notes on weather/site conditions present at the time of sampling.

Sampling frequency adhered to methodologies outlined in Wyoming's *Methods for Determining Surface Water Quality Condition and TMDL Prioritization* (Document #14-0367). Total coliform and *E. coli* samples were collected each year during the May 1st through September 30th timeframe. Since 2014, the WDEQ requires that a minimum of five samples be collected for a geometric mean and that they be separated by a minimum of ten days over a

60-day period. WDEQ recommends collecting more than five samples when resources allow. When more than five samples are collected, samples within ten day periods must be averaged before being used to calculate the 60-day geometric mean. For samples collected in 2013, the requirement was that five samples be collected within a 30-day period, 24 hours apart. Four of the five samples were to be taken in separate calendar weeks. Total coliform and *E. coli* samples constituted a volume of 100 mL per sample. Total coliform and *E. coli* samples were held on ice in a cooler and kept at <10 degrees Celsius as soon as collected and until the sample was processed.

Samplers also adhered to the *Manual of Standard Operating Procedures for Sample Collection and Analysis (SOP).* Blanks were a part of quality control (QC) and were required for all sampling activities. Blanks document that there is no sample contamination from the containers during custody, transportation and or pre-analysis preparation either in the field or in the laboratory. Blank generation was noted in the field log book and data sheets. Blanks were sampled using the same sealed and sterilized bottles as the water quality samples. The CCNRD WQ Laboratory used distilled water for *E. coli* blanks. Blanks from field use consisted of distilled water and certified acids as preservatives, and were sent to a third-party laboratory for testing. Field and laboratory blank collection methodologies follow SOP standards. Field blanks are collected at the first sampling site and remain in the cooler with the additional samples for the remainder of the day. Laboratory blanks are collected in the CCNRD WQ Laboratory and analyzed with the additional samples. A duplicate and a blank for both total coliform and *E. coli* were collected for each sampling day. Additionally, a duplicate turbidity sample was collected for each day of sampling. Field blanks and duplicates for ammonia, chloride, and field chemistry samples were collected ten percent of the time throughout the sampling season (May 1st through September 30th). Blanks sent to a third party lab included Total coliform/*E. coli* blanks submitted to Intermountain Laboratories, as well as ammonia, chloride, nitrate, and nitrite blanks submitted to the Wyoming Department of Agriculture's Analytical Laboratory. Photos, stream discharge, water temperature, pH, conductivity, total dissolved solids, and turbidity field measurements were collected at each sampling site and recorded in the field log book during each sampling day or event.

Sampling Results: 2013-2015

Water quality trends for 2013-2014 are summarized below; further information is located in a report in Appendix A; observances for the 2015 monitoring season are based on data provided in Appendix B of this report. Data from 2013-2014 were previously submitted with annual reports provided during Phase III.

Geomeans for E. coli concentrations summarized below for the 2013-2014 seasons and in the report provided in Appendix A were calculated as running geomeans. Two distinct geomeans were calculated for the 2015 season for the periods 6/15/2015-8/7/2015 and 6/29/2015-8/19/2015. In the following paragraphs, the chronic criterion of 126 organisms per 100 mL for E. coli geomeans is the most applicable method for assessing compliance with DEQ standards. The single-sample maximum concentration of 235 organisms per 100 mL is a guideline used for public health considerations in high-use swimming areas in the Wyoming. Much of the Belle Fourche River in Crook County would not be considered a high use area for swimming; the single-sample criterion is included in the discussion below for comparative purposes only.

E. coli

Sampling results from 2013 through 2014 indicate that water quality samples from the Belle Fourche River frequently exceeded the 60-day running geometric mean chronic criterion of 126 organisms per 100 mL, and occasionally exceeded the single-sample maximum concentration criterion of 235 organisms per 100 mL for *E. coli* bacteria over the summer recreation season. During the 2015 monitoring season, the single-sample maximum concentration for *E. coli* bacteria over the summer recreation season was frequently exceeded in May and June; this trend continued into August at sites BF3 and DC2N. At the uppermost site on the Belle Fourche River (BF2), sampling results indicate that *E. coli* concentrations increased between 2013 and 2014. *E. coli* bacteria

concentrations at BF2 never exceeded the chronic *E. coli* criterion in 2013. However, in 2014, BF2 exceeded the chronic *E. coli* criterion six times (using running geomeans). In 2015, BF2 did not exceed the chronic criterion. The sampling results at BF3N (on the Belle Fourche River above the confluence with Donkey Creek and below the Moorcroft wastewater treatment system) also indicate that *E. coli* bacteria concentrations increased between 2013 and 2014. The chronic 60-day geometric mean criterion revealed a jump from 36 percent to 100 percent exceedance over the 60-day periods from the 2013 to 2014 season. During the 2015 season, the chronic criterion at site BF3N was not exceeded. At BF3, directly below the confluence with Donkey Creek, sampling results indicate that *E. coli* concentrations exceeded the single-sample criterion twelve times (86 percent), in 2015 (the only year this site was sampled under Phase III). Both geomeans calculated for BF3 in 2015 reveal an exceedance of the chronic criterion. At BF5N (on the Belle Fourche River below the Keyhole Reservoir), sampling results indicate that *E. coli* concentrations exceeded the single-sample criterion seven times (28 percent) in 2013. The 60-day geometric mean criterion was exceeded only during the beginning of the season with a total of 50 percent exceedance; samples were not collected at this site in 2014 or 2015. At the most downstream site on the Belle Fourche River (BF8N), sampling results indicate that *E. coli* concentrations exceeded the 60-day geometric mean criterion during the first five 60-day periods in 2013 and were below the criterion for the remaining nine 60-day periods. Only four samples were collected at BF8N in all of 2014 and, as a result, a 60-day geometric mean was not calculated for this site, and only one sample exceeded the single-sample criterion for 2014. No samples were collected at BF8N during 2015.

At the site on Donkey Creek (DC2N), sampling results indicate that *E. coli* exceeded the 60-day geometric mean *E. coli* criterion during every 60-day period in 2013, all but one 60-day period in 2014, and both 60-day periods in 2015. The single-sample criterion for DC2N reveals a considerable increase in exceedances from 2013 to 2015 with 12 percent exceedance in 2013 to 64 percent in 2015.

Chloride

Chloride concentrations on the Belle Fourche River exhibited a seasonal pattern from 2013 to 2015 with elevated concentrations observed during the spring (especially during May) and fall (from August through September). During the 5-month monitoring periods for 2013 to 2015, sampling results at the BF3N and DC2N sites indicate that chloride concentrations on the Belle Fourche River and Donkey Creek never exceeded the aquatic life acute criterion. However, Site DC2N consistently showed higher concentrations of chloride than Site BF3N during the 2013-2015 sampling seasons. Although Donkey Creek is a Class 3B waterbody and is not subject to the chloride criteria nor listed for chloride, these results are important in that Donkey Creek appears to be contributing to elevated chloride concentrations in the Belle Fourche River.

Ammonia

Sampling results indicate that ammonia was not detectable in 81 percent of the water samples (34 out of 42) collected on the Belle Fourche River. The 19 percent of samples (8 out of 42), that had detectable levels of ammonia ranged from 0.1 to 0.3 mg N/L. No ammonia samples were identified as exceeding their respective pH-dependent acute criterion values during the May 1st to September 30th monitoring seasons for years 2013-2015 on the Belle Fourche River.

Similarly, ammonia was not detectable in 86 percent (36 out of 42) of samples collected on Donkey Creek. The 14 percent of samples (6 out of 42), that had detectable levels of ammonia ranged from 0.4 to 1.4 mg N/L. No ammonia samples were identified as exceeding their respective pH-dependent acute criterion values during the 2013 through 2015 monitoring period on Donkey Creek.

No ammonia samples were identified as exceeding their respective pH-dependent acute criterion values during the 2010 through 2015 monitoring periods on either waterbody. This finding is in-line with data collected for the TMDL study where all exceedances of the ammonia standard occurred during the winter.

Nitrate-Nitrogen and Nitrite-Nitrogen

Nitrate-nitrogen concentrations and nitrite-nitrogen concentrations were monitored during 2013-2014. During this time period, two samples exceeded the human health value criteria for nitrate-nitrogen on Donkey Creek; however, drinking water is not a designated use for Donkey Creek (Class 3). Overall, nitrate/nitrite concentrations followed a similar pattern in 2013 and 2014 with the highest concentrations observed in spring (especially May) and at the end of the growing season. The lowest concentrations were observed during the summer months, especially July and August. Due to a lack of consistent exceedances, nitrate-nitrogen concentrations and nitrite-nitrogen concentrations were not monitored in 2015.

Microbial Source Tracking (MST): Experiences with Next Generation Sequencing and qPCR Analysis

In the past, implementation of the *E. coli* TMDLs was slowed due to continued uncertainty over the primary pollutant sources. Microbial source tracking (MST), was identified as a tool that could assist and refine the source characterization processes. To focus Phase III resources and finances most effectively and efficiently, the CCNRD began exploring MST analysis as a tool to assist in identifying host sources so the appropriate BMPs could be implemented.

The benefit of using MST in the Belle Fourche River Watershed is that the CCNRD and other water resource managers will be able to direct efforts in controlling sources of *E. coli* where reasonable control is possible (e.g., human sewage, livestock management) and not at sources over which we have limited or no control (e.g., wildlife). Another advantage of using microbial source tracking is its ability to provide rapid results to assist in identifying sources so proper implementation can be conducted. The three host sources that the District concentrated their efforts on thus far are cattle, canines, and humans. In 2013-2014, samples were collected at three sites (BF8N, BF3N, and DC2N) for pyrosequencing analysis. A total of 42 samples were submitted to Research and Testing Laboratory in Lubbock, Texas for analysis during 2013-2014 (http://researchandtesting.com/). Because the laboratory was unable to provide the District with conclusive results (see *Source Tracking Capabilities of Next Generation Sequencing Technologies* in Appendix A for possible reasons), the laboratory opted not to charge the District for the time spent attempting to process the samples. In 2015, the District was able to use those funds to submit additional MST samples to Microbial Insights, Inc. (http://www.microbe.com/), out of Knoxville, Tennessee for analysis using qPCR (quantitative polymerase chain reaction) methodology. Samples were collected at sites BF3, BF3N, and DC2N one to two times a month between June 1st and September 30th (Appendix B). Of twenty samples collected, eight tests came back indicating low concentrations (up to 10^2 cells/mL), of ruminant genetic markers present in the samples at sites BF3 and DC2N. Given the sites sampled, the ruminant sources were narrowed down to wildlife or cattle. Human markers were present in a moderate concentration (10^3 to 10^5 cells/mL), for one sample collected at DC2N in August. Although dog markers were detected in some samples collected at DC2N, concentrations detected were not at a reportable limit. The Town of Moorcroft's wastewater treatment system appears to be functioning appropriately during the primary contact recreation season as the District did not detect any concentrations of human markers in MST samples collected downstream of the system at sites BF3N and BF3. The system has been considered a likely source of ammonia impairment for the listed segment of the Belle Fourche River upstream of the confluence with Donkey Creek. The other twelve results did not yield any reportable concentrations of genetic markers targeted under the study.

Throughout the project period, the CCNRD remained in direct contact with neighboring Campbell County Conservation District (CCCD) concerning the Donkey Creek portions of the watershed, which exists in both Crook

and Campbell County. Information learned from water quality monitoring regarding pollutant sources that may be derived from upstream areas in Campbell County will continue to be shared with the CCNRD.

Quality Assurance Reporting

The CCNRD maintained an up-to-date Sampling and Analysis Plan (SAP), which was most recently updated and approved by the Wyoming Department of Environmental Quality on July 27, 2015. Sampling and monitoring data for the timeframe of 2013 through 2015 was collected and analyzed by CCNRD staff (or contracted parties), and was subject to quality control and assurance review by DEQ, as well as by an independent third party verifier. Every one to two years, the DEQ performed a field sampling and laboratory audit to ensure the District was adhering to its SAP and to provide technical assistance as is needed by the District. Data collected by the CCNRD also underwent annual reviews by the DEQ for quality assurance/quality control purposes to verify the credibility of data collected and processed. These reviews entailed the submittal of data spreadsheets, raw data recorded, log books, bench sheets, and data results obtained by third party laboratories who processed samples collected. A quality assurance and quality control (QA/QC) review of the data was completed by Cathy Norris, WDEQ, for the Project timeframe of 2013 through 2015.

The Quality Assurance Project Plan (QAPP) adhered to by the CCNRD is written to meet the Quality Assurance/Quality Control measures of the Wyoming Department of Environmental Quality's "*Credible Data*" requirements listed in *Section 35, Chapter 1, Wyoming Surface Water Quality Standards*. The District's QAPP was also written to meet the United States Environmental Protection Agency (USEPA) requirements for water samples collected under volunteer water quality monitoring as well as the requirements of the Wyoming Enrolled Act 47, known as the *Credible Data Law*. The QAPP sets guidelines for quality control and quality assurance for all water quality monitoring projects proposed by the CCNRD. The current Belle Fourche River Watershed Assessment Sampling and Analysis Plan meets all the requirements described in this document and is available upon request.

SECTION 7.0 PARTNERS

CCNRD acknowledges and appreciates the ongoing support of the following conservation partners:

- The **Crook County Board of Commissioners**, who provided local leadership.
- The **Crook County Land Use Planning and Zoning Commission**, who possessed a wealth of County knowledge.
- The **Crook County Growth and Development**, who made available information regarding septic system installation, design and permitting documentation.
- The **Crook County Road and Bridge**, who gave invaluable knowledge regarding local access and County regulations.
- The **Crook County School Districts**, who supported the CCNRD information and education outreach programs.
- The **National Association of Conservation Districts**, who provided priceless support and assistance to the CCNRD.
- The **Wyoming Association of Conservation Districts**, who offered continuous leadership and knowledge to CCNRD staff.
- The **Campbell County Conservation District**, who willingly worked as a neighboring partner to help hone in on nonpoint source impairments.
- The **Weston County Natural Resource District**, who offered adjacent county knowledge and information.
- The **University of Wyoming Extension Service**, who provided outreach assistance.
- The **USDA National Resource Conservation Service**, who gave countless hours of program assistance, technical support, and partnership collaboration.
- The **USDA Farm Service Agency**, who bestowed landowner information and agency cooperation.
- The **USFS Bearlodge Ranger District**, who presented natural resource support.
- The **Bureau of Land Management**, who presented County-level natural resource management information.
- The **Bureau of Reclamation**, who presented management information on Keyhole Reservoir.

- The **Wyoming Department of Agriculture**, who made available guidance and program assistance.
- The **Wyoming Department of Environmental Quality**, who provided grant and project leadership and supervision, as well as financial support.
- The **Wyoming State Forestry**, who offered natural resource assistance.
- The **Wyoming Game and Fish**, who made available wildlife technical assistance.
- The **Environmental Protection Agency-Region 8**, who endowed overall grant and project support.

SECTION 8.0 INFORMATION AND EDUCATION

The role of the CCNRD is to educate, assist, and meet the needs of Crook County resource users. In Phase III, education and outreach were accomplished through a variety of delivery types with the goal of increasing water quality awareness within the Belle Fourche River Watershed. Outreach efforts assist the District in gaining community and landowner support for measures to reduce pollutant loading. Guidance and leadership from the District Board of Supervisors (who have acted consistently as a landowner advisory group throughout this project), was utilized to ensure a core group of community leaders were involved in directing educational efforts.

Sixteen educational programs, and two Watershed/BMP tours were conducted in Phase III and directly impacted 433 people, not to mention those reached in presentations that were delivered at various Basin Planning Meetings, monthly Board and County Commissioner meetings, and other watershed stakeholder meetings. Educational programs delivered in and out of the school setting focused on water quality and nonpoint source pollution management concepts, and were primarily targeted at Crook County youth. Educational programs delivered also focused on watershed science, pet waste pollution, river systems, and water conservation. Each program delivered identified the current status of the Belle Fourche River and Donkey Creek to increase awareness; students were given handouts and other material to help engage them in the lesson. Although these specific programs were designed for kids, adults were often present as well.

Tours conducted during Phase III offered insight, knowledge, and publicity of completed Best Management Practices. The tours not only showcased the benefits of BMPs installed; it provided an opportunity for local leaders to see and hear first-hand how landowners have been able to practice more sustainable range management while improving water quality.

Throughout Phase III, CCNRD staff presented program updates at monthly Board Meetings and County Commissioner Meetings and answered questions from local leaders and other community members regarding BMP implementation and mitigation tactics to reduce *E. coli* impairments. The CCNRD coordinated with County officials regarding potential septic system remediation projects, BMP projects, and offered free well water testing (for presence of bacteria) for concerned County residents. The CCNRD employees also provided updates regarding regional, state, and national water quality issues including the State's Categorical Use Attainability Analysis, and legislation regarding data collection and what defines credible data. District Board Meetings were open to the public; after each monthly meeting, minutes were submitted to the newspaper to provide the public information about what was discussed. Newspaper articles were also written over the course of the project period to describe the Belle Fourche River Watershed water quality and/or the local educational programs that were taught.

Initially, the CCNRD wanted to direct more focus during Phase III on a Pet Waste Campaign. District staff presented at various times on issues surrounding pet waste management in meetings; a waste management brochure previously developed continued to be disseminated throughout Phase III. However, the CCNRD fell short of launching an aggressive campaign. If MST data results from the Moorcroft area in 2015 are any indicator, however, pet waste may not be as big of a pollutant source as was previously thought. Nevertheless, pet waste

management will continue to be promoted by the District to better inform citizens of how they can help maintain or improve water quality in their communities.

Other outreach avenues in which little time was spent during Phase III included the development of new brochures targeted at landowners and updates to the District's web page. Although new brochures were not generated under Phase III, informational inserts about water quality and BMPs were included in letters sent to landowners in areas targeted for project implementation. District staff are currently working on website updates under the Phase IV agreement, including a Facebook page. Revisions are also planned for existing brochures; a few new ones are in the planning stages, including one focused on the BMP cost-share program, and one that addresses common concerns brought up when discussing monitoring with landowners.

Knowledge gained generally doesn't just stop; it continues to give. The CCNRD feels that increased interest in Phase III BMP projects were a direct result of outreach conducted during Phase II, as well as continued favorable experiences between landowners and the District during monitoring and BMP implementation activities. Due to relationships built under Phases II and III, the CCNRD applied for and was able to receive funding assistance for a fourth phase of BMP implementation, monitoring, and community outreach (currently underway). Approximately six months into Phase IV, the CCNRD has already obligated most of the funds available to cost-share on water quality improvement projects with interested landowners. Several outreach activities have also already been conducted, with more planned for both youth and adults in 2016.

SECTION 9.0 COMPLICATIONS

The CCNRD would like to expand water quality monitoring sites further out in the watershed so the District is better equipped to track water quality trends across the County. During Phase III, the focus was appropriately on monitoring the impaired segments of the Belle Fourche River and Donkey Creek. This will continue to be the primary focus of the District's monitoring efforts. Although the CCNRD is making headway with landowners regarding BMP implementation, other on-going efforts are needed to gain landowner trust and understanding regarding the collection of water quality data. At a minimum, the District will strive to maintain landowner permissions currently held. The sites monitored in 2015 will likely become long-term sites as they are strategically located within the listed stream segments above and below the confluence with Donkey Creek (in addition to the site located on Donkey Creek itself).

Lack of a flow meter and staff turnover in the middle of the 2015 water quality monitoring season resulted in additional expense to cover the technical assistance provided by RESPEC, Inc., and the Campbell County Conservation District. The CCNRD intends to have two recently hired staff become certified through the completion of State water quality training modules offered. Additionally, the District is exploring options for the purchase of a flow meter ahead of the 2016 monitoring season.

The Phase III project was amended three times; however, none were due to complications. Amendments consisted of accepting more 319 funds to complete additional BMP contracts and revisions to contract language.

SECTION 10.0 RECOMMENDATIONS

The CCNRD made great strides in building landowner interest and participation in implementing BMP projects. The District has also made a lot of progress in understanding different MST methodologies. Regarding the CCNRD's MST sampling work, the District is now at a juncture where decisions will have to be made regarding whether to continue sampling to determine the relative loading contributions of ruminants which were identified as sources during the 2015 season. The District may also opt to hold off on further sampling at this point and continue focusing its efforts on off-stream water improvement BMP projects and other practices designed to minimize livestock impacts to streams and riparian areas. The District plans to review other TMDLs developed

where wildlife were determined to be a significant pollutant loading source to identify approaches to load reduction efforts should that scenario prove to be the case in Crook County as well.

As noted in the previous section, the CCNRD successfully applied for and received funding assistance to complete Phase IV of the six implementation phases outlined to address TMDLs in the Belle Fourche River Watershed. During this time, the District intends to work closely with the DEQ to determine the most appropriate method for calculating load reductions. In hindsight, the District was ambitious in estimating a 10-15% load reduction by the close of Phase III. Although many projects have been implemented on or near the Belle Fourche River under Phase III, it would be premature at this point to state we've adequately assessed how well streams are recovering as a result of changes in grazing patterns and the reduced duration of exposure of cattle to riparian zones. Numerous factors must be considered when evaluating water quality improvements from BMP implementation. It can take several years for BMPs to become fully effective and for previously disturbed riparian areas to recover. Though current monitoring sites are strategically located in the impaired segment of Belle Fourche River, the CCNRD's monitoring program currently tracks water quality improvements on a watershed scale, not at a project scale. Fortunately, site-specific load reduction estimates were calculated to give the DEQ and other interested parties a reasonable idea of the extent to which fecal coliform, *E. coli*, and other pollutants have been reduced by BMPs installed. The CCNRD must also be mindful that progress in pollutant loading reductions in some subwatersheds may be offset by increasing pressure elsewhere in the greater Belle Fourche River Watershed. Increasing trends in *E. coli* concentrations in recent years, despite great strides in implementing BMPs, leads the District to believe more work needs to be done to determine the extent of sources contributing to the loading present in the Belle Fourche River. To address this concern, the District will continue working closely with the CCCD to compare data trends in the Donkey Creek and Stonepile Creek drainages as well as determining the extent of changes in the watershed above site BF2. The CCNRD will continue to emphasize building a stronger relationship with landowners to maintain trust and credibility-traits needed to further our monitoring and BMP project efforts.

The 319 Program has been a tremendous asset to the District. Without a mil levy, maintaining adequate resources to promote improved water quality and rangeland conditions would be extremely challenging. Technical and financial assistance provided to the County through the State's 319 Program has given area ranchers a huge advantage in being able to implement practices that are not only beneficial to their operations, but to the watershed (and public) as a whole. The CCNRD is impressed by and greatly appreciative of the Project Officers assigned to us. Whenever the District has technical or financial questions, the Phase III Project Officers have consistently provided knowledgeable advice in a timely manner.

SECTION 11.0 FINANCIAL SUMMARY

Table 5. Financial Summary

Task	Task Title	319 Funds Expended	Non-federal Match Expended	Total NPS Expenditures	Other Federal Funds Expended
1	Administration	$23,049.60	$19,954.74	$43,004.34	$2,135.41
2	Landowner Advisory Committee	$2,796.11	$620.12	$3,416.23	$43.31
3	Information and Education	$10,960.64	$7,785.60	$18,746.24	$2,389.64
4	Water Quality Monitoring	$16,900.36	$41,027.64	$57,928.00	$7,207.29
5	Microbial Source Tracking (MST)	$13,516.68	$550.00	$14,066.68	$0.00
6	BMP Implementation	$311,794.08	$152,438.80	$464,232.88	$1,335.59
7	Final Report	$3,112.53	$0.00	$3,112.53	$6,803.55
	Total	**$382,130.00**	**$222,376.90**	**$604,506.90**	**$19,914.79**

SECTION 12.0 ATTACHMENTS

APPENDIX A: WATER QUALITY MONITORING REPORTS
APPENDIX B: WATER QUALITY DATA
APPENDIX C: EDUCATION AND OUTREACH SUMMARY
APPENDIX D: IN-KIND AND CASH MATCH SUMMARY

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— Wyoming Department of Environmental Quality —

APPENDIX A

Water Quality Monitoring Report 2013-2014

CCNRD

Phase III

Belle Fourche River Watershed Plan

2012-2015

Grant: 9008630-12

Board of Directors

Chairman: Wayne Garman ***Vice Chairman***: Ted Parsons ***Treasurer***: Clinton Streeter ***Secretary***: Jennifer Hinkhouse ***Member***: Lily Altaffer

Final

WATER QUALITY MONITORING AND ASSESSMENT REPORT FOR THE BELLE FOURCHE RIVER WATERSHED CROOK COUNTY, WYOMING

Topical Report RSI-2561

prepared for

Crook County Natural Resource District
117 South 21st Street, P.O. Box 1070
Sundance, Wyoming 82729

October 2015

RESPEC

WATER QUALITY MONITORING AND ASSESSMENT REPORT FOR THE BELLE FOURCHE RIVER WATERSHED CROOK COUNTY, WYOMING

Topical Report RSI-2561

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October 2015

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1.0 INTRODUCTION AND BACKGROUND

The Crook County Natural Resource District's (CCNRD) long-term goal is to conserve and improve the quality and quantity of water resources in Crook County [CCNRD, 2010]. The CCNRD mission for the Belle Fourche River Watershed project is to support voluntary land-use management practices that, when implemented, will address human-caused water quality issues [CCNRD, 2009]. The CCNRD has been conducting water quality monitoring since 2001 under the guidance of a Board of Supervisors and a Sampling and Analysis Plan (SAP) approved by the Wyoming Department of Environmental Quality (WDEQ) [CCNRD, 2008].

Water quality monitoring performed by the U.S. Geological Survey (USGS) and the WDEQ in 1996, 1998, and 1999 led to 303(d)-impaired waters listings for the Belle Fourche River and Donkey Creek. Baseline data collected in 2001 verified fecal coliform bacteria impairments of the listed waterbodies and initiated the CCNRD's water quality monitoring program [CCNRD, 2008]. These listings prompted the CCNRD to establish the Upper Belle Fourche Watershed Advisory Group, which developed a grant proposal for Clean Water Act Section 319 funds to monitor water quality, develop a watershed plan, launch a best management practice (BMP) cost-share program, and perform information and educational outreach within the watershed. The Belle Fourche River Watershed Plan was completed in 2004 by the CCNRD, Belle Fourche River Watershed Advisory Committee, and Belle Fourche River Watershed Landowners with assistance from the Wyoming Association of Conservation Districts (WACD) [CCNRD, 2009].

The main goal of the CCNRD's watershed plan is to improve the water quality and, ultimately, delist the impaired segments of the Belle Fourche River. Conservation accomplishments and BMPs were implemented in the Belle Fourche River Watershed as part of this plan. The watershed plan addressed water quality monitoring, agricultural impacts, recreational impacts, urban and suburban impacts, funding resources, wildlife impacts, education, and information. The CCNRD monitored water quality on the impaired stream reaches from 2003 to 2005 and, in 2007, received additional 319 funding for ongoing water quality monitoring and to implement septic and agricultural BMPs. Between 2005 and 2009, the CCNRD monitored water quality at various sites on the Belle Fourche River and Donkey Creek for *Escherichia coli* (*E. coli*), bacteria, pH, temperature, specific conductivity, dissolved solids, turbidity, dissolved oxygen, ammonia, chloride, nitrate and nitrite [WWC Engineering, 2010].

In 2008, the CCNRD's proposal for the Belle Fourche River Watershed Plan Implementation Project, Phase II, was funded by the WDEQ. The goals of the CCNRD's Phase II project were to continue their efforts through ongoing watershed implementation to improve the water quality and meet state water quality standards within the watershed by reducing fecal coliform bacteria to remove the Belle Fourche River and Donkey Creek from the state of Wyoming's 303(d) list of impaired waterbodies [CCNRD, 2008]. The Phase II project continued monitoring on the upper and lower portions of the watershed to further isolate bacteria sources and allow for BMPs to be efficiently implemented [CCNRD, 2008]. The Phase II project's 319 funds supported the CCNRD's water quality efforts during the 2010, 2011, and 2012 monitoring seasons.

In 2013, the CCNRD was awarded a grant from the WDEQ for Phase III of the Belle Fourche River Watershed Plan Implementation. The main objective of the 3-year plan is to reduce bacterial loading to the Belle Fourche River, Donkey Creek, and tributaries within Crook County. The Phase III project supported water quality monitoring during 2013 and 2014 on the upper and lower portions of the watershed. Project effectiveness monitoring has provided data to evaluate whether or not BMP implementations or information/education efforts were successful.

1.1 WATER QUALITY STANDARDS

The following sections outline the Wyoming surface water quality standards as they are described in Chapter 1 of the Wyoming Water Quality Rules and Regulations pursuant to W. S. 35-11-101 through 1507 [WDEQ, 2015].

1.1.1 Key Terms

Acute Criterion – Criterion that constitutes the highest concentration of a physical or chemical parameter to which an aquatic community (i.e., macroinvertebrates) can be exposed for 1 hour without deleterious effects. According to the U.S. Environmental Protection Agency (EPA), unacceptable effects on freshwater organisms and their uses will not occur if this value is not exceeded more than once every 3 years [WDEQ, 2015].

Chronic Criterion – Criterion that represents the highest 4-day average concentration of a physical or chemical parameter to which an aquatic community can be exposed without deleterious effects. According to the EPA, unacceptable effects on freshwater organisms and their uses will not occur if this value is not exceeded more than once every 3 years [WDEQ, 2015].

Geometric Mean – A measure of central tendency used to summarize bacteria data given that bacteria can grow and die at exponential rates. The geometric mean is useful because it is not heavily influenced by large fluctuations in data points the way that a regular mean (arithmetic mean) can be.

Running Geometric Mean – The running 60-day geometric mean concentration is calculated for each 60-day window in the recreational season. Wyoming criteria require at least five samples in a 60-day period to calculate a 60-day geometric mean. Furthermore, samples collected within a 10-day period must be averaged before being used to calculate the geomean.

1.1.2 *E. coli*

Before 2013, the WDEQ used the geometric mean concentration of five samples collected over a **30**-day period to determine chronic *E. coli* impairments during the summer recreation season. In 2013, the WDEQ changed the geometric mean requirements to include a minimum of five samples collected during separate 24-hour periods for any **60**-day period [WDEQ, 2014a]. Furthermore, the five samples must also be separated by at least 10 days to be representative of the entire time period. When more than five samples are collected, samples within 10-day periods are averaged. The change from a 30-day period to a 60-day period was made to identify waters with chronic bacteria problems rather than those waters that have isolated incidences or short-term elevated bacteria concentrations.

In waters designated for primary contact recreation, the chronic criterion for *E. coli* is 126 organisms per 100 milliliters (mL) and is applicable from May 1 to September 30. Donkey Creek, Stonepile Creek, and the Belle Fourche River are designated for primary contact recreation. Additionally, the following single-sample maximum acute concentrations of *E. coli* bacteria shall apply during the summer recreation season on waters designated for primary contact recreation, based on a determination by the administrator as needed on a case-by-case basis [WDEQ, 2015]. High-use swimming areas include public beaches, reservoirs, and other popular recreational areas. The WDEQ typically only posts recreational-use advisories on high-use surface waters. Noncontact recreational activities (fishing/kayaking) are common on the Belle Fourche River and to a lesser extent on Donkey Creek; however, neither waterbody contains high-use swimming areas. The single-sample maximum value of 235 organisms per 100 mL is used in this report only for comparison purposes and does not imply that any water should be selected for a recreational advisory, point-source limitation, or included on the state's impaired water listings.

1. High-use swimming areas—235 organisms per 100 mL
2. Moderate full-body contact—298 organisms per 100 mL
3. Lightly used full-body contact—410 organisms per 100 mL
4. Infrequently used full-body contact—576 organisms per 100 mL.

1.1.3 Chloride

The WDEQ set the aquatic life acute value criterion for chloride at 860 milligrams per liter (mg/L) and the aquatic life chronic value criterion at 230 mg/L [WDEQ, 2014b]. For the purpose of this report, all chloride samples will be compared to the aquatic acute value criterion for single-sample analysis. These numeric criteria apply to Class 1, 2A, 2B, 2AB, and 2C waterbodies, and as a Class 2ABww waterbody, the Belle Fourche River is subject to the numeric criteria [Tetra Tech, Inc., 2013]. Donkey Creek is designated as Class 3 water, so it is not subject to the chloride criteria and is not listed for chlorides; however, observed data on Donkey Creek are displayed in tables and figures with the chloride criteria because Donkey Creek may affect loads in the Belle Fourche River.

1.1.4 Ammonia

The WDEQ set the acute ammonia criteria expressed in milligrams of ammonia nitrogen per liter (mg N/L) in Appendix C, Chapter 1, of the *Wyoming Water Quality Rules and Regulations* [WDEQ, 2015]. The acute ammonia criteria vary with temperature, pH, and fish species or fish life stage. Measured ammonia concentrations in this report will be compared to the acute ammonia criteria values contained in the first table in Appendix C, Chapter 1, of the *Wyoming Water Quality Rules and Regulations* [WDEQ, 2015]. These numeric criteria apply to Class 1, 2A, 2B, 2AB, and 2C waterbodies, and as a Class 2ABww waterbody, the Belle Fourche River is subject to the numeric criteria [Tetra Tech, Inc., 2013]. The acute ammonia criteria, or Criterion Maximum Concentration (CMC), vary by pH in accordance with Equation 1-1 when salmonids or other sensitive cold-water species are absent. Because salmonids and other sensitive cold-water species are absent from the Belle Fourche River (Class 2ABww), Equation 1-1 was used to calculate the acute ammonia criteria for this assessment report. The acute criterion is "the one hour average concentration of total ammonia nitrogen (in milligrams of nitrogen per liter) not to be exceeded more than once every 3 years" [WDEQ, 2015]. Because Donkey Creek is designated as Class 3 water, it is not

subject to the ammonia criteria and is not listed for ammonia, but its concentrations are displayed in tables and figures with the ammonia criteria because it may impact loads and concentrations in the Belle Fourche River, following the confluence.

$$\text{CMC} = \frac{0.411}{1+10^{7.204-pH}} + \frac{58.4}{1+10^{ph-7.204}} \tag{1-1}$$

1.1.5 Nitrate-Nitrogen and Nitrite-Nitrogen

The WDEQ set the human-health value criterion for "fish and drinking water" for nitrite-nitrogen on all Class 1, 2AB, and 2A waters as 1 mg/L [WDEQ, 2015]. Wyoming does not have a nitrate standard for aquatic life; however, the human-health value for nitrate-nitrogen is 10 mg/L [WDEQ, 2014a]. For the purpose of this report, all nitrate-nitrogen and nitrite-nitrogen samples will be compared to these criteria [WDEQ, 2015].

1.2 IMPAIRED WATERBODIES

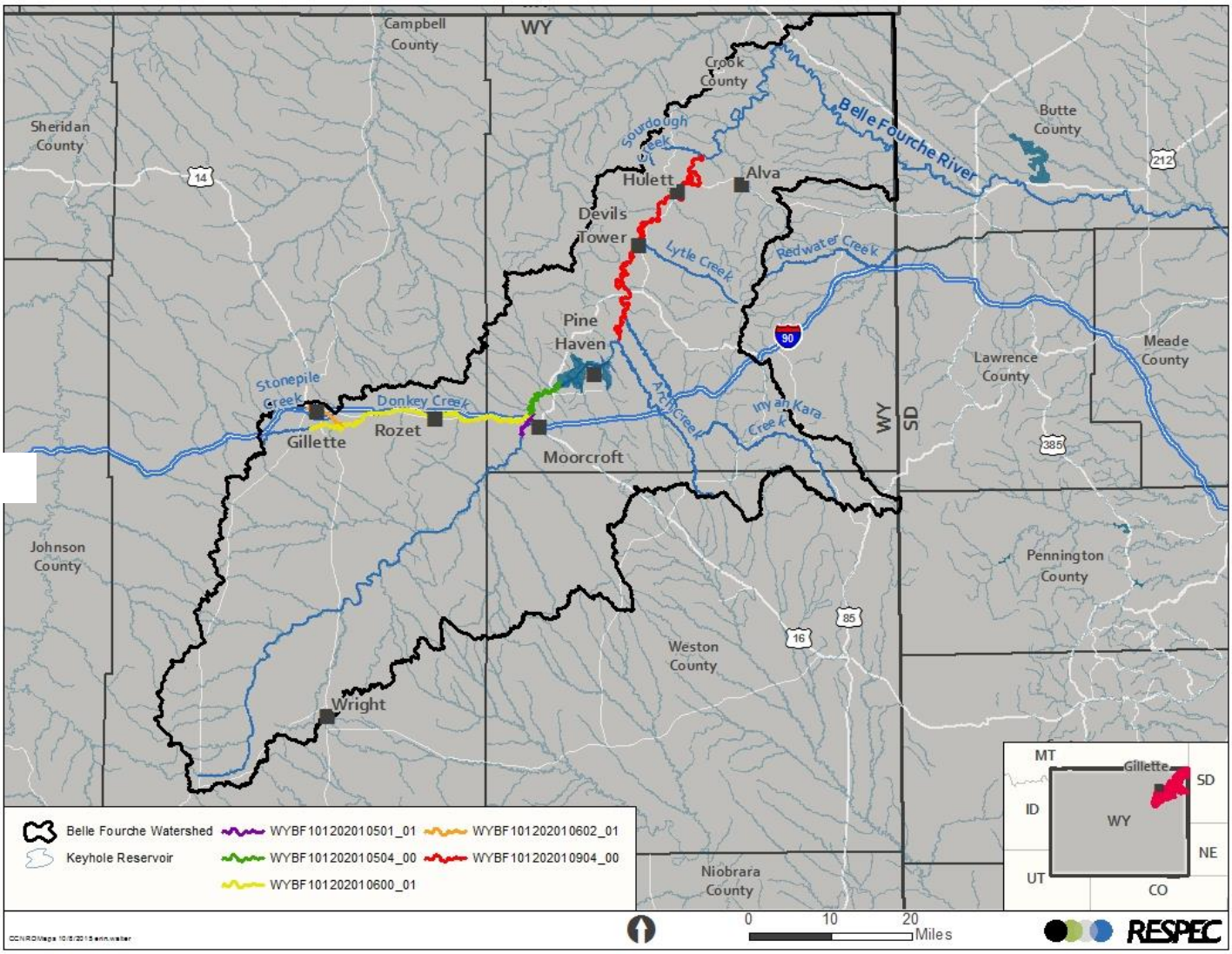
The WDEQ evaluated the water quality of the Belle Fourche River and the results indicate that most segments are in attainment of their designated uses [Tetra Tech, Inc., 2013]. However, three segments of the Belle Fourche River, along with Stonepile Creek and Donkey Creek, do not support their designated contact recreation uses because of elevated concentrations of fecal coliform or *E. coli* bacteria [Tetra Tech, Inc., 2013]. Additionally, segments of the Belle Fourche River do not support their designated fisheries life use because of elevated levels of ammonia and chloride [Tetra Tech, Inc., 2013]. Monitoring data collected by the USGS in 2009 and 2010 also indicated that the Belle Fourche River below Donkey Creek exceeded WDEQ's acute chloride criterion for aquatic life other than fish and that the concentration of ammonia occasionally exceeds the acute criterion protective of fisheries uses [WDEQ, 2014b].

Four stream segments within the CCNRD have been listed on the Wyoming Water Quality Assessment and Impaired Waters List or the 2014 Integrated 305(b) and 303(d) reports [WDEQ, 2014b]. Three of the listed stream segments are located on the Belle Fourche River and the other listing is located on Donkey Creek. Two of the three Belle Fourche River impaired reaches are listed for fecal coliform bacteria impairment only, while the other Belle Fourche River reach has been listed for fecal coliform, ammonia, and chloride impairments. The impaired reach on Donkey Creek has been listed for fecal coliform. Figure 1-1 illustrates the location of the impaired waters and Table 1-1 lists the impaired waters within the CCNRD. The Belle Fourche River confluence with Sourdough Creek is the Total Maximum Daily Load (TMDL) reach endpoint.

1.3 TOTAL MAXIMUM DAILY LOADS

In 2009, the WDEQ began monitoring to support the Belle Fourche River Watershed TMDL project. The development of seven TMDLs was initiated in 2009 for the 303(d)-listed waterbodies within the watershed [WDEQ, 2014b]. These include three for bacterial impairments on the Belle Fourche River: one for the ammonia and chloride impairments on the Belle Fourche River and one each for the fecal coliform listings on Donkey and Stonepile Creeks. *E. coli* data were used in the TMDLs for Donkey and Stonepile Creek; the WDEQ stopped listing waterbodies for fecal coliform in 2007 and began listing

Figure 1-1. Location of Impaired Waters in the Belle Fourche River Watershed.



305(b) Identifier	Waterbody	Class	Location	Miles	Impaired Beneficial Uses	303(d) Listing Parameter	List Date	TMDL Completed Date
WYBF101202010501_01	Belle Fourche River	2ABww	From the confluence with Donkey Creek to a point 6.2 miles upstream	6.2	Contact Recreation	Fecal Coliform	1996	2013
WYBF101202010504_00	Belle Fourche River	2ABww	From the confluence with Keyhole Reservoir upstream to the confluence with Donkey Creek	14.2	Contact Recreation	Fecal Coliform	1996	2013
WYBF101202010504_00	Belle Fourche River	2ABww	From the confluence with Keyhole Reservoir upstream to the confluence with Donkey Creek	14.2	Aquatic Life Warm Water	Ammonia	2008	2014
WYBF101202010504_00	Belle Fourche River	2ABww	From the confluence with Keyhole Reservoir upstream to the confluence with Donkey Creek	14.2	Aquatic Life Warm Water	Chloride	2008	2014
WYBF101202010904_00	Belle Fourche River	2ABww	From the confluence with Arch Creek downstream to the confluence with Sourdough Creek	60.7	Contact Recreation	Fecal Coliform	1996	2014
WYBF101202010602_01	Stonepile Creek	3B	From the confluence with Donkey Creek upstream to the junction of highways 14/16 and 59	7.6	Contact Recreation	Fecal Coliform	2002	2014
WYBF101202010600_01	Donkey Creek	3B	From the confluence with the Belle Fourche River upstream to Brorby Boulevard within the city of Gillette	61.4	Contact Recreation	Fecal Coliform	2000	2014

Table 1-1. Stream Segments Listed on the Wyoming Water Quality Assessment and Impaired Waters List (2014 Integrated 305(b) and 303(d) Report) Within the Crook County Natural Resource District

them for *E. coli* in 2008. The EPA recommends *E. coli* as the best indicator of health risk from water contact in recreational waters; fecal coliforms have been determined to be a poor health risk indicator [EPA, 2015].

In 2013, the *Belle Fourche River Watershed TMDLs for Pathogens, Ammonia, and Chloride* was finalized for the WDEQ [Tetra Tech, Inc., 2013]. The TMDL recommends implementation measures that could reduce ammonia, chloride, and *E. coli* bacteria loads in the Belle Fourche River and Donkey Creek. Priority sources of ammonia include the Moorcroft wastewater lagoons, the Gillette wastewater treatment facility, runoff from Gillette in relation to the application of deicing products, and runoff from Wyodak during high-flow events [Tetra Tech, Inc., 2013]. Deicing products in storm runoff were also identified as a primary source of chlorides to the Belle Fourche River during the winter. Available information was insufficient to identify the source(s) of the chloride impairment during the summer [Tetra Tech, Inc., 2013]. The largest contributors to the bacteria impairment on Donkey Creek included failing septic systems, livestock, wildlife and pet waste, and inflow from Stonepile Creek. Grab samples collected from Donkey Creek were consistently greater than the TMDL target; therefore, Donkey Creek was identified as a major contributor of *E. coli* to the Belle Fourche River. Grab samples collected from the segment of the Belle Fourche River from the confluence of Rush Creek to Keyhole Reservoir also exceeded TMDL targets with the exception of low flows [Tetra Tech, Inc., 2013]. Effluents from point sources were not expected to be significant contributors to the Belle Fourche River bacteria impairment. Bacteria loading from failing septic systems and animals were identified as priority sources, although the significance of each source is relatively unknown [Tetra Tech, Inc., 2013]. Bacteria concentrations in grab samples collected below Keyhole Reservoir were below TMDL standards, which indicates dilution in bacteria concentrations moving downstream toward the South Dakota border.

1.4 SUMMARY OF TOTAL MAXIMUM DAILY LOAD REDUCTIONS NEEDED

Tetra Tech, Inc. [2013] identified the following cumulative and flow zone load reduction for the impaired segments of the Belle Fourche River, Stonepile Creek, and Donkey Creek (Table 1-2).

Table 1-2. Required Flow Zone and Cumulative Load Reductions Identified for Impaired Streams as Identified in the Belle Fourche River Watershed TMDLs [Tetra Tech, Inc., 2013]

	Constituent	Season	Flow Zone					Cumulative Reduction (%) Amount/Day
			High (0–10) (%)	Moist (10–40) (%)	Midrange (40–60) (%)	Dry (60–90) (%)	Low (90–100) (%)	
Upper Belle Fourche River *(from an undetermined location upstream of Donkey Creek to Keyhole Reservoir and from the confluence with Donkey Creek to a point 6.2 miles upstream)*	Ammonia	Summer	0	0	0	0	0	No Reduction
		Winter	0	84	0	94	90	89% or 651.35 lbs/day
	Chloride	Annual	0	10	0	19	44	24% or 23,234 lbs/day
	E. coli	PCR	82	38	0	0	0	79% or 1.54E+12 counts/day
		SCR	—	0	0	0	0	No Reduction
Lower Belle Fourche River *(from Arch Creek to Sourdough Creek)*	*E. coli*	PCR	85	0	0	0	0	82% or 7.35E+11 counts/day
		SCR	—	0	0	0	0	No Reduction
Donkey Creek	*E. coli*	PCR	88	57	20	54	50	77% or 6.92E+11 counts/day
		SCR	—	—	—	—	—	No data available
Stonepile Creek	*E. coli*	PCR	73	70	41	93	—	77% or 5.48E+11 counts/day
		SCR	0	55	—	0	13	42% or 2.49E+11 counts/day

PCR = Primary Contact Recreation
SCR = Secondary Contact Recreation

2.0 WATERSHED CHARACTERISTICS

For the purposes of this water quality monitoring assessment report, the project area is illustrated in Figure 2-1.

2.1 RESOURCE SETTING

The Belle Fourche River Watershed encompasses over 3,225 square miles (approximately 2,063,773 acres) in northeastern Wyoming and covers parts of Campbell, Weston, and Crook Counties. The headwaters of the Belle Fourche River lie in the southern portion of Campbell County. From its headwaters, the Belle Fourche River flows in a northeasterly direction and enters the Keyhole Reservoir just north of Moorcroft, Wyoming. Downstream of the Keyhole Reservoir, the Belle Fourche River continues to flow in a northeasterly direction until it enters the state of South Dakota.

Elevations in the watershed range from 6,436 feet on Warren Peaks summit, 5,407 feet at the headwaters of the Belle Fourche River, 4,134 feet at the Belle Fourche River and Donkey Creek confluence, 3,363 feet at the northernmost reach of the river, and 3,107 feet at the Wyoming-South Dakota state line. The majority (82 percent) of the land in the watershed is privately owned. Approximately 18 percent is public land managed by the U.S. Forest Service, state of Wyoming, U.S. Bureau of Land Management, U.S. Bureau of Reclamation, and National Park Service [CCNRD, 2009]. Several incorporated and unincorporated communities (or developed areas) are located within the watershed, including Alva, Carlile, Colony, Devils Tower, Gillette, Hulett, Moorcroft, Pine Haven, Rozet, Sleepy Hollow, and Wright. Key landscape features in the watershed include the Belle Fourche River, Black Hills National Forest, Devils Tower National Monument, and Thunder Basin National Grassland.

Table 2-1 provides land cover, land use, and associated areas for the watershed according to the 2006 National Land Cover Dataset (NLCD). Vegetative cover in the watershed primarily consists of 52 percent grassland and/or herbaceous land, 31 percent shrub and/or scrub, and 13 percent evergreen forest. The geology of the Belle Fourche River in Crook County varies greatly from interbedded, eroded shales and sandstones along the Belle Fourche River, to limestone and intrusive igneous and metamorphic rocks in the Bearlodge Mountains [CCNRD, 2009]. The watershed is dominated by alluvial and sedimentary formations including Morrison Shale, Pierre Shale, Fox Hills Sandstone, and Spearfish Siltstone [CCNRD, 2009]. Soils within the project area are typically (1) well-drained soils formed on alluvium on terraces and flood plains; (2) well-drained soils derived from sedimentary rock; (3) clay shale, sandstone shale, and sandstone on nearly level to very steep uplands; and (4) well-drained soils derived from siltstone, shale, and limestone, on mountains, and formed in material weathered from siltstone, sandstone, and limestone and rock outcrop on uplands [CCNRD, 2009].

Crook County averages over 24 inches of annual precipitation. The average snowfall is 65 inches per year, and the average temperature is 42 degrees Fahrenheit (42°F). Table 2-2 lists meteorological sites and their corresponding precipitation totals over each monitoring period and annual totals within Crook County in 2013 and 2014. Figure 2-2 shows monthly precipitation totals for the meteorological sites during the summer recreation season over the monitoring period from 2013 to 2014.

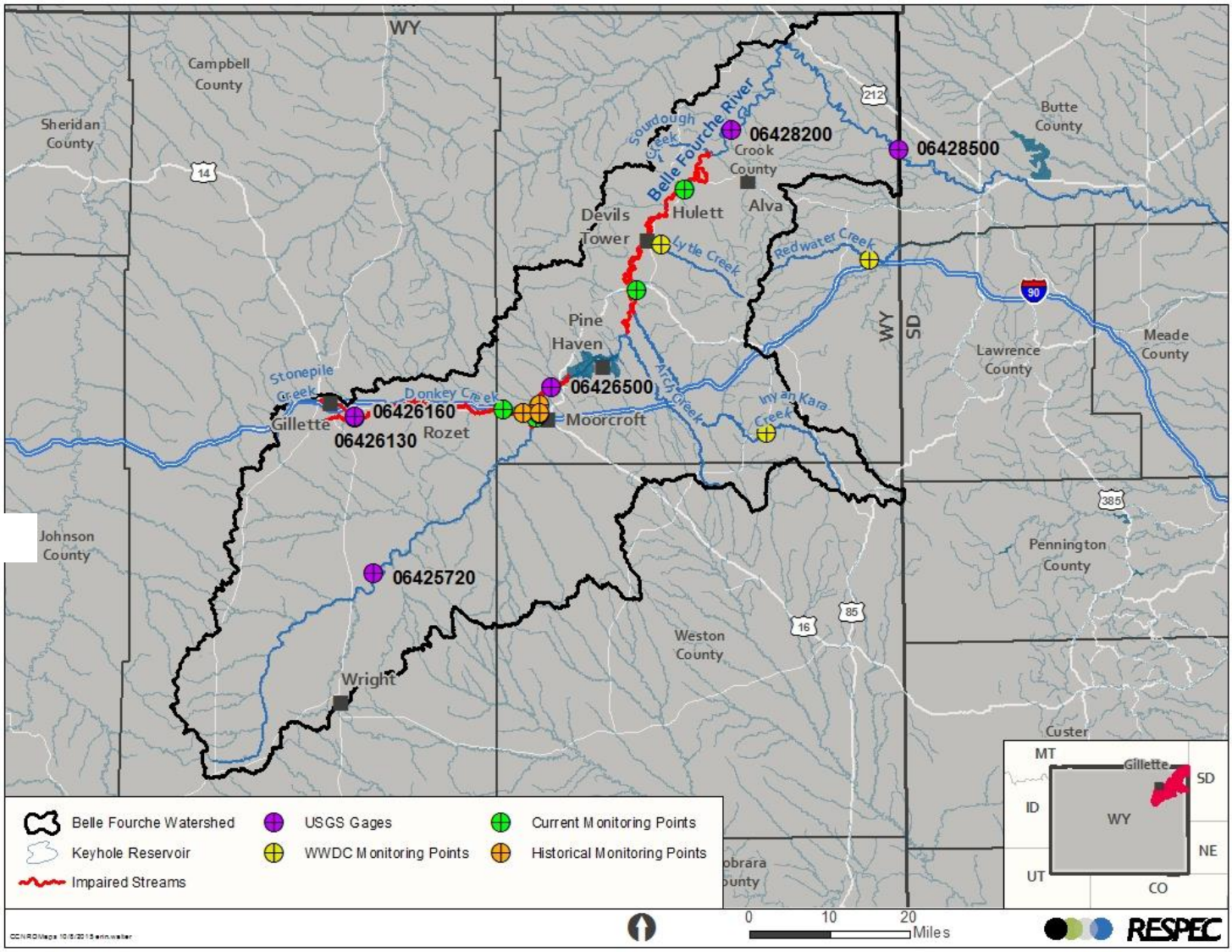


Figure 2-1. Location of Monitoring Points in the Belle Fourche River Watershed.

Table 2-1. 2006 National Land Cover Dataset Land Use for the Belle Fourche River Watershed

Classification	Description	Area (acres) (%)	Percent of Study Area (%)
Grassland and Herbaceous	Gramanoid or herbaceous vegetation, generally greater than 80 percent of total vegetation cover. These areas are not subject to tilling but are used for grazing.	1,081,075	52.4
Shrub and Scrub	Shrubs less than 16 feet tall with canopy typically greater than 20 percent of total vegetation. This class includes shrubs and trees in early successional stages or stunted from environmental conditions.	638,763	31.0
Evergreen Forest	Trees greater than 16 feet tall, and greater than 20 percent of total vegetation cover. More than 75 percent of the tree species maintain their leaves all year. Canopy is never without green foliage.	264,328	12.8
Barren Land (Rock/Sand/Clay)	Bedrock, desert pavement, scarps, talus, slides, volcanic material, glacial debris, sand dunes, strip mines, gravel pits and other earthen material. Vegetation accounts for less than 15 percent of total.	16,871	0.8
Developed, Open Space	A mixture of some constructed materials, but mostly vegetation in the form of lawn grasses. Impervious surfaces account for less than 20 percent of cover. These areas commonly include large-lot, single-family housing units, parks, golf courses, and vegetation planted in developments for recreation, erosion control, or aesthetics.	14,090	0.7
Cultivated Crops	Production of annual crops and also perennial woody crops. Crops accounts for greater than 20 percent of total vegetation. This class also includes land being tilled.	11,698	0.6
Pasture and Hay	Grasses, legumes, or mixtures planted for livestock grazing or the production of seed or hay crops on a perennial cycle. Pasture/hay vegetation accounts for greater than 20 percent of total vegetation.	7,563	0.4
Woody Wetlands	Forests or shrublands account for greater than 20 percent and the soil is periodically covered with water.	7,188	0.3
Deciduous Forest	Trees greater than 16 feet tall and greater than 20 percent of vegetation cover. More than 75 percent of tree species shed foliage in response to seasonal change.	4,327	0.2
Developed, Low Intensity	A mixture of constructed materials and vegetation. Impervious surfaces account for 20 to 49 percent of total	5,273	0.3

	cover. These areas commonly include single-family housing units.		
Emergent Herbaceous Wetlands	Perennial herbaceous vegetation accounts for greater than 80 percent of vegetative cover and the soil or substrate is periodically covered with water.	4,698	0.2
Open Water	Open water, usually less than 25 percent cover of vegetation or soil.	4,463	0.2
Developed, Medium Intensity	A mixture of constructed materials and vegetation. Impervious surfaces account for 50 to 79 percent of the total cover. These areas commonly include single-family housing units.	3,026	0.1
Other	Areas with less than 0.1 percent of the study area.	410	0.1
Total		**2,063,773**	**100.0%**

Table 2-2. Meteorological Sites Within Crook County [Western Regional Climate Center, 2014]

Meteorological Site	2013 Precipitation (in)		2014 Precipitation (in)	
	Summer Recreation Season[(a)]	Annual	Summer Recreation Season[(a)]	Annual
Dillinger (482580)	9.78	17.15	11.13	15.93
Devils Tower (482466)	20.07	30.41	12.54	20.50
Hulett (484760)	16.82	27.51	11.43	19.05
Gillette (483855)	13.98	21.50	13.94	20.77

(a) May 1 through September 30.

2.2 WATER QUANTITY

Peak flows typically occur between March and June, and low flows typically occur in November and December [CCNRD, 2009]. Discharge measurements for water years 2013 and 2014 were obtained from the USGS for six gaging stations as a means of comparison with observed water quality data on the Belle Fourche River, Donkey Creek, and Stonepile Creek within the project area [USGS, 2015a; 2015b]. Stonepile Creek was included because it contributes significant flow to Donkey Creek. These six gaging stations house automated gaging equipment, and real-time data display options are available via the Wyoming and South Dakota USGS Water Science Centers. Additionally, Wyoming Water Development Commission (WWDC) staff worked with RESPEC to install three additional temporary gaging stations in April 2013 and 2014. Transducers were retrieved in November 2013 and reinstalled in the spring of 2014. Data were collected throughout the spring and summer of 2014 before the gages were removed in November 2014. These gages were equipped with pressure transducers with built-in dataloggers that were programmed to collect water depth at 15-minute intervals. The data collected at these sites provided insight to the

hydrologic processes on three previously unmonitored streams, two of which discharged upstream of water quality monitoring points on the Belle Fourche (along Lytle Creek and Inyan Kara Creek) and a third site on Redwater Creek that discharges downstream of the selected water quality monitoring points (Figure 2-1).

The USGS gaging station farthest downstream on the Belle Fourche River (06428500) lies in South Dakota near the Wyoming-South Dakota state line. The next USGS gaging station upstream (06428200) is located on the Belle Fourche River near Alva, Wyoming. North of Moorcroft, Wyoming, USGS Gaging Station 06426500 lies directly upstream of the Keyhole Reservoir. The farthest upstream gaging station on the Belle Fourche River (Station 06425720) is located below the confluence of Rattlesnake Creek and the Belle Fourche River. Discharge measurements for Donkey Creek were obtained from USGS Gaging Station 06426130, as well as data from Stonepile Creek (06426160), both located near Gillette, Wyoming (Figure 2-1).

Table 2-3 summarizes the six USGS and three WWDC gaging stations within the project area. Figures A-1 through A-9 in Appendix A display discharge rates of the gaging stations over the monitoring period. The figures illustrate seasonal variation in observed discharge rates that can be attributed to spring runoff and precipitation events.

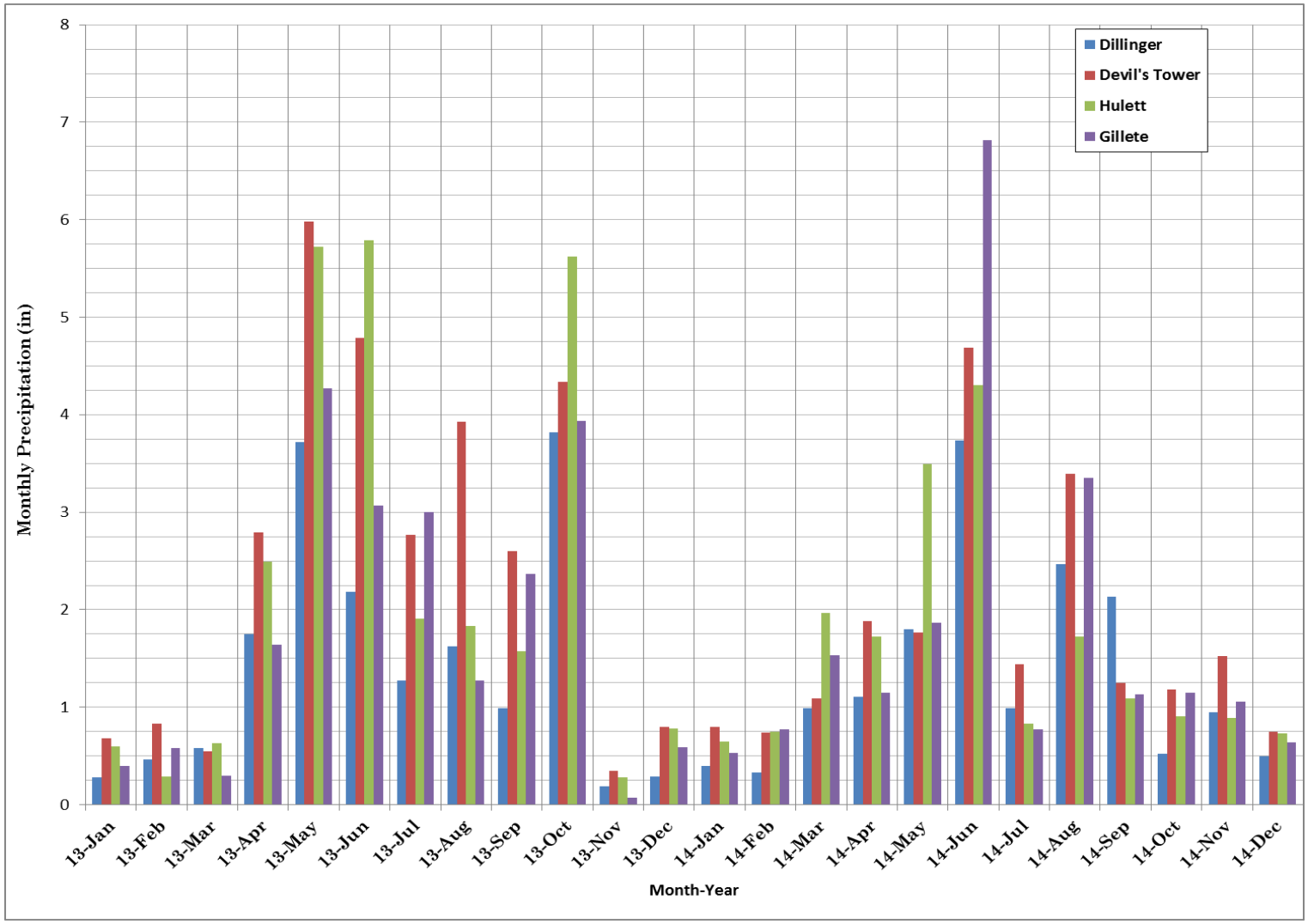


Figure 2-2. Monthly Precipitation Totals During the 2013 and 2014 Summer Recreation Season for Selected Meteorological Sites Within the Belle Fourche River Watershed.

Table 2-3. Selected U.S. Geological Survey and Wyoming Water Development Commission Gaging Stations Within the Belle Fourche River Watershed

Gaging Station	Name	County
USGS 06425720	Belle Fourche River Below Rattlesnake Creek Near Piney, WY	Campbell
USGS 6426130	Donkey Creek Near Gillette, WY	Campbell
USGS 06426160	Stonepile Creek at Mouth Near Gillette, WY	Campbell
USGS 06426500	Belle Fourche River Below Moorcroft, WY	Crook
USGS 06428200	Belle Fourche River Near Alva, WY	Crook
USGS 06428500	Belle Fourche River at WY-SD State Line	Butte (SD)
WWDC Inyan Kara Creek	Inyan Kara Creek West of Inyan Kara Bridge	Crook
WWDC Lytle Creek	Lytle Creek Near Devils Tower National Monument	Crook
WWDC Redwater Creek	Redwater Creek South of Crook County Road 115	Crook

2.3 WATER QUALITY

Water quality sampling results from the CCNRD's monitoring sites located on the Belle Fourche River and Donkey Creek are summarized in Section 2.3.1 and Section 2.3.2. Section 2.3.1 contains a summary of previous water quality results and findings from past monitoring within the project area, while Section 2.3.2 contains a description and summary of current water quality monitoring results from 2013 and 2014.

2.3.1 Past Monitoring Sites

2.3.1.1 *U.S. Geological Survey and Wyoming Department of Environmental Quality*

From 1990 to 1999, the USGS and the WDEQ sampled bacteria on two sites located on the Belle Fourche River: at USGS Gaging Station 06426500 downstream of Moorcroft and USGS Gaging Station 06428050 downstream of the town of Hulett [Clark and Norris, 2000]. The USGS and WDEQ determined that fecal coliform bacteria concentrations were excessive at both monitoring locations, and maximum concentrations of 17,000 organisms per 100 mL were observed at 06428050 and 14,000 organisms per 100 mL at 06426500 [Clark and Norris, 2000].

In 1998, the WDEQ completed monitoring assessments for various water quality parameters at seven sites on two reaches of the Belle Fourche River from the confluence of Raven Creek downstream to the confluence of Donkey Creek. The assessments identified elevated chloride concentrations, as well as excessive nutrients below the mouth of Donkey Creek, which led to a recommendation to continue monitoring to identify potential sources [WDEQ, 2004a]. The WDEQ performed another assessment to determine sources of ammonia below Keyhole Reservoir from the confluence of Barlow Creek downstream to the confluence of Sourdough Creek. The results of this assessment indicated that the town

of Hulett's Waste Water Treatment Facility (WWTF) effluent discharge was a potential nutrient source [WDEQ, 2004b]. The results of both assessments conducted by the WDEQ indicated that the Belle Fourche River was supporting its assigned beneficial uses, with the exception of contact recreation [WDEQ, 2004b]. The WDEQ did not perform bacteria sampling through either of the assessments described above, because the CCNRD's grant proposal for Section 319 funding was awarded to address bacteria sources within the watershed in 2000.

2.3.1.2 *Crook County Natural Resource District*

The CCNRD has been conducting water quality monitoring since 2001 under the guidance of their Landowner Advisory Committee and a SAP approved by the WDEQ [CCNRD, 2008]. Monitoring data collected in 2001 verified fecal coliform bacteria impairments of the listed waters and initiated the CCNRD's water quality monitoring program [CCNRD, 2008]. In 2003 and 2004, the CCNRD performed water quality monitoring on the Belle Fourche River and Donkey Creek, and sampling data indicated that *E. coli* bacteria concentrations exceeded the summer recreation season criterion of 126 organisms per 100 mL on the Belle Fourche River upstream of Keyhole Reservoir as well as on Donkey Creek [EDE Consultants, 2005]. Moreover, Donkey Creek had higher *E. coli* bacteria concentrations than observed on the Belle Fourche River during the same period [EDE Consultants, 2005].

From 2005 to 2009, the CCNRD monitored 13 locations on the Belle Fourche River and Donkey Creek during the summer recreation season (May 1 through September 30). During this period, CCNRD monitored water quality for *E. coli* bacteria, pH, temperature, specific conductivity, dissolved solids, turbidity, and dissolved oxygen [WWC Engineering, 2010]. In 2008, when sections of the Belle Fourche River were found to have higher than acceptable levels of ammonia and chloride by WDEQ, the CCNRD began sampling for ammonia, chloride, nitrate, and nitrite [CCNRD, 2009].

During this period, water quality sampling results indicated that the Belle Fourche River's *E. coli* bacteria concentrations exceeded the summer recreation season *E. coli* chronic criterion of 126 organisms per 100 mL on monitoring sites located above and below Keyhole Reservoir [WWC Engineering, 2010]. These exceedances were based on the old criterion using a 30-day geometric means. From 2005 to 2009, the geometric mean summer recreation season *E. coli* bacteria criterion of 126 organisms per 100 mL was exceeded 27 times on the Belle Fourche River [WWC Engineering, 2010]. Ammonia and chloride water quality data collected in 2008 and 2009 on the Belle Fourche River had no exceedances of the acute ammonia criteria [WWC Engineering, 2010].

Water quality data collected on Donkey Creek from 2005 to 2009 indicated that 60 percent of the *E. coli* bacteria samples exceeded chronic criterion of 126 organisms per 100 mL [WWC Engineering, 2010]. Although Donkey Creek is not subject to chloride or ammonia criteria (Class 3 waterbody), chloride samples were compared to the aquatic life acute value, 860 mg/L; no samples collected exceeded this criteria [WWC Engineering, 2010]. In addition, ammonia concentrations on Donkey Creek did not exceed the pH-dependent acute ammonia criteria values [WWC Engineering, 2010].

Between 2010 and 2012, the CCNRD conducted regular sampling at seven monitoring locations throughout the summer recreation season (May 1 through September 30). These locations are shown in Figure 2-3. Three monitoring sites were used on Donkey Creek over the monitoring period, and four sites were used along the Belle Fourche River. A full summary of results and recommendations from this monitoring effort are available in the 2013 water quality monitoring and assessment report for the Belle Fourche River Watershed [CCNRD, 2013].

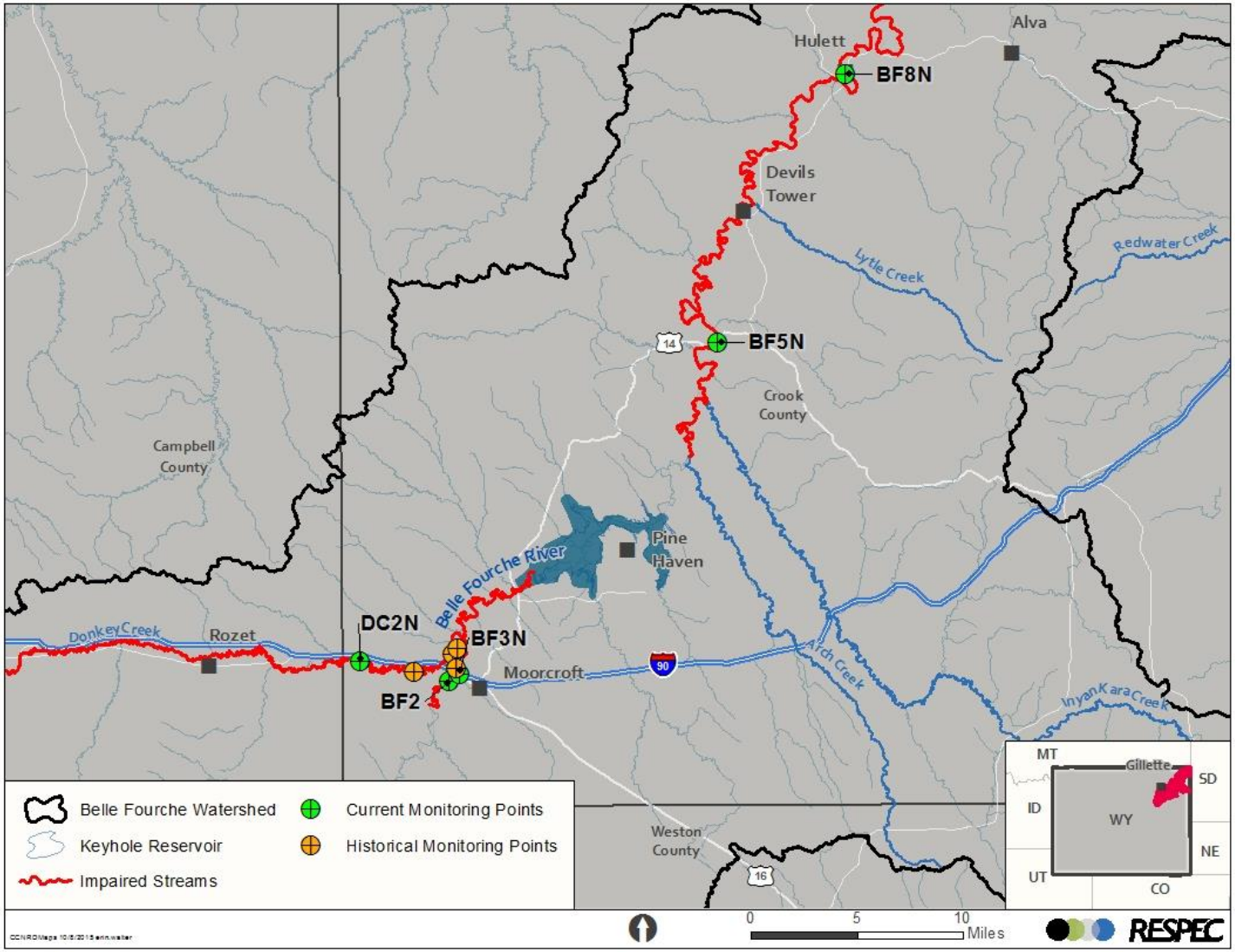


Figure 2-3. Crook County Natural Resource District 2010–2014 Water Quality Monitoring Sites.

2.3.2 Current Monitoring Sites

Between 2013 and 2014, the CCNRD sampled at five monitoring locations during the summer recreation season (May 1 through September 30), including four sites that were monitored previously (BF2, BF3N, BF8N, and DC2N) and one new site (BF5N). All monitoring locations are depicted in Figure 2-3.

2.3.3 Current Monitoring Results

Three segments on the Belle Fourche River and one on Donkey Creek are listed on the 2012 Wyoming's impaired waters list [WDEQ, 2014b]. Three segments on the Belle Fourche River (WYBF101202010501_01, WYBF101202010504_00, and WYBF101202010904_00) are impaired because of fecal coliform bacteria. Segment WYBF101202010504_00 on the Belle Fourche River is also listed as impaired because of ammonia and chloride. Segment WYBF101202010600_01 on Donkey Creek is listed as impaired because of fecal coliform bacteria [WDEQ, 2014b]. Because of the various impairment listings on the Belle Fourche River and Donkey Creek, water quality data results for 2013 and 2014 will be presented by each pollutant.

2.3.4 *E. coli* Bacteria

From 2013 through 2014, the CCNRD collected and analyzed 198 water quality samples for *E. coli* bacteria during the summer recreation season (May 1–September 30). Of these 198 samples, the CCNRD collected 150 samples at four monitoring sites (BF2, BF3N, BF5N, and BF8N) located on the Belle Fourche River and 48 samples at one monitoring site (DC2N) located on Donkey Creek. Figure 2-4 depicts the running 60-day geometric mean concentrations for the 150 samples collected at the sites located on the Belle Fourche River (BF2, BF3N, BF5N, and BF8N). Figure 2-5 displays the running 60-day geometric mean concentrations for the samples collected at the Donkey Creek monitoring site (DC2N) in comparison with BF3N located on the Belle Fourche River upstream of the confluence with Donkey Creek.

Only four samples were collected at BF8N in 2014; therefore, a 60-day geometric mean was not calculated for this site. For purposes only related to this report, the water quality single-sample concentrations from the 2013 and 2014 monitoring seasons were compared to the single-sample maximum concentration criterion of 235 organisms per 100 mL for *E. coli* bacteria. This single-sample criterion has been included only as a visual comparison in the plots, illustrated in Figures 2-6 through 2-11, that display single-sample concentrations and discharge associated with each sampling location during the monitoring period. These plots help in recognizing the seasonal variation in bacteria concentrations and discharge on the Belle Fourche River and Donkey Creek. On several sampling days, discharge from storm events or spring runoff exhibited increased bacteria concentrations.

Table 2-4 summarizes the exceedance under single-sample criterion as well as the 60-day geometric mean chronic criterion for sample seasons 2013 and 2014. The number of single samples that exceed 235 organisms/100 mL and 60-day geomean periods that exceed the chronic criteria 126 organisms/100 mL as well as the percent exceedances are tabulated and calculated for each site. Although BF8N was only sampled four times in 2014, the number of exceedances are included for the single-sample evaluation.

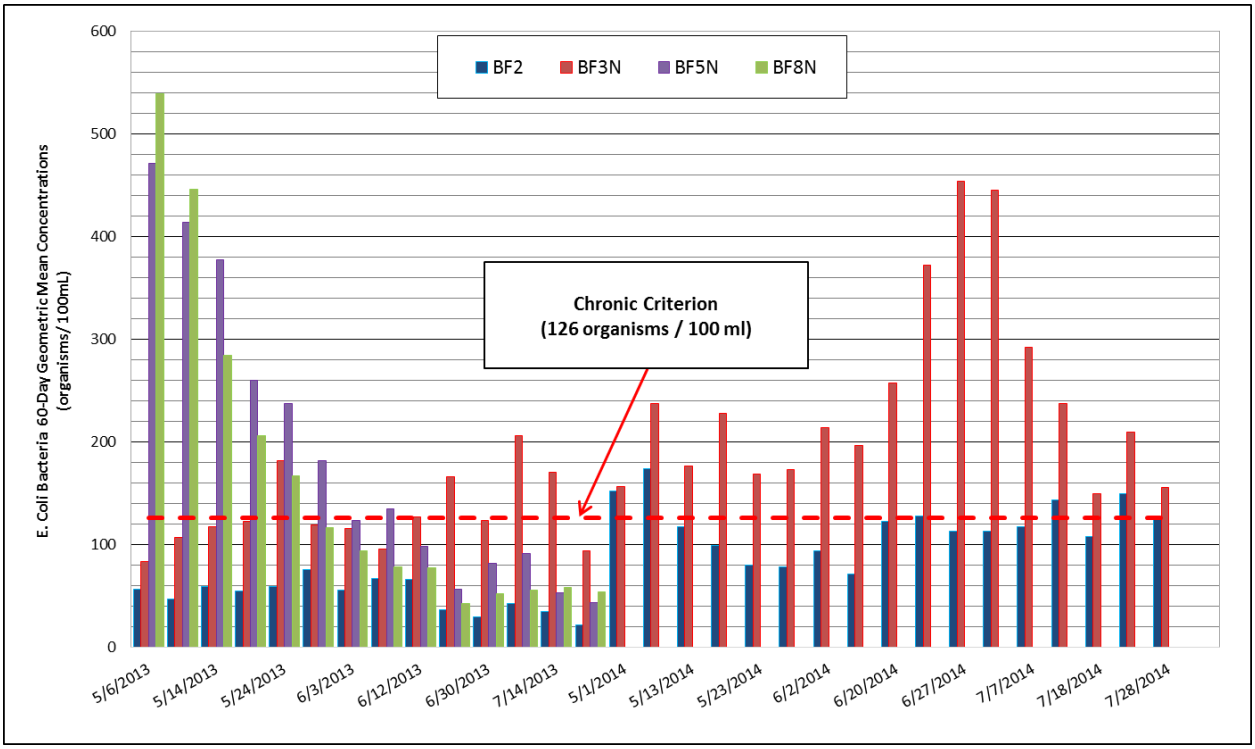


Figure 2-4. *E. coli* 60-Day Running Geometric Mean Concentrations at BF2, BF3N, BF5N, and BF8N From 2013 Through 2014.

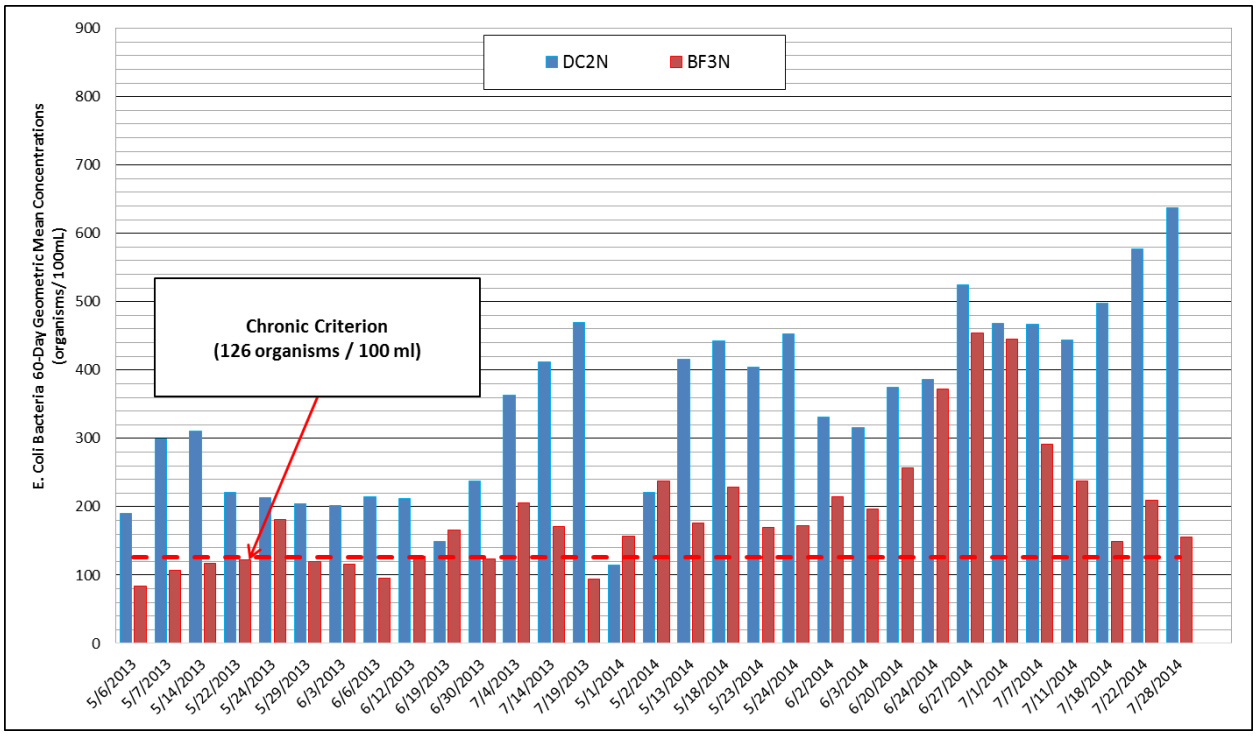


Figure 2-5. 60-Day Running *E. coli* Geometric Mean Concentrations at DC2N and BF3N From 2013 Through 2014.

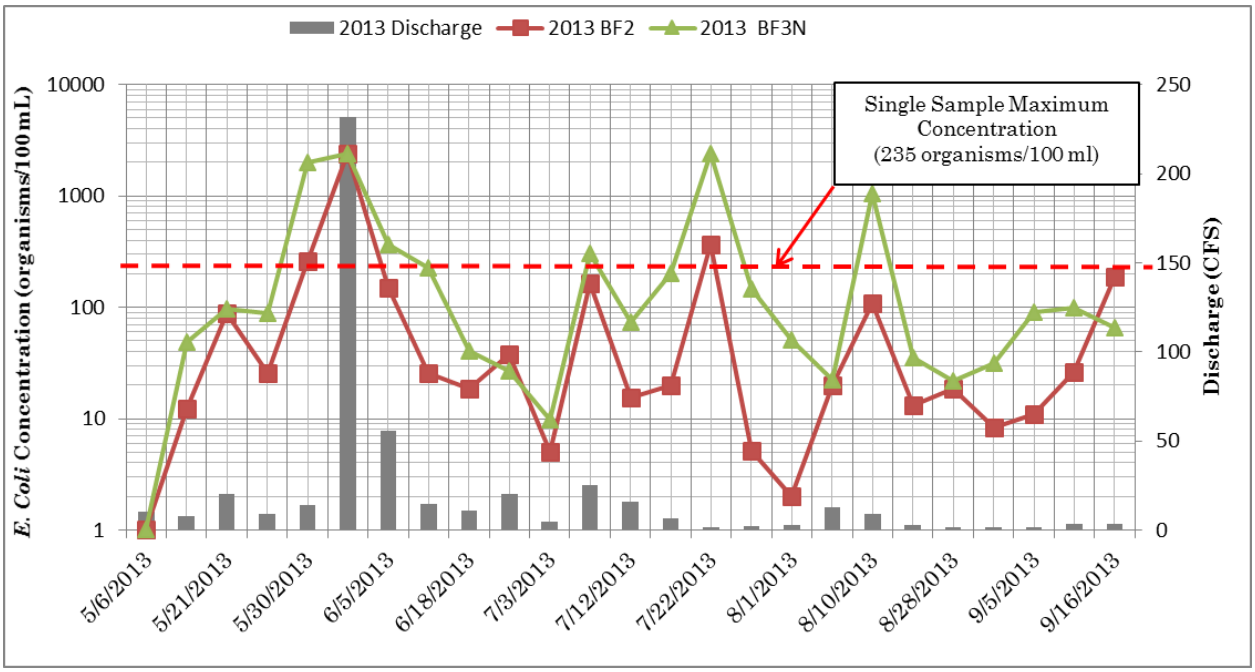


Figure 2-6. *E. coli* Single-Sample Concentrations at BF2 and BF3N Along With Discharge at USGS Site 06426500 in 2013.

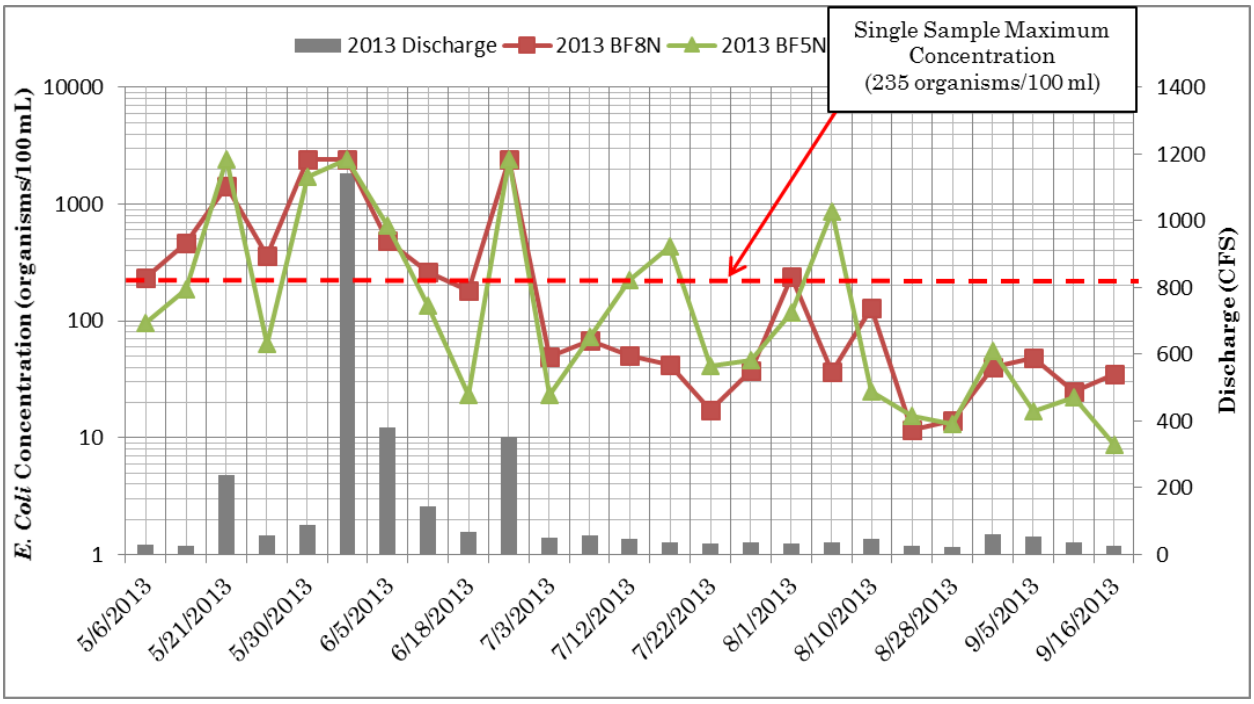


Figure 2-7. *E. coli* Single-Sample Concentrations at BF8N and BF5N Along With Discharge at USGS Site 06428200 in 2013.

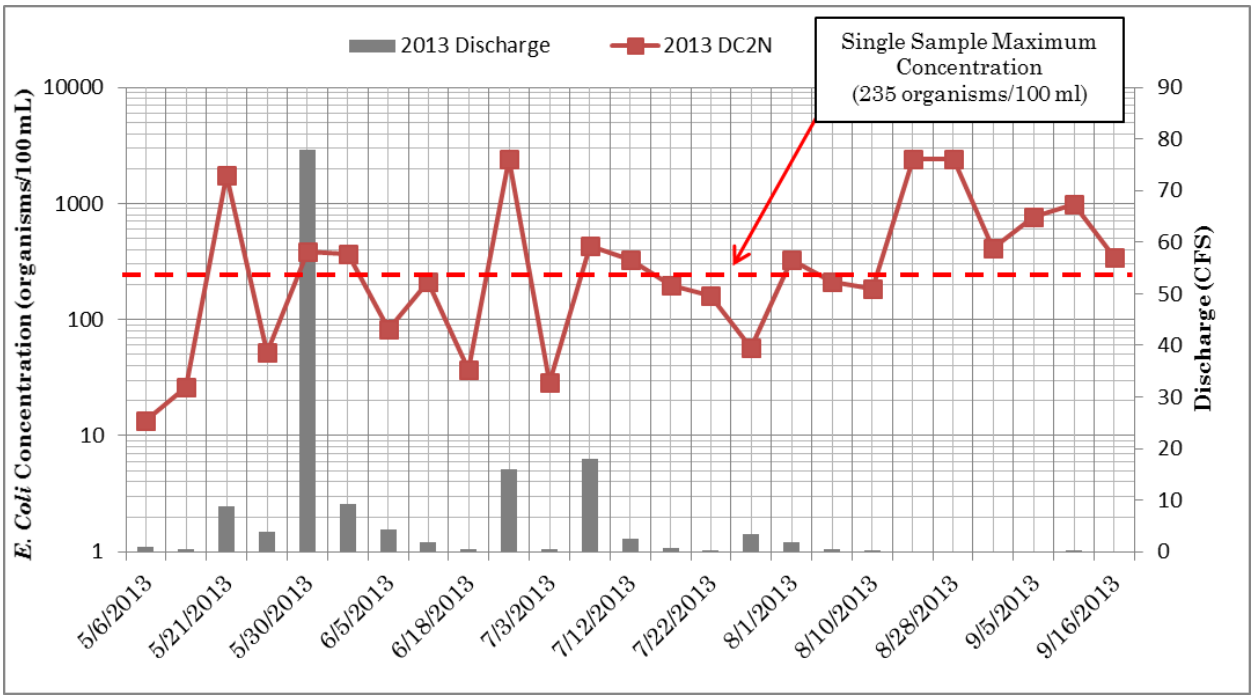


Figure 2-8. *E. coli* Single-Sample Concentrations at DC2N and Discharge at USGS Site 06426130 in 2013.

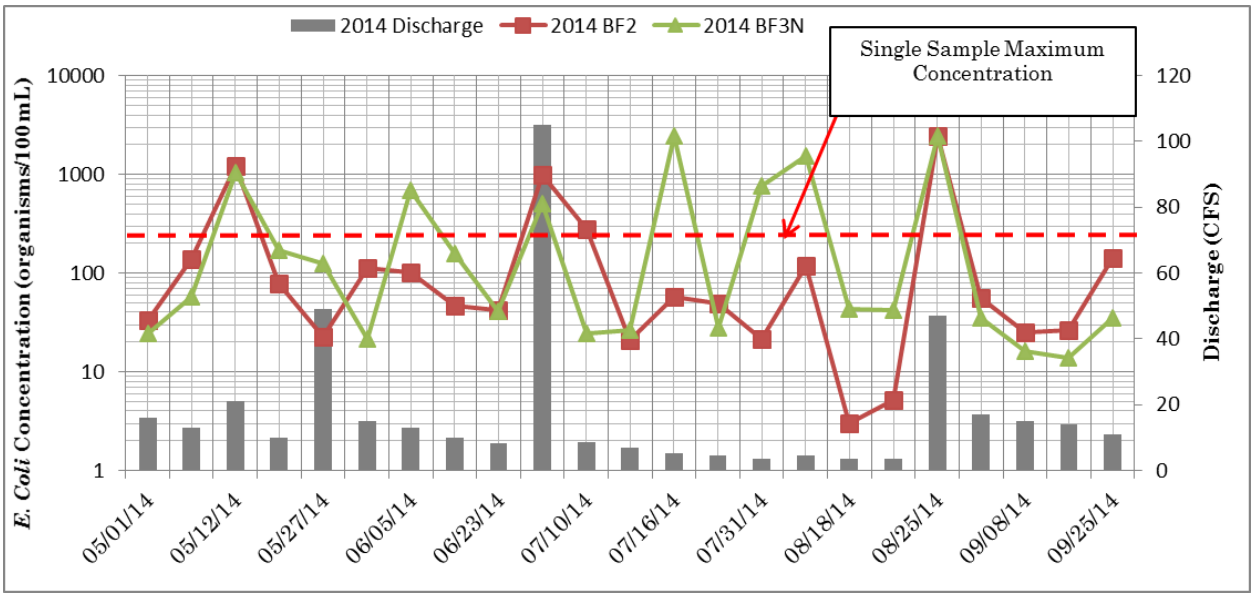


Figure 2-9. *E. coli* Single-Sample Concentrations at BF2 and BF3N and Discharge at USGS Site 06426500 in 2014.

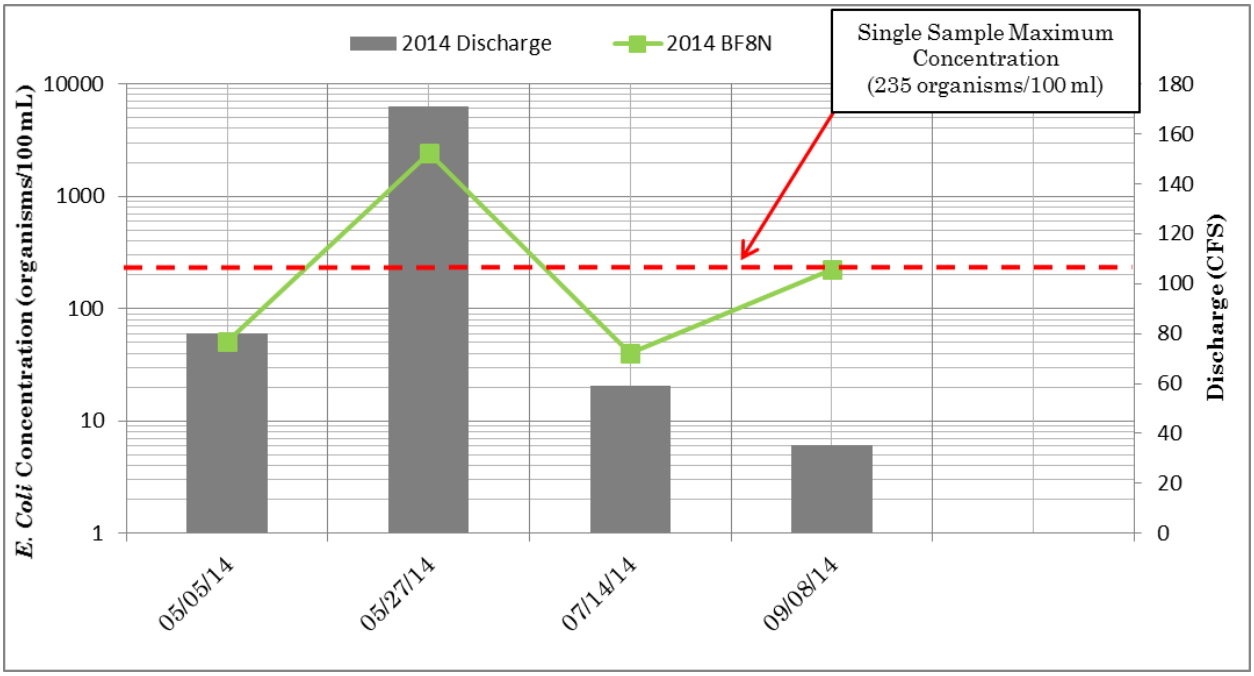


Figure 2-10. *E. coli* Single-Sample Concentrations at BF8N and Discharge at USGS Site 06428200 in 2014.

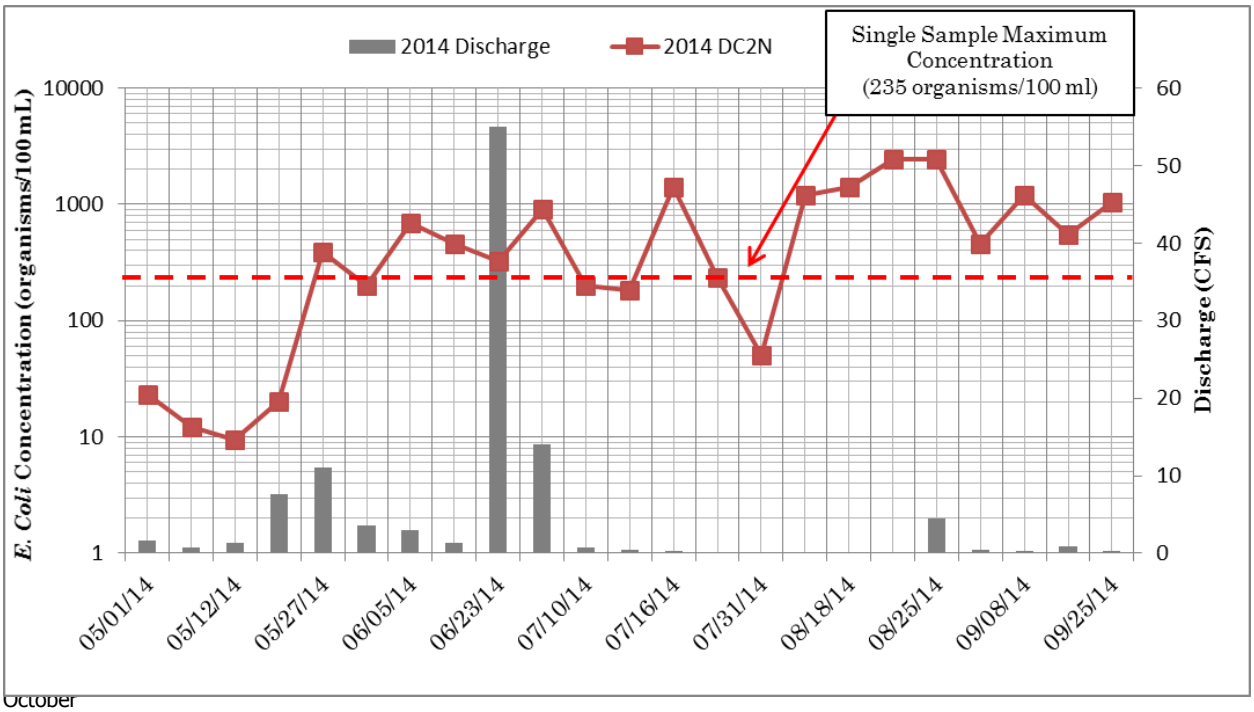


Figure 2-11. *E. coli* Single-Sample Concentrations at DC2N and Discharge at USGS Site 06426130 in 2014.

Table 2-4. *E. coli* Single-Sample Criteria and 60-Day Running Geometric Mean Chronic Criteria Exceedance and Percent Exceedance for 2013 at Sites BF2, BF3N, BF5N, DC2N, and BF8N as Well as 2014 Sampling Season at BF2, BF3N, DC2N, and BF8N

Single-Sample Criteria					60-Day Running Geometric Mean – Chronic Criteria				
Site	2013: 25 Total Samples		2014: 23 Total Samples		Site	2013: 14 Total Samples		2014: 17 Total Samples	
	Exceedance	% Exceedance	Exceedance	% Exceedance		Exceedance	% Exceedance	Exceedance	% Exceedance
BF2	3	12	4	17	BF2	0	0	6	35
BF3N	6	24	7	30	BF3N	5	36	17	100
BF5N	7	28			BF5N	7	50		
DC2N	13	52	15	65	DC2N	5	36	16	94
BF8N	10	40	1	25[a]	BF8N	14	100		

(a) Only four samples were collected from BF8N in 2014.

2.3.5 Chloride

From 2013 through 2014, the CCNRD collected and analyzed 60 water samples for chloride at one site on the Belle Fourche River (BF3N) and one site on Donkey Creek (DC2N). Chloride concentrations were compared to the aquatic life acute chloride criterion of 860 mg/L. Samples collected on the Belle Fourche River and Donkey Creek never exceeded the chronic chloride criterion over the 2-year monitoring period during the collection period (primary recreation season). Figure 2-12 displays observed chloride concentrations at DC2N and BF3N in 2013 and 2014.

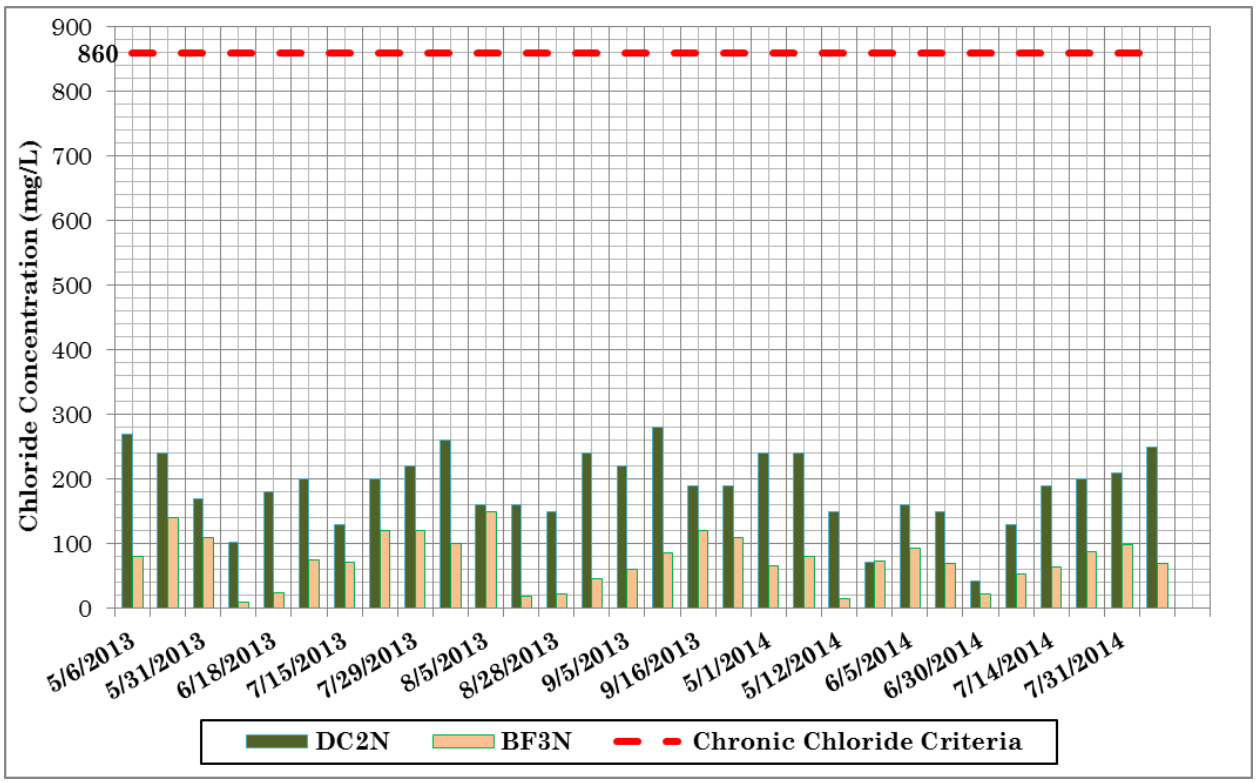


Figure 2-12. Chloride Concentrations at DC2N and BF3N in 2013 and 2014.

2.3.6 Ammonia

From 2013 through 2014, the CCNRD collected and analyzed 60 water samples for ammonia at one site on the Belle Fourche River (BF3N) and one site on Donkey Creek (DC2N). The sampling results have been compared to the pH-dependent values of the acute criteria for ammonia. The results indicate that 88 percent of the ammonia concentrations sampled were nondetectable (<0.1 milligrams ammonia nitrogen per liter [mg N/L]) by laboratory methods. The greatest concentration observed during the monitoring period was 0.90 mg N/L at BF3N on May 21, 2013, and is provided in Table 2-5. Field measurements on that day indicate a pH of 8.39, which correlates to an acute ammonia criterion of 3.95 mg N/L for salmonids absent. Over the monitoring period from May through September, no ammonia samples have been identified as exceeding their respective pH-dependent acute criterion values. Figure 2-13 displays single-sample ammonia concentrations observed during the 2013 through 2014 monitoring period.

Table 2-5. Mean Ammonia Concentrations Calculated for Water Quality Samples Collected on the Belle Fourche River and Donkey Creek From 2013 Through 2014

Monitoring Site (Year)	Location	Mean Ammonia Concentration (mg N/L)	Ammonia Concentration Range (mg N/L)
BF3N (2013)	Belle Fourche River 300 meters downstream of I-90 below Moorcroft and upstream of the confluence of Donkey Creek	0.114	<0.1–0.9
DC2N (2013)	Donkey Creek upstream of the confluence with the Belle Fourche River. 5 miles west of Moorcroft on Donkey Creek Road	<0.1	<0.1
BF3N (2014)	Belle Fourche River 300 meters downstream of I-90 below Moorcroft and upstream of the confluence of Donkey Creek	0.0625	<0.–0.2
DC2N (2014)	Donkey Creek upstream of the confluence with the Belle Fourche River. 5 miles west of Moorcroft on Donkey Creek Road	0.1125	<0.–0.4

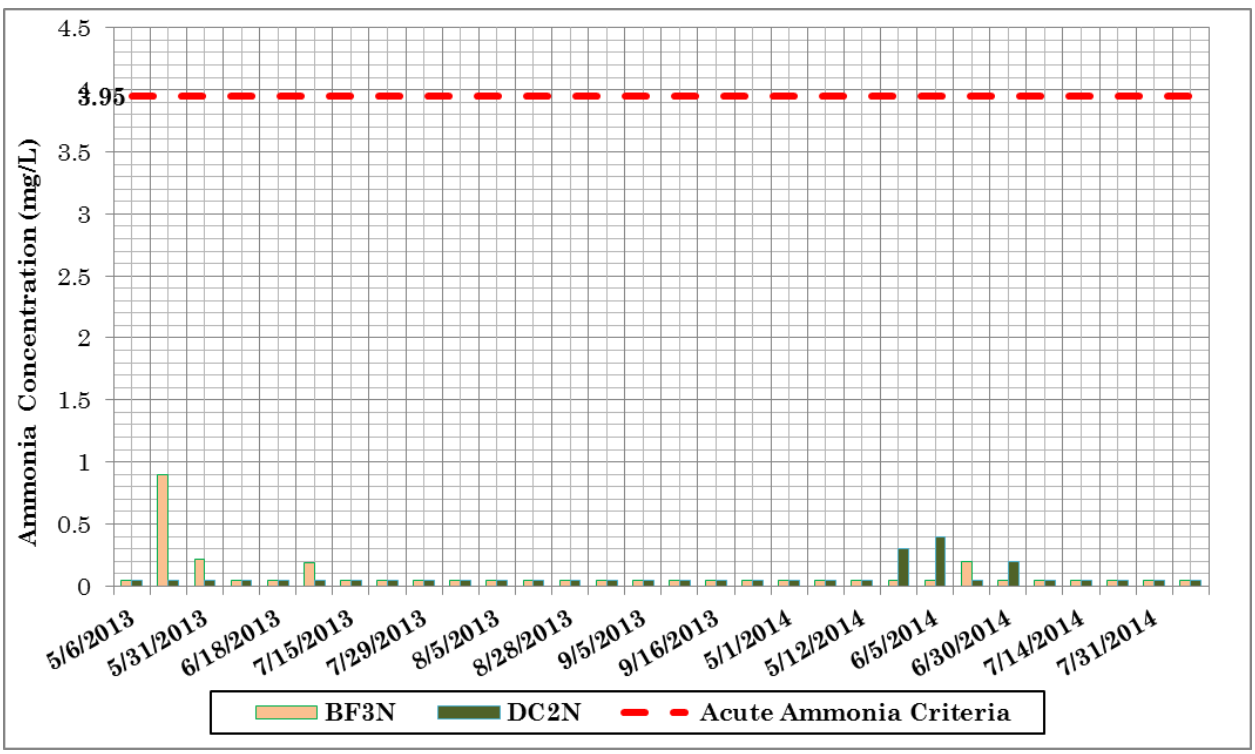


Figure 2-13. Single-Sample Ammonia Concentrations Observed Over the 2013 Through 2014 Monitoring Period.

2.3.7 Nitrate-Nitrogen and Nitrite-Nitrogen

From 2013 through 2014, the CCNRD collected and analyzed 72 water quality samples for nitrate-nitrogen and 72 samples for nitrite-nitrogen from one site on the Belle Fourche River (BF3N) and one on

Donkey Creek (DC2N). Nitrate-nitrogen concentrations were compared to the human-health value for "fish and drinking water" criterion of 10 mg/L. Nitrite-nitrogen concentrations were compared to the human-health value for "fish and drinking water" criterion of 1 mg/L. During the monitoring period, two samples collected at Site DC2N exceeded the human-health value criteria for nitrate-nitrogen, including a value of 14.0 mg/L on May 1, 2014, and a value of 15 mg/L on September 15, 2014. The next greatest nitrate-nitrogen concentration observed during the monitoring period was 8.8 mg/L at Site DC2N on May 5, 2014 (Figure 2-14). The greatest nitrite-nitrogen concentration observed was 0.15 mg/L at Site DC2N on May 21, 2013 (Figure 2-15). Overall, nitrate/nitrite concentrations followed a similar pattern in 2013 and 2014 with the highest concentrations observed at the beginning of the growing season (especially May) and at the end of the growing season. The lowest concentrations were observed during the summer months, especially during July and August.

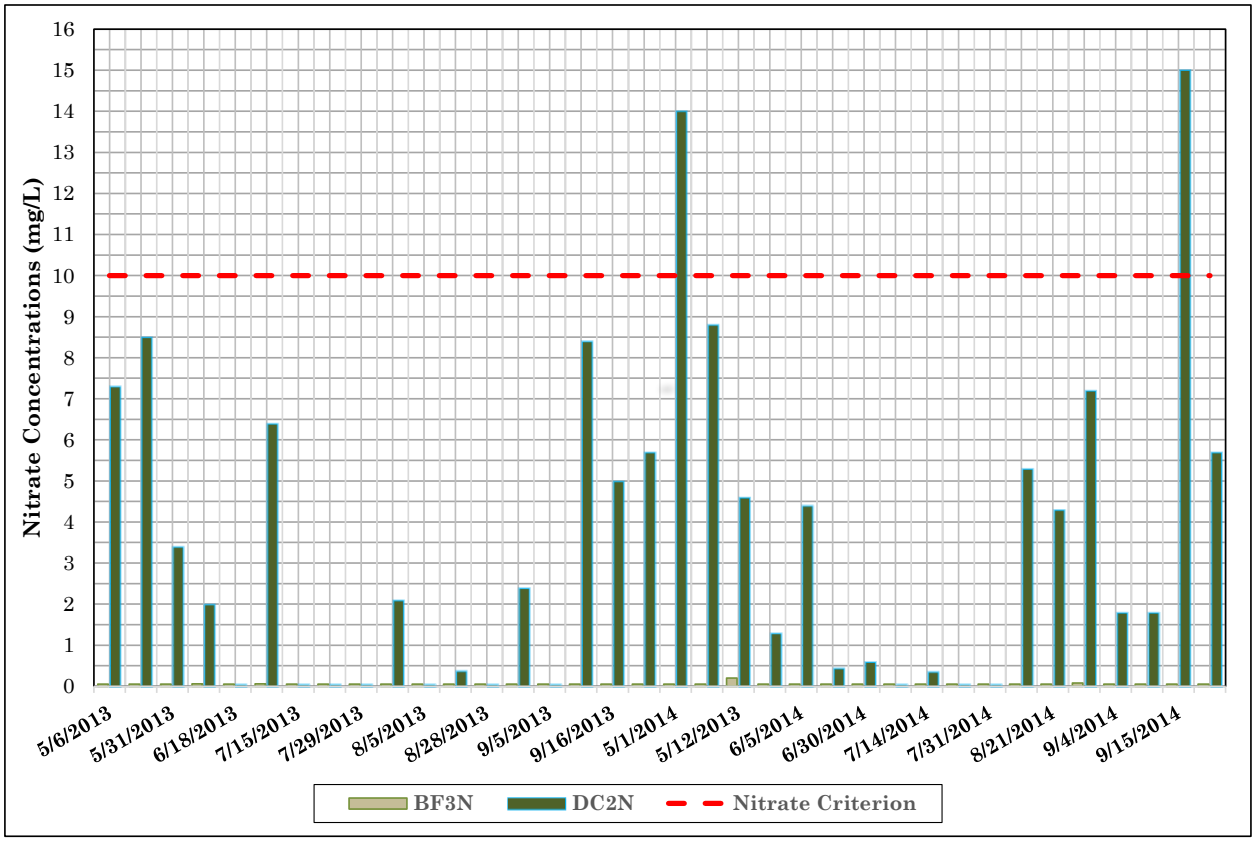


Figure 2-14. Nitrate Concentrations for BF3N and DC2N in 2013 and 2014.

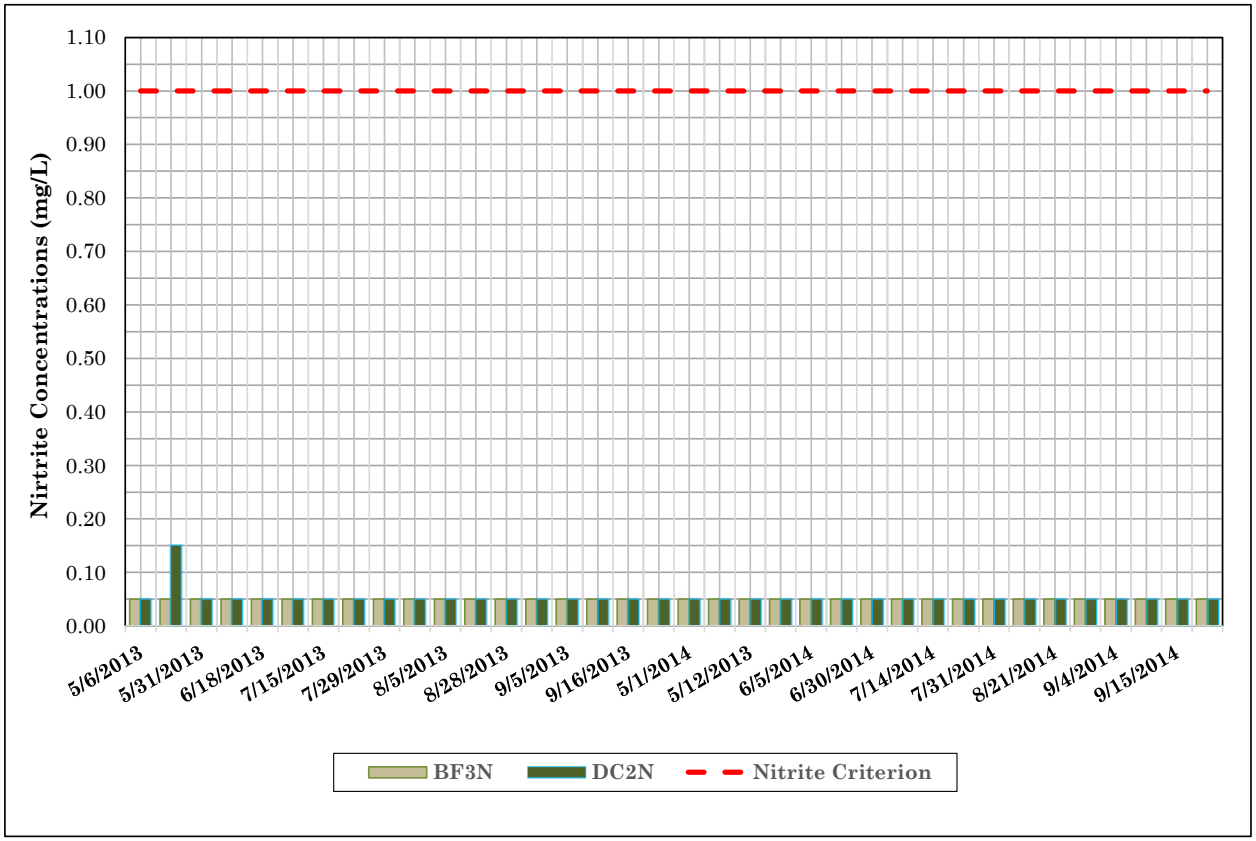


Figure 2-15. Nitrite Concentrations at BF3N and DC2N in 2013 and 2014.

3.0 ANALYSIS

3.1 SUMMARY

The CCNRD monitored water quality in 2013 and 2014 during the summer recreation season from May 1 through September 30 and collected and analyzed 198 water quality samples for *E. coli* bacteria from the Belle Fourche River and Donkey Creek. The CCNRD also collected and analyzed 60 samples for chloride, 60 samples for ammonia, and 72 samples each for nitrate-nitrogen and nitrite-nitrogen from the Belle Fourche River and Donkey Creek. The examination was performed on the provisional data for *E. coli* bacteria, chloride, ammonia, nitrate-nitrogen, and nitrite-nitrogen. Additionally, field measurements included pH, temperature, conductivity, dissolved solids, dissolved oxygen, and turbidity. The WDEQ performed quality assurance/quality control (QA/QC) reviews of the provisional water quality data.

Sources of *E. coli* bacteria, chloride, ammonia, nitrate-nitrogen, and nitrite-nitrogen concentrations in the Belle Fourche River and Donkey Creek are discussed in detail within Tetra Tech, Inc. [2013]. Priority sources identified in the TMDL included failing septic systems; wastewater treatment facilities; livestock, pet, and wildlife waste; and winter runoff following the application of deicing products [Tetra Tech, Inc., 2013].

3.1.1 *E. coli* Bacteria

Sampling results from 2013 through 2014 indicate that water quality samples from the Belle Fourche River frequently exceeded the 60-day running geometric mean chronic criterion of 126 organisms per 100 mL and occasionally exceeded the single-sample maximum concentration criterion of 235 organisms per 100 mL for *E. coli* bacteria over the summer recreation season. Figure 2-4 summarized the 60-day geometric means for all sampling sites during this monitoring period. Of these 198 samples, the CCNRD collected 150 samples at four monitoring sites (BF2, BF3N, BF5N, and BF8N) located on the Belle Fourche River and 48 samples at one monitoring site (DC2N) on Donkey Creek. At the uppermost site on the Belle Fourche River (BF2) sampling results indicate that *E. coli* concentrations increased between 2013 and 2014. *E. coli* bacteria concentrations at BF2 never exceeded the chronic *E. coli* criterion in 2013; however, in 2014 BF2 exceeded the criterion six times (35 percent of the 60-day geometric means). The sampling results at BF3N (on the Belle Fourche River above the confluence with Donkey Creek) also indicate that *E. coli* bacteria concentrations increased between 2013 and 2014. The percent exceedance for single-sample criteria increased from 24 percent in 2013 to 30 percent in 2014. However, the chronic 60-day geometric mean criterion reveal a jump from 36 percent to 100 percent exceedance over the 60-day periods from the 2013 to 2014 season. At BF5N (on the Belle Fourche River below the Keyhole Reservoir), sampling results indicate that *E. coli* concentrations exceeded the single-sample criterion seven times (28 percent) in 2013. The 60-day geometric mean criterion were exceeded only during the beginning of the season with a total of 50 percent exceedance; samples were not collected at this site in 2014. At the most downstream site on the Belle Fourche River (BF8N), sampling results indicate that *E. coli* concentrations exceeded the 60-day geometric mean criterion during the first five 60-day periods in 2013 and were still below the criterion for the remaining nine 60-day periods. Only four samples were collected at BF8N in all of 2014 and, as a result, a 60-day geometric mean was not calculated for this site and only one sample exceeded the single-sample criterion for 2014.

At the site on Donkey Creek (DC2N), sampling results indicate that *E. coli* exceeded the 60-day geometric mean *E. coli* criterion during every 60-day period in 2013 and all but one 60-day period in 2014 as illustrated in Figure 2-5. The single-sample criterion for DC2N reveals a slight increase in exceedance from 2013 to 2014 with 12 percent exceedance in 2013 to 17 percent in 2014.

To help further understand the variability of samples, a load duration curve (LDC) was developed for each site, as depicted in Figures 3-1 through 3-5. An LDC visually represents exceedances of the water quality standard. The observed water quality samples are paired with flow measurements at the time they were collected to determine a load in the stream. The *x*-axis of an LDC displays the percent of time that a flow is equaled or exceeded while the *y*-axis displays the load (concentration × flow). Flows are grouped into high (0–10 percent), moist (10–40 percent), midrange (40–60 percent), dry (60–90 percent), and low (90–100 percent) flows. This instantaneous load is then compared to the maximum load that a stream can assimilate (blue line) without exceeding water quality standards. Data points above the blue line indicate exceedances of the standard. To reiterate, the 235 organisms per 100 mL is used as a reference only and this standard is not intended to apply to the Belle Fourche River or Donkey Creek. Exceedances in the high and moist range are often associated with indirect loads caused by precipitation events. Dry and low flow exceedances are attributed to deposition directly into a stream. Samples were further categorized by grouping them by month to help understand potential seasonal trends.

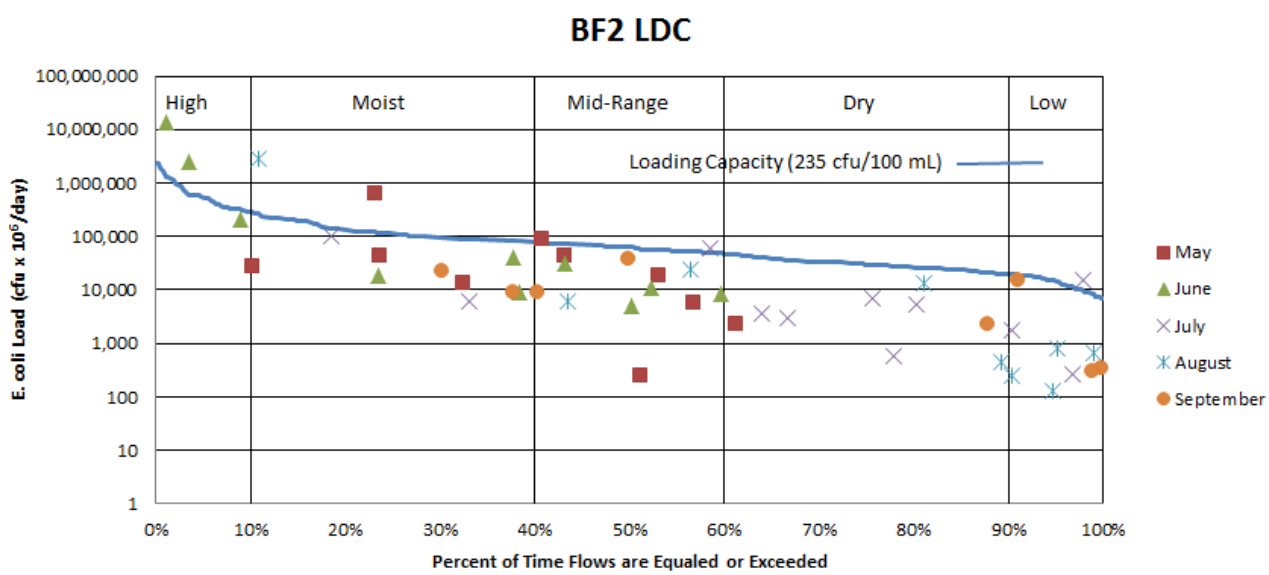


Figure 3-1. Load Duration Curve Displaying Data Collected at Site BF2 During the 2013 and 2014 Monitoring Seasons.

The LDC for Site BF2 (illustrated in Figure 3-1) indicates exceedances of the water quality standard predominantly in the high and moist flow regimes, although the number of samples is limited. Exceedances were primarily in May, June, and July. The LDC for BF3N, displayed in Figure 3-2, indicates exceedances occurring throughout the recreational season except September and throughout all flow regimes, which indicates both direct and indirect loading sources. Both BF5N and BF8N sites, depicted in Figures 3-3 and 3-4, demonstrate exceedances primarily at higher flows in May and June. At the Donkey

Creek site (DC2N) displayed in Figure 3-5, exceedances occurred during all recorded flow regimes and during all months.

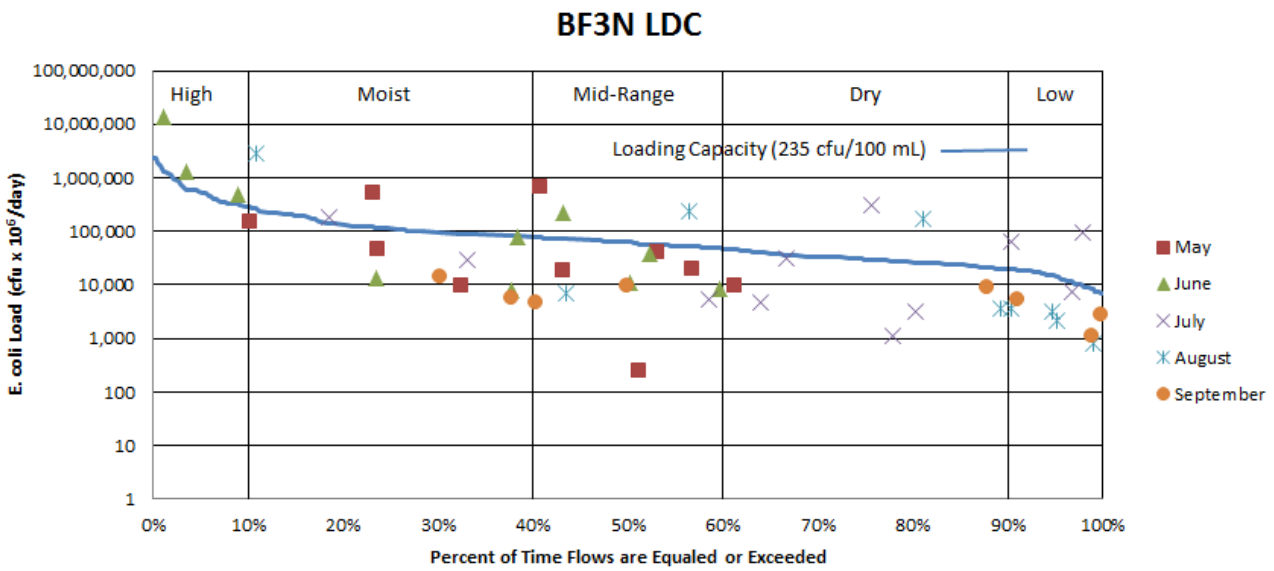


Figure 3-2. Load Duration Curve Displaying Data Collected at Site BF3N During the 2013 and 2014 Monitoring Seasons.

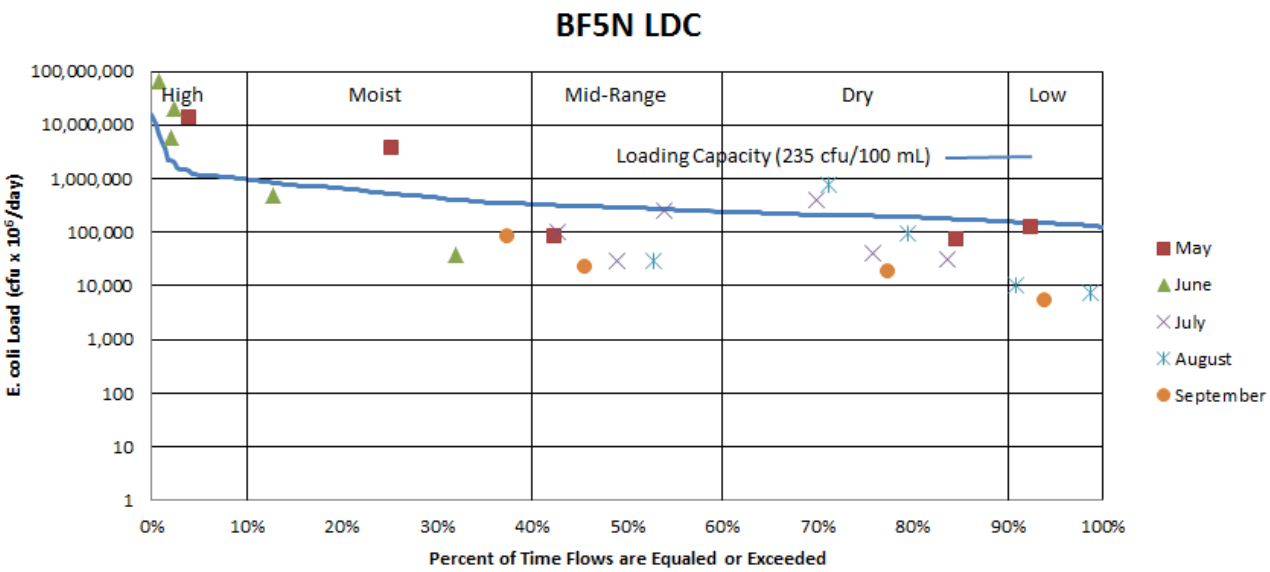


Figure 3-3. Load Duration Curve Displaying Data Collected at Site BF5N During the 2013 and 2014 Monitoring Seasons.

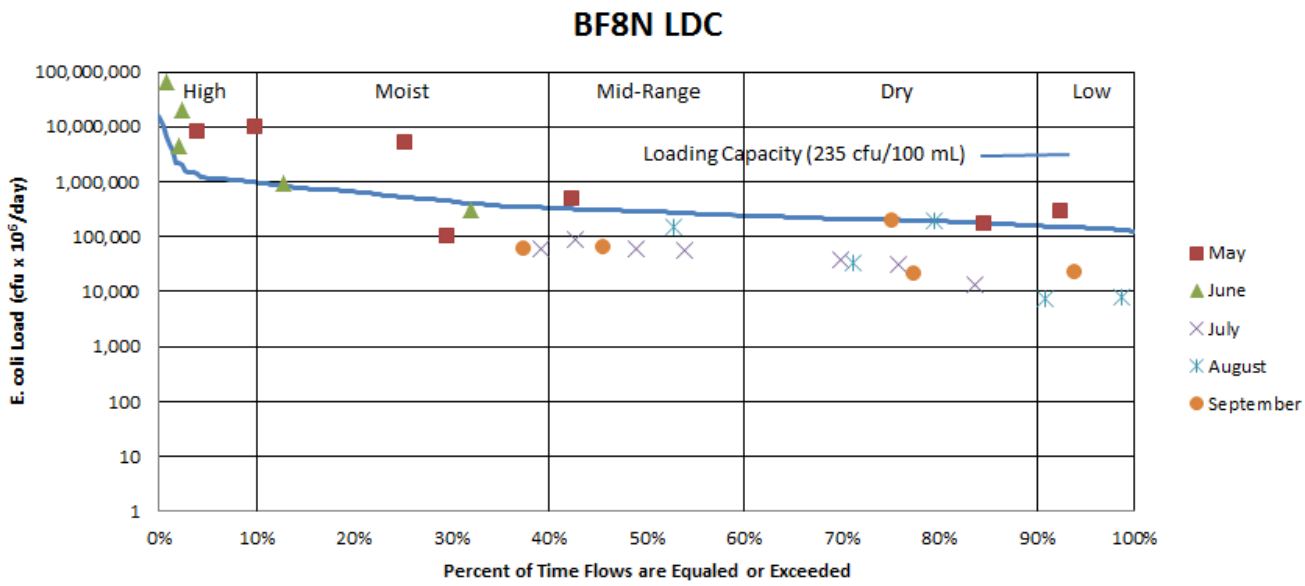


Figure 3-4. Load Duration Curve Displaying Data Collected at Site BF8N During the 2013 and 2014 Monitoring Seasons.

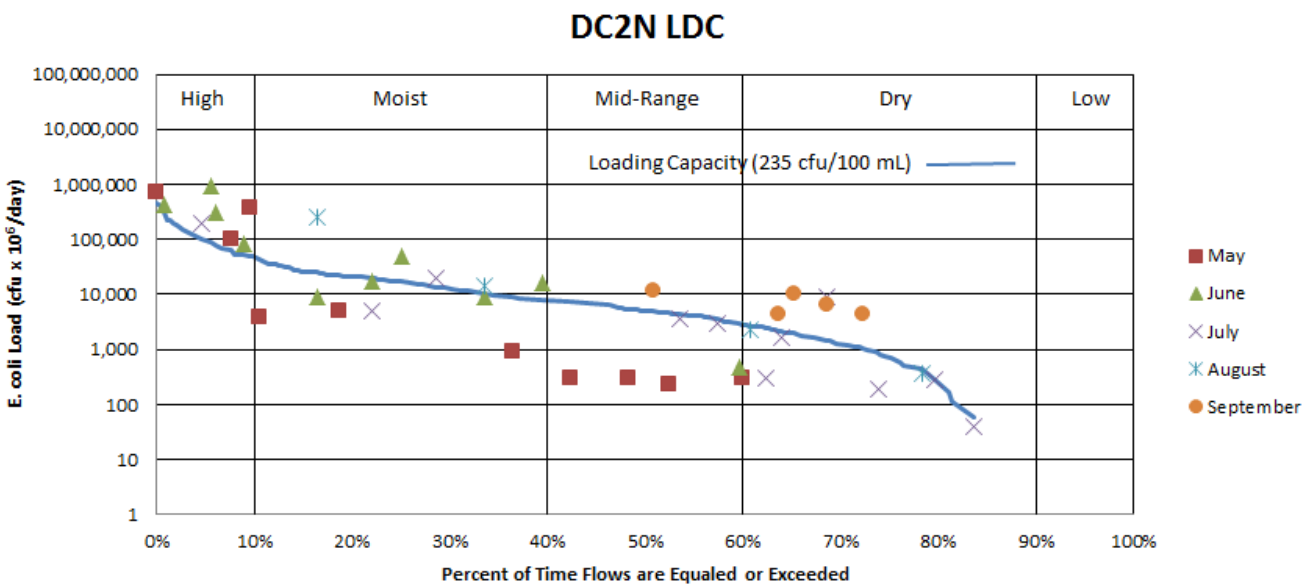


Figure 3-5. Load Duration Curve Displaying Data Collected at Site DC2N During the 2013 and 2014 Monitoring Seasons.

The LDC for Site BF2 (illustrated in Figure 3-1) indicates exceedances of the water quality standard predominantly in the high and moist flow regimes, although the number of samples is limited. Exceedances were primarily in May, June, and July. The LDC for BF3N, displayed in Figure 3-2, indicates exceedances occurring throughout the recreational season except September and throughout all flow regimes, which indicates both direct and indirect loading sources. Both BF5N and BF8N sites, depicted in Figures 3-3 and 3-4, demonstrate exceedances primarily at higher flows in May and June. At the Donkey

Creek site (DC2N) displayed in Figure 3-5, exceedances occurred during all recorded flow regimes and during all months.

3.1.1 Chloride

Chloride concentrations exhibited a seasonal pattern in 2013 and 2014 with elevated concentrations observed during the spring (especially during May), and fall (from August through September). During the 5-month monitoring period for 2013 and 2014, sampling results at the BF3N and DC2N sites indicate that chloride concentrations on the Belle Fourche River and Donkey Creek never exceeded the aquatic life acute criterion. However, Site DC2N does reveal higher concentrations of chloride than Site BF3N during the entire 2013 sampling season, and all but one sample date in 2014. Although Donkey Creek is a Class 3B waterbody and is not subject to the chloride criteria nor listed for chloride, these results are important in that Donkey Creek is potentially contributing to elevated chloride concentrations in the Belle Fourche River.

Sources of chloride identified within the watershed included deicing agents, dust-suppressant solutions, oil treater and/or coal mine effluent, irrigation return flows, groundwater, point sources, and natural sources/processes [Tetra Tech, Inc., 2013]. Higher chloride samples support the evaluations that show that Donkey Creek is a source of chloride to the Belle Fourche River, while the elevated chloride loads from Stonepile Creek are the major source of chloride load to Donkey Creek [Tetra Tech, Inc., 2013].

3.1.2 Ammonia

Sampling results indicate that ammonia was not detectable in 87 percent of the water samples (26 out of 30) collected on the Belle Fourche River. The 13 percent of samples (4 out of 30) that had detectable levels of ammonia ranged from 0.19 to 0.9 mg N/L. No ammonia samples were identified as exceeding their respective pH-dependent acute criterion values during the 2013 through 2014 monitoring period on the Belle Fourche River.

Similarly, ammonia was not detectable in 90 percent (27 out of 30) of samples collected on Donkey Creek. The 10 percent of samples (3 out of 30) that had detectable levels of ammonia ranged from 0.2 to 0.4 mg N/L. No ammonia samples were identified as exceeding their respective pH-dependent acute criterion values during the 2013 through 2014 monitoring period on Donkey Creek.

Since 2010, 185 ammonia samples have been collected on the Belle Fourche River or Donkey Creek. No ammonia samples were identified as exceeding their respective pH-dependent acute criterion values during the 2010 through 2014 monitoring periods on either waterbody. This finding is in-line with data collected for the TMDL study where all exceedances of the ammonia standard occurred during the winter.

Sources of ammonia to the Belle Fourche River include the Moorcroft wastewater lagoon, the Gillette WWTF, the Wright Water and Sewer District, runoff from Gillette following deicing applications, and runoff from the city of Wyodak, especially during high-flow events [Tetra Tech, Inc., 2013]; however, based on the data reviewed for the TMDL, the most likely source of the impairment is the Moorcroft wastewater lagoon [Tetra Tech, Inc., 2013], which contributes elevated ammonia loads to Rush Creek just above the confluence of the Belle Fourche River. In addition, ammonia loads are likely enhanced by Donkey Creek causing the impairments on the Belle Fourche River because of the Gillette WWTF, runoff

from Gillette following deicer applications, and the Wyodak Power Plant with elevated loads likely only reaching the listed segment when the loads are very large or during high-flow conditions with fast-moving water [Tetra Tech, Inc., 2013]. The ammonia discharged from the Gillette WWTF, Crestview Estates, Wyodak Power Plant, and Wright is assumed to convert to nitrate and other nitrogen-species through in-stream processes before reaching USGS Gage Station 06426500 on the Belle Fourche River. This assumption is based on synoptic ammonia data collected by Campbell County Conservation District (CCCD) on Donkey Creek [Tetra Tech, Inc., 2013].

3.1.3 Nitrate-Nitrogen and Nitrite-Nitrogen

Nitrate-nitrogen concentrations and nitrite-nitrogen concentrations were compared to the human- health value for "fish and drinking water" criterion of 10 mg/L and 1 mg/L, respectively. During the monitoring period, two samples exceeded the human health value criteria for nitrate-nitrogen on Donkey Creek; however, drinking water is not a designated use for Donkey Creek (Class 3). The greatest nitrite-nitrogen concentration observed was 0.15 mg/L at Site DC2N on May 21, 2013. Overall, nitrate/nitrite concentrations followed a similar pattern in 2013 and 2014 with the highest concentrations observed in spring (especially May) and at the end of the growing season. The lowest concentrations were observed during the summer months, especially July and August.

3.2 RECOMMENDATIONS

Data collected by the CCNRD over the 2013 and 2014 summer recreation seasons provided a good indication of the status of the water quality in the Belle Fourche River and Donkey Creek within Crook County. Based on monitoring results from the 2013 through 2014 monitoring seasons, significant contributing *E. coli* sources are likely present between monitoring locations BF2 and BF3N. Monitoring sites on Donkey Creek continued to exhibit *E. coli* concentrations that exceed water quality criteria. The CCNRD's past monitoring efforts collected water quality data to adequately identify potential sources and delineate priority implementation areas within the watershed. Recommended future efforts should focus on the following aspects:

- Maintaining long-term, permanent monitoring sites
- Coordinating with Campbell County on water quality monitoring sites and schedules
- Working with point- and nonpoint-source contributors to implement BMPs and monitor implementation effectiveness
- Updating the CCNRD SAP as needed
- Reducing the frequency of ammonia sampling. Recent results have indicated that no significant problem exists, so periodic checks should be sufficient. Nitrogen sampling frequency could also be reduced, especially on the Belle Fourche River, for the same reason.

3.2.1 Long-Term Monitoring Sites

Future water quality sampling and associated flow monitoring is recommended to allow for comparisons between sampling sites and to further identify trends of pollutant concentrations/loads. New sampling sites could be added in close proximity to implementation projects to aid in determining implementation

effectiveness. The following current monitoring sites are recommended for continued use in future, long-term monitoring efforts within Crook County:

- Site DC2N to obtain consistent concentration/loading data in Donkey Creek
- Site BF2 to obtain consistent concentration/loading data on the Belle Fourche River for the watershed area above the town of Moorcroft and above the confluence with Donkey Creek
- Site BF3N or a nearby site on the Belle Fourche River to maintain consistent concentration/loading data on the Belle Fourche River below the town of Moorcroft to understand impacts that the town may have on the River
- Site BF5N to obtain consistent concentration/loading data on the Belle Fourche River above Site BF8N and below the confluence of Inyan Kara and Arch Creeks
- Site BF8N to maintain a record of overall impacts of the project below Keyhole Reservoir, to provide a means of comparison with upstream segments, and to obtain consistent concentration/loading data on the Belle Fourche River above the town of Hulett and below the confluence of Black Tail Creek
- Site BF3 or a nearby site on the Belle Fourche River just downstream the confluence of Donkey Creek to isolate what impact Donkey Creek has on the Belle Fourche River.

3.2.2 Water Quality Monitoring Coordination

Coordination could occur between the CCNRD, the CCCD, the city of Gillette, the WDEQ, and the USGS along with discharge permit holders to share sampling methods, review recent results, and discuss future plans regarding each entity's water quality monitoring activities and sample collection within the watershed.

3.2.3 Sampling and Analysis Plan Update

The CCNRD updated their SAP, which was approved by WDEQ in 2014. This document will continue to guide the CCNRD in achieving monitoring goals [CCNRD, 2014]. The 2014 SAP update included plans to contract with a certified laboratory to verify total coliform and *E. coli* concentrations as well as verification of blanks and duplicates. With the 2014 update, CCNRD has also been collecting stream velocity and discharge measurements using a flow meter as well as adding microbial source tracking (MST) to their sampling procedure. We recommend that the SAP be updated whenever significant changes are made in sampling or testing equipment, methods, or locations.

Lastly, we recommend that the CCNRD continue to perform monitoring efforts at the same diligent and detailed level as they have in the past. The careful and thorough attention to detail in field sampling procedures, techniques, and data recording performed by the CCNRD assists greatly in interpreting water quality data and reporting the results.

4.0 REFERENCES

Crook County Natural Resource District, 2008. *Belle Fourche River Watershed, Watershed Plan Implementation Project–Phase II,* prepared for the Crook County Natural Resource District, Sundance, WY.

Crook County Natural Resource District, 2009. *Belle Fourche River Watershed Plan*, prepared for the Crook County Natural Resource District, Sundance, WY.

Crook County Natural Resource District, 2010. *Crook County Natural Resource District, Annual Report 2008–2009, Annual Plan of Work 2009–2010*, prepared for the Crook County Natural Resource District, Sundance, WY.

Crook County Natural Resource District, 2013. *Water Quality Monitoring and Assessment Report for the Belle Fourche River Watershed*, prepared for the Crook County Natural Resource District, Sundance, WY.

Crook County Natural Resource District, 2014. *Sampling and Analysis Plan Belle Fourche River Watershed Plan, Phase III Wyoming Nonpoint Source Section 319*, prepared for the Crook County Natural Resource District, Sundance, WY.

Clark, M. L. and J. R. Norris, 2000. *Occurrence of Fecal Coliform Bacteria in Selected Streams in Wyoming, 1990–1999,* prepared by the Wyoming Department of Environmental Quality, Water-Resources Investigations, Cheyenne, WY, for the U.S. Geological Survey, Reston, VA.

EDE Consultants, 2005. *Belle Fourche River/Donkey Creek 319 Watershed Assessment-Draft 2003–2004 Indicator Bacteria Monitoring Report,* prepared by EDE Consultants, Sheridan, WY, for the Crook County Natural Resource District, Sundance, WY.

Environmental Protection Agency, 2015. "5.11 Fecal Bacteria," *epa.gov*, retrieved March 25, 2015, from *http://water.epa.gov/type/rsl/monitoring/vms511.cfm*

Tetra Tech, Inc., 2013. *Belle Fourche River Watershed TMDLs for Pathogens, Ammonia, and Chloride,* prepared by Tetra Tech, Inc., Pasedena, CA, for the Wyoming Department of Environmental Quality, Cheyenne, WY.

U.S. Geological Survey, 2015a. **"**Water Resources of Wyoming and Montana," *usgs.gov*, retrieved May 15, 2015, from *http://wy-mt.water.usgs.gov/*

U.S. Geological Survey, 2015b. **"**Water Resources of South Dakota," *usgs.gov*, retrieved May 16, 2015, from *http://sd.water.usgs.gov/*

WWC Engineering, 2010. *Upper Belle Fourche Watershed Data Analysis Report 2005-2009,* prepared by WWC Engineering, Sheridan, WY, for the Crook County Natural Resource District, Sundance, WY.

Western Regional Climate Center, 2014. "Western Regional Climate Center, Coop Sites," *dri.edu*, retrieved March 12, 2015, from *http://www.wrcc.dri.edu/summary/Climsmwy.html*

Wyoming Department of Environmental Quality, 2004a. *Monitoring and Assessment Report, Belle Fourche River-WYBF10120201-004*, prepared for the Wyoming Department of Environmental Quality, Cheyenne, WY.

Wyoming Department of Environmental Quality, 2004b. *Beneficial Use Reconnaissance Monitoring and Assessment Report, Belle Fourche River- WYBF10120201-009*, prepared for the Wyoming Department of Environmental Quality, Cheyenne, WY.

Wyoming Department of Environmental Quality, 2014a. "Wyoming Surface Water Quality Standards Chapter 1,".*state.wy.us*, retrieved April 14, 2015, from *http://soswy.state.wy.us/Rules/RULES/9176.pdf*

Wyoming Department of Environmental Quality, 2014b. *Wyoming Water Quality Assessment and Impaired Waters List (2012 Integrated 305(b) and 303(d) Report),* prepared for the Wyoming Department of Environmental Quality, Cheyenne, WY.

Wyoming Department of Environmental Quality, 2015. "Wyoming Water Quality Rules and Regulations," *state.wy.us*, April 15, 2015, from *http://soswy.state.wy.us/Rules/RULES/6547.pdf*

APPENDIX A
U.S. GEOLOGICAL SURVEY DISCHARGE GRAPHS FOR SELECTED GAGING STATIONS IN THE BELLE FOURCHE RIVER WATERSHED

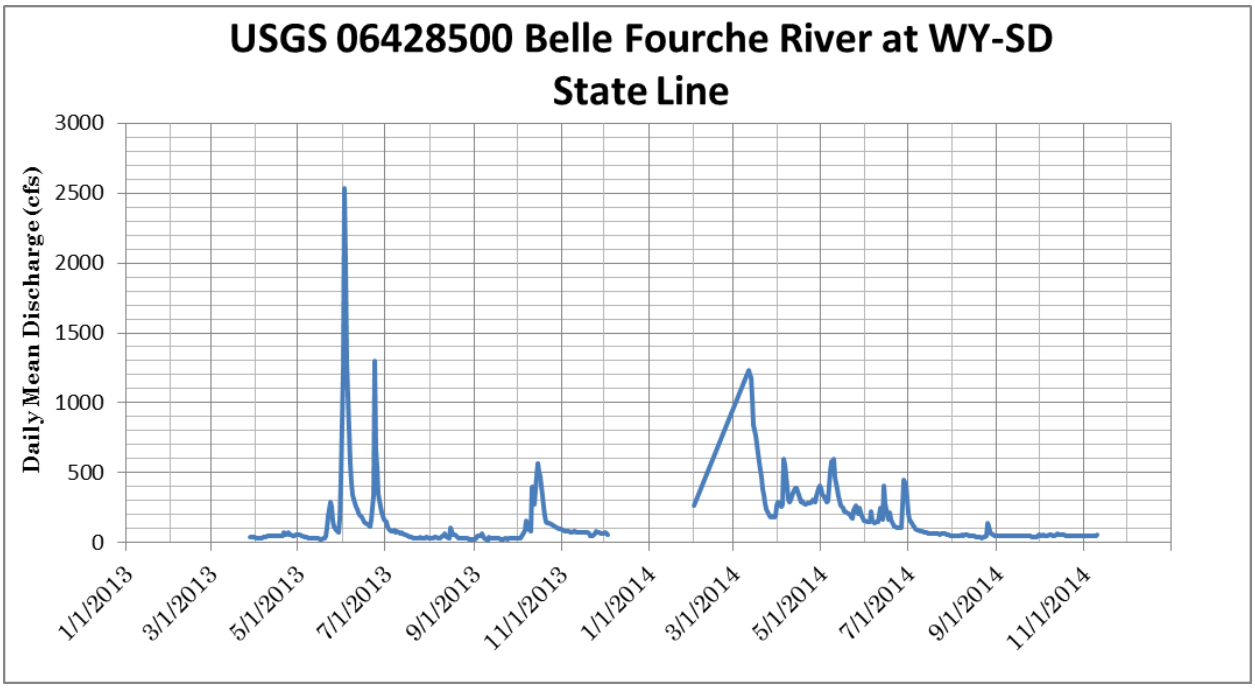


Figure A-1. Discharge Rates at USGS Gaging Station 06428500 Over the Monitoring Period. Gaps indicate periods of ice or no data.

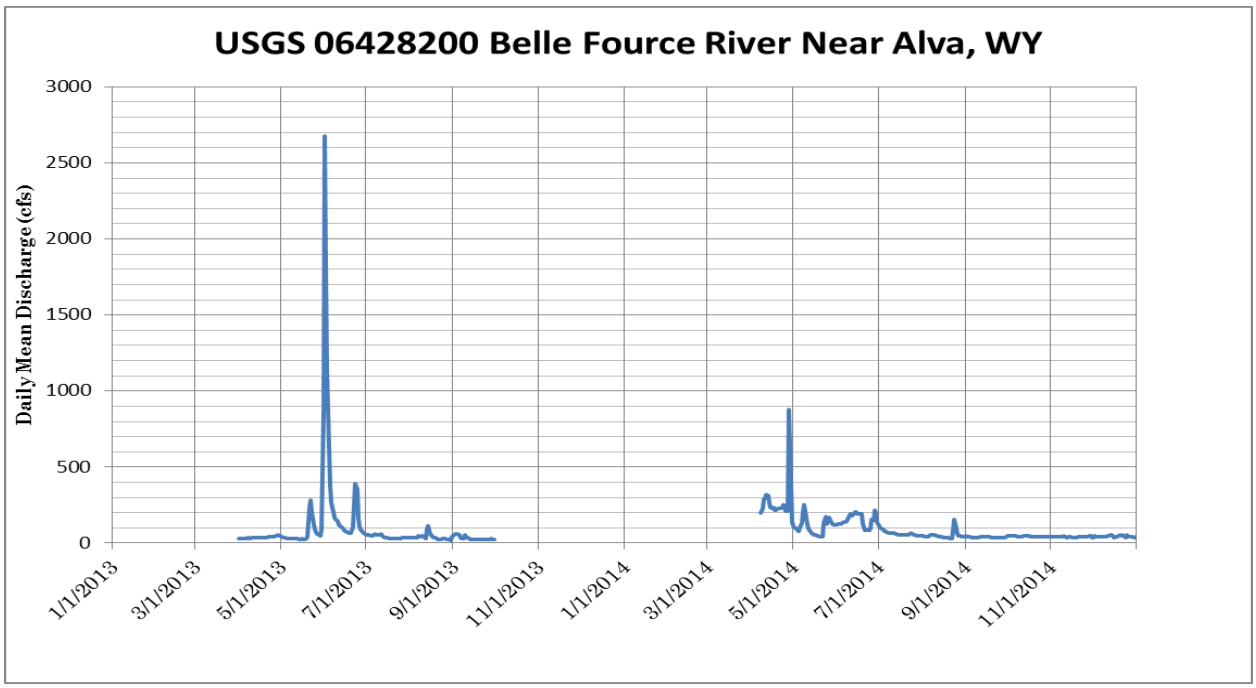


Figure A-2. Discharge Rates at USGS Gaging Station 06428200 Over the Monitoring Period. Gaps indicate periods of ice or no data.

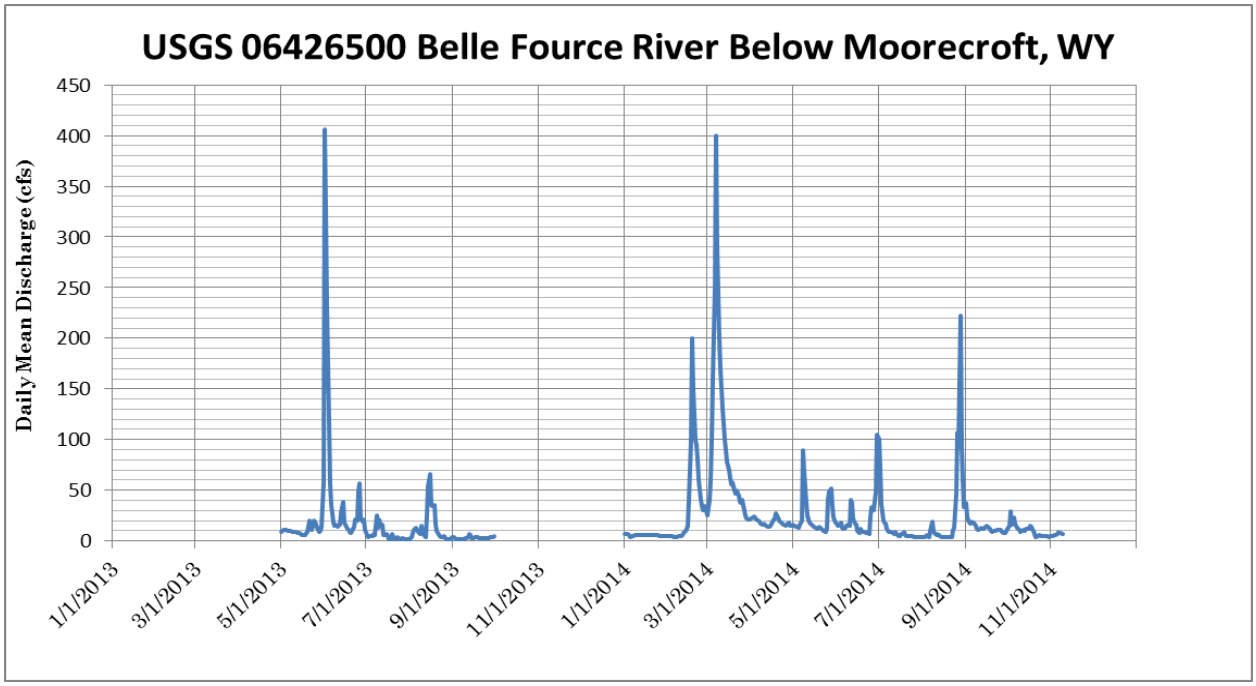


Figure A-3. Discharge Rates at USGS Gaging Station 06426500 Over the Monitoring Period. Gaps indicate periods of ice or no data.

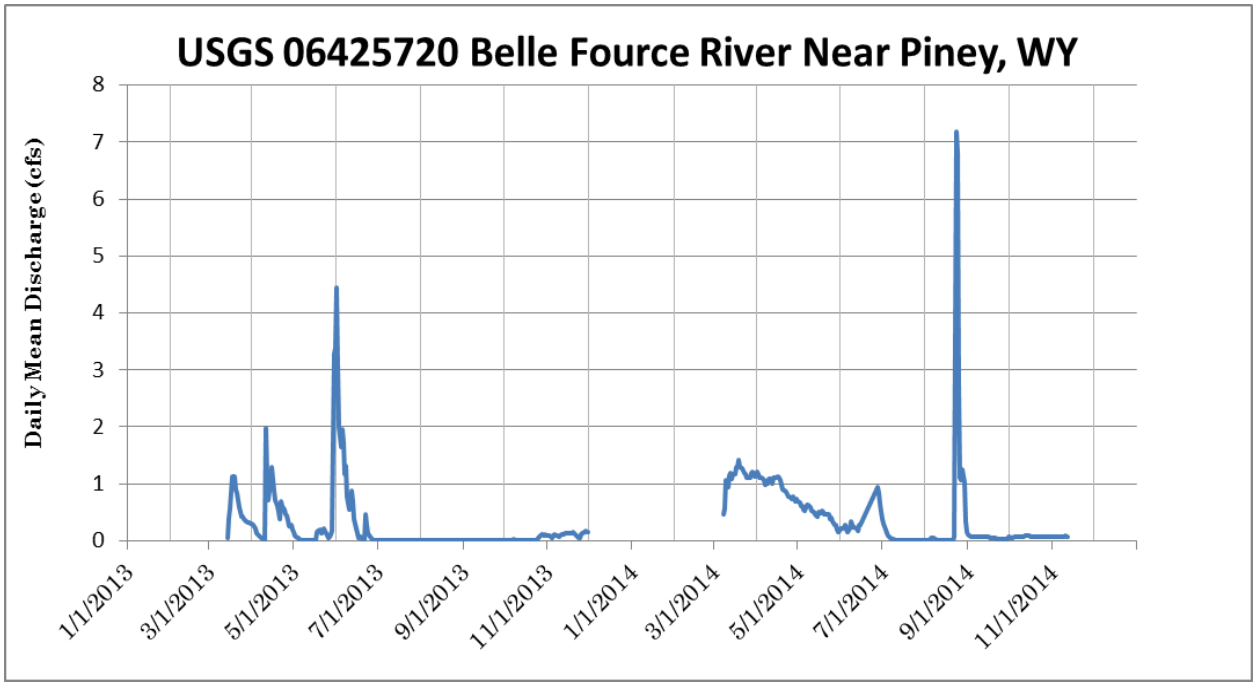


Figure A-4. Discharge Rates at USGS Gaging Station 06425720 Over the Monitoring Period. Gaps indicate periods of ice or no data.

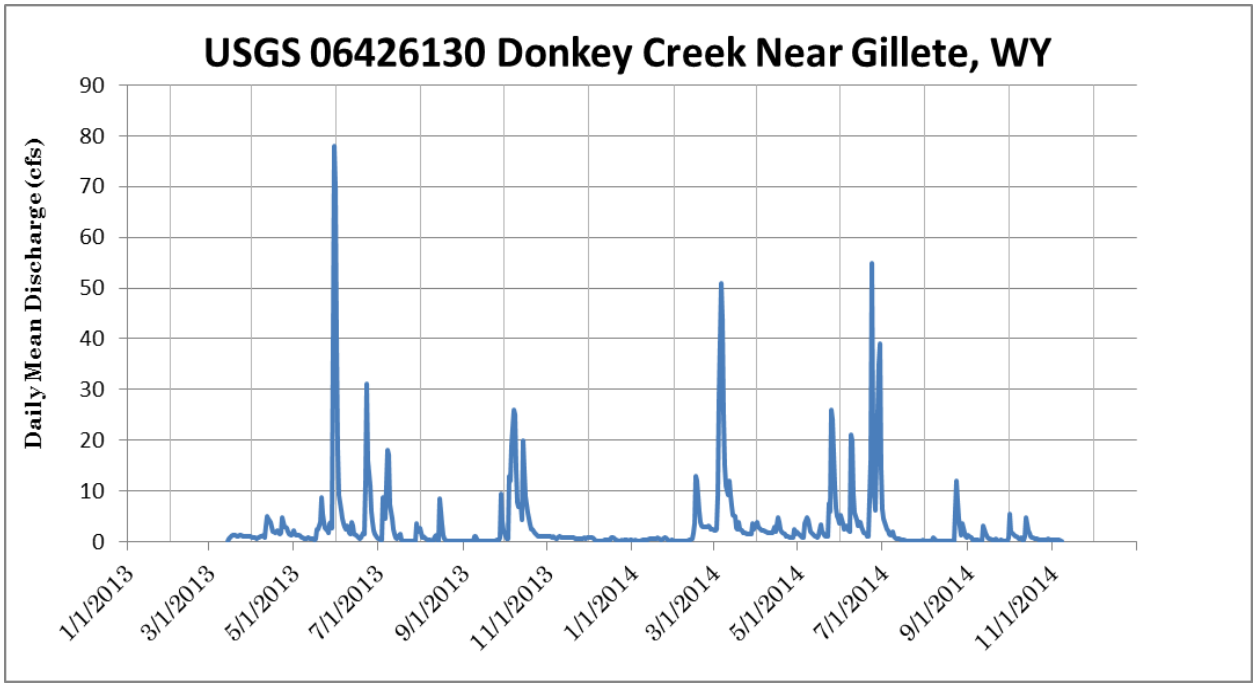


Figure A-5. Discharge Rates at USGS Gaging Station 06426130 Over the Monitoring Period.

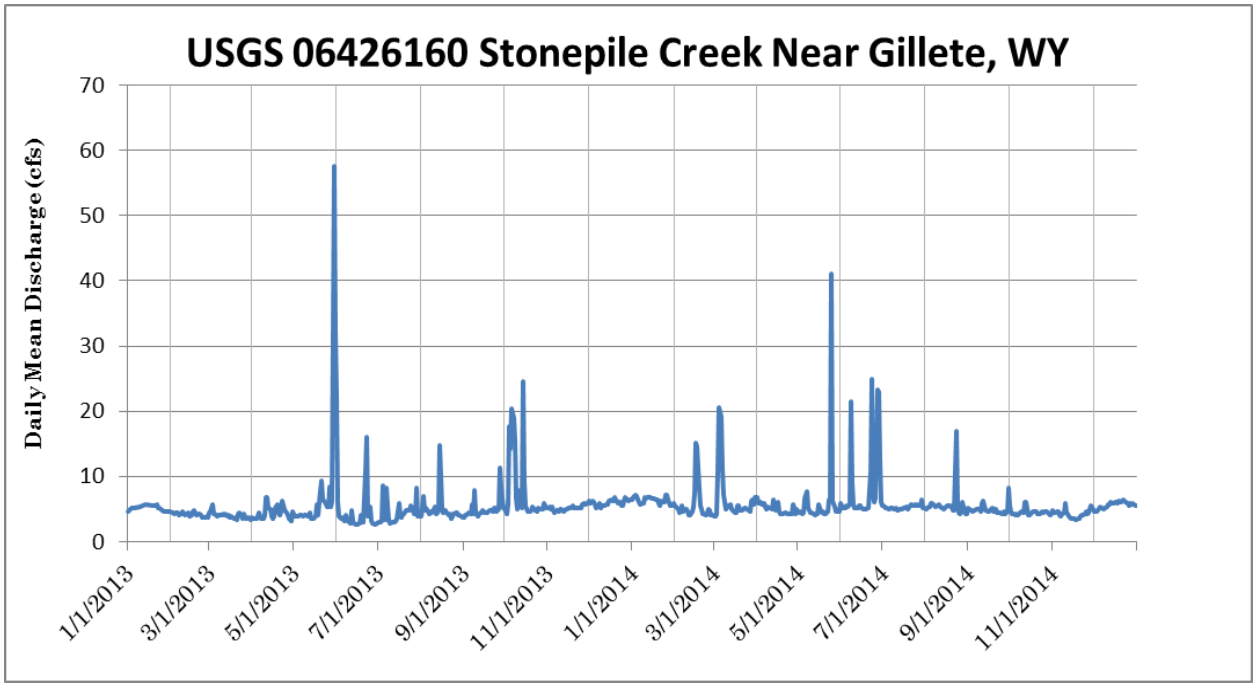


Figure A-6. Discharge Rates at USGS Gaging Station 06426160 Over the Monitoring Period.

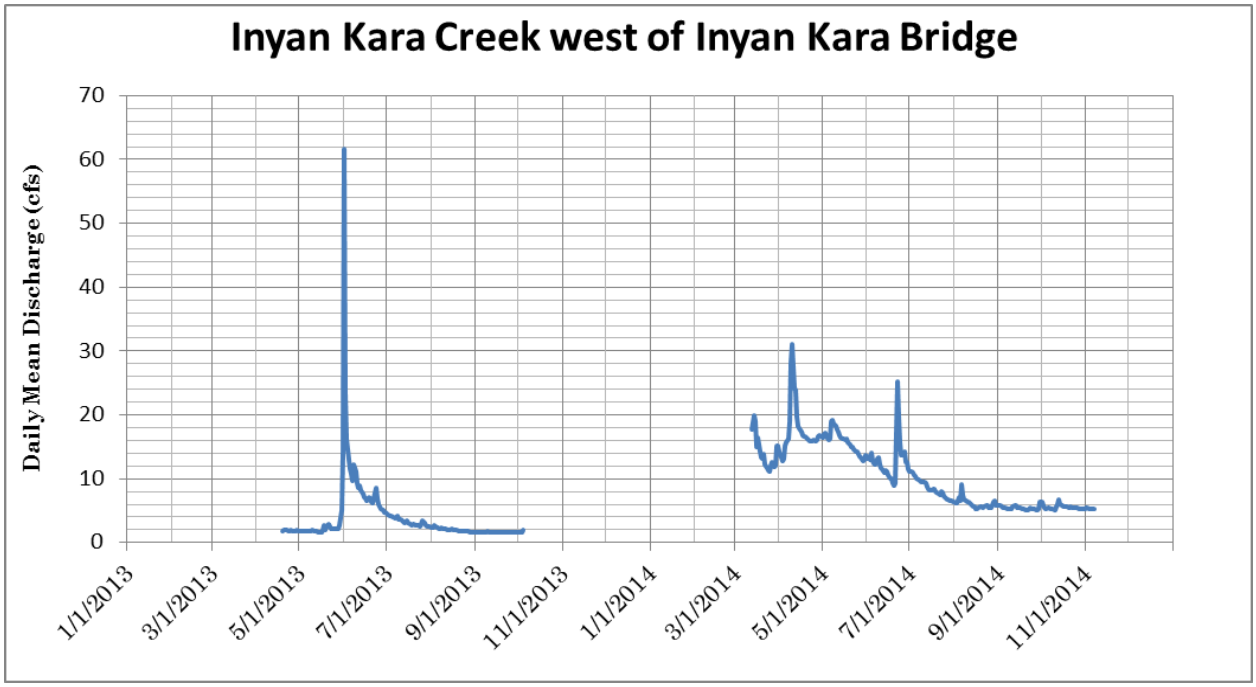


Figure A-7. Discharge Rates at Inyan Kara Creek Over the Monitoring Period. Gaps indicate periods of ice or no data.

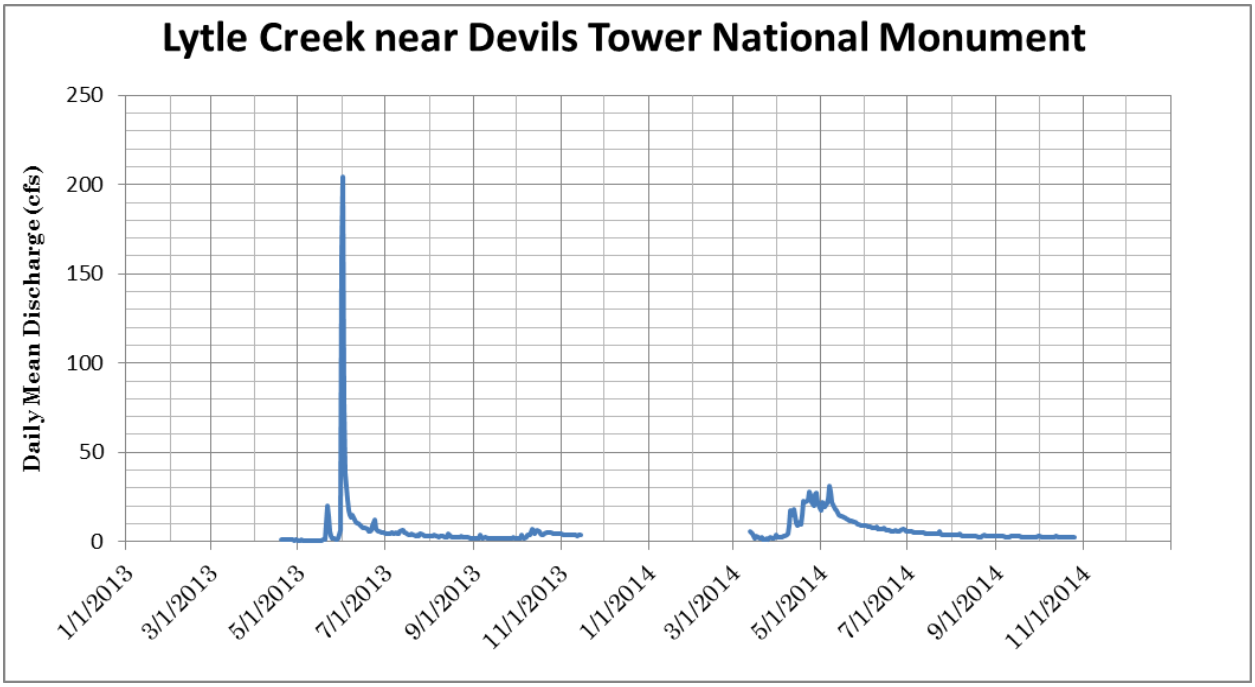


Figure A-8. Discharge Rates at Lytle Creek Over the Monitoring Period. Gaps indicate periods of ice or no data.

Source Tracking Capabilities of Next Generation Sequencing Technologies

Background

Next generation sequencing (NGS) technologies, characterized by high throughput sequencing platforms and multiplexed sample strategies, have become a standard tool in identifying the microbiotic structure of nearly every type of microbiome. This tool has been shown to be a more effective tool in clinical diagnostics than standard culture approaches (Dowd et al. 2008, Handelsman 2004, Streit and Schmitz 2004). It has performed robustly in identifying environmental microbiomes, and is even being used in NASA's rover project to identify what microorganisms are present on the rovers before they are sent to Mars. This knowledge enables researchers to subtract those organisms from any data the rover collects on the planet surface (La Duc et al. 2014, 2012). More relevantly, NGS has been employed in microbial source tracking (MST), using host specific organisms to identify the potential sources of bacterial contamination (Gomi et al. 2014, Prystajecky et al. 2014, Kildare et al. 2007, Ballesté and Blanch 2011, Bernhard and Field 2000).

The microbiome of an individual organism changes based on a number of variables, including health, age, parentage, diet, and reproductive state. However, there are groups of organisms that are found consistently within members of the same host species and not in others. Some of these species specific microbes are also physiologically obligate anaerobes. Effectively, this means they do not reproduce, or do not reproduce well, in aerobic environments. These two characteristics (anaerobic and species specific) make them excellent indicators of source organisms. Some of these microbes are bacterial (primarily in the Bacteroidetes; Kildare et al. 2007, Bernhard and Field 2000) and others are eukaryotic (*Giardia* and *Crytposporidium*; Prystajecky et al. 2014). The biggest drawback to using these organisms as MST markers is their lower abundance in the water column vs naturally occurring environmental bacteria. Appropriate sampling, concentrating water, using specific primers, and generating enough sequences are all necessary for successful source identification.

Methods

Samples were received and extracted one of two ways. First, 3-5ml of sample were spun down, 1ml at a time, in 1.7ml tubes at max speed for 10min. The resulting supernatant was removed and the pellet was extracted using the MoBio powersoil extraction kit. Alternatively, samples

were poured through a 0.2nm filter using a vacuum manifold, the filter was then used as the substrate for DNA extraction. Isolated DNA was amplified using a variety of primers and barcoded uniquely for sequencing (see Table 1) in a standard amplification process. Amplicons were visualized using eGels (Life Technologies, Grand Island New York) and pooled in equimolar ratios using amplicon strength as an indicator of concentration. Pools were cleaned and size selected using Agencourt AMPure XP beads (BeckmanCoulter, Indianapolis, Indiana). Cleaned pools were sequenced on the Illumina MiSeq, Roche 454, or LifeTech PGM platforms (San Diego, California; Branford, Connecticut; Grand Island, New York, respectively). Resulting reads were demultiplexed, denoised, checked for chimeras, and compared to a curated bacterial database using a custom bioinformatics pipeline.

Results

One assay (universal 16S) showed known species specific commensal gut bacteria (Table 2). All the associated bacteria were linked with human microbiomes. The 16S primers designed to be more specific did not pick up the organisms they were designed to amplify. Additionally, the *E. coli* and *Bifidobacteria* specific primers did not amplify even positive controls.

Conclusion

Despite multiple examples in the literature, Research and Testing was not able to adequately identify sources of potential contamination by using NextGeneration sequencing. There are a number of putative reasons for this failure.

1) There are no contaminates present
2) Field sampling was not appropriate
3) Targets are in low numbers
4) Primers used for amplification were not sensitive enough to the target species
5) Samples were not sequenced to enough depth with general 16S primers

We believe the first and second answers are the least likely. Both of these could be eliminated as a potential explanation if other forms of testing showed contamination. The second answer may be partially correct if the field sampling was not appropriate for this method, however a review of the literature seems to indicate it is appropriate. The actual answer probably lies in a combination of the last 3 answers. The most promising techniques use primers that are very specific to *Escheria* or *Bifidobacteria* and are able to identify strains within those genera. However, using those techniques, we were unable to get any of the samples to amplify (not even our positive controls). The other primer sets, though more sensitive to certain organisms, probably were not able to overcome the low abundance of target DNA. Additional sequencing

depth may have helped in this regard, in that rare populations would be better able to be detected, however, they would still be in very low percentages. When organisms are identified in low percentage, the confidence level goes down relative to when they are identified as a larger proportion of the overall sample. The ideal situation would be to use one of the highly specific assays (e.g. *Escheria* or *Bifidobacteria*) and generate a large number of reads.

Future Directions

The use of Next Generation sequencing shows high promise, based on research available in the literature as well as some of the initial results (showing human associated bacteria). However, at this time we cannot confidently deliver results adequate to identify sources of water contamination. We intend to continue working on developing this type of assay as we believe the science is sound, however this assay development is being done *pro bono* and so the timing is significantly slowed. In the interim we would recommend exploring other possibilities for Microbial Source Tracking that may be already established or working. Should the abilities of this technology change, or we find a solution to getting the taxa specific assays to work we will certainly contact the Crook County NRD.

Bacteria >10% abundance (*<10%,**<1%)	Identification	Primers***
Anabaena	cyanobacteria	1,2,3**,4**
Bacteroides coagulans	anaerobic gut bacteria (human and mammals)	1
Bacteroides vulgatus	anaerobic gut bacteria (human and mammals)	1
Citreicella	sulfur oxidizer found in water	1
*Clostridium***	gut and soil associated	1,2,3,4
Corynebacterium	environmental	1,3**,4**
Cyanobium sp	cyanobacteria	1,2,3*,4**
Escherichia	Gut associated	3*,4**
Enterobacter hormaechei	Human Associated	1
Exiguobacterium	environmental (including water)	1,2,3**
Faecalibacterium prausnitzii	Human Associated	1
Flavobacterium	environmental (including water)	1,2,3
Herbaspirillum	environmental (including water)	2,3*,4**
Planococcus	water associated	1,2,3
Planomicrobium	water associated	2,3*
Pseudomonas	ubiquitous	2,3*,4*
Pseudonocardia	Ant and plant associated	2
Sporichthya	environmental (mainly soil)	1,2
*Subduligranulum***	Human Associated	1
Synechococcus	cyanobacteria	1,2,3**

*other vertebrate associated taxa were associated at less than 10%

**Primer names are as follows: “1” 28F-519R, “2” 28YMF3-510R, “3” 27aF-338R, “4” 357wF-785R

References

Ballesté E, Blanch AR (2011) Bifidobacterial diversity and the development of new microbial source tracking indicators. Appl Environ Microbiol 77:3518-3525

Bernhard AE, Field KG (2000) A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S RNA. Appl Environ Microbiol 66:4571-4574.

Dowd SE, Sun Y, Wolcott RD, Domingo A, Carroll JA (2008) Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned Salmonella-infected pigs. Foodborne Pathog Dis 5:459-472.

Gomi R, Matsuda T, Matsui Y, Yoneda M (2014) Fecal source tracking in water by next generation sequencing technologies using host-specific *Escherichia coli* genetic markers. Environ Sci Tech 48:9616-9623.

Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68:669-685.

Kildare BJ, Leutenegger, CM, McSwain BS, Bambic DG, Rajal VB, Wuertz S (2007) 16S rRNA-based assays for quantitative detection of universal, human-, cow-, and dog-specific fecal *Bacteroidales*: a Bayesian approach. Water Res 41:3701-3715.

La Duc MT, Vaishampayan P, Nilsson HR, Torok T, Venkateswaran K (2012) Pyrosequencing0derived bacterial, archaeal, and fungal diversity of spacecraft hardware destined for Mars. Appl Environ Microbiol 78(5912-5922).

La Duc MT, Venkateswaran K, Conley CA (2014) A genetic inventory of spacecraft and associated surfaces. Astrobiology 14:15-23.

Prystajecky N, Huck PM, Schreier H, Isaac-Renton JL (2014) Assessing *Giardia* and *Cryptosporidium* spp. as a microbial source tracking tool for surface water: application in a mixed use watershed. Appl Environ Microbiol 80:2328-2336.

Streit WR, Schmitz RA (2004) Metagenomics: the key to the uncultured microbes. Curr Opin Microbiol 7:492-498.