SECTION 319 NONPOINT SOURCE POLLUTION CONTROL PROGRAM

ASSESSMENT/PLANNING PROJECT FINAL REPORT

PERSISTENCE OF E. COLI IN STREAM SEDIMENTS AND THE IMPACT ON WATER QUALITY

By

Rachel McDaniel, Adjunct Assistant Professor,

South Dakota State University

July 1, 2020

This project was conducted in cooperation with the State of South Dakota and the United States Environmental Protection Agency, Region 8.

Grant # 9981850602016

Executive Summary

PROJECT TITLE: Persistence of E. coli in stream sediments and the impact on water quality

GRANT Number: 9981850602016 Grant Source: South Dakota DENR

Initiation Date: 06/30/16 Expiration Date: 07/31/20

Funding

Funding source	Original Budget	Actual expenditure
US EPA Section 319 Grant	$188,965.00	$188,965.00
Match (cash/in-kind)	$125,976.00	$125, 762.39

Accomplishments Summary

A common cause of water quality impairment in South Dakota is bacteria, including *E. coli*. E. coli was responsible for poor water quality in over 2,000 miles of streams in South Dakota. Recreational waters, such as the Big Sioux River, are a concern for public health as high *E. coli* concentrations are indicators of fecal pollution. Skunk Creek (Sk) which connects to the Big Sioux River is a major contributor of bacterial pollution to the Big Sioux River, and was therefore selected as the study site.

The overall goal of this project was to evaluate *E. coli* attachment to particles of different sizes and estimate the impact of attachment on *E. coli* transport in streams during high flows. *E. coli* fate and transport are difficult to predict and this information may be incorporated into existing or future models to estimate *E. coli* concentrations in streams as well as contribute to the development of management practices to reduce transport.

Overview of Major Results

There is no significant difference in processing sediment samples for *E. coli* within 8 hours and 24 hours after sample collection in the majority of cases (4/5), indicating that a 24 hour sample processing time is likely acceptable

All five sites evaluated for *E. coli* variability had right skewed data distributions, indicating that the median would be a better measure of central tendency than the mean. In addition, the edge of the stream had 4 to 925 times more *E. coli* than the middle of the stream, depending on the site; however, there was no significant difference between the edge concentrations and the middle concentrations, likely due to the high variability.

All sites showed significant correlations between particle sizes less than 0.075 mm (silt and clay particles) and *E. coli* concentrations, indicating that sediment composition should be considered when creating a sampling regime. However, the direction of correlation was not consistent.

Three of the sites had significantly positive correlations while two sites had a significantly negative correlation.

Sample size analysis results indicate that a minimum of five samples are required to have a 90% probability of the median falling within the 95% confidence interval. More statistical analyses of the data are required before making a final sample size recommendation.

Monthly sediment sampling and sampling surrounding storm events began in 2017. Results indicate the highest concentrations occurred at the cattle crossing during August, the hottest part of the summer.

At the end of each storm event evaluated, *E. coli* concentrations increased within sediments for at least a brief period. This may be a result of wash-in from the surrounding pasture land.

Table of Contents

List of Figures

List of Tables

1.0 Introduction

Waterbody Description

Project Location	
Watershed(s)	Skunk Creek
303(d) Listed Stream	Yes
HUC (8 digit)	10170203
Counties	Moody, Lake, and Minnehaha
Coordinates of project location	N 43°46’44’’ W 96°51’41’’

Skunk Creek flows from Brandt Lake to the Big Sioux River and covers portions of Lake, Moody, and Minnehaha counties. The length of Skunk Creek is approximately 74 miles and the watershed is about 373,000 acres. Precipitation averages about 25 inches per year. The primary land use is agricultural including both cropland and rangeland. Cattle have the potential to be a major contributor to bacterial impairments.

The 2014 Water Quality Assessment Report lists Skunk Creek as impaired for E. coli, fecal coliform, and TSS. Limited Contact Recreational and Warmwater Marginal Fish Life uses are not supported in Skunk Creek. To address these issues, best management practices (BMPs) have been implemented along the creek including Riparian Area Management (RAM) and Seasonal RAM (SRAM). These systems are aimed at minimizing fecal bacteria loading from cattle by reducing or eliminating their time in the stream as well as providing a buffer between grazing lands and the stream to reduce overland transport.

Reservoirs of E. coli can be found in stream sediments, providing a potential source for water quality impairments. Monitoring for E. coli concentrations in the sediment of Skunk Creek began in 2014. Current data show a range of E. coli concentrations from 10 to 24,200 MPN (Most Probable Number method)/g sediment.

Water Quality problems

Nearly 70% of the streams assessed from 2008 through 2013 in South Dakota did not support at least one of their designated beneficial uses (DENR, 2014). The primary causes for nonsupport were total suspended solids (TSS) and bacteria including Escherichia coli (E. coli). E. coli alone was responsible for poor water quality in over 2,000 miles of streams in South Dakota (EPA, 2015). Livestock, wildlife, and crop production are listed as the top three probable sources of impairment in the state.

What is not considered is the reservoir of fecal bacteria within stream sediments which can often have much higher concentrations of fecal bacteria than the water column. For example, van Donsel and Geldreich (1971) found 100 to 1000 times more fecal coliform in bottom sediments than the water column. In addition, fecal bacteria have been shown to survive and persist in stream sediments for extended periods of time (Flint, 1986, Davies et al., 1995).

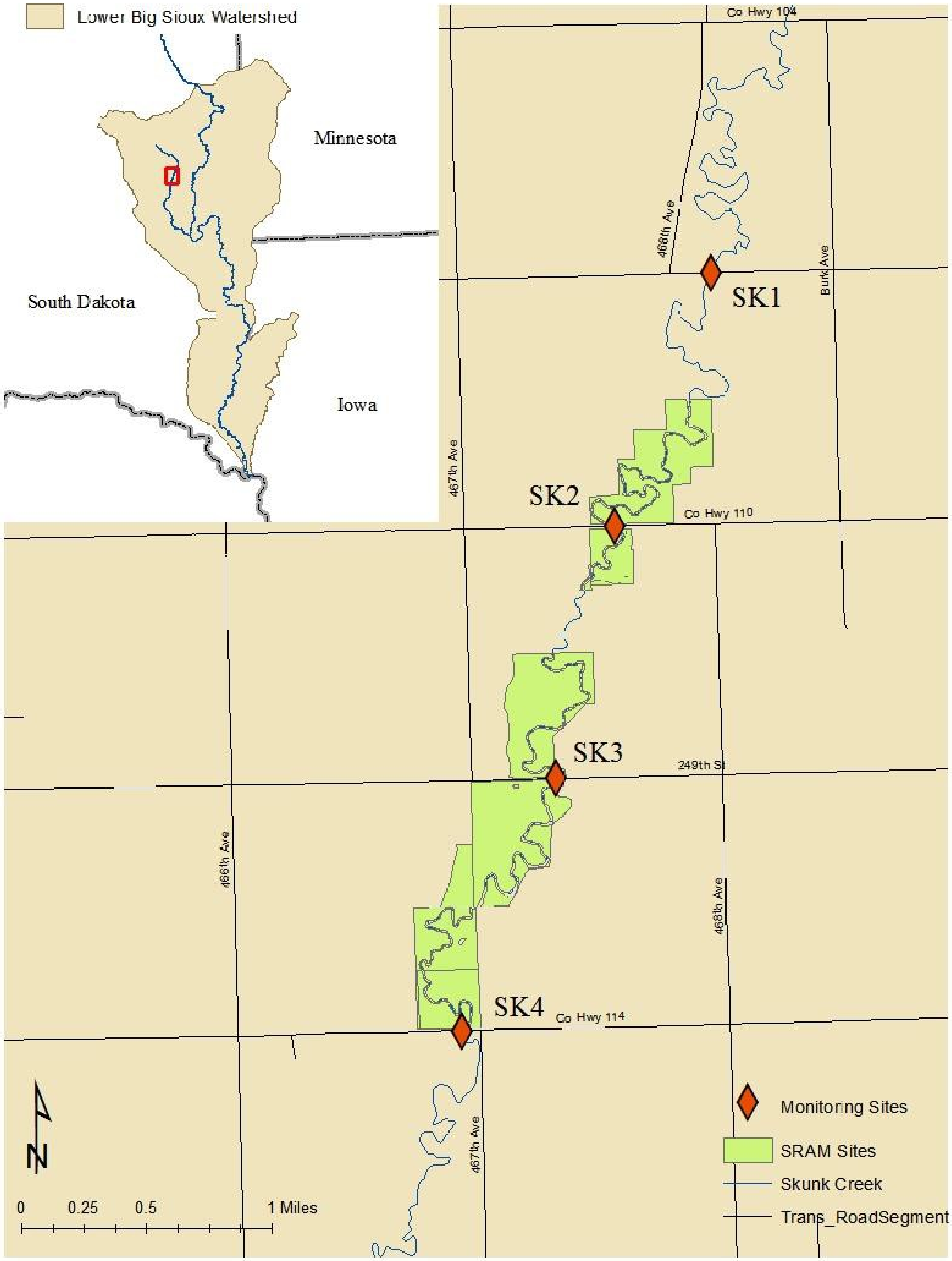
Sediment composition, including texture and organic matter, can affect survival of fecal bacteria, but the results have not been consistent. Several studies show smaller particles and more organic matter are associated with higher fecal bacteria concentrations (Garzio, 2009; Niewolak, 1998; Ferguson et al., 1996; Irvine and Pettibone, 1993); however, Cinotto (2005) found higher concentrations of E. coli in sand.

Disturbances of stream bottom sediments (e.g. cattle crossing the stream or storm events) can transport bacteria from these sediment reservoirs into the water column, thus contributing to water quality impairments. In one study, an artificial flood event was created by releasing water from a reservoir, preventing external input of E. coli into the system (i.e. in runoff). The results showed an increase in E. coli concentrations from 100 to 13,000 MPN 100mL-1 (Nagels et al., 2002), far above the South Dakota limited contact recreation single sample standard of 2,000. Current data show that E. coli stores from stream sediments in Skunk Creek can range from an average of 83 to over 12,000 cfu (colony forming units)/g, indicating that the sediment may be a significant source of E. coli to the water column in some areas of South Dakota.

The information obtained from this study can be used statewide to provide a standard sampling method for E. coli concentrations in stream sediments. It will also provide information on the stability of E. coli reservoirs in South Dakota's streams, the potential impacts of storms on instream E. coli reservoirs, the potential recovery of E. coli reservoirs, and the impact of SRAM on sediment E. coli reservoirs.

Although the information can be used throughout the state, the study focused on Skunk Creek. Skunk Creek is listed as impaired for E. coli and fecal coliform. A TMDL published in 2004 indicates a required storm flow reduction of 95% for fecal coliform in Skunk Creek; however, a more recent TMDL for the Big Sioux River determined that Skunk Creek will need to meet stricter standards because of its high contribution to the Big Sioux River.

Figure 1 -Location of Skunk Creek monitoring sites within the Lower Big Sioux Watershed. Green areas are SRAM sites while orange diamonds are the monitoring locations.



2.0 Project Goals, Objectives, and Activities

The goals and objectives of this project are twofold:

1. Develop a standard sampling procedure for *E. coli* in stream sediments; and
2. Using the standard procedure, evaluate the persistence of in-stream sediment stores of *E.coli* and their implications for water quality

Goal 1: Develop a standard sampling procedure for E. coli in stream sediments

Task 1: Literature Review

A literature review was conducted at the start of the project to identify and summarize commonly accepted practices for sediment sampling and processing. The following is an outline of the resulting literature review with general recommendations for sediment sampling and processing. A final document will be compiled containing this information and summarizing the results of the sampling analysis.

- Collection typically occurs from the top few centimeters.
  - Most *E. coli* present in the top few centimeters (Garzio-Hadzick et al., 2010; Pachepsky et al., 2011)
  - Resuspension of *E. coli* also occurs from the top few centimeters
  - **Recommendation**: Collect sediment sample from the top 3 cm
- Collection occurred with a variety of containers/methods
  - Scoop, bottles, sediment corer, etc.
  - **Recommendation:** A sediment core would provide the most accurate sample collection, providing both an opportunity for measuring the top 3 cm and the sediment density for mass to volume conversion. However, this collection method has been proven difficult due to equipment not retaining the sample and seizing of equipment due to sediment particles. Therefore, it is recommended to use a simpler sediment collection method such as a scoop or shovel to collect the sample.
- Dilution ratios of 1 gram sediment to 10 mL dilution solution (1:10) and 1 gram sediment to 11 mL dilution solution (1:11) were commonly used in the literature.
  - The dilution solution was commonly purified water or phosphate buffer solution.
  - **Recommendation**: 1:10 dilution ratio using 1 gram sediment to 10 mL of phosphate buffer solution. The buffer solution maintains osmotic pressure for the bacteria, making it the recommended dilution solution.
- Processing method:
  - **Recommendation:** Shake diluted samples in an orbital shaker for 45 min to free loosely attached *E. coli* and resuspend *E. coli* into the purified water. Use the supernatant for sample processing.
- Estimating density for mass to volume conversion and comparison to water sample results.

- Most literature did not discuss the method used for sediment mass to volume conversion, so publication authors were contacted to determine the method used.
- Possible conversion methods include assuming an average sediment density, measuring sediment density, and using Archimedes' Principle.

<u>Task 2: Temporal stability of *E. coli* in sediment sampled</u>

150 samples were processed to assess the temporal stability of *E. coli* in the sediment sample as well as the *E. coli* variability in stream bottom sediments. Samples were processed in triplicate (standard procedure) at two time periods, 8 hours and 24 hours after sample collection, and with multiple dilutions for accurate colony counts. This resulted in over 1,800 plates processed for the stability analysis.

Samples were collected in a five by five sample grid (Figure 1), for a total of 25 samples at each of five locations including four sites at Skunk Creek (SK1, SK2, SK3, SK4) and one site on Six Mile Creek (SM). Samples were collected by scooping the top few, approximately three, centimeters of sediment into a sterilized bottle and transporting them to the McDaniel lab at SDSU on ice. Each sample was diluted with one gram of sediment to ten mL of phosphate buffer water, shaken with an orbital shaker for 45 minutes, and the supernatant collected for sample processing. Samples were processed using standard membrane filtration on modified mTEC agar, which specifically selects for *E. coli*.

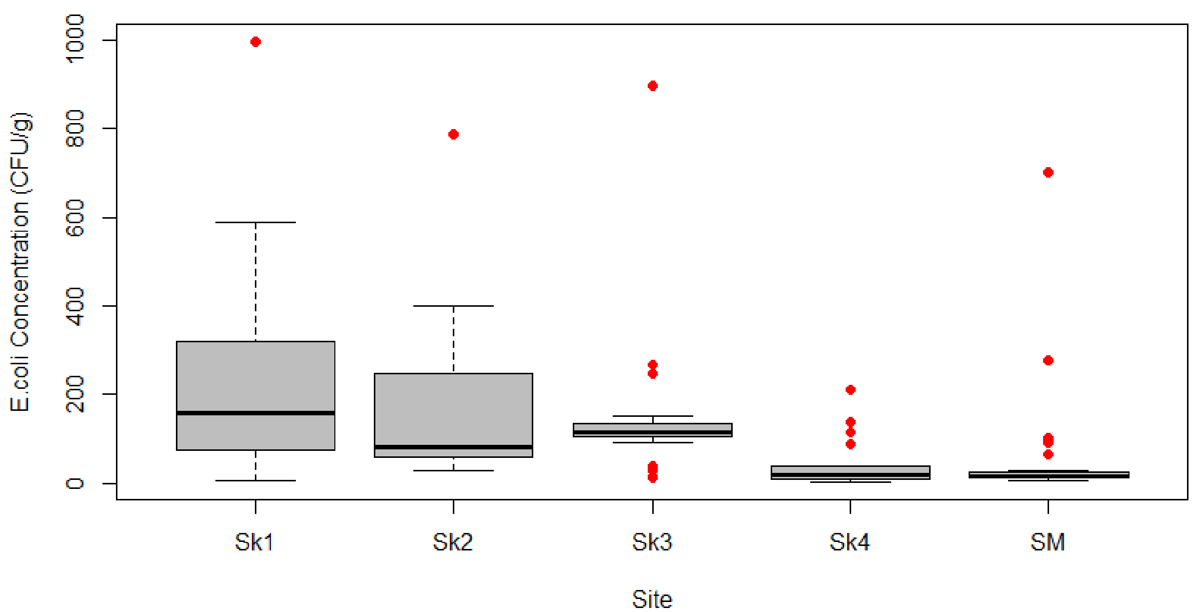
Figure 2: A five by five sample grid was used to assess *E. coli* variability in streambed sediments and the temporal stability of *E. coli* in sediment samples.

The Wilcoxon Signed Rank test was used to assess the significant difference between the 8 hour and 24 hour sample processing times. This test is specifically used for paired observations. No significant differences in *E. coli* concentrations were found in samples processed within 8 hours and 24 hours of sample collection at sites SK1, SK3, SK4, and SM; however, a significant difference was observed in processing time for SK2. Therefore, the sample processing time can be extended to 24 hours without significant impacts on results in the majority of cases, 80% of those analyzed.

<u>Task 3: *E. coli* Variability in Stream Bottom Sediments</u>

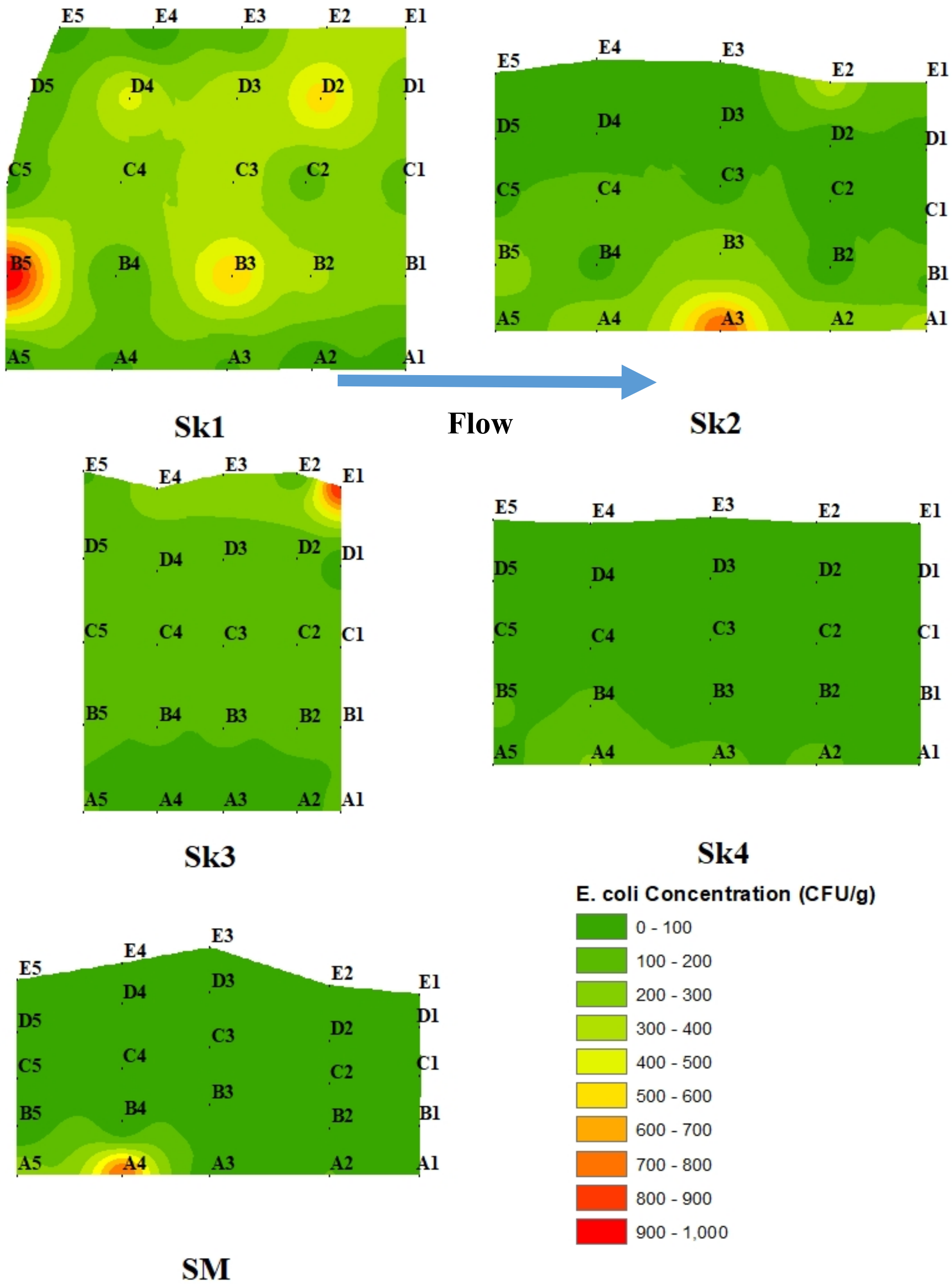
The same samples that were collected for the temporal stability were used to assess the *E. coli* variability in stream bottom sediments. Table 1 shows the overall statistics for the *E. coli* concentrations at each of the five sites (SK1, SK2, SK3, SK4, and SM) that have been analyzed. Briefly, the highest variability was found at SK2 (Figure 2), while the lowest was found at SK4. The measures of central tendency (mean and median) were about one order of magnitude lower for SK4 and SM than SK2 and SK3. All measures of central tendency were also above the single sample maximum (SSM) standard for limited contact recreation waters when the standard is converted to Colony Forming Units per gram of water - CFU/gH_2O (11.78 CFU/gH_2O). We had originally planned to use a sediment corer to obtain the sediment density and convert “gSediment” to “100mL”; however, issues with the corer made it challenging to use for sediment collection. To address this issue, we are currently looking into using Archimedes’ Principle as an alternative method of unit conversion.

Figure 3: Box plots for the 25 samples at each location show the highest *E. coli* variability was found at SK1, while the lowest was observed at SM.



The following are *E. coli* concentration maps for the five sites that have been analyzed (Figure 4). Each of the maps are oriented so the stream flow is from left to right with the stream banks located on the top and bottom of the figures. All sites showed a minimum of one sample with much higher *E. coli* concentrations than the majority of the others, resulting in a consistent right skewed distribution of the 25 samples at each of the five sites. With the consistent skewed distribution, the median is preferred as the measure of central tendency. The mean is more affected by extreme data points, such as those that are present in skewed distributions, thus causing a greater likelihood of over or under representing the data set. In this case, with right skewed data, there would be greater likelihood of representing the *E. coli* concentrations in the stream bottom sediments with a value that is higher than the actual center of the data and, therefore, over-represent the potential sediment source. As a result, t is recommended to use the median *E. coli* concentration when presenting the results. One challenge with this approach is comparing the data to other studies, as traditionally, results are presented with the mean.

Figure 4: Areal view of *E. coli* concentrations at the five monitoring sites. Rows A and E were along the stream bank and the water flowed from column 5 to column 1. All sites showed pockets of high *E. coli* concentrations resulting in right skewed distributions.



Task 4: Examine variability between sample locations

In addition, we assessed the stream cross section for any significant differences between sample locations, specifically samples collected at the edge versus the middle of the stream. The maps indicate that the high *E. coli* concentrations are typically found at the edge of the stream, near the bank. In fact, the edge of the stream contained 2 to 8 times more bacteria than the middle of the stream on average, with the exception of the cattle crossing at SK1 (Table 1). However, no significant difference was found at any of the sites between *E. coli* concentrations from the edge and middle of the stream. Higher variability was also observed in all sites with the exception of SK1.

Table 1: The edge of the stream on average has higher E. coli concentrations and variance than the middle of the stream.

Statistic	Location	*E. coli* concentration: Edge (CFU g^{-1})	*E. coli* concentration: Middle (CFU g^{-1})	Edge÷Middle
Mean	Sk1	107	329	0
	Sk2	263	89	3
	Sk3	194	116	2
	Sk4	67	31	2
	SM	132	17	8
Median	Sk1	58	267	0
	Sk2	217	66	3
	Sk3	118	115	1
	Sk4	24	16	1
	SM	43	16	3

Sample Size Analysis

Preliminary statistical analyses of each of the four sample sites has been conducted to begin the determination of the number of samples required for assessing *E. coli* concentrations in stream bottom sediments. Two methods were used to assess the number of samples to adequately represent the sties: (1) a bootstrapping, and (2) a modified equation for non-parametric sample size. To begin the sample size analysis, bootstrapping on the 25 samples from four out of the five locations (SK2, SK3, SK4, SM) was completed to determine the 95% confidence interval for the mean and median of the data sets. Next, the data was resampled to assess the probability of the mean and median for various sample sizes falling within the 95% confidence interval. In general, the median had a higher probability of falling within the confidence interval for sample sizes less than about 16, confirming that the median is a better representation of the data. The analysis from each location indicates that at least five samples are required to have a 90% probability of the median falling within the 95% confidence interval.

Equation 1 was used to calculate the sample size for each location in this study.

$$\text{sample size, n} = [\frac{Z_{\frac{\alpha}{2}} * \sigma}{E}]^2 \quad (1)$$

where, $Z_{\alpha/2}$ is the critical value, σ is the population standard deviation, and E is the margin of error. This equation is usually used for parametric tests (i.e. the data are normally distributed). The data is nonparametric, so an additional 15% was added to adjust per literature recommendations (Lehmann et al., 1998).

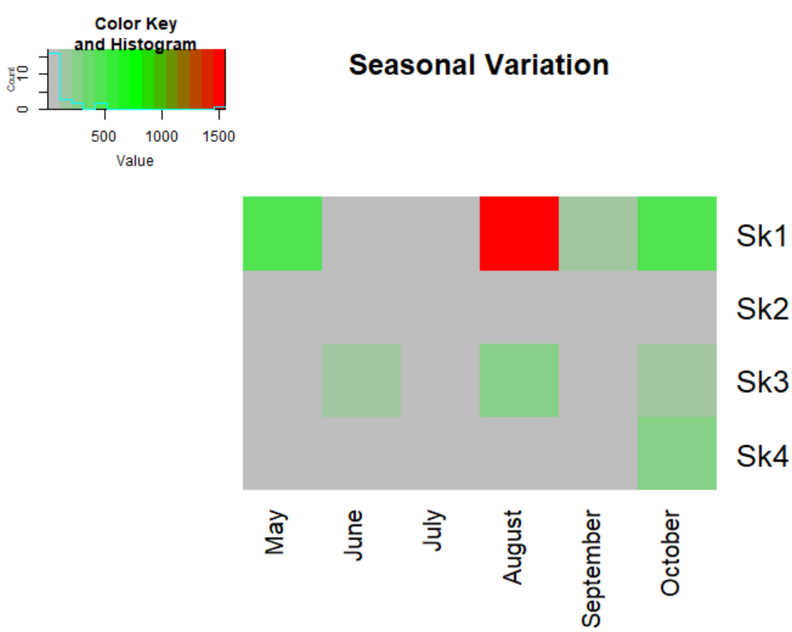
Goal 2: Using the standard procedure, evaluate the persistence of in-stream sediment stores of E.coli and their implications for water quality

Outcome 2

Task 1: Monthly Sampling

Monthly sampling began in 2017, but was challenging due to periodically high flows and soft bottom sediments. Sampling was conducted on accessible sites from May through October in both 2017 and 2018. The concentration of bacteria was found at SK1, the cattle crossing site during August (Figure 5), the hottest period. In 2017, there was a significantly greater concentration of bacteria in the sediment during the late season (August, September, and October) than the early season (May, June, July) at SK1. However, no differences between the early and late season were observed at SK2, Sk3, and SK4.

Figure 5: Concentrations of *E. coli* within streambed sediments at Skunk Creek throughout the 2017 season.



Task 2: Sampling surrounding storm events

Samples were collected surrounding several storm events in 2017. One challenge the research team faced was additional storm events prior to when the 7-day post storm sampling could occur. Instead, we examined the depletion of *E. coli* in stream bottom sediments over a series of storm events to assess intra-seasonal variability of sediment *E. coli* reservoirs. We found by the end of a storm event or series of storm events, *E. coli* concentrations increased for at least a brief period of time, potentially due to wash-in from the storm events.

Outcome 3

Task 1: Particle Size in stream bottom sediments

Skunk Creek was dominated by sand sized particles whereas Six Mile Creek had more gravel. The relationship between the particle size and *E. coli* concentration is discussed in Outcome 4.

Task 2: Attachment rates

We began assessing attachment rates of *E. coli* to sediment particles in 2018. The beginning of the season focused on identifying an appropriate method. Fractional filtration resulted in too thick of sediment to allow for *E. coli* growth on the media. Instead, we used a settling method that partitioned between *E. coli* attached to settleable particles and those that are unattached or attached to very fine, clay particles.

Attachment rates for *E. coli* in the sediment ranged from 37% to 78% for all sites during the two-month period of analysis (September and October 2018). Sk4 had the least amount of attachment, while Sk2 and Sk3 had the highest attachment rates for September and October, respectively. Higher attachment rates were observed in October for all three sites monitored during this month. Data for October 2018 are missing for Sk1 due to lack of site accessibility. The average stream flow (USGS 06481480) during the day of sample collection was eight times higher in October (93.7 cfs) than September (11.5 cfs). This higher flow may have contributed to the higher attachment rates seen in October. For one, the higher flow may have washed out more of the loosely attached or free *E. coli* from the sediment reservoirs. Secondly, higher attachment rates are often observed in the water column during high flows than baseflow conditions (Characklis et al., 2005; Soupir et al., 2010). In addition, the attachment rates were higher for the sediment than previously reported for the water column (Amegbletor, 2018) at this location. A study by Amegblator, (2018) reported average attachment rates in the water column were 19% during baseflow and 25% in storm flow at Sk2, whereas the average measured attachment rate for sediment at Sk2 found in this study was 67%.

Outcome 4

Task 1: Particle Size and *E. coli* Concentrations

All sites evaluated for sample variability showed significant correlations between particle sizes less than 0.075 mm (silt and clay particles) and *E. coli* concentrations. Three out of the five sites analyzed had significant positive correlations (SK2, SK4, and SM) while SK1 and SK3 showed a significant negative correlation between sediment particle size and *E. coli* concentrations (Table 2). While the direction is inconsistent, the fact that all sites showed significant correlations indicates that variations in stream bottom sediment composition should be considered when sampling as it has a significant impact on *E. coli* concentrations.

Table 2: Correlations between particle size and E. coli concentrations were significant for all sites. SK2, SK4, and SM all showed significant positive correlations, while SK3 had a significant negative correlation.

Site	R
Sk1	-0.56
Sk2	0.62
Sk3	-0.48
Sk4	0.7
SM	0.64

Outcome 5

Task 1: Estimate shear stress during storm events

The shear stress was calculated using data from the nearby USGS gauging station for the storm events analyzed in 2017.

No significant correlations ($p > 0.05$) were observed between the E. coli concentrations, water quality parameters (turbidity and water temperature), and hydrologic factors (flow, shear stress) (Table 3).

Table 3: Statistical analysis of E. coli concentration, water quality parameters, and hydrologic factors.

	Turbidity	TC	MC	FVF	SF	CU
Flow (m^3s^{-1})	-0.47	NS	NS	NS	NS	NS
Water	0.99	NS	NS	NS	NS	NS
Turbidity	NA	NS	NS	NS	NS	NS
Bed Shear	-0.32	NS	NS	NS	NS	NS

NTU = Nephelometric Turbidity Unit, TC = Total *E. coli*, MC = Medium and Coarse Silt, FVF = Fine and Very Fine Silt, SF = Settleable Fraction (MC + FVF) NS = Not Significant, NA = Not Applicable CU= Clay and Unattached.

Outcome 6

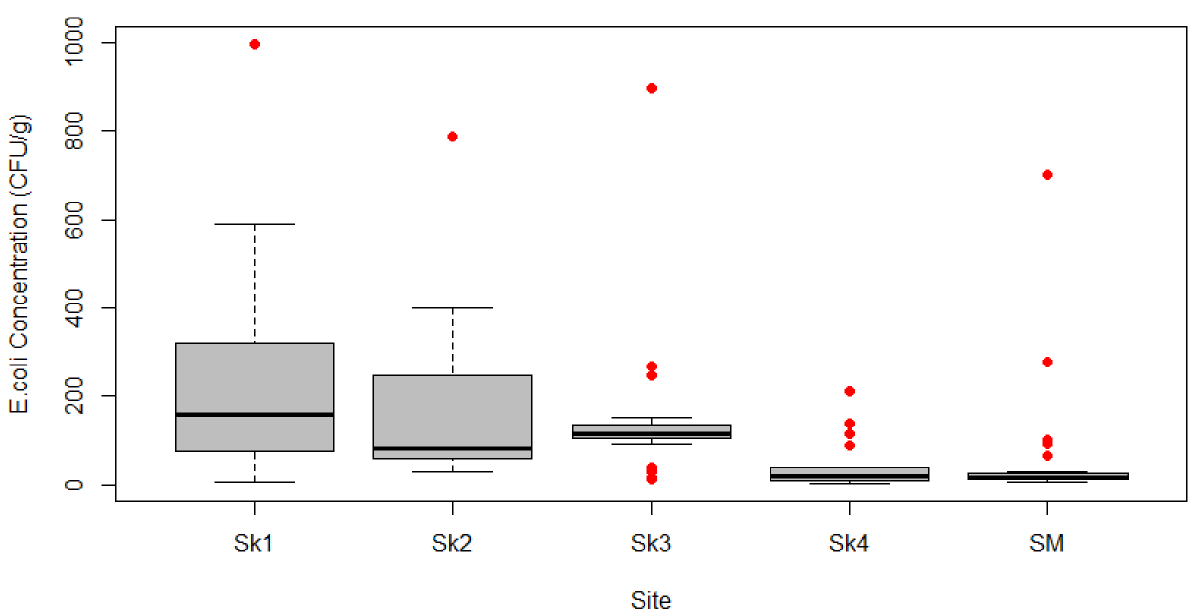
The minimum concentration measured at all five sites was 4 CFU g-1 located at Sk4 and the maximum concentration was 997 CFU g-1 at Sk1. Along Skunk Creek, the concentrations were highest at Sk1. Sk2 and Sk3 showed similar concentrations and Sk4 had the lowest concentrations. The high concentrations found at Sk1 are unsurprising given the accessibility of the site to cattle. Previous research has also found that cattle access to streams can result in *E. coli* concentrations in the sediment that are several fold higher than seen in areas with other land uses (Bragina et al., 2017; Stephenson and Rychert, 1982).

All measures of central tendency were over five times higher at Sk1 than Sk4. The reductions observed between Sk1, the cattle crossing, and Sk4, located three miles downstream, were significant ($p < 0.05$). The three miles between the two sites are almost entirely enrolled in SRAM, a best management practice to reduce fecal indicator bacteria (FIB) concentrations in the stream. This provides support for the theory that riparian management strategies, such as SRAM, can reduce FIB concentrations in sediments in addition to the water column reductions observed by many previous studies (Bragina et al., 2017; Smolders et al., 2015; Parkyn, 2004).

Table 4: Table of statistics for *E. coli* concentrations in CFU g^{-1} for all sites. The highest and lowest *E. coli* concentrations were observed at Sk1 and Sk4, respectively.

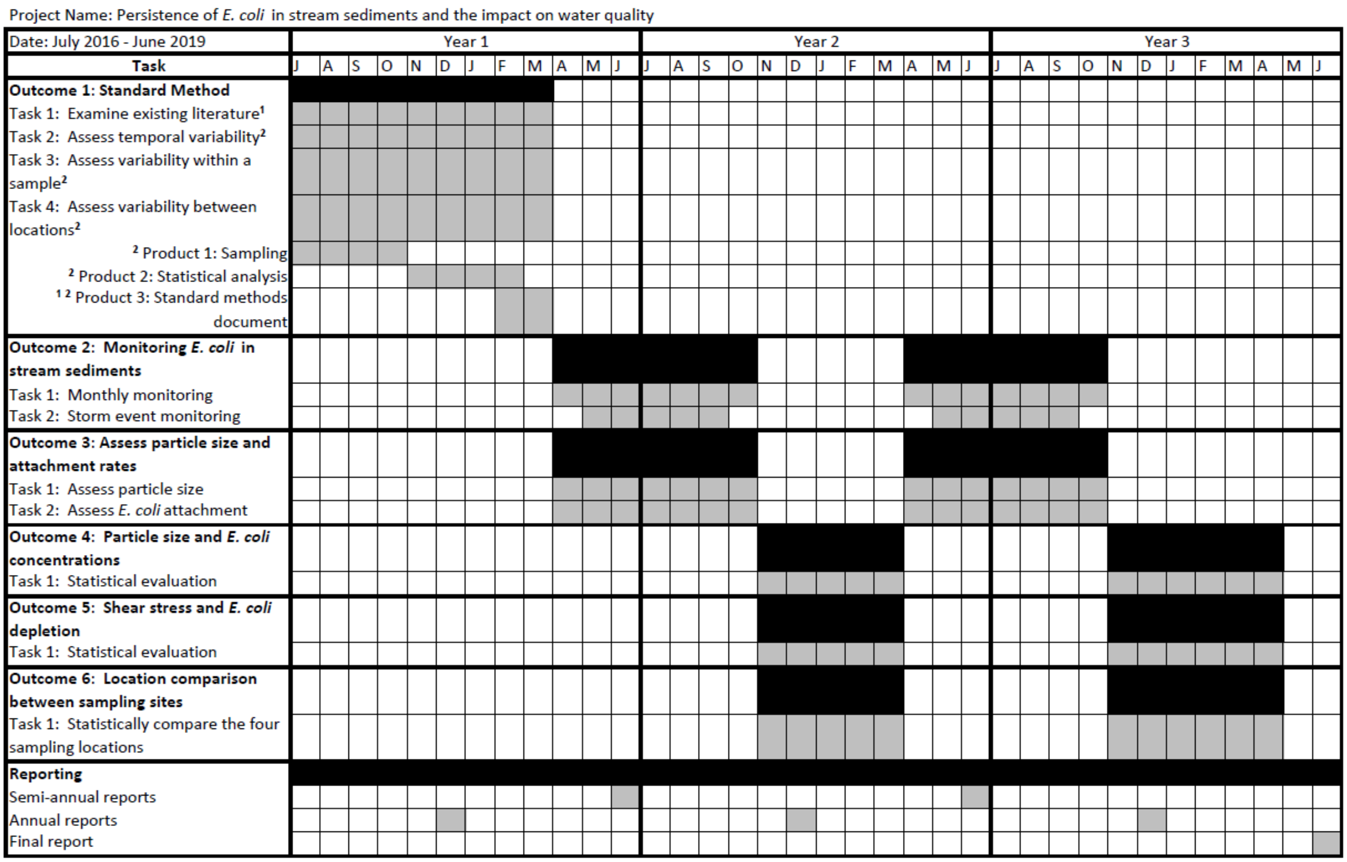
	SK1	SK2	SK3	SK4	SM
Min	8	31	14	4	7
Max	997	788	899	212	701
Mean	240	158	147	45	63
Geomean	135	105	105	24	25
Median	157	84	115	19	17
Std Dev	230	171	167	55	144

Figure 6- E. coli concentrations at all five monitoring sites varied from four to nearly 1,000 CFU g^{-1}. Outliers are indicated by red points.



2.1 Planned and actual milestones, Products, and Completion Dates

Figure 7 - Planned and Actual Milestones



2.2 Evaluation of Goal Achievement and Relationship to the State NPS Management Plan

2.3 Supplemental Information

News articles

1. **Understanding E. coli behavior important for improving water quality**

   https://www.sdstate.edu/news/2019/03/understanding-e-coli-behavior-important-improving-water-quality

2. **Precision testing shows danger in Big Sioux**

   https://www.argusleader.com/story/news/2016/02/20/precision-testing-shows-danger-big-sioux/80572266/

3. **Understanding microbial fate and transport resulting from manure application**

   2018 North American Manure Expo

   https://www.manureexpo.org/uploads/4/1/3/4/41345563/3._understanding_microbial_fate_and_transport_resulting_from_manure_application.pdf

Invited Presentations

McDaniel, R., S. Salam*, L. Amegbletor*, B. Bleakley, Z. Gu, and M. Hummel. (2018). Microbial contamination: Sediment sources, attachment, and development of a source tracking biosensor. Poster presentation at the National Nonpoint Source Training Workshop, Colorado Springs, CO

Salam, S.*, R. McDaniel, and B. Bleakley. (2018). Monitoring the seasonal variability of E. coli levels in streambed sediment and the evaluation of the effect of Seasonal Riparian Area Management (SRAM). Poster presentation at the Eastern South Dakota Water Conference, Brookings, SD.

Salam, S.*, R. McDaniel, and B. Bleakley. (2018). Assessment of seasonal variability, antibiotic resistance, and the impact of storm events on streambed E. coli concentrations in Skunk Creek, South Dakota. Oral presentation at the ASCE-EWRI Conference, Minneapolis, MN

R. McDaniel, B. Bleakley, S. Mardani*, S. Salam*, and L. Amegbletor*. (2018). E. coli fate and transport in South Dakota Environments: An update on current research. Oral presentation at the April EDWDD Board Meeting, Egan, SD

Salam, S.*, R. McDaniel, and B. Bleakley. (2017). Variability of E. coli in stream bottom sediments and the implications for sediment sampling. Oral presentation at the ASABE Annual International Meeting, Spokane, WA

Salam, S.*, R. McDaniel, and B. Bleakley. (2017). Variability, antibiotic sensitivity, and the influence of particle size on E. coli in Eastern South Dakota's streambed sediment. Oral presentation at the 2017 AWRA Annual Water Resources Conference, Portland, OR

McDaniel, Rachel. (2016). Contaminant fate and transport in South Dakota waters: Current projects and future directions. Oral presentation at the October EDWDD Board of Directors Meeting in Brookings, SD.

R. McDaniel E. coli transport in South Dakota streams. UCOWR and NIWR conference June 21-23, 2016 Hilton Pensacola Beach Hotel Pensacola Beach, FL https://ucowr.org/wp-content/uploads/2018/08/2016_Conference_Program.pdf

Peer-Reviewed Journal Articles

Salam, Sadia, Rachel McDaniel, Bruce Bleakley, Louis Amegbletor, and Sara Mardani (In-review), Variability of E. coli in streambed sediment and its implication for sediment sampling. Journal of Contaminant Hydrology.

Theses and Dissertations

Amegbletor, Louis, "Evaluating E. Coli Particle Attachment and the Impact on Transport During High Flows" (2018). Electronic Theses and Dissertations. 2629. https://openprairie.sdstate.edu/etd/2629

Salam, Sadia, "Variability of E. coli in Streambed Sediments and its Implication for Water Quality" (2019). Electronic Theses and Dissertations. 3632. https://openprairie.sdstate.edu/etd/3632

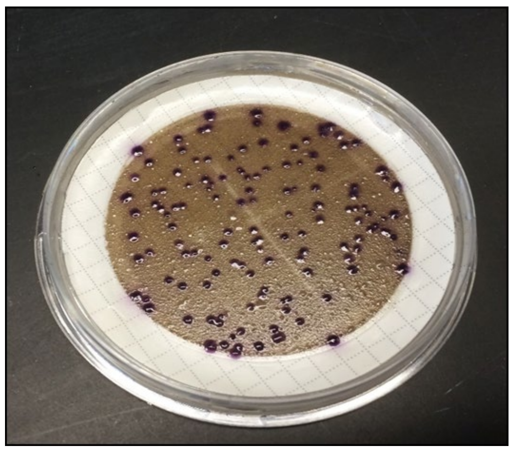
3.0 Monitoring Results

1. Provide a document detailing the sampling and testing procedure for evaluating E. coli concentrations in stream sediments.
   To accomplish this goal, past research work was reviewed. For sediment sample collection, previous researchers used a Grab sampler or sterile bottles or tubes. Most of the researchers collected samples from the top few centimeters because bacteria is prevalent in the top few centimeters [(Garzio-Hadzick et al., 2010) and (Pachepsky et al., 2011)].

   For processing sediment samples, a 1:10 or 1:11 dilution ratio was commonly used. For dilution, purified water or phosphate buffer solution were used. Samples were usually processed by membrane filtration technique or most probable number (MPN) technique.

   Based on previous research, sediment samples were collected from the top three centimeters by using sterilized wide mouth bottles. 25 samples were collected by creating a 5X5 grid formation from each location. The monitoring sites were Skunk Creek (Sk2, Sk3 and Sk4). After sample collection, the samples were transferred to the laboratory in a cooler for sample processing. The time between water sample collection and analysis should be as short as possible to limit changes in microbial populations within the sample. However, sediments have been shown to be a more stable source of bacteria than water (Pachepsky and Shelton, 2011) with long survival times (Haack, 2017; Haller et al., 2009a; Garzio-Hadzick et al., 2010). No information is available on potential changes to FIB concentrations in sediment samples during storage; therefore, a comparison between short (i.e. < 8-hour) and long (~24-hour) storage time was conducted to determine the temporal stability of E. coli in sediment samples and the resulting uncertainty in storing these samples in a refrigerated (~ 4°C or, 37 °F) environment.

Figure 8 - E. coli grown using membrane filtration



2. Monitor E. coli concentrations in stream sediments and the water column during a range of flow conditions
   Sediment samples were collected by creating a five-by-five sample grid at each site for a total of 25 samples. Samples were processed two times, within 8-hour and 24-hour of sample collection, to assess the temporal stability of E. coli in sediment samples. E. coli concentrations in the sediment ranged from 4 to 997 CFU g-1 (8.9×10^2 to 2.1×10^5 CFU 100 mL-1) (Fig 3). All the Skunk Creek sites were dominated by sand particles, with the D50 value ranging from 0.32 to 0.35 mm, while the SM site was dominated by gravel particles (D50=6.72 mm). The Spearman correlation showed a significant correlation between particle size and E. coli concentration in bed sediment; however, the direction of the correlation was inconsistent between sites.

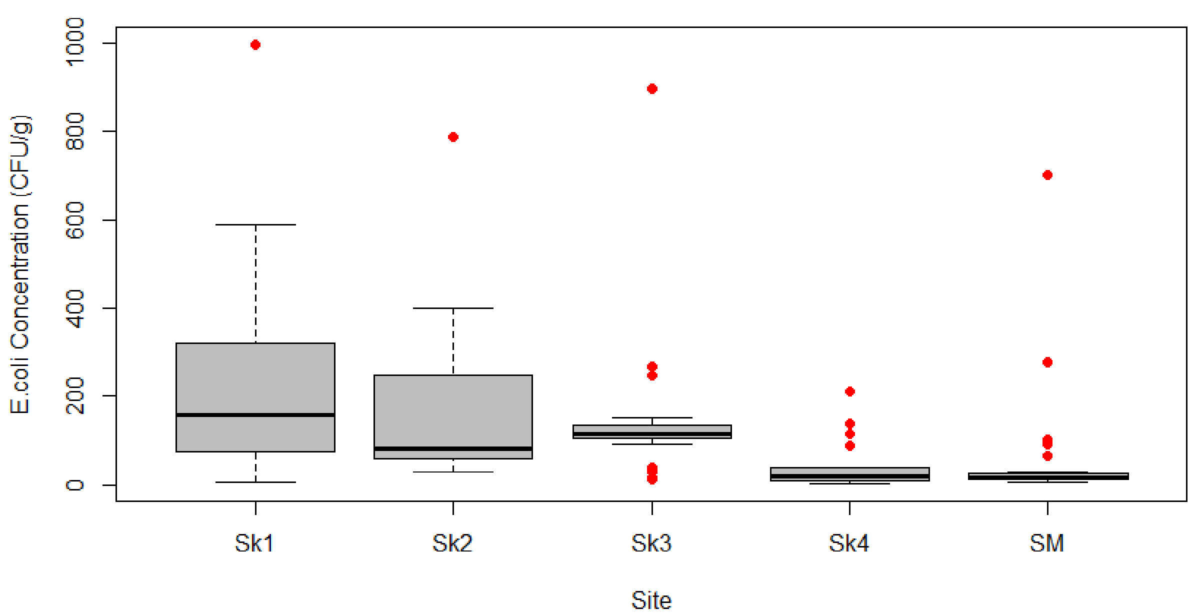
Table 5: Statistics for samples at four sample locations were calculated. The median and mean concentrations of *E. coli* for all sample sites was above the single sample maximum (SSM) concentration for limited contact recreation when the SSM is converted from a volume to a mass basis (11.78 CFU/gH_2O).

	Sk1	Sk2	Sk3	Sk4	SM
Min	7.6	30.55	13.89	4	7.3
Max	996.5	788.23	899.31	212	701.4
Mean	240.1	158.47	146.85	45	63
Median	156.9	83.68	115.28	19	17.4
Std Dev	230.4	170.6	167.0	55.5	144.3

Table 6 - Statistics for E. coli concentrations in CFU g^{-1} for all sites. The highest and lowest E. coli concentrations were observed at Sk1 and Sk4, respectively

	Sk1	Sk2	Sk3	Sk4	SM
Min	8	31	14	4	7
Max	997	788	899	212	701
Mean	240	158	147	45	63
Geomean	135	105	105	24	25
Median	157	84	115	19	17
Std Dev	230	171	167	55	144

Figure 9- E. coli concentrations at all five monitoring sites varied from four to nearly 1,000 CFU g^{-1}. Outliers are indicated by red points.



3. All five sites evaluated for *E. coli* variability had right skewed data distributions, indicating that the median would be a better measure of central tendency than the mean. In addition, the edge of the stream had 4 to 925 times more *E. coli* than the middle of the stream, depending on the site; however, there was no significant difference between the edge concentrations and the middle concentrations, likely due to the high variability

Figure 10 - *The aerial view of E. coli concentrations at the five monitoring sites shows pockets of high E. coli concentrations at all sites. Rows A and E are the banks of the stream and the flow is moving from column 5 to column 1.*

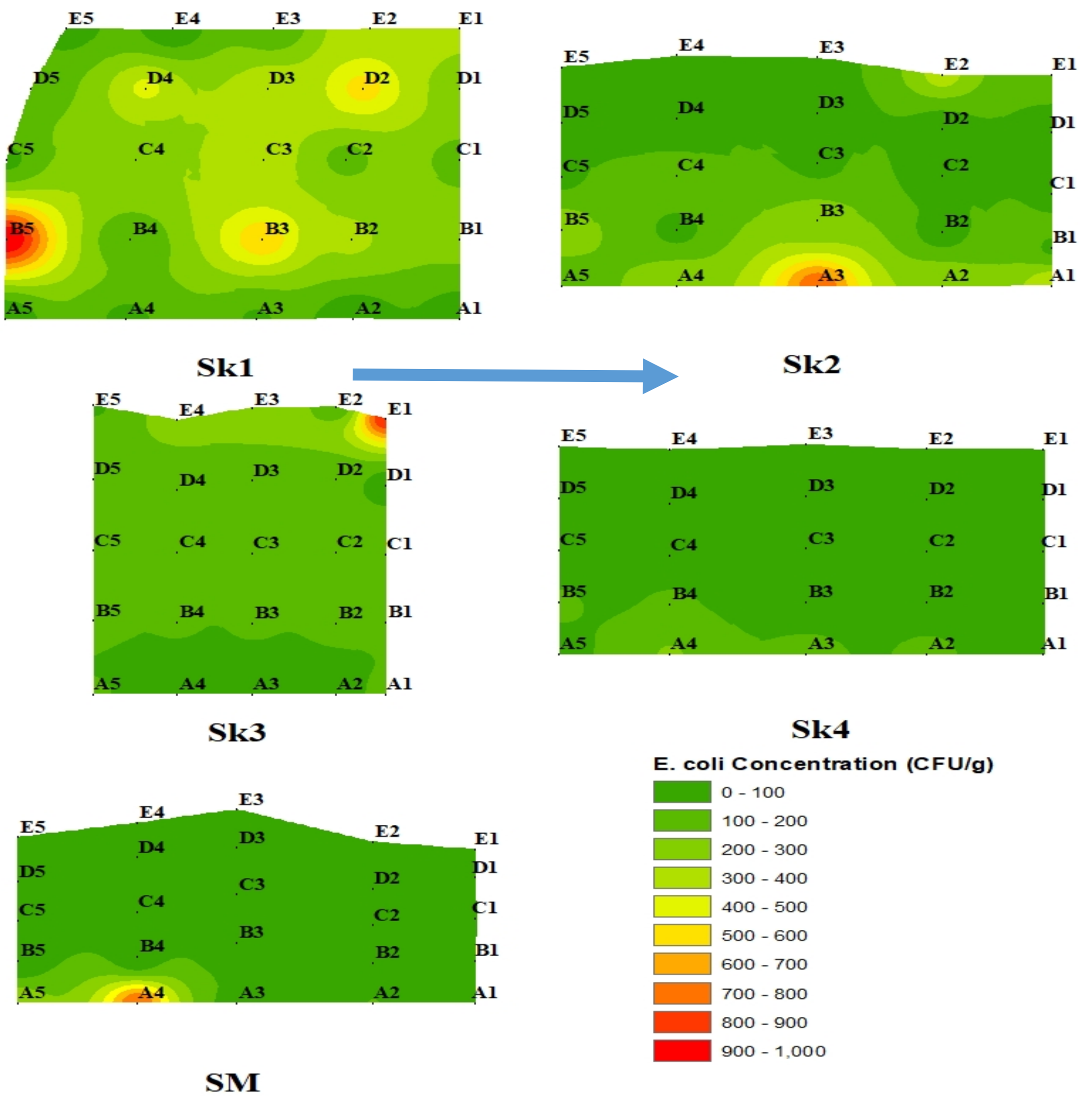


Figure 11: The E. coli variability map at site (a) Sk2, (b) Sk3, (c) Sk4 and (d) SM

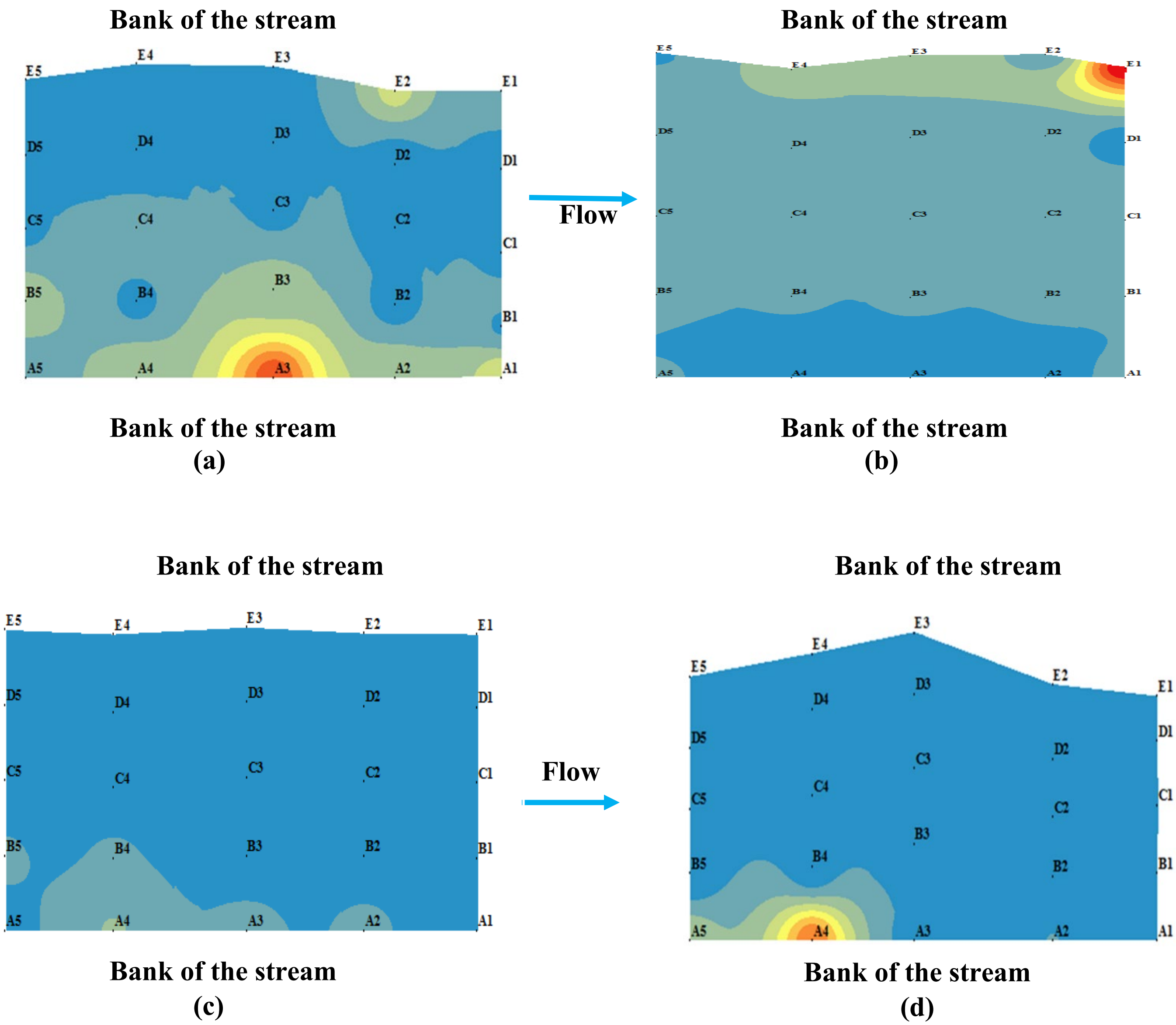


Table 7: The edge of the stream on average has higher E. coli concentrations and variance than the middle of the stream.

Statistic	Location	*E. coli* concentration: Edge (CFU g^{-1})	*E. coli* concentration: Middle (CFU g^{-1})	Edge÷Middle
Mean	Sk1	107	329	0
	Sk2	263	89	3
	Sk3	194	116	2
	Sk4	67	31	2
	SM	132	17	8
Median	Sk1	58	267	0
	Sk2	217	66	3
	Sk3	118	115	1
	Sk4	24	16	1
	SM	43	16	3

4. All sites showed significant correlations between particle sizes less than 0.075 mm (silt and clay particles) and *E. coli* concentrations, indicating that sediment composition should be considered when creating a sampling regime. However, the direction of correlation was not consistent. Three of the sites had significantly positive correlations while two sites had a significantly negative correlation.

   The majority of the three miles between Sk1 and Sk4 are managed using Seasonal Riparian Area Management, which restricts cattle access to the stream during the recreation season. Sk1 and Sk3 had the highest measured E. coli concentrations, with median concentrations of 53 and 85, respectively. Sk2 and Sk4 had significantly lower E. coli concentrations, with median concentrations of 23 and 21, respectively. Attachment rate of E. coli to settleable particles (> 0.004 mm) ranged from 37% to 78% and was highest at Sk2 and Sk3. This study examined the fate and attachment of E. coli to various particle sizes as well as their impact on water quality during both storm and baseflow events within an impaired stream.

   The study also assessed the relationship between water quality hydrologic variables and E. coli in predicting E. coli concentrations. Unattached dominated the total E. coli concentration across both storm events (60 to 97% of the total E. coli) and baseflow samples (62 to 97% of the total E. coli). With unattached E. coli forming the majority of the total E. coli concentration, further analysis to test the assumption that the total bacteria concentration can be modeled as free-living was performed. The unattached E. coli were significantly different in three out of eight storm events, or 38% of storm events.

Figure 12 - The distribution of the (a) settleable (attached) and (b) unattached E. coli over storm and base-flow events.

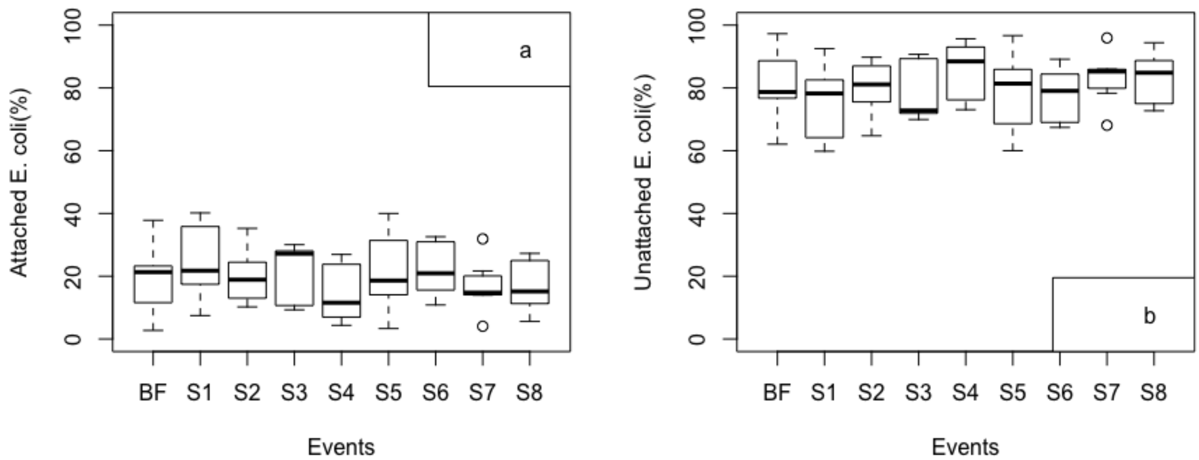


Table 8- Sediment E. coli concentration (CFU g^{-1}) statistics for the two-year monitoring period from 2017 to 2018

	Sk1	**Sk2**	**Sk3**	**Sk4**
Min	1	0	0	0
Max	2.7×10^3	1.4×10^4	1.4×10^6	1.7×10^3
Mean	2.3×10^2	4.1×10^2	2.6×10^4	1.5×10^2
Geomean	53	23	57	21
Median	53	16	115	26
Std Dev	5×10^2	2.1×10^3	1.6×10^5	3.3×10^2

Table 9 - The data of E. coli concentrations in sediment from May to October for a two year period compared to the standard for limited contact recreation of 1178 CFU 100 mL^{-1} (3.07 log_{10} CFU 100 mL^{-1}). All data were log_{10} transformed for better representation of the data. Here, E. coli concentration is expressed as log_{10} CFU 100 mL^{-1}.

Year	2017				2018			
Month	Sk1	Sk2	Sk3	Sk4	Sk1	Sk2	Sk3	Sk4
May	2.6	1.4	0	0.5	0.9	0	0.4	0
June	1.6	0.3	2.3	0.5	1.9	1.2	1.7	1.7
July	1.8	1.2	1.1	1.1	1.8	0.8	1.9	0.1
August	3.2	1.4	2.5	1.6	0.8	1.3	4.4	1.6
September	2.2	1.6	1.7	1.3	1.1	0.9	1.5	1.0
October	2.7	1.7	2.1	2.4	No Data	3.2	3.3	2.9
Scale Bar			Lowest		Standard		Highest	

Table 10- Microbial Source tracking number for 'General Bacteroidetes' and 'Ruminant Fecal' in all four monitoring sites for both sediment and water samples. Site Sk2 showed highest marker quantified for both markers and for both water and sediment. Here, 'Detected but Not Quantifiable' is abbreviated 'DNQ'.

Monitoring Site	**Marker Quantified (× 105 copies/ 100 mL)**			
	GenBac3		Rum-2-Bac	
	Sediment	Water	Sediment	Water
Sk1	30.7	6	8.8	0.3
Sk2	381	10	26.2	1
Sk3	14.4	1.9	3.9	0.2
Sk4	7	5.6	DNQ	0.6

Table 11- *Statistical summary table of percent organic content for sediment samples taken at the four sites during six months of recreational period (May to October) in 2018.*

	Sk1	**Sk2**	**Sk3**	**Sk4**
Min	0.4	0.8	0.6	0.9
Max	11.6	21.9	28.8	21.7
Mean	4.8	4.2	8.2	6.1
Geomean	2.9	2.6	5.3	4.2
Variance	13.2	25.7	46.7	30.6

5. The results show the highest concentrations of E.coli occurred at the cattle crossing during August, the hottest part of the summer.

Figure 13: Seasonal variation of log10 median *E. coli* concentration from May to October for Sk1, Sk2, Sk3, and Sk4 in 2017 and 2018. Missing data are represented in white.

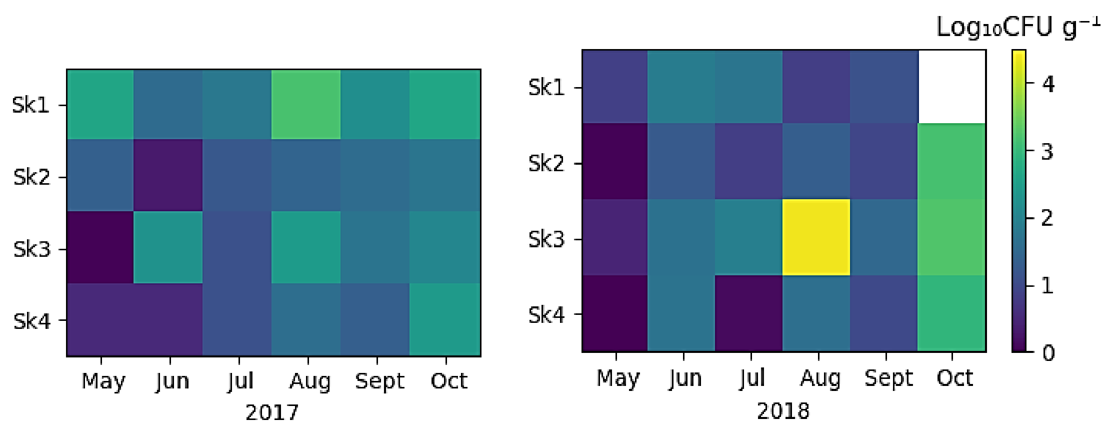


Table 12 - Skunk Creek Stream Flow, 2017-18

Stream flow (cfs)			
year	Mean	Early season	Late
	May- October	May –July	August- October
2017	18.5	33.4	3.5
2018	151.7	104.7	78.4

4.0 Public Involvement and Coordination

4.1 State Agencies

The South Dakota Department of Environment and Natural resources provided help with surveying at site Sk2.

4.2 Federal Agencies

4.3 Local Governments, Industry, Environmental and Other Groups, Public-At-Large

The Moody County Conservation District helped coordinate with the landowners for sampling purposes.

The Big Sioux River flows through Sioux Falls, SD, the largest city in the state, and is used for recreational purposes. However, water quality in the river, including E. coli, does not meet water quality standards. The poor water quality of the river has become a major concern. In response, the city of Sioux Falls has initiated an annual conference, the Big Sioux Water Summit, to inform local stakeholders of the current issues and progress in the watershed as well as discuss potential solutions to the water quality problems.

4.4 Other Sources of Funds

This research was also supported by USDA Hatch projects SD00H604-15 and SD00H452-14 courtesy of the SDSU Agricultural Experiment Station.

5.0 Aspects of the Project That Did Not Work Well

Additional work should include measurements from sites with different characteristics and land uses to determine the consistency of the results. It is recommended that longer-term monitoring as well as monitoring in more varied stream conditions be conducted to determine the consistency of these results.

Due to the high volume of snow melt and record amounts of precipitation in 2019, Skunk Creek was out of its banks throughout the year. Access to the stream was not possible. As a result, only a few samples could be collected. A special streambed grab sampler was purchased to take sediment samples without entering the stream. Unfortunately, high currents resulted in overturning of the sampler while collecting samples. Also, the streambed sediments proved to be too sandy/gravelly for proper sample collection. Small rocks and sand would jam the sampler in the open position.

No samples were collected in FY 2020 due to restrictions from the COVID-19 pandemic.

6.0 Future Activity Recommendations

- It is recommended that additional work be performed on monitoring the sediment E. coli in different stream reaches with different geographical locations or pollution sources as well as attachment rate analyses for Fecal Indicator Bacteria in sediment samples during different hydrological conditions.
    - **Sediment Sample collection Recommendation**: Collect sediment sample from the top 3 cm. A sediment core would provide the most accurate sample collection, providing both an opportunity for measuring the top 3 cm and the sediment density for mass to volume conversion. However, this collection method has been proven difficult due to equipment not retaining the sample and seizing of equipment due to sediment particles. Therefore, it is recommended to use a simpler sediment collection method such as a scoop or shovel to collect the sample
    - Sediment sample processing for E.coli: 1:10 dilution ratio using 1 gram sediment to 10 mL of phosphate buffer solution. The buffer solution maintains osmotic pressure for the bacteria, making it the recommended dilution solution.
    - Shake diluted samples in an orbital shaker for 45 min to free loosely attached *E. coli* and resuspend *E. coli* into the purified water. Use the supernatant for sample processing.
- The high sampling uncertainty and sample size analysis implies that a single grab sample may not be able to adequately represent *E. coli* concentrations in the sediment without substantial error. It is recommended that longer-term monitoring as well as monitoring in more varied stream conditions be conducted to determine the consistency of these results.

7.0 Literature Cited

Garzio-Hadzick, A., Shelton, D.R., Hill, R.L., Pachepsky, Y.A., Guber, A.K., Rowland, R., 2010. Survival of manure-borne E. coli in streambed sediment: effects of temperature and sediment properties. Water Res 44, 2753–2762. https://doi.org/10.1016/j.watres.2010.02.011

Haack, S., 2017. Fecal Indicator Bacteria and Sanitary Water Quality [WWW Document]. United States Gelogical Surv. URL https://mi.water.usgs.gov/h2oqual/BactHOWeb.html (accessed 1.4.17).

Haller, L, Amedegnato, E., Pote, J., Wildi, W., 2009a. Influence of Freshwater Sediment Characteristics on Persistence of Fecal Indicator Bacteria. Water Air Soil Pollut. 203, 217–227. https://doi.org/10.1007/s11270-009-0005-0

Nagels, J.W., Davies-Colley, R.J., Donnison, A.M., Muirhead, R.W., 2002. Faecal contamination over flood events in a pastoral agricultural stream in New Zealand. Water Sci. Technol. 45, 45–52.

Pachepsky, Y.A., Shelton, D.R., 2011. Escherichia Coliand Fecal Coliforms in Freshwater and Estuarine Sediments. Crit. Rev. Environ. Sci. Technol. 41, 1067–1110. https://doi.org/10.1080/10643380903392718
Pandey, P., Soupir, M., 2014. Assessing Linkages between E. coli Levels in Streambed Sediment and Overlying Water in an Agricultural Watershed in Iowa during the First Heavy Rain Event of the Season. Trans. ASABE 1571–1581. https://doi.org/10.13031/trans.57.10371

Sherer, B.M., Miner, J.R., Moore, J.A., Buckhouse, J.C., 1992. Indicator bacterial survival in stream sediments. J. Environ. Qual. 21, 591–595.

Cinotto, P.J. (2005). Occurrence of fecal-indicator bacteria and protocols for identification of fecal contamination sources in selected reaches of the West Branch Brandywine Creek, Chester County, Pennsylvania. USGS Scientific Investigations Report 2005-5039.

Davies, C.M., J.A.H. Long, M. Donald, and N.J. Ashbolt. (1995). Survival of fecal microorganisms in marine and freshwater sediments. *Applied and Environmental Microbiology* 61(5): 1888-1896.

DENR. (2014). The 2014 South Dakota Integrated Report for Surface Water Quality Assessment. Retrieved Sept. 2015 at < http://denr.sd.gov/documents/14irfinal.pdf>.

EPA. (2015). South Dakota Water Quality Assessment Report. Accessed Sept. 2015 at < http://ofmpub.epa.gov/waters10/attains_state.control?p_state=SD>.

Ferguson, C.M., B.G. Coote, N.J. Ashbolt, and I.M. Stevenson. (1996). Relationships between indicators, pathogens and water quality in an estuarine system. *Water Research* 30: 2045-2054.

Flint, K.P. (1987). The long-term survival of *Escherichia coli* in river water. *Journal of Applied Bacteriology* 63: 261-270.

Garzio, A. (2009). Survival of *E. coli* delivered with manure to stream sediment. Environmental Science and Policy Honors Thesis. University of Maryland, College Park.
Irvine, K.N. and G.W. Pettibone. (1993) Dynamics of indicator bacteria populations in sediment and river water near a combined sewer outfall. *Environmental Technology* 14: 531-542.

Nagels, J.W., R.J. Davies-Colley, A.M. Donnison, and R.W. Muirhead. (2002). Faecal contamination over flood events in a pastoral agricultural stream in New Zealand. *Water Science and Technology* 45(12): 45-52.

Niewolak, S. (1998). Total viable count and concentration of enteric bacteria in bottom sediments from the Czarna Hancza River, Northeast Poland. *Polish Journal of Environmental Studies* 7: 295-306.

Soupir, M.L., S. Maostaghimi, and N.G. Love (2008). Method to partition between attached and unattached *E. coli* in runoff from agricultural lands. *JAWRA* 44(6): 1591-1599

Van Donsel, DJ and EE Geldreich. (1971). Relationships of Salmonellae to fecal coliforms in bottom sediments. *Water Research* 5: 1079-1087

Soupir, M., Mostaghimi, S., Dillaha, T., 2010. Attachment of Escherichia coli and enterococci to particles in runoff. J Env. Qual 39, 1019–1027. https://doi.org/10.2134/jeq2009.0296
Characklis, G.W., Dilts, M.J., Simmons 3rd, O.D., Likirdopulos, C.A., Krometis, L.A., Sobsey, M.D., 2005. Microbial partitioning to settleable particles in stormwater. Water Res 39, 1773–1782. https://doi.org/10.1016/j.watres.2005.03.004

Amegbletor, Louis, "Evaluating E. Coli Particle Attachment and the Impact on Transport During High Flows" (2018). Electronic Theses and Dissertations. 2629. https://openprairie.sdstate.edu/etd/2629