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## Temporal and Spatial Evaluation of the Relationship between Beaver Dam Analogs (BDAs) and E. coli Levels

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## ABSTRACT

### Temporal and Spatial Evaluation of the Relationship between Beaver Dam Analogs (BDAs) and *E. coli* Levels

By

Jordan E. Daniel

April 29, 2021

**INTRODUCTION:** Beaver Dam Analogs (BDAs) have been installed along Mill Creek to help improve water quality. As a feeder creek into the impaired creek Nancy Creek, it would be beneficial to improve water quality in Mill Creek. Nancy creek is listed on Georgia's 303(d) list as an impaired stream. The EPA classifies a stream as impaired if the *E. coli* levels are consistently over 2.1 Log<sub>10</sub>/100mL, among other standards.

**AIM:** Compare the level of *E. coli* in the water before the beaver dam analogs were installed to the level of *E. coli* in the water after the beaver dam analogs were installed. In addition, the spatial difference of *E. coli* levels was also investigated.

**METHODS:** Fifteen water samples were collected each week from the same sites that were marked by stakes on the bank of the creek. The water samples were then processed through membrane filtration. The filter was plated on BioRad RAPID'E. coli 2™ medium and incubated at 37 degrees Celsius for 18 – 24 hours. Once the plates were removed, the colonies were counted. The data was collected from March 2018 to April 2021.

**RESULTS:** A One-Way ANOVA test was conducted on the data through the program GraphPad Prism 8. When comparing the dates over time it showed that the *E. coli* levels did differ significantly over time. This adds evidence that the beaver dam analogs (BDAs) do impact the *E. coli* levels. However, while the ANOVA test showed a significant difference the majority of the data was over the EPA's water quality limit of 2.1 Log<sub>10</sub>/100mL. Another One-Way ANOVA was run spatially (site-to-site), and it was found that there was no significant difference in the *E. coli* levels between the fifteen sites.

**DISCUSSION:** Overall, the data showed that the beaver dam analogs were not effective in improving *E. coli* levels of Mill Creek. While there was a significant difference between the *E. coli* levels between dates, there were data points above the EPA's limit of 2.1 Log<sub>10</sub>/100mL. While the beaver dam analogs did not improve water quality, they also did not decrease water quality. Therefore, the beaver dam analogs had relatively no change on Mill Creek's water quality.

Beaver Dam Analog's (BDAs) effect on *E. coli* levels in Mill Creek temporally and spatially

by

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B.S., CLAYTON STATE UNIVERSITY

A Thesis Submitted to the Graduate Faculty  
of Georgia State University in Partial Fulfillment

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Temporal and spatial evaluation of the relationship between beaver dam analogs (BDAs) and *E. coli* levels

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Author's Statement Page

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\_\_\_\_ Jordan E. Daniel \_\_\_\_\_

Signature of Author

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## Chapter 1: Introduction

### **1.1 Beavers as a key stone species**

The North American Beaver (*Castor canadensis*) has a range throughout North America, except for Florida and the desert (NHPBS, n.d.). Beavers are known as ecosystem engineers for their ability to create dams, which create wetlands for various species to live in. The dams they create can also help wetlands in a variety of different ways, from stabilizing water levels, water quality improvement, and slow erosion, to name a few (Law et al., 2017). With the addition of beavers, a wetland can be restored and support a variety of wildlife.

Blue Heron Nature Preserve was and still is home to beavers. In an attempt to restore the wetlands ecosystem within the nature preserve, human-made beaver dams, called beaver dam analogs, were constructed in 2019 to improve stream health and attract beavers to take up permanent residence in the creek.

### **1.2 Mill Creek**

Mill Creek is an urban creek that is located at the Blue Heron Nature Preserve in the City of Buckhead in Georgia. It was once a millpond that was breached at the beginning of the 1990s to create space for urban development. This creek is 1.6 miles long and is a tributary creek for Nancy creek that flows into the Chattahoochee River and then to the Gulf of Mexico. Situated between two neighborhoods, affected by nonpoint pollution, which means that there are multiple possible sources of pollution flowing into the creek, with any one source being difficult to pinpoint. There is runoff from the nearby homes, waste from the streets, and debris from the people who walk through the nature preserve trails.

The EPA deems a stream impaired if the waterway violates the recreational water quality standards consistently (USEPA, 2012). Nancy Creek, the waterway that Mill Creek feeds into, is classified as impaired due to the creek exceeding the fecal coliform bacteria standard and for biota impacts to fish. One way to help improve a stream is to strengthen its tributaries, implementing beaver dam analogs in Mill Creek.

### **1.3 Beaver Dam Analogs**

Beaver dam analogs (BDA) are human-made beaver dams designed to look as close as possible to naturally made beaver dams. These dams are used to help restore stream quality and to attract beavers back to the stream. The construction of eight beaver dam analogs along Mill creek is designed to help

create ponds within the creek and regulate water quality. The Blue Heron Nature Preserve built these sites by driving poles into the ground and winding smaller branches between the poles. Since their initial construction, additional natural items have been added such as wood, rocks, and other natural components.

The stream that Mill Creek flows into, Nancy Creek, is an impaired waterbody for exceeding the limit of fecal coliform bacteria. Therefore, with the addition of beaver dam analogs (BDAs), the goal of Blue Heron Nature Preserve is to help increase stream health by providing a low-cost structure, non-invasive method that could one day be adopted by beavers. These BDAs would hopefully restore the Blue Heron Nature Preserve wetlands and help the site to continue to support the animal diversity it has attained.

#### **1.4 Research Aims and Hypothesis**

This research will use the fecal indicator *E. coli*. The overall goal is to determine if the beaver dam analogs create a significant change in *E. coli* levels in Mill Creek. We will also look at the variability of *E. coli* before the beaver dam analogs and after the beaver dam analogs.

**Aim 1:** Compare the level of *E. coli* present in the water before the beaver dam analogs to the amount present in the water after the beaver dam analogs were installed.

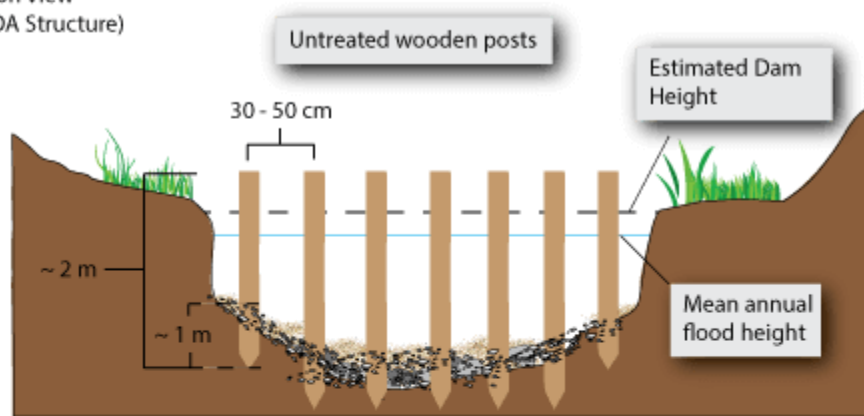
**Hypothesis 1:** *Installation of the beaver dam analogs will alter E. coli levels in Mill Creek*

**Aim 2:** Examine the change of *E. coli* spatially (between sites) to see if there are any sites that have improved more than other sites.

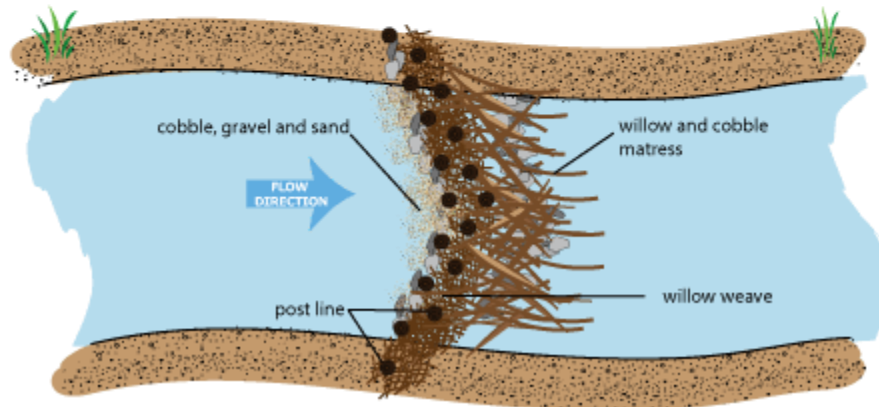
**Hypothesis 2:** *E. coli levels will indicate no significant change spatially.*

Figure 1.1 Example of Beaver Dam Analog Plans

Cross Section View  
(Generic BDA Structure)



Plan View  
(Convex Primary Dam)



## Chapter II: Literature Review

### **2.1 Effects of beavers of stream quality**

Beavers can have a significant impact on a stream. The pooling affect that the beaver dams create are the reason that the beaver dams are effective in improving stream health. Bouwes et al. (2018), a study done in Oregon that showed the implementation of beaver dam analogs attracted the natural beaver population to the artificial dams and improved the stream quality and ecology. The beavers that were attracted to the BDAs started to maintain the artificial dams and create their own dams in the process. The creation of BDAs led to an increase in beaver activity in the study area and in other parts of the creek that were not a part of the study. In terms of habitat response, the water table rose, the temperatures remained constant or lowered, and the channels showed variability in-depth (Bouwes et al., 2018). This study also monitored the fish population in the creek with the BDA and natural beaver dams; while the juvenile fish weight decreased, the survival rate increased by 52%, and there were more juvenile steelhead trout than in past years (Bouwes et al., 2018).

In another study by Catalan et al. (2015), they examined the effect of beavers on dissolved organic matter (DOM) quality from building new artificial beaver dams in a stream in Sweden. Dissolved organic matter is important in the environment because it contributes to the carbon cycle; it mainly originates in the disturbed soil surrounding the water where the new artificial beaver dams were placed. While the study could not definitively claim whether the DOM significantly increased or significantly decreased, the dissolved oxygen content decreased as the researchers sampled down the stream (Catalán et al., 2016). This decrease provides some evidence of the organic matter being used within the stream. Finally, when comparing the new artificial dam segments to older beaver dam segments, there showed to only be a substantial increase for only a limited period of time (Catalán et al., 2016).

Another study showed that beaver dams were able to help lower the nitrate concentration in a stream in New York. Denitrification is the process that microbes undergo to remove nitrate from the environment (Klotz, 2010). This act occurs mainly in sediment, which the beaver dams provide a surface area of sediment (Klotz, 2010). The study by Klotz (20120) showed the fine sediment in the beaver dams created an environment for more microorganisms to remove nitrate from the soil and water. The pooling effect caused by the beaver dams allows for the water to remain in one area longer, which means that a greater number of nitrates can be removed from the water (Klotz, 2010).

## **2.2 *E. coli* as a fecal indicator**

*Escherichia coli* is a commonly used bacterial indicator due to the availability of affordable, fast, sensitive, specific, and easier-to-perform detection methods (Odonkor and Ampofo, 2019). The Environmental Protection Agency (EPA) has advised that a freshwater recreational quality criterion should not exceed 126 CFU/100 mL (USEPA, 2012). *E. coli* is found in mammalian feces and has the potential to cause disease in humans and can cause stomach cramps, vomiting, and diarrhea. It can survive in water for 4 to 12 weeks; therefore, it is an acceptable measure of water quality due to its ability to be easily detected and that it lasts in the stream to get an accurate count of *E. coli* levels (Edberg et al., 2000).

There is a natural fluctuation in *E. coli* levels in an urban nonpoint source pollution creek due to several factors. One of the main factors contributing to this is rainfall and river discharge. A study done by Schilling et al. (2009) showed that *E. coli* levels are highest during the region's period of greatest rainfall intensity. Another factor that affected the streams' *E. coli* levels was the stream's flow rate (Schilling et al., 2009). When the stream is at its lowest flow rate, it provides more time for the water to contact the soil, and the microbes in the soil help filter and remove the *E. coli*. The purpose of the beaver dam analogs would be to help lower the flow rate and hold water longer in the pooling region to create a longer contact time between the water and the soil.

## Chapter III: Methods

### **3.1 Primary Data Collection**

Water samples were collected weekly from fifteen sites from Mill Creek located in Blue Heron Nature Preserve. To ensure that the sampling sites were consistent between the weeks, a stake was placed on the bank at each site to mark where to collect each sample at. Each sample was collected from the thalweg via a sterile Whirl-Pak®. Roughly 4 ounces were collected in each Whirl-Pak®. The samples were collected from March 2018 to April 2021, with some gaps due to beaver dam analog installation and the COVID-19 pandemic.

The creek is situated downhill from a residential area on west side, and on the other side is the walking trail for Blue Heron Nature Preserve, wetlands, and another residential area. Site two, which is the first site in the creek, is located just a few feet downstream of the first BDA and is downstream from a pedestrian bridge; the water is collected as it flows from the BDA. Then the creek flows through a shallow, pebbled creek bed, with the sides of the creek being incised by over a meter. Another BDA was installed, and site three is collected a few feet after this second BDA. Then the creek flows to a third BDA, where site three is situated after the BDA. The creek flows into another shallow creek bed where site one, the first control site is located in the shallow open water. Further downstream, the creek widens, and site five is collected from the downstream side of the fourth BDA. Site six is located a few feet after the fifth BDA and is the location of the most significant bank erosion as the creek diverted around the dam. This causes major erosion along the cut bank. Further downstream, the creek deepens, and site seven, eight, and nine samples are taken in the deepest parts of the creek. Site nine is also near a deer feeder that was placed by one of the residences. Site 10 is collected just before the sixth BDA. The stream then flows to three consecutive dams, which are roughly twenty to thirty feet from each other; site 11 is located after the sixth BDA, site 12 is located after the seventh BDA, and site thirteen is located after the eighth BDA. Further downstream, the creek narrows, and site fourteen is in shallow open water. The creek then gets very shallow, and just before the creek joins into Nancy Creek, site fifteen is located about twenty feet from where the water flows over the Mill dam.

To analyze the amount of *E. coli* in each water sample, the EPA has approved membrane filtration (MF) to be an effective method of quantification. This method is useful for samples of large volumes. Using the membrane filtration technique, the water sample is placed in a funnel with a 47 mm 0.45 µm pore size cellulose filter (Milipore Corporation) filter on the bottom, and a flask with a vacuum

attachment is placed underneath. The sample is pulled through the membrane. Two different volumes below 100 mL, the usual volumes measured were 5mL and 25mL, of each sample were run each time; therefore, a total of thirty samples were run each filtration day.

The membrane is then placed on a RAPID *E.coli* 2 (BioRad Corp) agar for water testing, which selects for and differentiates *E. coli*. The plates were then incubated for 18 to 24 hours at 44.5 degrees Celsius. The *E. coli* is grown as a pink to violet color due to the plates detecting the presence of  $\beta$ -D-Glucuronidase (GLUC) and  $\beta$ -D-Galactosidase (GAL). The colonies were counted, and then, using the formula below, each sample was converted to CFU/100mL.

$$\frac{\text{Total number of colonies formed on plate}}{\text{Total volume filtered}} * 100 = \text{CFU}/100\text{mL}$$

### 3.2 Data Analysis

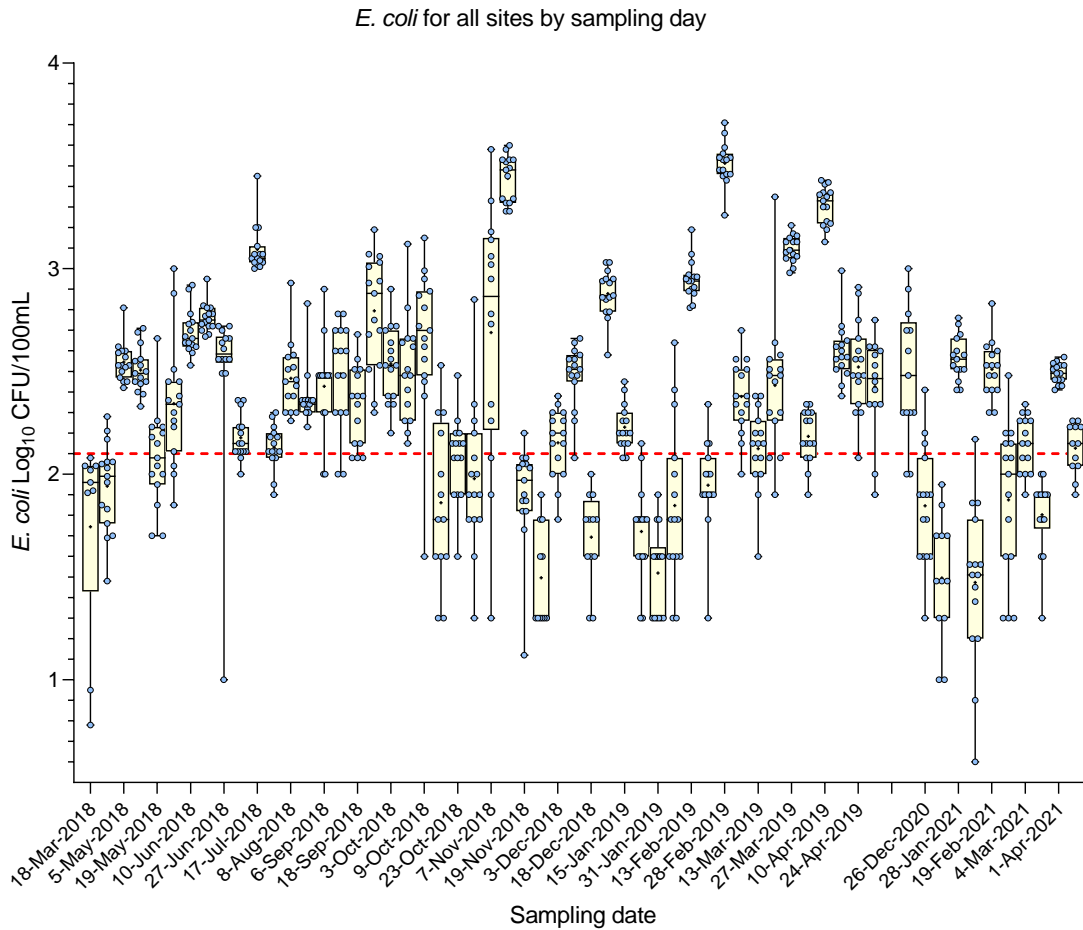
The data was collected, sorted, and converted in Microsoft Excel. Microsoft Excel was also used to calculate the average CFU/100mL for each site and for each sample day for the whole sampling period. Data was log transformed to normalize the distribution. Graphpad8 Prism was used to conduct the statistical analysis and to create the graphs. A one way-ANOVA was used to compare the sites before the BDA was implemented and after the BDA was implemented. An alpha value of 0.05 was used to determine the significance level. In the graphs, the horizontal red dotted line indicates the cut-off for acceptable *E. coli* levels in the water as determined by the EPA. It is set at a 2.1Log10CFU/100mL.



## Chapter IV: Results

### 4.1 *E. coli* trends over all the sites by the data the sample was collected.

Graph 4.11 *E. coli* trends for all sites by date



**Figure 4.1** above shows the temporal trends of *E. coli* for Mill Creek. It is the log-transformed mean for each site that is displayed by the date it was collected. The dotted red line represents the EPA's acceptable level of *E. coli* of 126 CFU/100mL or 2.1 Log<sub>10</sub>/100mL. The BDA's were installed on July 28<sup>th</sup>, 2018.

Over that sampling time, March 2018 to April 2021, the mean level of *E. coli* was 2.33 Log<sub>10</sub>/100mL with a standard deviation of 0.4692 Log<sub>10</sub>/100mL. Overall, the sample site means ranged from 1.4 to 3.5 Log<sub>10</sub>/100mL, with the median at 2.3 Log<sub>10</sub>/100mL.

**Table 4.11**

	<b>SS</b>	<b>DF</b>	<b>MS</b>	<b>F (DFn, DFd)</b>	<b>P value</b>
Treatment (between columns)	181.5	58	3.129	F (58, 796) = 53.79	P<0.0001
Residual (within columns)	46.31	796	0.05818		
Total	227.8	854			

**Table 4.11** displays the One-Way ANOVA test that ran in GraphPad Prism 8. The ANOVA was conducted to determine if there was a difference between the levels of *E. coli* from all sampling sites by date. The ANOVA test was run using a 95% confidence interval,  $\alpha = 0.5$ . The P-value <0.0001 determined by the ANOVA test was less than the alpha value, which means that the average *E. coli* level differences between dates are statistically significant.

#### **4.2 Spatial analysis**

**Graph 4.2 *E. coli* trends by site**

*E. coli* for all sampling day by site

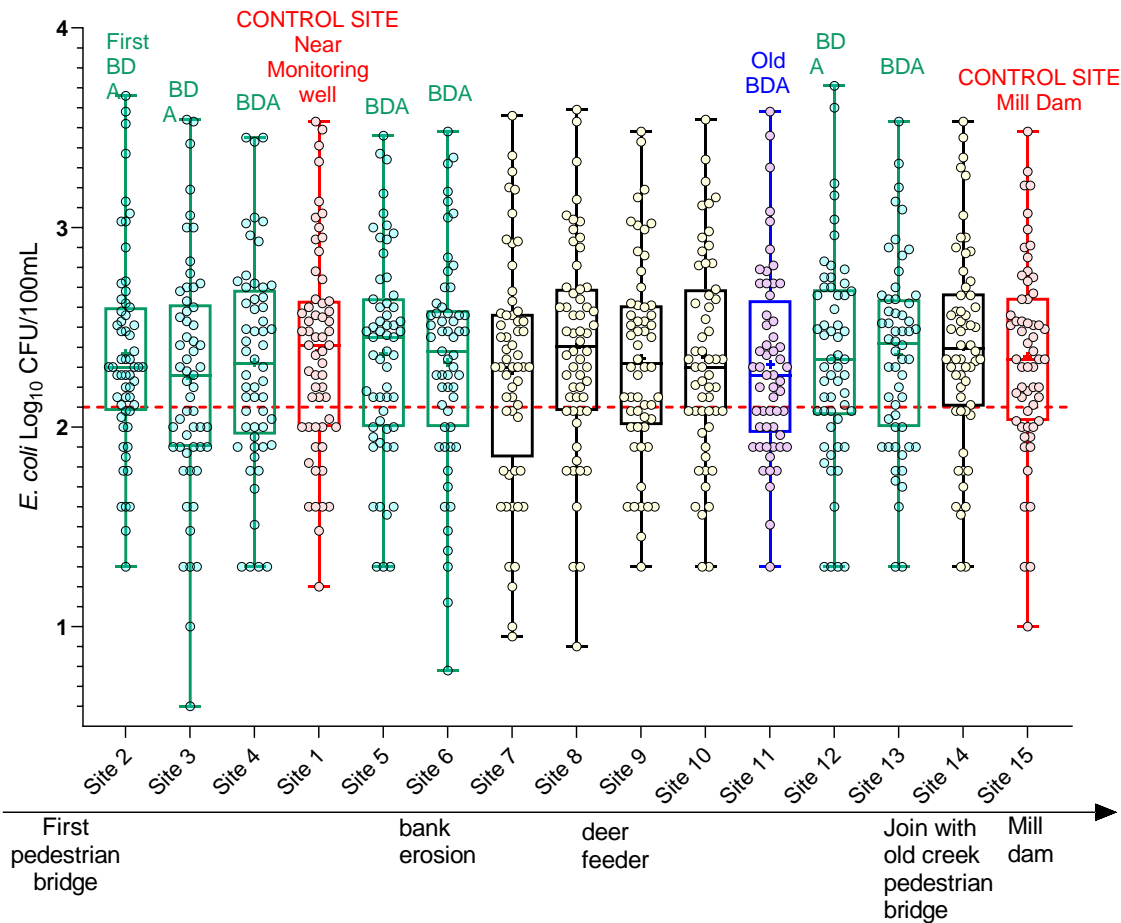


Figure 4.2 displays the *E. coli* trends spatially (site-to-site). This box and whisker plot shows that the majority of the sample means are above the EPA's recreational water quality standards, suggesting that all the sites could be classified as impaired. The EPA's standard of an acceptable level of *E. coli* of 126 CFU/100mL or 2.1 Log<sub>10</sub>/100mL is designated on the graph by the dotted red line. The sites do not deviate significantly from our two controls. The two control sites were chosen because site one is near the creeks monitoring well and site fifteen is at the end of the creek where we would expect *E. coli* to be washed down to.

**Table 4.2**

	<b>SS</b>	<b>DF</b>	<b>MS</b>	<b>F (DFn, DFd)</b>	<b>P value</b>
Treatment (between columns)	1.494	14	0.1067	F (14, 840) = 0.3961	P=0.9763
Residual (within columns)	226.3	840	0.2694		
Total	227.8	854			

**Table 4.2** shows the One-Way ANOVA table performed on the data to determine if any of the sites significantly differ from each other. The ANOVA test was run at a 95% confidence interval,  $\alpha=0.05$ . The P-value of 0.9763 is greater than the alpha value, which means that the sites *E. coli* levels are not significantly different between sites.

## Chapter V: Discussion

### **5.1 Discussion of Research Questions**

The purpose of this research was to determine if the beaver dam analogs resulted in changes in levels of *E. coli* in Mill Creek. Overall, the data shows that Mill Creek could be classified as an impaired creek due to the *E. coli* levels for each site (for the majority of the data, were over the EPA's standard and that there has been no significant change in *E. coli* levels since the installation of the beaver dam analogs. Therefore, while my hypothesis that the beaver dam analogs will alter the *E. coli* level is supported and the level of *E. coli* changed, it did not lower enough to be within the EPA's guidelines. While the data shows that the *E. coli* levels do differ significantly over the temporal data, the levels are sometimes above and below the EPA's standard of 2.1 Log<sub>10</sub>/100mL. There are possible seasonal differences in *E. coli* levels, but more data during different seasons would be needed to determine a definite pattern.

The second research question addressed in this research was: Is there a difference spatially between the sampling sites. The data shows that there is no significant difference in the *E. coli* levels between the fifteen sites. The mean for all fifteen sites is above the EPA's recreational water quality standard, meaning that all sites could be classified as impaired and are generally not safe for primary contact. The sites were above this level during most sampling events. The BDA sites were also similar to the two control sites, which suggests that BDAs may not be significantly affecting the levels of *E. coli* at Mill Creek.

Overall, it seems that the beaver dam analogs have not significantly changed the level of *E. coli* in Mill Creek from before their installation. It is also important to note that while the beaver dam analogs have not improved the *E. coli* levels, they have not worsened the *E. coli* levels. Another thing to note is that while the water quality has not changed significantly, the bank around the water has. When the beaver dam analogs are not built properly, it causes the water to divert around the dams, eroding the cut bank. This has caused an issue on the nature preserve as site six erosion is near a walking path and the worry is that the erosion will destroy the walking trail. There is a positive correlation between sediment erosion and *E. coli* levels (Tiefenthaler et al., 2010). During the last two weeks of sampling, April 1st and April 8th, a construction crew was working on-site six to fix the beaver dam analog and to fix the step bank to a slope. This will hopefully fix the problem of the creek creating the oxbow.

## 5.2 Study Strengths and Limitations

The study's main strength was that the data was collected for at least one year so that we can start to see if there are any temporal patterns. The consistency of the collection and processing methods also helps provide a strong base for the research.

The limitation of the study is that some days the colony numbers were too numerous to count (TNTC) which meant that these samples were re-run at a different volume and if it was still TNTC the data point was excluded. There was also the issue of some of the sites were unable to be sampled on certain days. Another issue is the gap in the data that was created due to COVID-19 shutting down the lab for a time. Lastly, this study did not take into account for precipitation that could affect the *E. coli* levels in the stream.

## 5.3 Implications of Findings

The results of the data show that the beaver dam analogs installation has not resulted in a significant change in *E. coli* levels. This means that Mill Creek could be considered an impaired creek and that, as a feeder creek to Nancy Creek, it still may contribute nonpoint source pollution to other streams.

It is also important to note that currently, no beavers have taken up residency of the beaver dam analogs, so these dams will need to be monitored for when beavers do take up residency. The presence of beavers may cause a change in *E. coli* levels because they will maintain the dams and have the dams functioning properly to reduce the *E. coli* levels.

## 5.4 Recommendations and Future Steps

The Blue Heron Nature Preserve needs to invest more time into building the beaver dam analogs properly to prevent the cut bank that is forming around some of the beaver dam analogs. Once site six was repaired properly, it started to create the pool necessary for beavers and stopped the erosion that was causing the cut bank.

For future studies at Mill Creek, it would be beneficial to incorporate other measures of water quality. Some of these could include pH, dissolved oxygen, and other water quality indicators. It would also be beneficial to monitor the relationship between rainfall and *E. coli* levels to see if there is a relationship. Lastly, it may be useful to monitor for antibiotic-resistant *E. coli*.

## 5.5 Conclusion

The *E. coli* levels are significantly different temporally, however, there are times where the mean *E. coli* level is above the EPA's recreational water quality standard. The data also shows that the sites do not differ in *E. coli* levels between the fifteen sites and all the sites look similar to the controls. Overall, there is no significant improvement of water quality associated with the beaver dam analogs when examining *E. coli* levels in Mill Creek. There would be a possibility of change if beavers were to take up residency in the beaver dam analogs due to their ability to maintain the dams and have it function properly. Further studies would need to be done to test for more water quality factors and examine the impact of beavers on the water quality.

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### Images

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