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Evaluation of naturally occurring and anthropogenic contamination in Missouri streams

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Department:

EVALUATION OF NATURALLY OCCURRING AND ANTHROPOGENIC CONTAMINATION IN MISSOURI STREAMS

by

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ABSTRACT

The goal of this study is to observe the values and variability of water quality parameters and benthic macroinvertebrates in watersheds with very little anthropogenic impact and to compare these values with those acquired in watersheds with more anthropogenic impact. The following five HUC 12-digit watersheds had very little anthropogenic impact and were considered “pristine”: Rogers Creek, Mill Creek, Middle West Fork-Black River, Bee Fork, and Ottery Creek. Five largely urban sub-basins were also considered; these basins are: Grand Glaize Creek, Glaize Creek, Sugar Creek, Hominy Creek, and Grindstone Creek. For each watershed, both water quality parameters and benthic macroinvertebrates were sampled. The macroinvertebrate samples were used to calculate the biotic index for each stream using the Missouri Department of Natural Resources method, percent Ephemeroptera, Plecoptera, Trichoptera, and the Hilsenhoff Family Biotic Index to help better determine long-term stream health. Water quality parameters were also analyzed to identify seasonal changes and patterns between the streams. Correlation matrices were constructed to determine significant correlations between water chemistry parameters at the pristine steams, the urban streams, and when considering all streams as one sample set. Welch ANOVA was additionally performed to determine which streams were statistically part of the same population. For most water quality parameters, the pristine streams tended to be grouped as one population, while the urban streams were often separated into two populations. The reason for differing populations is most likely related to land use/land cover and varying activities in each watershed.

ACKNOWLEDGMENTS

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1. INTRODUCTION

1.1. BACKGROUND

Deterioration of water quality in surface water and groundwater is an important concern both locally and globally. To best manage our water resources, it is important to have reliable information on water quality on a range of scales. Water quality is affected by a combination of anthropogenic factors (e.g. agriculture practices, wastewater/industrial influent, urban runoff) and natural factors (e.g. precipitation, temperature, bedrock type, terrain, soil type, and wildlife) (Carpenter et al., 1998; Smith at el., 2013). These factors influence the biochemical and hydrological processes that affect the water quality in a watershed, so it is often difficult to determine the extent to which different factors are responsible for variation of water quality.

Water quality can be evaluated using standard analyses such as temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), turbidity, nutrient concentrations, and other parameters. Temperature measures the amount of heat in the water and is important for analyzing aquatic environments and indicating the source of water. Natural temperature readings in Missouri streams typically range from 0°-24°C (Brown and Czarnezki, 2003). pH measures how acidic or basic the water is and unusual pH measurements can indicate pollution from mining activity or chemical imbalances. Acceptable pH values for most invertebrates and fish range from 6.5-9.0 in Missouri streams (Brown and Czarnezki, 2003). Electrical conductivity (EC) is determined by the amount of dissolved ions in the water. EC is often controlled by the geology, the size of the watershed, urban and agricultural runoff, wastewater, and source of water in a stream (overland flow or baseflow). Dissolved oxygen (DO) measures the amount of oxygen dissolved in the water and is essential for assessing aquatic environment health in streams since most aquatic animals need certain levels of DO to survive. Generally, higher levels of DO are indicative of healthy, stable streams. Natural readings for DO in Missouri streams typically range from 5-15 mg/L (Brown and Czarnezki, 2003). Turbidity measures the suspended solids which are undissolved in the water and is also known as the clarity of the water. High turbidity in a stream can be problematic for sustaining aquatic life.

Stream nutrients such as nitrate and phosphate both measure organic matter and fertilizer materials in water. High concentrations of nitrate and phosphate can be caused by runoff from fertilizers, water treatment facilities, animal feeding operations, and animal waste. Natural reading of both nitrate and phosphate range from 0.0-2.0 mg/L in Missouri streams. Chloride is often associated with runoff from road salts and urban pollution. High concentration of chloride in water can have a negative impact on aquatic ecosystems. The Department of Environmental Management (DEM) has determined the acceptable chloride concentrations for freshwater organisms. The acceptable concentration limit to prevent immediate exposure effects is 860 mg/L, while 230 mg/L is the acceptable limit to prevent long-term effects on organism health (Herron and Green, 2012).

Water chemistry parameters all provide valuable information about water quality but are seldom gathered continuously at most sites and can fluctuate greatly with time. Thus, these data can give an indication of water quality at the time of measurement but are not always available with sufficient temporal resolution to understand the long-term health of a body of water or to evaluate fluctuations in water quality over time. A longer-term assessment of surface water quality can be obtained by evaluating aquatic organisms, primarily benthic forms. By analyzing the type and number of benthic life forms present, the condition of a stream over the span of a few months can be estimated (De Castro-Catala, 2015). Streams with a higher population and diversity of benthic macroinvertebrates indicate better water quality and provide a suitable habitat for other aquatic organisms. Benthic macroinvertebrates are usually in the water, so it is ideal to use these populations to assess the long-term health of a stream. Several methods exist for calculating a water quality rating based on macroinvertebrate surveys; some methods are more sensitive to changes in nutrient loads in the stream (Lawes et al., 2018), while others may investigate the relation of deposited sediment to invertebrate communities (Zweig, Leanna D. and Rabeni, Charles F., 2001). However, there seems to be no clear consensus on the effectiveness of different analysis method for evaluating water quality.

Many researchers have studied how changes in the land use/land cover (LULC) and different human activities have affected water quality (Ferrell 2001; Hallidat et al., 2012). Urbanization and agricultural activities can both negatively impact water quality (Ngoye and Machiwa, 2004). Generally, industrial and urban land uses are associated with heavy metals, industrial chemicals, and organic pollution, while agricultural land use often results in elevated levels of nitrogen, phosphorus, fecal bacteria, and pesticides in stream water (Malmqvist and Rundle, 2002). As watersheds become more urban, studies have shown that stream health usually degrades significantly (Wang et al., 2008). Urban areas have a high percentage of impervious ground cover, and these surfaces can cause increased pollutant loads. An Australian study found that pH, EC, and DO values all increased in study areas where the imperviousness increased (Hatt et al., 2004). Several researchers have established regression models to develop relationships between water quality parameters and LULC in watersheds (Ferrier et al., 2001; Li et al., 2009; Kang et al., 2010). These models indicate that nutrient and fecal coliform concentrations within urban watersheds are often higher than those in non-urban watershed during storm cycles as well as under base-flow conditions. Other studies have found that nitrate loads tend to be higher in urban riparian zones when compared to rural areas (Groffman et al., 2002).

1.2. OBJECTIVES

One of the goals of this study is to provide data on variations in water quality in watersheds with little anthropogenic impact. Understanding the natural levels of contamination in streams can provide regulators with baseline levels of pollutants and can help to determine what surface water contamination is caused by human activity and what occurs naturally. Having a baseline of the natural contamination in Missouri streams can assist regulators in developing total maximum daily load thresholds while also helping to evaluate the extent to which non-point sources are contributing to local water quality degradation.

Another goal is to develop a better understanding of how water quality parameters change seasonally in watersheds with relatively little anthropogenic impact. This knowledge will assist water resource managers in determining what fluctuations in water quality can be expected naturally and which may be the result of human activity. This information can assist in providing background data for water quality parameters in the absence of significant anthropogenic impact.

The third goal of this study is to evaluate the impact of various water quality parameters on benthic macroinvertebrate health and to determine which methods of evaluating macroinvertebrate health best correlate with different water quality parameters. The results of this analysis will help regulators to determine which parameters may be most impacting macroinvertebrate health in streams and to use targeted macroinvertebrate analyses to predict water quality parameters. Since macroinvertebrates are usually present in a stream, macroinvertebrate health may be a better indicator of long-term water quality than infrequent physicochemical monitoring.

2. STUDY AREA

2.1. PRISTINE STREAMS

Pristine data were acquired at five 12-digit watersheds in southeastern Missouri with little urban development or agriculture. These watersheds include Rogers Creek, Mill Creek, Middle West Fork – Black River, Bee Fork, and Ottery Creek as shown in Figure 2.1.

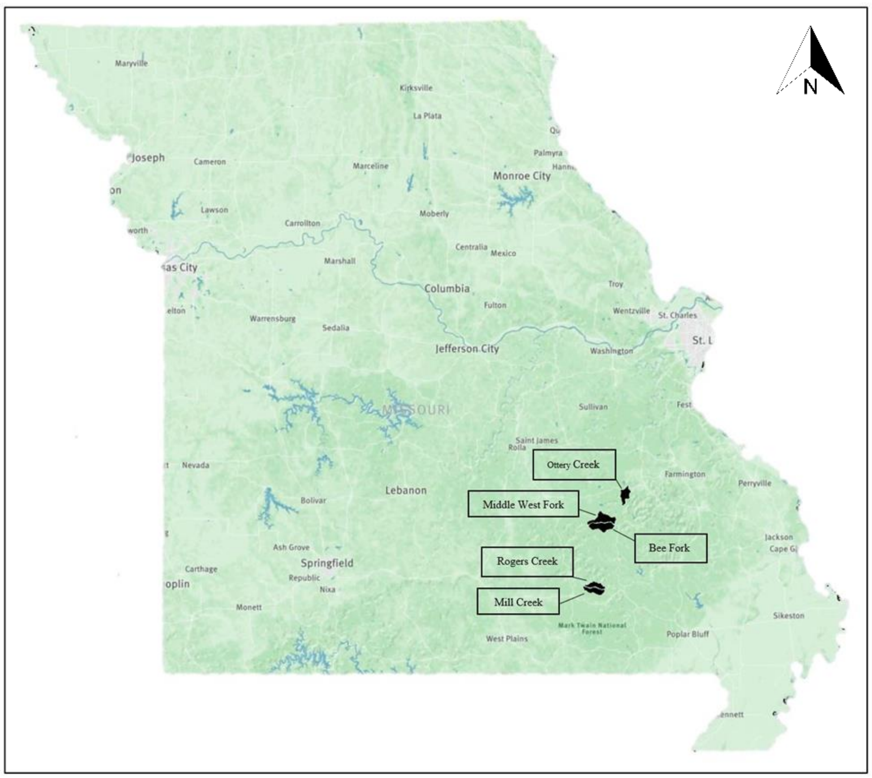

Figure 2.1. Location of the Pristine Watersheds

The pristine watersheds were chosen for this study because they have little human activity and encompass no large urban areas, allowing for the collection of water quality data in less impaired environments. Urban and agricultural activity, such as row crops and livestock production, are also minimal in these watersheds. While each watershed has slight differences in land use/land cover (LULC), the land is predominantly forested. Figures 2.2 to 2.6 show the land use for each watershed while Table 2.1 summarizes the data.

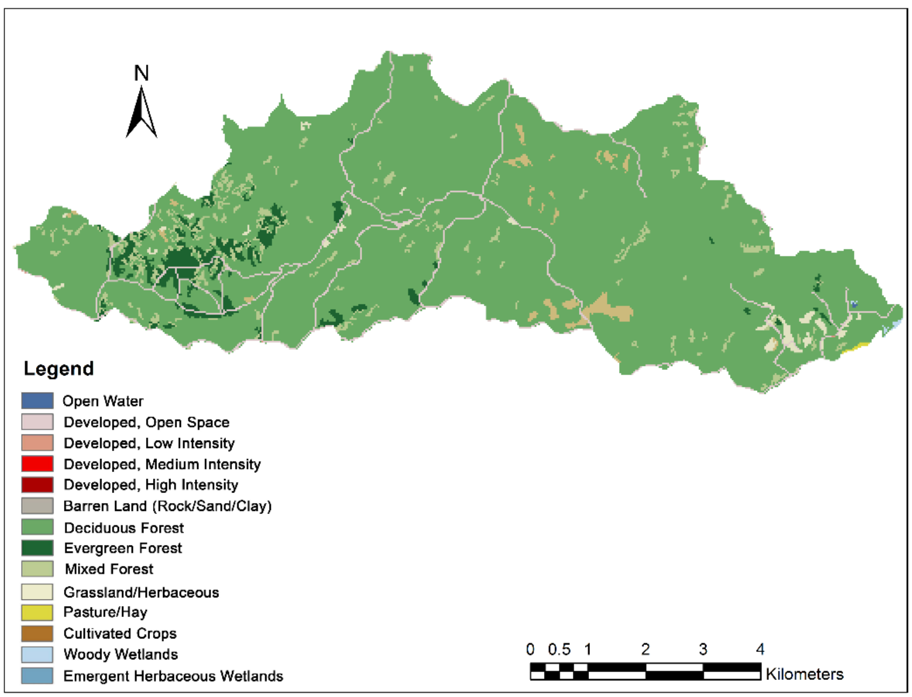

Figure 2.2. LULC for Rogers Creek

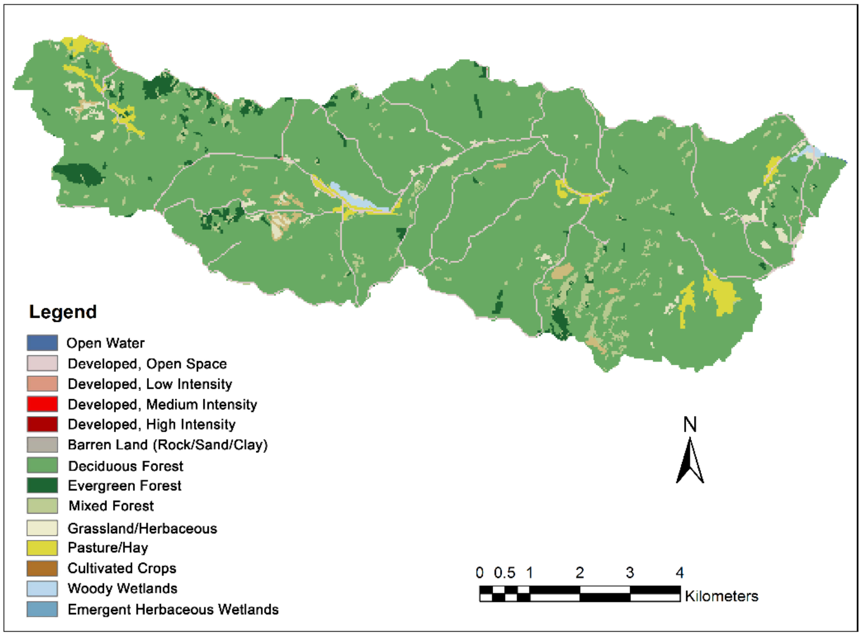

Figure 2.3. LULC for Mill Creek

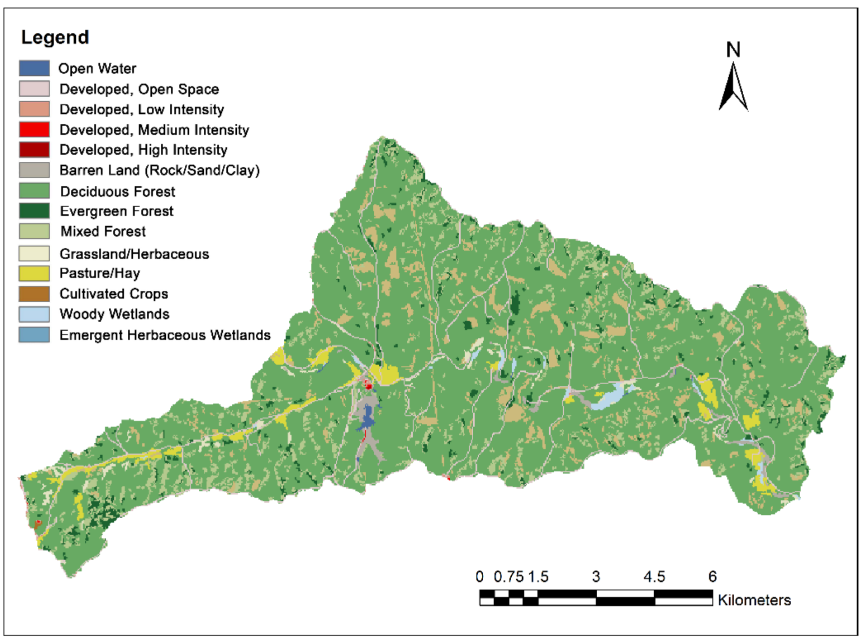

Figure 2.4. LULC for Middle West Fork

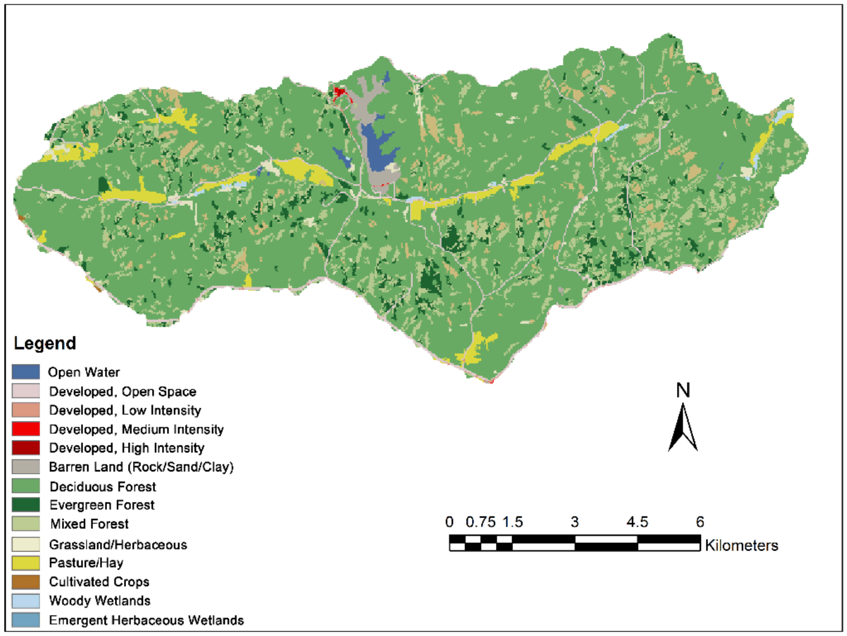

Figure 2.5. LULC for Bee Fork

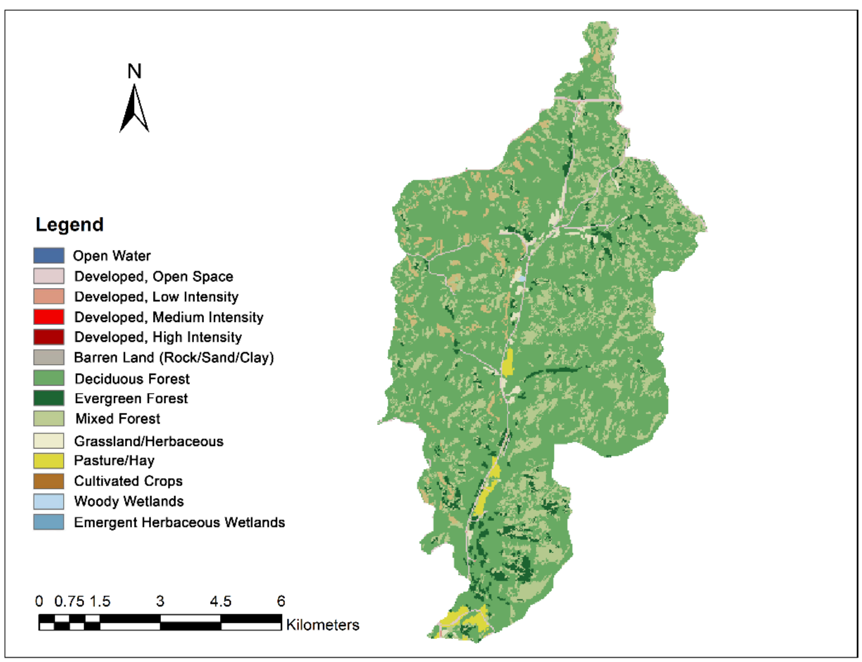

Figure 2.6. LULC for Ottery Creek

Table 2.1. LULC Percentages for Pristine Streams

		Land use Land cover %			
Stream #	Watershed	Urban	Forest	Pasture/Hay	Cultivated Crops
1	Rogers Creek	3.4	94.42	0.05	0
2	Mill Creek	3.51	91.78	2.06	0
3	Middle West Fork	3.67	85.2	2.33	0.02
4	Bee Fork	3.1	87.18	3.4	0.03
5	Ottery Creek	1.91	93.64	1.12	0

The topography of the five pristine watersheds is moderately hilly with the average slope percent of each watershed being: Rogers Creek (15.5%), Mill Creek (14.6%), Middle West Fork (18.9%), Bee Fork (15.9%), and Ottery Creek (16.8%). Most of the watersheds are covered with organic-rich soil, with Ottery Creek also having a gravely silt loam soil texture. Table 2.2 displays the breakdown of each watershed into percent sand, silt, and clay. The watersheds are majority silt, followed by sand then clay. Throughout the watersheds, the bedrock is primarily dolomite with small areas containing rhyolite and sandstone. According to Model My Watershed (Stroud Water Research Center, 2017) the average annual precipitation ranges 109.22 to 119.38 cm in all watersheds, and the greatest precipitation occurs during the months of March, April, May, and June. The average annual temperature throughout the watersheds ranges from 13°C to 14°C. Table 2.3 summarizes the precipitation and temperature data.

Table 2.2. Breakdown of Pristine Stream Soil Texture

Stream #	Watershed	% Sand	% Silt	% Clay
1	Rogers Creek	26.04	57.76	16.19
2	Mill Creek	26.92	56.13	16.94
3	Middle West Fork	26.13	56.22	17.65
4	Bee Fork	31.6	51.69	16.54
5	Ottery Creek	22.73	57.06	20.21

Table 2.3. Summary of Annual Precipitation and Temperature

Stream #	Watershed	Mean Annual Precipitation (cm)	Mean Annual Temperature (°C)
1	Rogers Creek	116.50	13.7
2	Mill Creek	116.89	13.7
3	Middle West Fork	114.09	13.1
4	Bee Fork	113.79	13.1
5	Ottery Creek	111.71	13.0

Pristine watersheds were chosen to be as similar as possible to better understand the natural variations in water quality while minimizing confounding factors. While similar sites were desired, the necessity of minimizing anthropogenic impact required some variations in watershed properties such as area. Rogers Creek, Mill Creek, and Ottery Creek are all between 11,000 and 17,000 acres while Middle West Fork and Bee Fork are slightly larger having a size of 23,000 to 25,000 acres.

2.2. URBAN STREAMS

Urban data were acquired at five streams located in two HUC 12-digit watersheds. Grand Glaize Creek Watershed is located near St. Louis, Missouri and was broken down into three sub-basins which contain Grand Glaize Creek, Glaize Creek, and Sugar Creek, (Figure 2.7). Middle Hinkson Creek Watershed is located near Columbia, Missouri, and was broken down into two sub-basins. One basin encompasses Hominy Creek, and the other includes Grindstone Creek, (Figure 2.8).

These streams were chosen for this study because they have a significantly higher percentage of urban area (Figures 2.9 – 2.13) when compared to the five pristine streams, but have fairly similar topography, soils, geology, and climate, and thus can assist in providing information about anthropogenic impacts on water quality. While each sub-basin has slightly different land uses and land cover, all are mainly urban. Table 2.4 summarizes the LULC data for the urban streams.

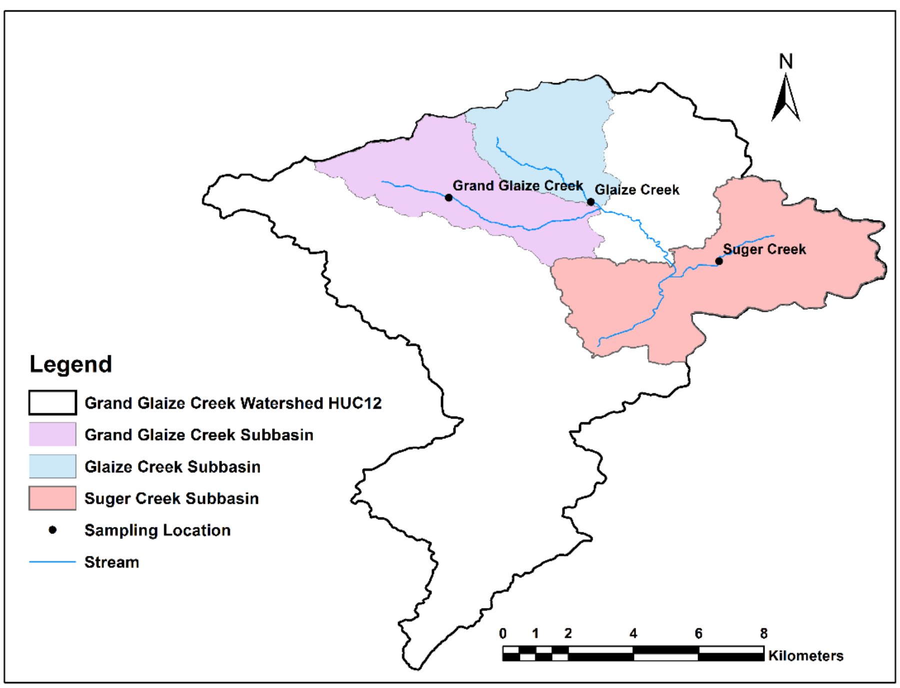

Figure 2.7. Grand Glaize Creek Watershed and Sub-basins

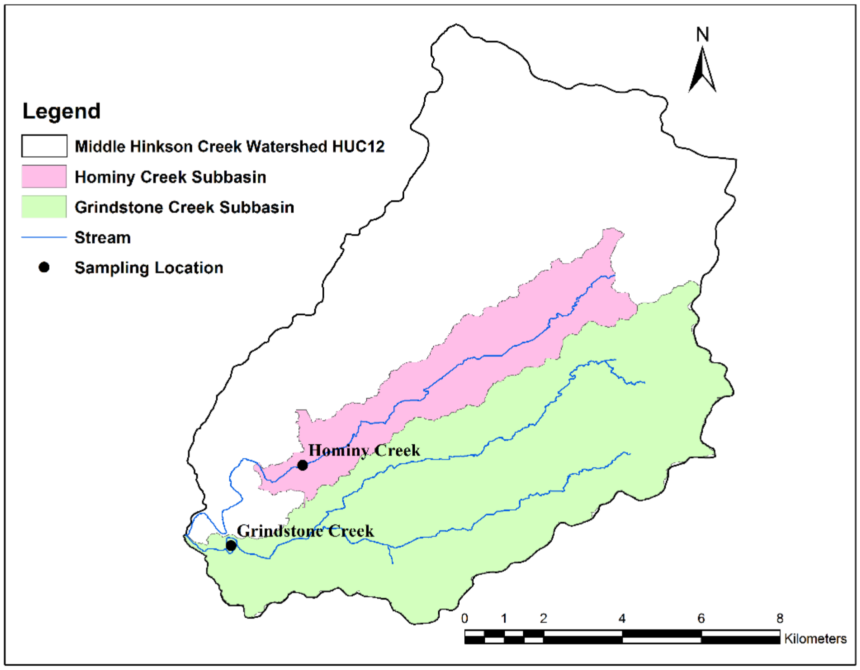

Figure 2.8. Middle Hinkson Watershed and Sub-basins

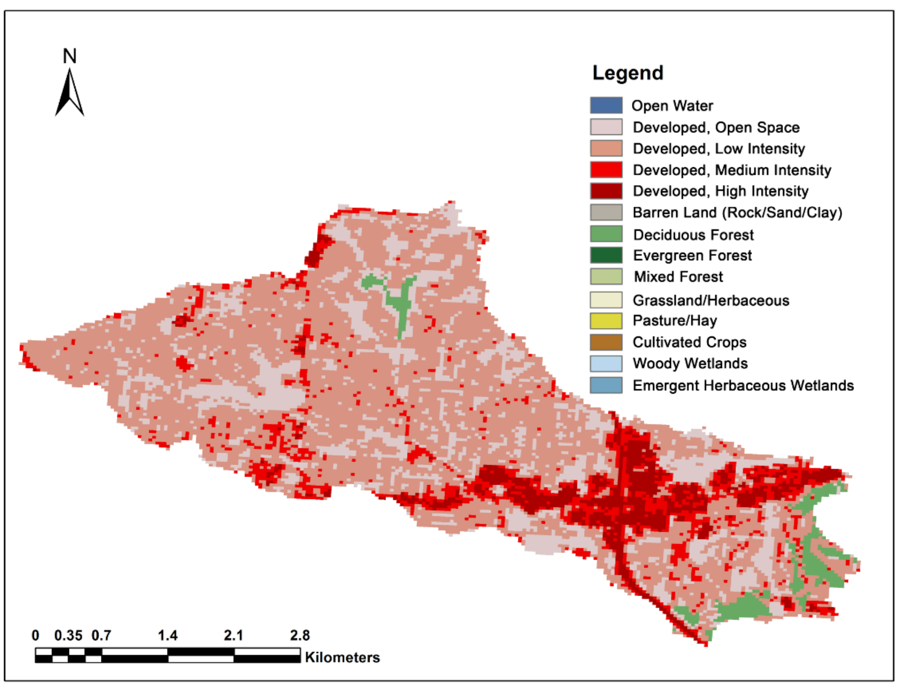

Figure 2.9. LULC for Grand Glaize Creek

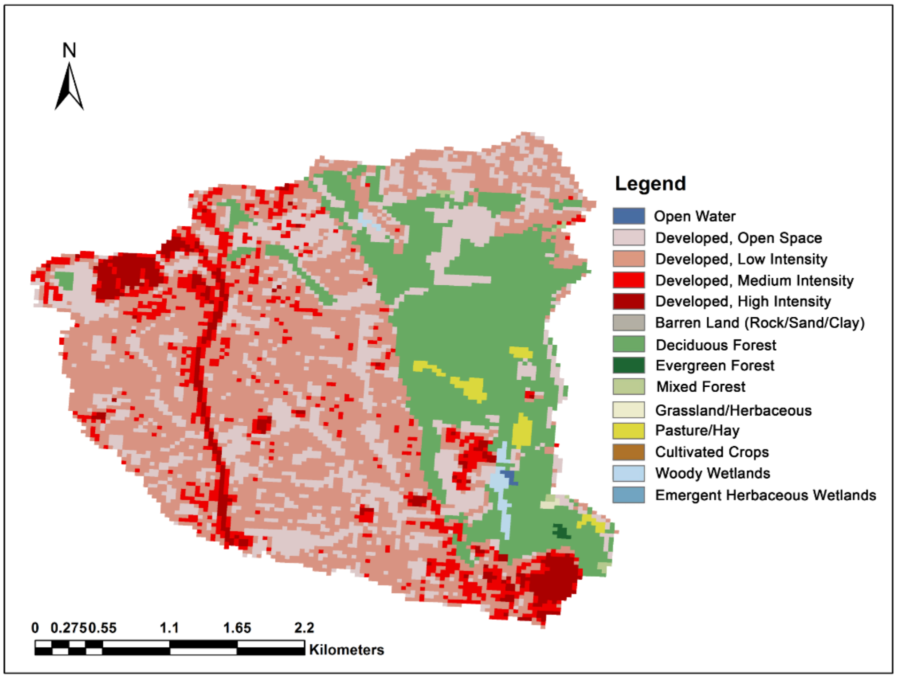

Figure 2.10. LULC for Glaize Creek

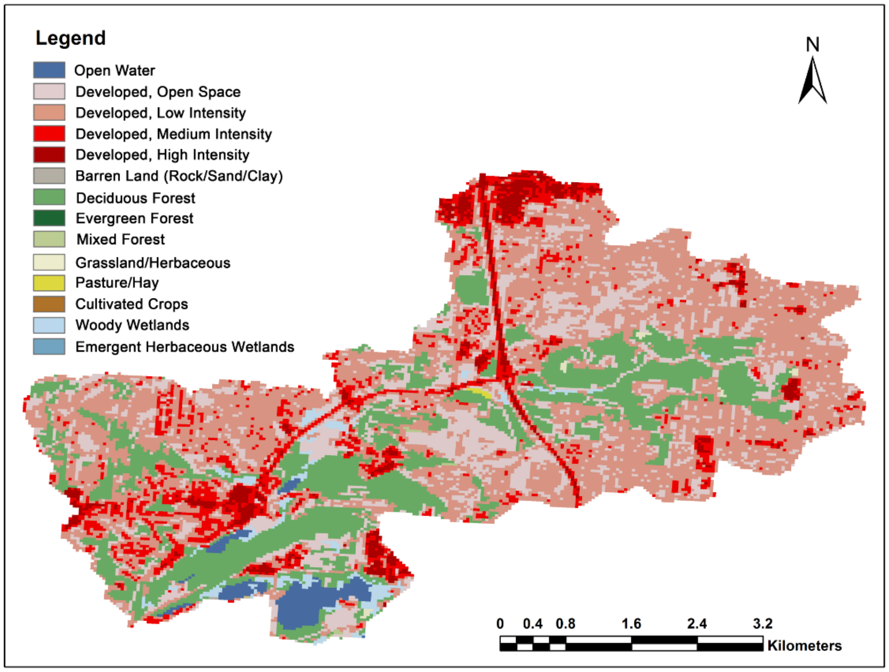

Figure 2.11. LULC for Sugar Creek

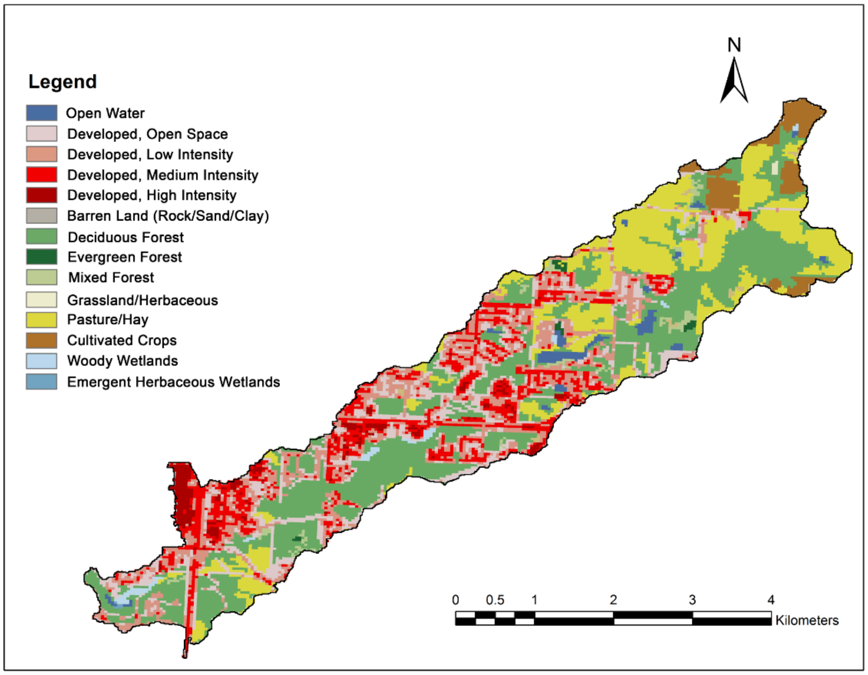

Figure 2.12. LULC for Hominy Creek

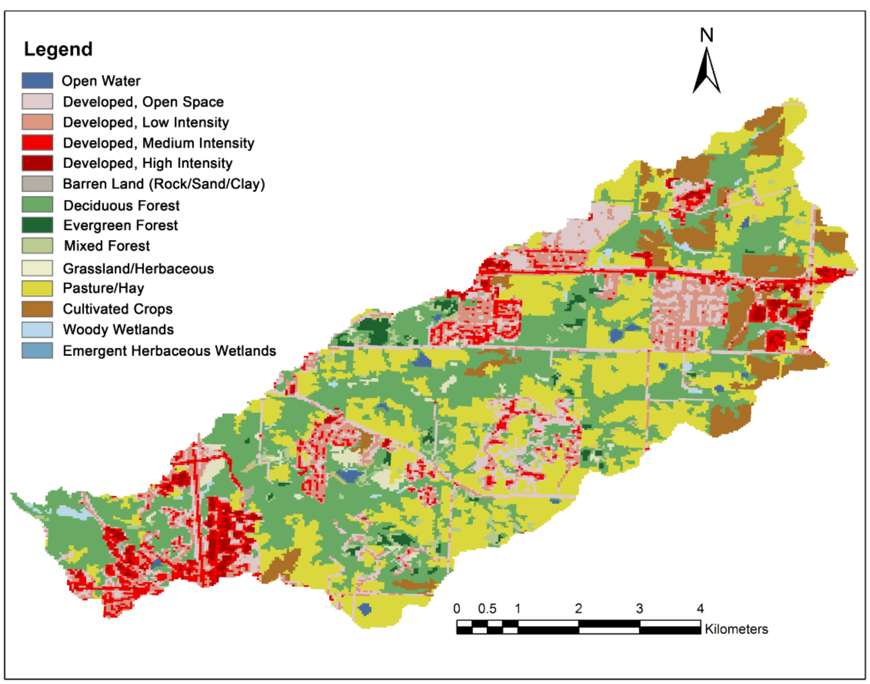

Figure 2.13. LULC for Grindstone Creek

Table 2.4. LULC Percentages for Urban Streams

		Land use Land cover %			
Stream #	Sub-basin	Urban	Forest	Pasture/Hay	Cultivated Crops
6	Grand Glaize Creek	96.99	3.01	0	0
7	Glaize Creek	74.55	23.8	0.07	0.95
8	Sugar Creek	74.94	21.15	0.15	0.06
9	Hominy Creek	46.21	30.38	0.08	20.62
10	Grindstone Creek	36.48	30.06	1.28	30.56

The topography of the five subbasins is moderately flat with the average slope percent of each watershed being: Grand Glaize Creek (8.4%), Glaize Creek (8.3%), Sugar Creek (10.8%), Hominy Creek (5.1%), and Grindstone Creek (5.0%). Grand Glaize Creek, Glaize Creek, and Sugar Creek all have a soil texture composed of clay loam and silty loam while Hominy Creek and Grindstone Creek are composed only silty loam. Table 2.5 shows the sub-basin soil texture in percent sand, silt, and clay. Like the pristine watersheds, the five urban sub-basins are also predominantly silt. However, the urban subbasins contain higher percentages of clay when compared to the pristine watersheds. The urban sub-basins also contain very small amounts of sand. The bedrock of the sub-basins is mostly limestone. Glaize Creek and Sugar Creek have small areas of shale, while Hominy Creek and Grindstone Creek are nearly half limestone and half shale. According to Model My Watershed (Stroud Water Research Center, 2017) the average annual precipitation ranges from 101.6 to 127 cm for all the urban subbasins with the greatest precipitation occurring during the months of April, May, June, and September. The average annual temperature throughout the subbasins ranges from 12.5°C to 13.5°C as seen in Table 2.6.

Table 2.5. Breakdown of Urban Stream Soil Texture

Stream #	Sub-basin	% Sand	% Silt	% Clay
6	Grand Glaize Creek	11.95	64.01	24.03
7	Glaize Creek	6.14	69.06	24.27
8	Sugar Creek	10.39	64.88	24.71
9	Hominy Creek	14.96	51.99	16.54
10	Grindstone Creek	14.06	53.67	32.23

Table 2.6. Summary of Annual Precipitation and Temperature

Stream #	Sub-basin	Mean Annual Precipitation (cm)	Mean Annual Temperature (°C)
6	Grand Glaize Creek	103.09	13.5
7	Glaize Creek	125.96	13.5
8	Sugar Creek	102.99	13.5
9	Hominy Creek	101.90	12.7
10	Grindstone Creek	101.90	12.7

3. METHODS

3.1. SITE SELECTION

3.1.1. Pristine Streams. Model My Watershed (Stroud Water Research Center, 2017) was used to select the locations of the five pristine streams for this study. USGS Subwatershed unit (HUC-12) boundaries were first overlain on the state of Missouri and then the National Land Cover Database layer was added to display the LULC for each watershed. After finding five desired watersheds with low anthropogenic and agricultural activity, Google Earth (McClendon, 2013) was used to determine where roads intersected the stream near the mouth of the watershed so that sampling sites would be accessible by vehicle.

3.1.2. Urban Streams. The Stream Team Interactive Map by the Missouri Stream Team (Stream Team Program, 2017) was used to find the five urban streams for this study. This interactive map shows sites where stream team employees and volunteers have collected water quality data which have been stored in a public database. Streams near larger urban cities were first narrowed down to those that had similar discharge values to the five pristine streams (using data which were collected in the fall of 2017). After determining that a stream had similar discharge to the pristine streams, it was next checked to ensure that the database had both water chemistry and macroinvertebrate data available for the site. Once five urban streams were chosen, Google Earth was again used to find safe and convenient sampling locations.

3.2. SAMPLE COLLECTION

Water quality measurements, including water chemistry samples and discharge values, were taken at least once per month for the five pristine streams during September 2017, October 2017, February 2018, March 2018, April 2018, May 2018, June 2018, July 2018, and August 2018. Each sampling location for the pristine streams was near the mouth of the watershed so that runoff from the entire watershed could be characterized.

Water quality data for the urban streams were collected during October 2018 by two Missouri S&T field teams. The Stream Team Interactive Map database (Stream Team Program, 2017) was used to acquire the past stream monitoring data at the five urban streams to be used in conjunction with the more recently collected data.

3.2.1. Water Chemistry Samples. At each site the following water chemistry parameters were measured: temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), turbidity, nitrate, phosphate, and chloride. Chloride was not measured in October 2017 and November 2017 at the five pristine streams since the necessary equipment was not available at the time of sampling. During each sampling campaign, temperature, pH, EC, DO, and chloride were measured in-situ with a YSI PRO multimeter probe. Turbidity was measured in the field using a Hach 2100Q Portable Turbidimeter. The sample tube was filled with water to the line, sealed, then wiped down to remove any fingerprints and dirt before being analyzed. Bacteria samples for *E. coli* and other coliforms were collected in Whirl-Pak® bags and stored on ice for later lab analysis. Water samples were collected in polyethylene bottles and also stored on ice for later lab analysis of nitrate and phosphate concentrations.

Samples for bacteria testing were analyzed using Coliscan® Easygel® agar and petri dishes. Samples were incubated at 35°C for 24-36 hours. After the incubation period, E. coli concentrations were counted and recorded. Nitrate and phosphate concentrations were analyzed using a Hach DR 3900 Spectrophotometer based on the manufacture's recommendation. Nitrate concentrations were evaluated using the chromotropic acid method (Hach Method 10020) while phosphate concentrations were evaluated using the ascorbic acid method (Hach Standard Procedure 8048).

3.2.2. Discharge. Discharge was determined using the USGS Pygmy Current Meter Model 6205 (Rickly Hydrological Co., 2019) when possible; streams with too low of a flow were measured using a floating object test (Intermountain Environmental, Inc., 2015). Each stream's width and depth were measured using a tape measure and a depth-rod. A minimum of 20 depth measurements were taken at each stream, with larger stream channels having additional measurements. When the floating object test was implemented, the floating object was dropped in three different locations across the channel and the measurements were averaged to improve accuracy.

3.2.3. Benthic Macroinvertebrates. Benthic macroinvertebrates were collected at the five pristine streams during October 2017, April 2018, and October 2018 and at the urban streams during October 2018. The rest of the macroinvertebrate data for the urban sites were acquired from the Missouri Stream Team database. Invertebrates were collected with a 1000-micron kick net at each site. The net was placed downstream of a riffle zone and weighed down with large rocks. A 3-foot by 3-foot area immediately upstream of the net was disturbed by stirring up the bottom of the streambed. Once the area was sufficiently agitated, the net was carefully lifted out of the water to avoid loss of water over the sides. The net was then moved to dry land where the benthic macroinvertebrates were picked off for approximately 20 to 40 minutes by the field team. All invertebrates were identified in the field rather than being preserved and taken back to the lab for future identification. After being identified in the field, the invertebrates were recorded then released back into the stream. This process was performed at each site three times consecutively for each sampling campaign. Macroinvertebrates were sampled moving from downstream to upstream to assure that the population had been accurately assessed, but not over sampled (Sarver, 2018).

3.2.3.1. Water quality rating. The water quality rating (WQR) was designed as a way to assign a numerical weight to the benthic macroinvertebrate data, which is qualitative. The water quality rating for all pristine and urban streams was calculated using the method used by the Missouri Department of Natural Resources (Sarver, 2018), where a higher diversity of invertebrates results in a higher water quality rating. Invertebrates are categorized into three groups which include: pollution sensitive, somewhat pollution tolerant, and pollution tolerant. Pollution sensitive invertebrates are extremely sensitive to pollution; therefore, it is typical to only find these organisms in streams with excellent water quality. Somewhat pollution tolerant invertebrates are more common and can survive in streams which are moderately polluted. Pollution tolerant invertebrates are the only organisms that can be found in all water systems, healthy or impaired. If a certain taxon is present in any of the three net sets, its value is added to the overall water quality rating, as seen in Equation (1).

$$WQR = 3x + 2y + 1z \tag{1}$$

In the equation, WQR is the water quality rating, x is the number of sensitive taxa present, y is the number of somewhat tolerant taxa present, and z is the number of tolerant taxa present. The water quality rating is reflective of the presence or absence of certain species, not the total number of organisms collected in the net. Table 3.1. displays the description of each water quality rating for Missouri streams from 0-23.

Table 3.1. Water Quality Rating for Missouri Streams

Water Quality Rating	
>23	Excellent
18-23	Good
12-17	Fair
<12	Poor

3.2.3.2. Percent EPT. EPT taxa richness is the total number of distinct taxa within the **E**phemeroptera (mayflies), **P**lecoptera (stoneflies), and **T**richoptera (caddisflies) orders, which are pollution sensitive invertebrates. These macroinvertebrate orders are generally intolerant to higher levels of pollution in streams and therefore, EPT taxa richness will increase with increasing stream health (Weber, 1973). The total number of EPT organisms divided by the total number of organisms in a sample is known as the percent EPT (Table 3.2).

Table 3.2. Percent EPT

EPT%	
>50%	Good
50% - 25%	Moderate
<25%	Poor

3.2.3.3. The biotic index. The Hilsenhoff Biotic Index (HFBI) was calculated (Equation 2) based off Hilsenhoff's Family Biotic Index (Hilsenhoff, 1987).

$$BI = \sum_{i=1}^{S} \frac{TV_i \, Ni_i}{N_t} \tag{2}$$

where S is the number of taxa in the sample, TV_i is the pollution tolerance value of the ith species taxon, and N_t is the total number of macroinvertebrates in the sample (Lenat, 1993). This method for determining the biotic index was modified from Hilsenhoff's Biotic Index (Hilsenhoff, 1977) and focused on the family-tolerant values rather than individual taxa. Tolerance values developed by Missouri Department of Natural Resources (Sarver, 2005) were used in this study and range from 0 (very intolerant) to 10 (very tolerant) with higher values indicating increased tolerance to pollution. These tolerance values were used in Hilsenhoff's method to calculate the biotic index for this study. The HFBI is scored from 0 to 10 (Table 3.3), with 0 indicating excellent water quality and 10 indicating extremely poor water quality (Hilsenhoff, 1988).

Table 3.3. Biotic Index and Pollution Levels

Biotic Index	Water Quality	Organic Pollution
<3.75	Excellent	Pollution Unlikely
3.76 - 4.25	Very Good	Possible Slight Pollution
4.26 - 5.00	Good	Some Pollution Probable
5.01 - 5.75	Fair	Fairly Substantial Pollution
5.76 - 6.50	Fairly Poor	Substantial Pollution Likely
6.51 - 7.25	Poor	Very Substantial Pollution
7.26 - 10.00	Very Poor	Severe Pollution Likely

3.2.4. Statistical Analyses. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software. Water quality data were first tested to determine if they were normally distributed. By examining the skewness, kurtosis z-values, and the Shapiro-Wilk test p-values, it was concluded that not all water quality parameters were distributed normally. Consideration was given to transforming the data to achieve normal distributions; however, even if the data were normally distributed, they would still have had unequal sample sizes and would have violated normal ANOVA assumptions. Instead, non-parametric ANOVA methods were used.

4. RESULTS AND DISCUSSION

4.1. PRISTINE WATERSHEDS

4.1.1. Boxplots. A boxplot was made in SPSS for each water quality parameter (Figures 4.1 to 4.10) to visually compare the statistical distributions across the five pristine streams. The pristine streams numbers are labelled as: Rogers Creek (1), Mill Creek (2), Middle West Fork – Black River (3), Bee Fork (4), and Ottery Creek (5).

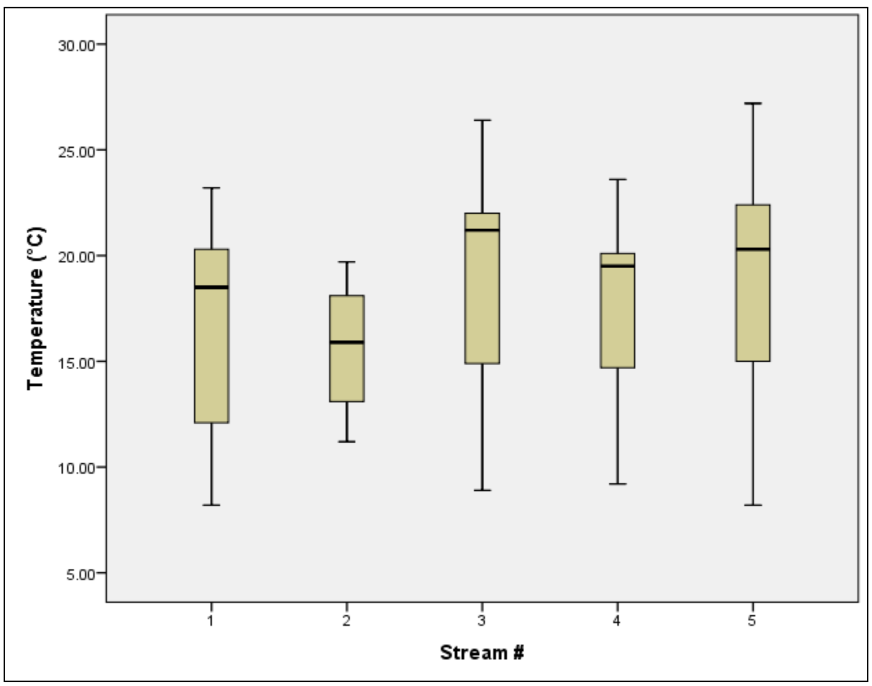

Figure 4.1. Boxplot Displaying Pristine Stream Temperature Variation

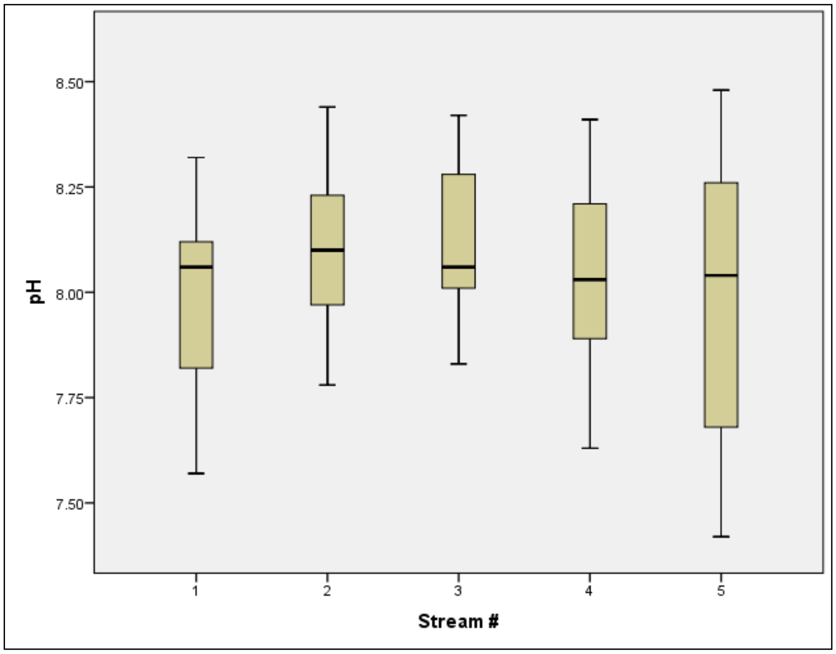

Figure 4.2. Boxplot Displaying Pristine Stream pH Variation

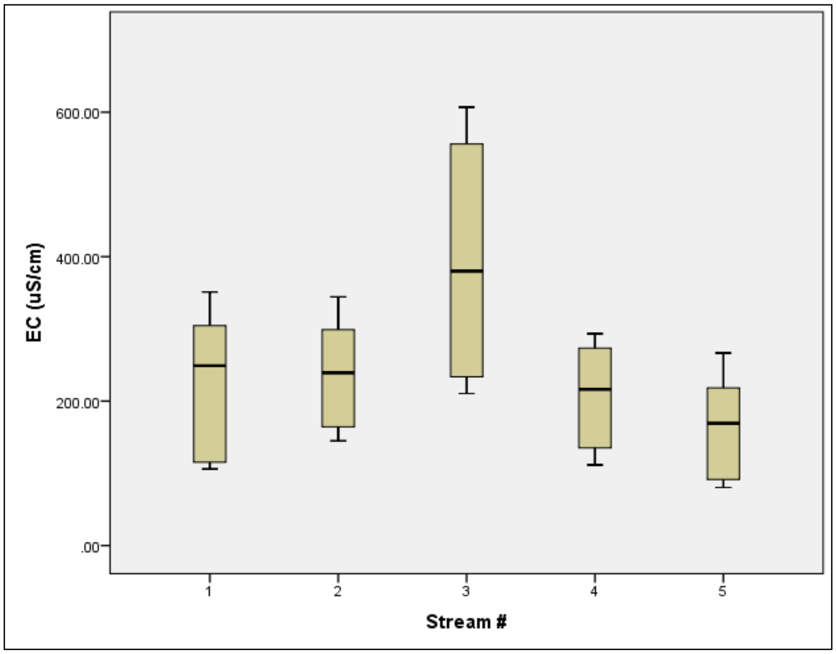

Figure 4.3. Boxplot Displaying Pristine Stream EC Variation

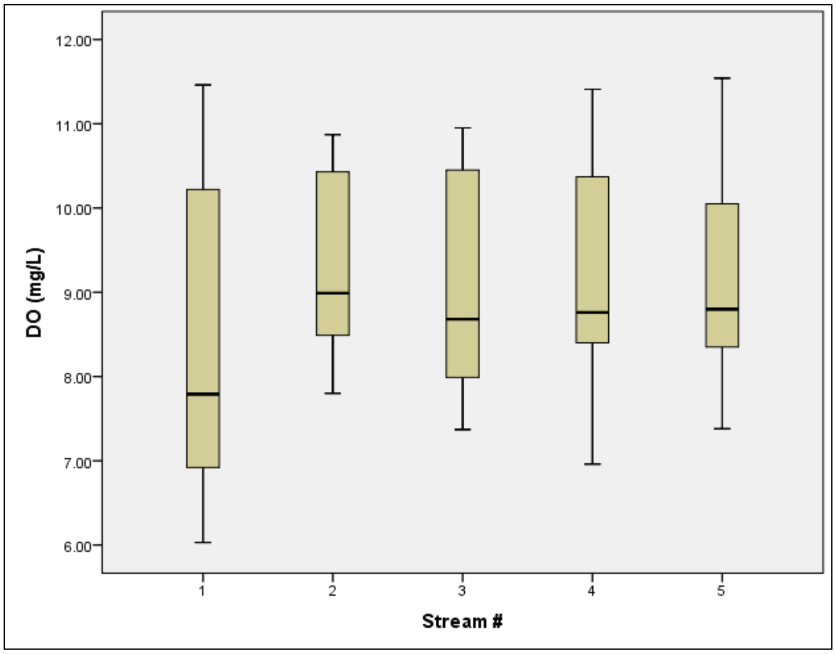

Figure 4.4. Boxplot Displaying Pristine Stream DO Variation

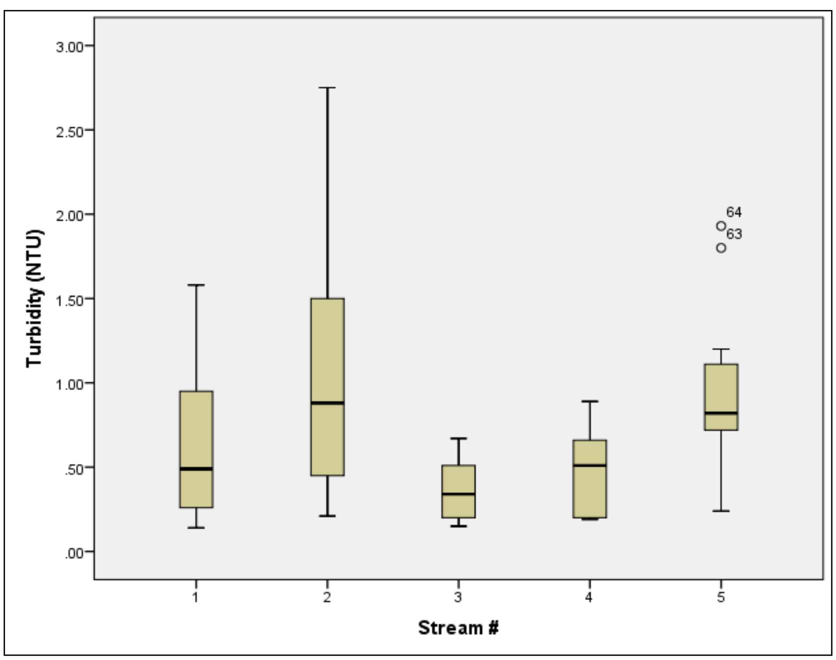

Figure 4.5. Boxplot Displaying Pristine Stream Turbidity Variation

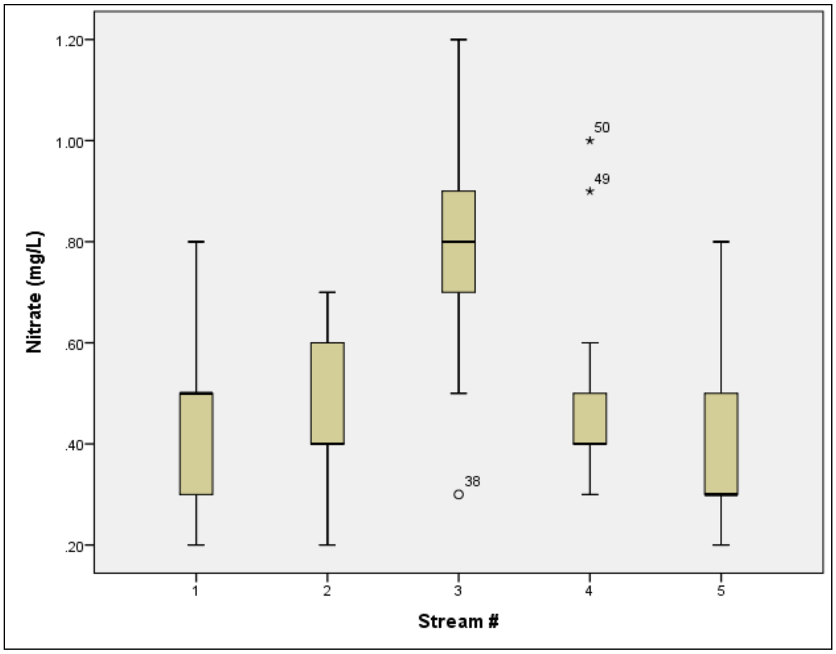

Figure 4.6. Boxplot Displaying Pristine Stream Nitrate Variation

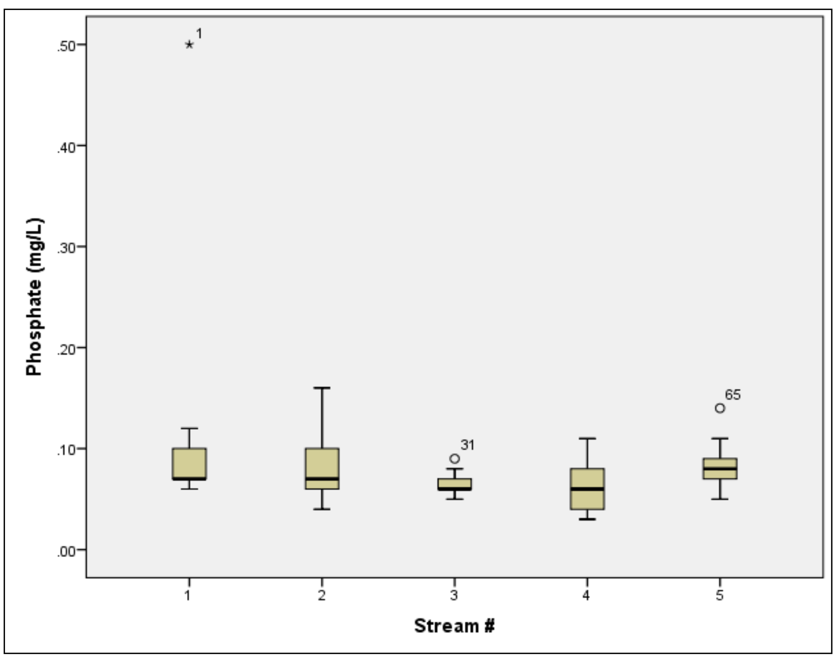

Figure 4.7. Boxplot Displaying Pristine Stream Phosphate Variation

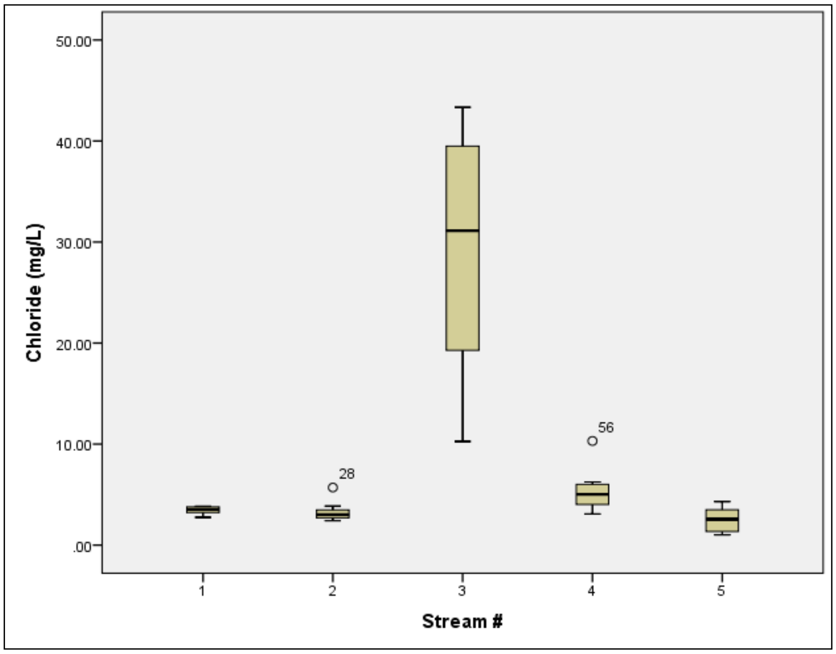

Figure 4.8. Boxplot Displaying Pristine Stream Chloride Variation

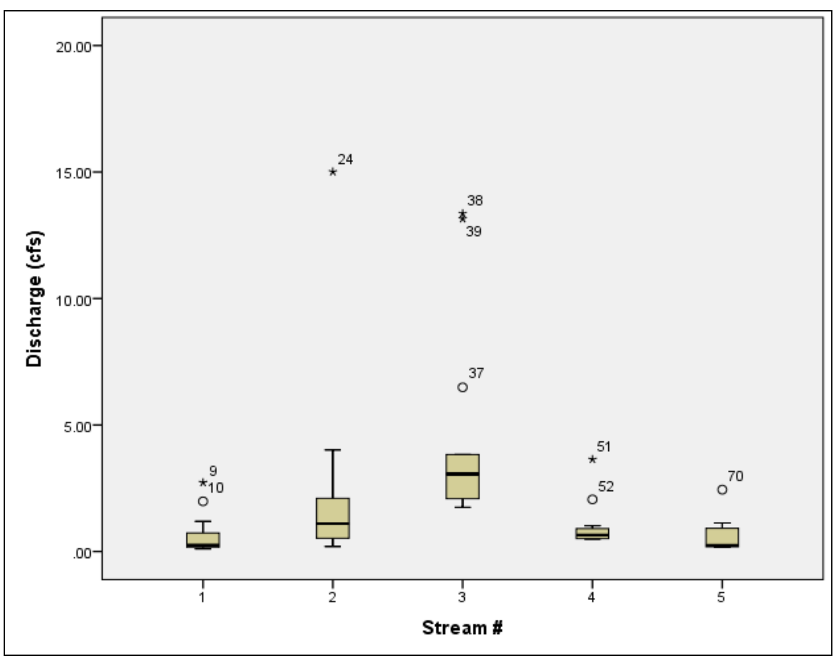

Figure 4.9. Boxplot Displaying Pristine Stream Discharge Variation

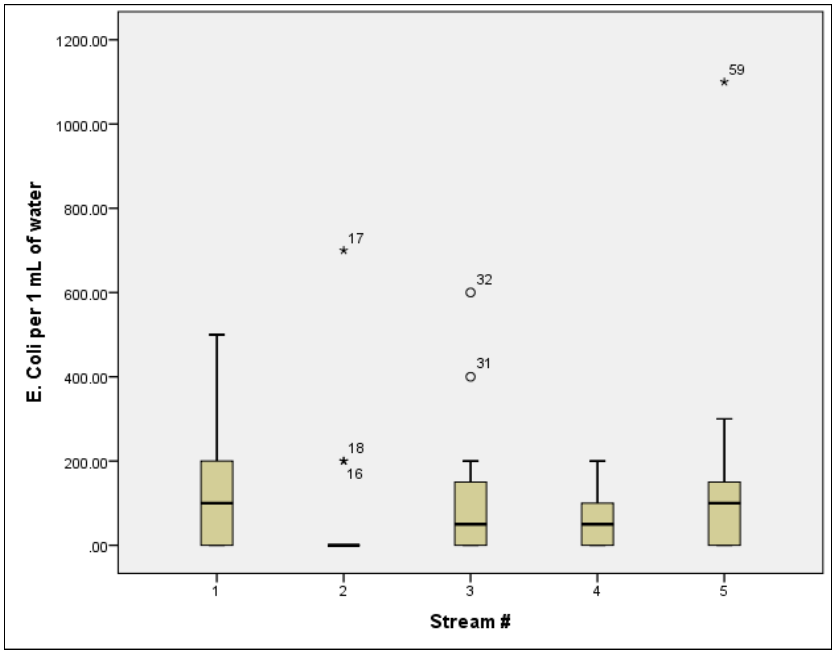

Figure 4.10. Boxplot Displaying Pristine Stream *E.coli* Variation

The temperature plot shows only small differences between the five pristine streams. The medians and variability are approximately the same for all streams, although Stream 2 has a lower average temperature and slightly lower variability. Medians are also similar for pH and are nearly constant. Stream 5 has great variability in pH, but the median value is still similar to that of the other pristine streams. DO is relatively similar between Streams 2,3,4, and 5 while Stream 1 has a significantly lower median and also has higher variability. It is typical to see lower DO values in warmer water, and Stream 1 is one of the streams with overall higher water temperature. Stream 3 displays large differences compared to all other streams for EC, nitrate, and chloride. It exceeds the other streams in terms of median values as well as variability for these water chemistry parameters. Increased anthropogenic activity near Stream 3 could account for the increase in EC, nitrate, and chloride. Streams 2 and 5 show noteworthy spikes in turbidity and have medians slightly above the remaining streams. Stream 2 additionally displays variability much greater than the rest of the streams when it comes to turbidity. Phosphate has minimal differences between the streams with nearly identical medians. Overall values of phosphate across all streams are extremely low. While Stream 3 stands out amongst the other streams with respect to chloride values, the remaining streams all have similar medians with low variability. Streams 2 and 3 display large spikes on the discharge plots that are most likely a result of flooding during April 2017 and May 2017. Even with these spikes, the average discharge of all of the streams is low, within 5 cubic feet per second.

4.1.2. Correlation Matrix A correlation matrix was calculated to determine if any of the pristine stream water quality parameters were significantly related. Table 4.1 shows the correlations that are statistically significant at alpha = 0.05 and alpha = 0.01 for a two-tailed test. While the matrix appears to show numerous correlations between the water chemistry indicators, many of them have R^2 values that are quite low, and therefore do not actually represent a strong correlation. This can be observed for the following: temperature and pH, temperature and turbidity, turbidity and chloride, and nitrate and phosphate. A positive correlation was observed between temperature and EC. This correlation is expected since warmer streams tend to have higher amounts of dissolved inorganic material in them. Another expected correlation is seen between temperature and DO. DO is known to be temperature dependent, so this negative correlation means that higher DO is seen in colder waters. Another correlation is observed between EC and DO. This relationship is most likely caused by temperature, which affects both EC and DO as stated above. As temperature decreases, EC values decrease in streams, while DO values increase. This could explain the negative correlation between EC and DO at the pristine watersheds, since truly causative factors for this relationship seem unlikely. A positive correlation between DO and discharge is also present as well as expected. Faster moving water tends to have higher DO values, whereas more stagnant water will have lower values. A weak correlation is seen between DO and turbidity that is probably a function of discharge. Since both DO and turbidity tend to increase with increasing discharge, the correlation between DO and turbidity probably reflects this sensitivity to discharge rather than being a truly causative relationship. A negative correlation is noted between EC and turbidity. Higher turbidity will reflect more surface runoff, while EC values are usually impacted by the dissolved calcium and magnesium ions in the groundwater. Groundwater EC will usually be higher than surface water EC. Therefore, when more of the discharge comes from surface water, turbidity is higher. When there is more groundwater, there will be higher EC. The inverse relationship between EC ad turbidity probably reflects the higher EC observed during lower discharge and the higher turbidity observed during higher discharge. Nitrate and chloride have an unexpected positive correlation that might be explained as the result of human impacts. Greater human activity would lead to increased values of both nitrate and chloride. *E. coli* shows a positive correlation with EC and phosphate as well as a negative correlation with DO. The correlation between *E. coli* and phosphate is mostly likely also a result of human impact. Areas of greater human activity will tend to see increased phosphate values and higher *E. coli* counts in streams. The correlation between E. coli and DO is expected because, generally, streams with higher E. coli counts will have less dissolved oxygen.

Table 4.1. Correlation Matrix for Pristine Stream Water Quality Parameters

		Temp	pH	EC	DO	Turbidity	E. Coli	Nitrate	Phosphate	Chloride	Discharge
Temperature (°C)	Pearson Correlation	1	.393**	.462**	-.764**	-.448**	.182	-.149	.123	.130	-.105
	Sig. (2-tailed)		.001	.000	.000	.000	.156	.236	.328	.423	.404
pH	Pearson Correlation	.393**	1	.071	-.069	-.087	-.143	.026	-.056	.247	.138
	Sig. (2-tailed)	.001		.573	.584	.491	.267	.836	.659	.124	.271
EC (uS/cm)	Pearson Correlation	.462**	.071	1	-.542**	-.504**	.287*	.225	.035	.703**	-.039
	Sig. (2-tailed)	.000	.573		.000	.000	.024	.071	.781	.000	.759
DO (mg/L)	Pearson Correlation	-.764**	-.069	-.542**	1	.564**	-.319*	.243	-.072	-.029	.278*
	Sig. (2-tailed)	.000	.584	.000		.000	.012	.051	.569	.857	.025
Turbidity (NTU)	Pearson Correlation	-.448**	-.087	-.504**	.564**	1	-.171	-.030	-.003	-.320*	.228
	Sig. (2-tailed)	.000	.491	.000	.000		.184	.812	.983	.044	.068
E. Coli per 1 mL water	Pearson Correlation	.182	-.143	.287*	-.319*	-.171	1	-.152	.278*	.201	-.210
	Sig. (2-tailed)	.156	.267	.024	.012	.184		.240	.029	.213	.102
Nitrate (mg/L)	Pearson Correlation	-.149	.026	.225	.243	-.030	-.152	1	-.265*	.550**	.169
	Sig. (2-tailed)	.236	.836	.071	.051	.812	.240		.033	.000	.178
Phosphate (mg/L)	Pearson Correlation	.123	-.056	.035	-.072	-.003	.278*	-.265*	1	-.199	-.078
	Sig. (2-tailed)	.328	.659	.781	.569	.983	.029	.033		.217	.539
Chloride (mg/L)	Pearson Correlation	.130	.247	.703**	-.029	-.320*	.201	.550**	-.199	1	.230
	Sig. (2-tailed)	.423	.124	.000	.857	.044	.213	.000	.217		.154
Discharge (cfs)	Pearson Correlation	-.105	.138	-.039	.278*	.228	-.210	.169	-.078	.230	1
	Sig. (2-tailed)	.404	.271	.759	.025	.068	.102	.178	.539	.154	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4.2. URBAN STREAMS

4.2.1. Correlation Matrix. A correlation matrix was created to determine if any of the urban stream water quality parameters were significantly related. Table 4.2 shows the correlations that are statistically significant at alpha = 0.05 and alpha = 0.01 for a two-tailed test. A negative correlation is observed between temperature and EC. This is expected since EC is temperature dependent and warmer streams tend to have higher levels of EC. Temperature and DO also display an expected negative correlation since DO is another parameter which is somewhat temperature dependent. Generally, colder streams will have higher DO than warmer streams. A negative correlation is present between temperature and chloride which can be explained by the fact that in winter (when streams tend to be colder) more salt is put on the roads. Therefore, during winter months, runoff will have greater amounts of chloride entering streams. A positive correlation is observed between pH and EC that could potentially be explained by pH being greatly controlled by rock chemistry. The more calcium and magnesium dissolved, the higher the pH will be, leading also to higher EC. The correlation between pH and chloride has an R^2 value that is so low the correlation is assumed to be spurious. This is also an explanation for the low R^2 value between DO and nitrate. A negative correlation is observed between EC and turbidity, which is probably a function of discharge, as explained in the pristine stream section above. The last correlation seen is a positive one between EC and chloride which is anticipated since greater salt in streams will also result in higher EC values.

Table 4.2. Correlation Matrix for Urban Stream Water Quality Parameters

		Temp	pH	EC	DO	Turbidity	Nitrate	Phosphate	Chloride	Discharge
Temperature (°C)	Pearson Correlation	1	-.038	-.404**	-.208*	.168	.092	-.099	-.305**	-.062
	Sig. (2-tailed)		.678	.000	.023	.087	.327	.542	.005	.654
pH	Pearson Correlation	-.038	1	.308**	-.032	-.072	-.098	-.064	.285**	-.070
	Sig. (2-tailed)	.678		.001	.730	.473	.302	.699	.009	.617
EC (uS/cm)	Pearson Correlation	-.404**	.308**	1	.106	-.273**	-.042	.063	.619**	-.257
	Sig. (2-tailed)	.000	.001		.254	.005	.659	.701	.000	.061
DO (mg/L)	Pearson Correlation	-.208*	-.032	.106	1	.083	.256**	.093	.079	.161
	Sig. (2-tailed)	.023	.730	.254		.408	.007	.572	.487	.240
Turbidity (NTU)	Pearson Correlation	.168	-.072	-.273**	.083	1	.003	.234	-.228	.350*
	Sig. (2-tailed)	.087	.473	.005	.408		.978	.177	.055	.014
Nitrate (mg/L)	Pearson Correlation	.092	-.098	-.042	.256**	.003	1	-.050	.127	-.166
	Sig. (2-tailed)	.327	.302	.659	.007	.978		.764	.270	.235
Phosphate (mg/L)	Pearson Correlation	-.099	-.064	.063	.093	.234	-.050	1	.129	.206
	Sig. (2-tailed)	.542	.699	.701	.572	.177	.764		.489	.427
Chloride (mg/L)	Pearson Correlation	-.305**	.285**	.619**	.079	-.228	.127	.129	1	-.176
	Sig. (2-tailed)	.005	.009	.000	.487	.055	.270	.489		.292
Discharge (cfs)	Pearson Correlation	-.062	-.070	-.257	.161	.350*	-.166	.206	-.176	1
	Sig. (2-tailed)	.654	.617	.061	.240	.014	.235	.427	.292	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4.3. COMBINED WATERSHEDS

4.3.1. Summary Statistics. Table 4.2 displays the summary statistics for the water quality parameters for both pristine and urban streams. When focusing only on the pristine streams, little variation is seen between streams for pH, turbidity, nitrate, and phosphate measurements. Urban stream analysis shows little difference between streams for pH, nitrate, and phosphate values. Table 4.2. also allows comparisons to be made between pristine and urban stream data. The largest variations between stream types were seen in EC, turbidity, and chloride measurements. The urban steams have a larger quantity of inorganic dissolved material as well as suspended solids, which results in larger ranges of EC and turbidity values. High turbidity values could be explained from the possibility of the urban streams having a faster runoff velocity over land, meaning that more sediment is eroded and runs off into the streams. The difference in chloride concentrations can be attributed to urban pollution and most likely a greater amount of runoff from road salting at the urban streams. EC, turbidity, and chloride are all heavily influenced by LULC at each stream while pH and DO are not. While it seems unexpected to see higher values of DO in the urban streams, it is important to notice that on average the urban streams are colder and that higher DO values are typical in cooler water. It is also important to note that both pristine and urban streams have low concentrations of nitrate and phosphate due to a lack of agricultural activity throughout the streams.

Table 4.3. Summary Statistics Comparing Water Quality Parameters

Variable	Pristine Streams					Urban Streams				
	N	Minimum	Maximum	Mean	Std. Deviation	N	Minimum	Maximum	Mean	Std. Deviation
Temperature (°C)	65	8.2	27.2	17.448	4.9375	127	0.00	30.00	13.4587	7.42576
pH	65	7.42	8.48	8.0526	.24412	122	7.20	11.00	8.1815	.56083
EC (uS/cm)	65	80.4	607.0	246.185	125.4501	126	274.1	2000.0	971.291	425.1215
DO (mg/L)	65	6.03	11.54	9.0018	1.38332	120	2.00	19.00	9.4248	3.35123
Turbidity (NTU)	65	.14	2.75	.6877	.54214	107	0.00	160.00	18.3979	28.16936
Nitrate (mg/L)	65	.2	1.2	.511	.2278	115	0.000	1.500	.30422	.258510
Phosphate (mg/L)	65	.03	.50	.0820	.05861	40	.09	1.84	.4145	.32490
Chloride (mg/L)	40	1.01	43.34	8.7850	11.64775	84	2.00	891.00	152.5281	132.92547
Discharge (cfs)	65	.101	15.010	1.83908	2.940318	56	0.00	8.60	1.2504	1.90473

4.3.2. Boxplots. A boxplot was made for each water quality parameter (Figures 4.11 to 4.19) to visually compare statistical distribution across the five pristine streams and the five urban streams. To generate the boxplots, the data for the pristine and urban streams were first grouped into seasons. Seasons were classified as: Fall (September and October), Winter (February), Spring (March, April, and May), and Summer (June, July, and August). Sampling was not done in November, December, and January at the pristine streams, so therefore these months are absent. At each stream, pristine and urban, the average measurement for each water quality parameter in each season (when more than one data campaign was conducted per season) was calculated and then the "average" measurements for each of the five streams were plotted. The x-axis displays the season and stream type. For example, Fall-P displays the fall data from pristine streams while Fall-U displays fall data from the urban streams.

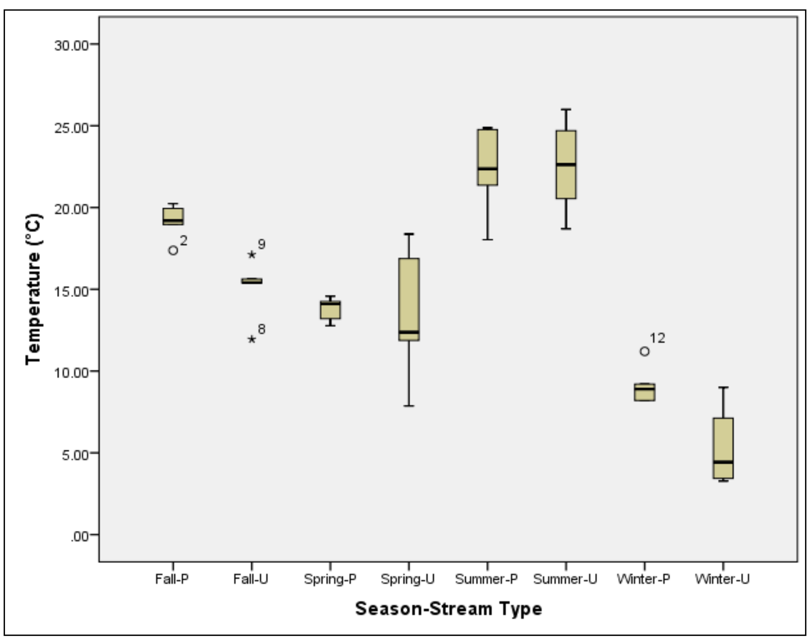

Figure 4.11. Boxplot Displaying Temperature Variation

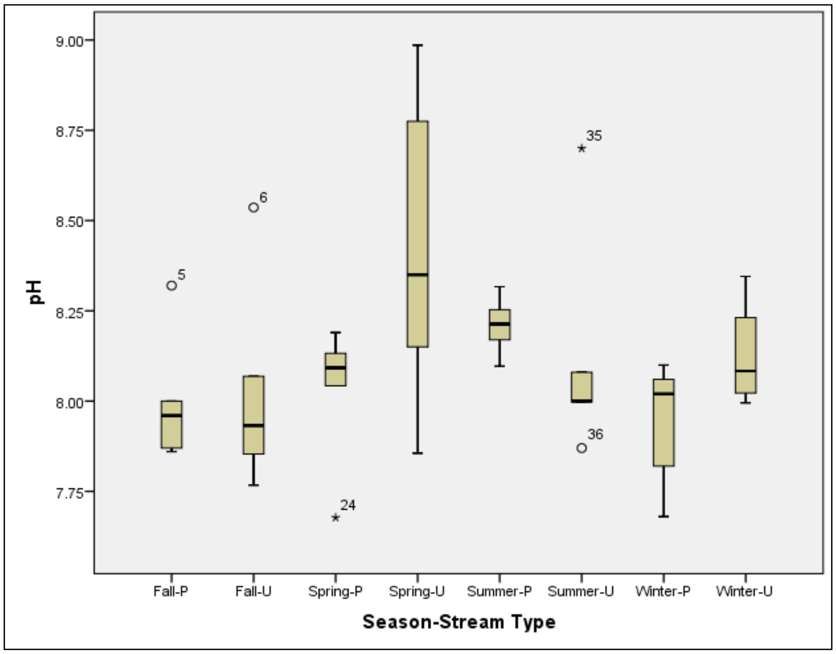

Figure 4.12. Boxplot Displaying pH Variation

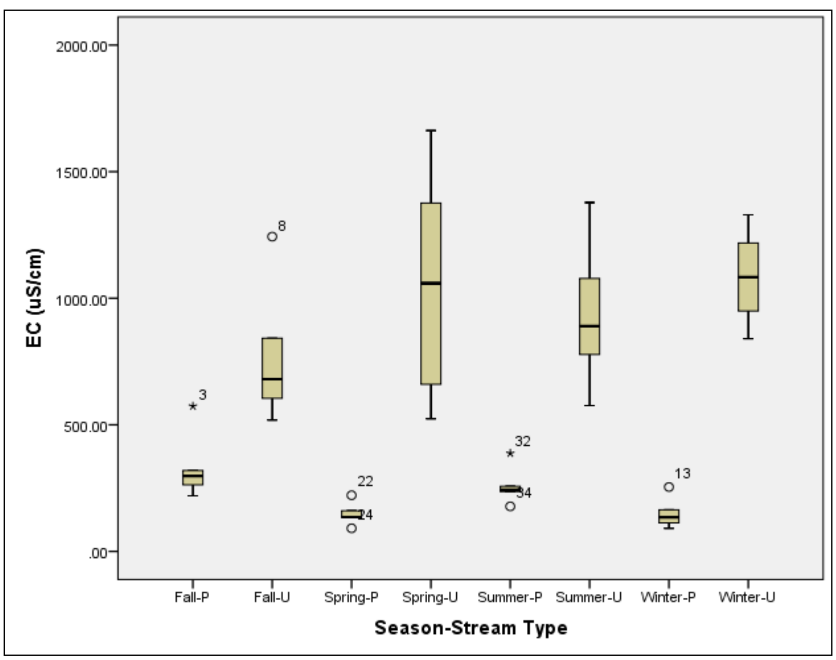

Figure 4.13. Boxplot Displaying EC Variation

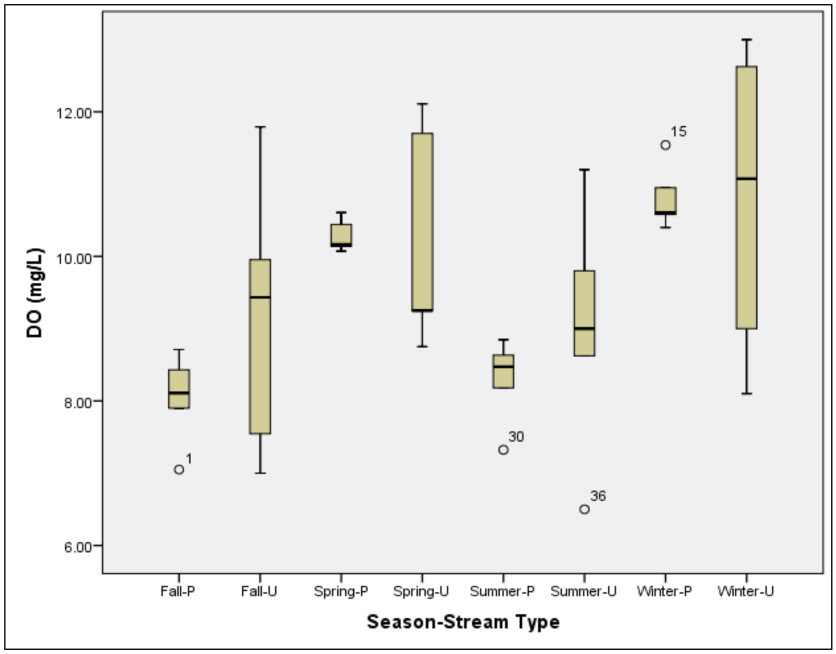

Figure 4.14. Boxplot Displaying DO Variation

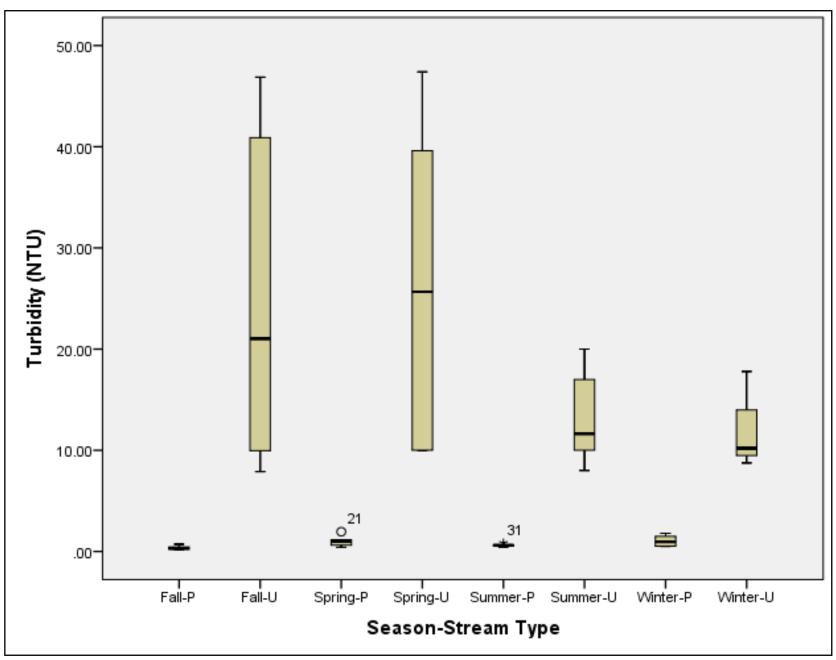

Figure 4.15. Boxplot Displaying Turbidity Variation

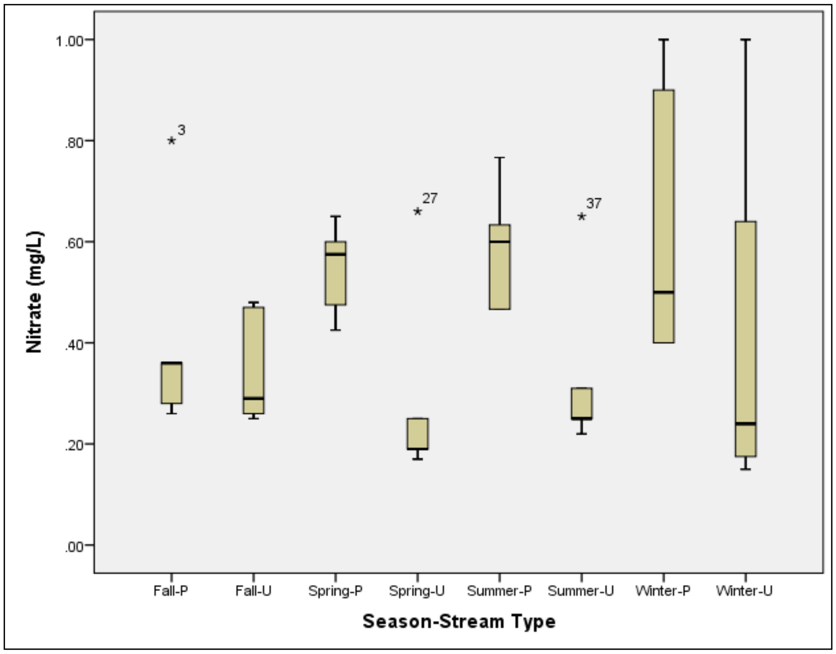

Figure 4.16. Boxplot Displaying Nitrate Variation

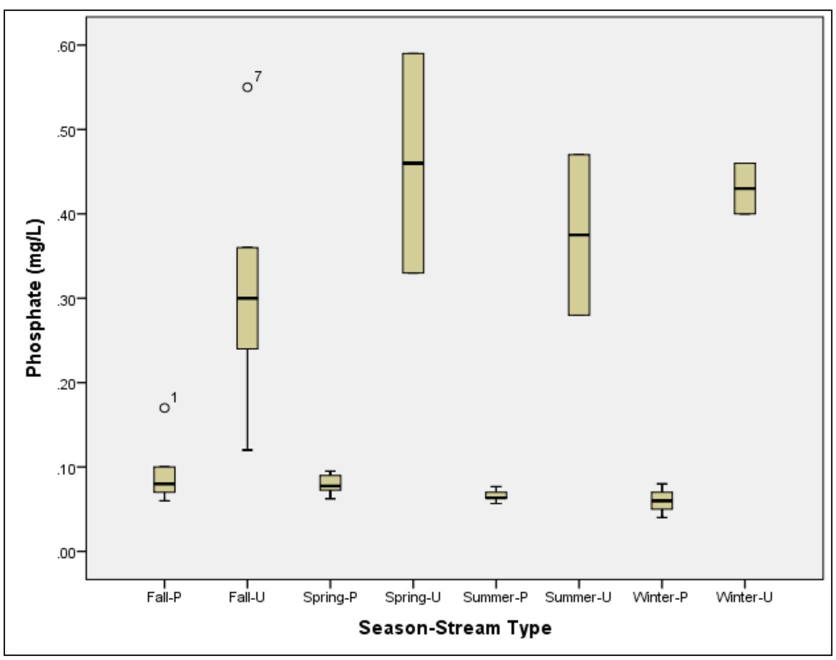

Figure 4.17. Boxplot Displaying Phosphate Variation

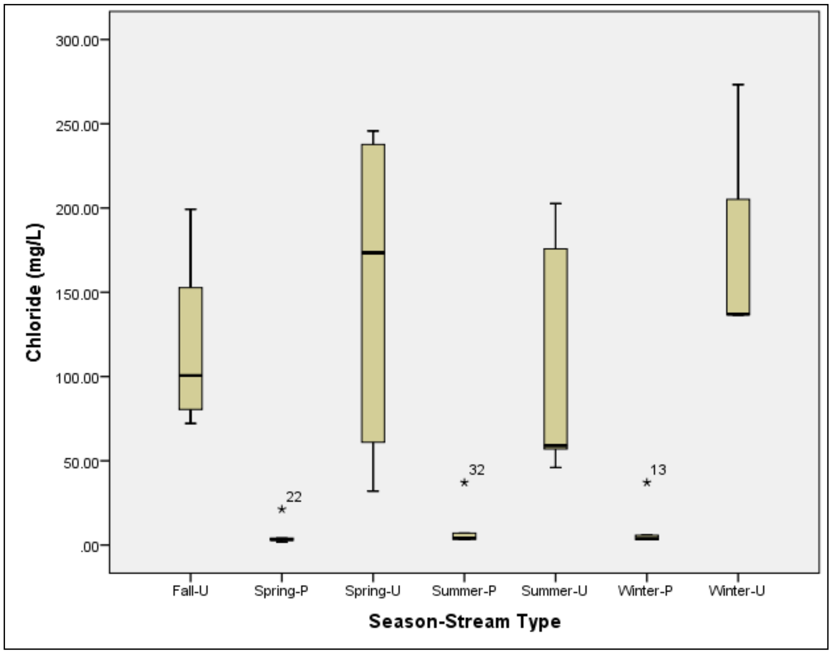

Figure 4.18. Boxplot Displaying Chloride Variation

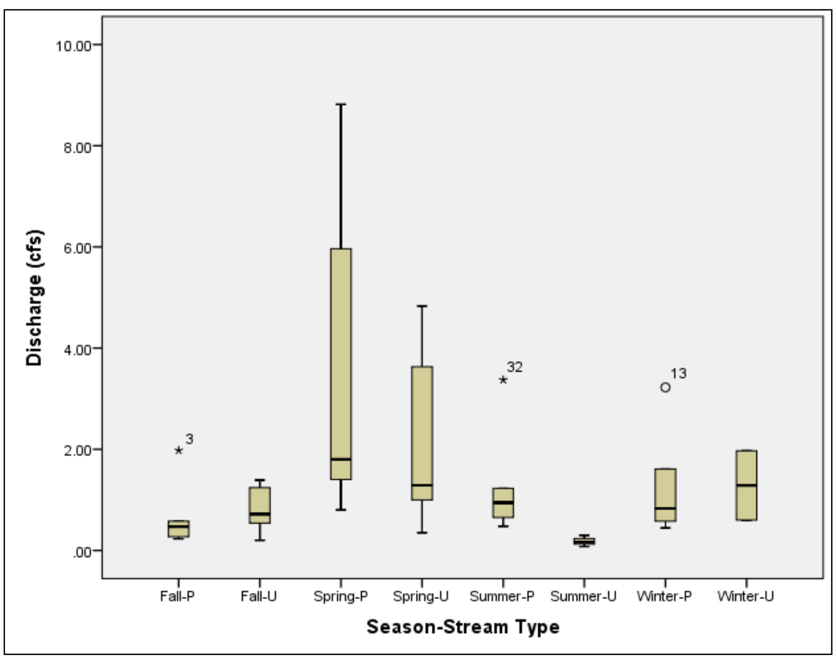

Figure 4.19. Boxplot Displaying Discharge Variation

Differences in temperature among sites could potentially be explained by streams having slightly different latitudes. The urban streams are all 108 km or more miles north of the pristine streams, so the average air temperature is colder in these watersheds. pH values for all pristine and urban streams are approximately the same and have median values that differ by 0.5 or less. The higher variability at the urban streams during the spring could be caused by runoff having higher levels of pollutants from the previous winter months. Overall, the pristine streams have lower EC values and much lower variability when compared to the urban streams. The pattern of means (how each change with season) of the pristine streams appears to be the complete opposite to the pattern of means of the urban streams. EC and discharge values were analyzed to determine whether dilution could be the mechanism causing larger EC values. However, that hypothesis was disproven, so it is assumed that the higher EC values are related to the total ionic load varying throughout the year. DO tracks temperature and it is expected to see higher DO in colder streams. The median values do not appear to change with LULC; however, variability does. Variability in the urban streams is most likely due to varying channel roughness between the streams. The urban streams are additionally very shallow, so it is expected to see higher DO in shallow streams where discharge is low. Very low turbidity values and variability in the plots shows that the pristine streams have much cleaner water than the urban streams. While turbidity is highest in the urban streams during fall and spring, there is no consistent seasonal pattern between turbidity measurements. Additionally, no pattern is seen between discharge and turbidity. It is typical to see higher turbidity values at streams with the greatest discharge; however, in this study the highest turbidity values are seen in streams which have intermediate discharge. To better understand the turbidity values, more temporal resolution is needed along with additional data points.

Typically, higher nitrate values are expected to be seen during the spring and the fall when fertilizer is supplied. Overall, nitrate values are low among all pristine and urban streams; however, high nitrate values at the pristine streams are seen in spring, summer, and winter, with lower values in the fall. It is expected that there is simply more animal life in the streams during the spring and summer which attributes to these raised values. It is unclear what causes raised values during the winter. Median values of nitrate are similar across the urban streams and variability is low during both spring and summer. This information leads to the conclusion that agricultural activity near the urban streams is very low. Phosphate values remain low at the pristine streams and are much greater at the urban streams. The highest values at the urban streams occur during spring, summer, and winter. Overall, there appears to be no significant pattern between the pristine and urban streams. It is important to note that chloride was not measured at the pristine steams during the fall and therefore is left out of this boxplot. The pristine streams all show low variability in chloride values when compared to the urban streams. The greatest variability at the urban streams occurs during fall, winter, and summer. Positive skewness is also seen at the urban streams during the same months. During the summer months, a few streams are highly skewing the chloride values; however, the median of the values remains low. The highest chloride values are seen in winter and spring which is typical due higher amounts of road salt in runoff near the urban streams. The pristine discharge plot displays an expected pattern where the lowest discharge is in the fall and the highest is in the spring. This pattern is not observed with the urban data.

The lack of pattern is most likely due to the fact that the urban data was collected in different years than the pristine data, so there is unmatched temporal resolution.

4.3.3. Correlation Matrix. A correlation matrix was made to determine if any of the water quality parameters were significantly related among all pristine and urban streams. Table 4.4 shows the correlations that are statistically significant at alpha = 0.05 and alpha = 0.01 for a two-tailed test. While the matrix appears to show numerous correlations between the water chemistry indicators, many of them have R^2 values that are quite low, and therefore do not actually represent a strong correlation. This can be observed for the following: pH and chloride, nitrate and phosphate, DO and nitrate, chloride and discharge, EC and nitrate, and EC and discharge. A negative correlation between temperature and EC is observed. This is surprising since warmer streams will have higher levels of EC or more dissolved inorganic solids, not colder. However, there is a strong correlation between EC and chloride (0.743). An explanation could be that as temperatures drop, more salt will eventually get into the water and that causes a high correlation between EC and chloride. Therefore, the higher EC values seen as temperature drops is related to the amount of chloride being added to streams. While the R^2 value was very low for the correlation between temperature and DO, the trend still follow what is anticipated. Higher DO is expected to be seen in colder water. The negative correlation between temperature and phosphate was not expected. A very conservative explanation for this correlation could be that more people wash their cars in the winter and the soap from washing is impacting the phosphate values at the urban streams. Another expected correlation is that between temperature and chloride. When temperatures are colder (winter) more salt is used on the roads which can be carried to streams through runoff, so streams have higher chloride levels. A positive correlation is observed between pH and EC that could potentially be explained by pH being greatly controlled by rock chemistry. The more calcium and magnesium dissolved, the higher the pH will be, leading also to higher EC. Phosphate and EC displays a positive correlation which is expected since increases phosphate levels will also results in higher EC in streams. Turbidity and phosphate display a strong positive correlation. This correlation is not significant in the pristine correlation matrix or the urban correlation matrix. It was only seen when the stream data was combined. Overall, the pristine steams show little sensitivity over seasons with respect to turbidity. However; the urban sites continually have higher turbidity values. Essentially there are just two groups of data with a line drawn between them and the correlation is most likely spurious. A positive correlation between phosphate and chloride is most likely due to human factors. An increase in both phosphate and chloride will be seen in areas of increased human activity.

4.3.4. Analysis of Variance. Analysis of Variance or ANOVA tests are commonly used to assist in comparing two or more data sets to see if they are statistically different from each other (Sullivan). An ANOVA test can determine whether samples are from the same population, but it does not specifically state which of the samples are different; it simply concludes that there are population differences. To find out which samples are different, a posteriori or post hoc test needs to be performed (Laerd Statistics, 2018). Since the data from this study are not normally distributed and have unequal sample sizes, the Welch ANOVA test was used to determine whether all 10 streams (pristine and urban) are from the same population (Frost, 2018).

Table 4.4. Correlation Matrix for Combined Stream Water Quality Parameters

		Temp	pH	EC	DO	Turbidity	Nitrate	Phosphate	Chloride	Discharge
Temperature (°C)	Pearson Correlation	1	-.017	-.401**	-.284**	.038	.133	-.297**	-.331**	-.044
	Sig. (2-tailed)		.813	.000	.000	.626	.075	.002	.000	.631
pH	Pearson Correlation	-.017	1	.293**	-.026	-.002	-.111	.052	.265**	.014
	Sig. (2-tailed)	.813		.000	.730	.979	.140	.600	.003	.876
EC (uS/cm)	Pearson Correlation	-.401**	.293**	1	.092	.082	-.271**	.581**	.743**	-.184*
	Sig. (2-tailed)	.000	.000		.219	.290	.000	.000	.000	.045
DO (mg/L)	Pearson Correlation	-.284**	-.026	.092	1	.079	.201**	.050	.044	.170
	Sig. (2-tailed)	.000	.730	.219		.312	.007	.617	.630	.064
Turbidity (NTU)	Pearson Correlation	.038	-.002	.082	.079	1	-.145	.535**	-.004	.105
	Sig. (2-tailed)	.626	.979	.290	.312		.063	.000	.970	.266
Nitrate (mg/L)	Pearson Correlation	.133	-.111	-.271**	.201**	-.145	1	-.321**	-.132	.084
	Sig. (2-tailed)	.075	.140	.000	.007	.063		.001	.154	.364
Phosphate (mg/L)	Pearson Correlation	-.297**	.052	.581**	.050	.535**	-.321**	1	.526**	-.143
	Sig. (2-tailed)	.002	.600	.000	.617	.000	.001		.000	.200
Chloride (mg/L)	Pearson Correlation	-.331**	.265**	.743**	.044	-.004	-.132	.526**	1	-.226*
	Sig. (2-tailed)	.000	.003	.000	.630	.970	.154	.000		.047
Discharge (cfs)	Pearson Correlation	-.044	.014	-.184*	.170	.105	.084	-.143	-.226*	1
	Sig. (2-tailed)	.631	.876	.045	.064	.266	.364	.200	.047	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

ANOVA has a specific hypotheses structure consisting of a null hypothesis and an alternative hypothesis. The null hypothesis is that all the streams, pristine and urban, are from the same group for each water quality parameter. The alternative hypothesis is that all the streams are not from the same group when analyzing each water quality parameter. The Welch ANOVA test produces a p-value and if $p \leq 0.05$, the null hypothesis is rejected (Frost, 2018). If the null hypothesis is rejected, it is concluded that not all streams are from the same group. The Games-Howell post hoc test was run in accordance with the Welch test to determine which specific streams are not from the same population after the null hypothesis is rejected. The Games-Howell post hoc test was used because it is implements multiple comparisons while not requiring the data to have equal variances (Frost, 2018).

For each water quality parameter, the streams were separated into groups that are statistically from the same population. When analyzing some water chemistry parameters, many differences were present which lead to numerous significant stream groups. To better understand how those groups were determined, a matrix (Tables 4.5 to 4.9) was made to visually show the differences amoung stream groups. On each matrix, the cells highlighted in blue show the statistically significant differences between streams.

When looking at temperature, it was determined that all streams are from the same the same population since there were no differences between any of the streams. When analyzing pH, two distinct groups were observed. Grand Glaize Creek (Stream 6) showed a significant difference between all five pristine streams as well as with two of the other urban streams. Therefore, Stream 6 was placed into its own group while the other nine streams were all placed into the same group. Streams were separated into numerous groups with respect to EC. First, all the pristine streams were analyzed to see if they were from the same population. Streams 1,2,4, and 5 were all determined to be statistically the same; however, Stream 3 displayed significant differences with pristine streams 4 and 5 as seen in Table 4.5 Next, the urban streams were analyzed the same way. Streams 6 and 7 were found to be from the same population and so were Streams 9 and 10. Stream 8; however, was not able to be placed with either of those two groups due to conflicts with Streams 7, 9, and 10. Therefore, for EC five distinct groupings were made. Group 1 contains Stream 1, 2, 4, 5; Group 2 contains Stream 3; Group 3 contains Streams 6 and 7; Group 4 contains Streams 9 and 10; Group 5 contains only Stream 8.

Table 4.5. Matrix Displaying Differences Between Streams for EC

1,1	1,2	1,3	1,4	1,5	1,6	1,7	1,8	1,9	1,10
	2,2	2,3	2,4	2,5	2,6	2,7	2,8	2,9	2,10
		3,3	3,4	3,5	3,6	3,7	3,8	3,9	3,10
			4,4	4,5	4,6	4,7	4,8	4,9	4,10
				5,5	5,6	5,7	5,8	5,9	5,10
					6,6	6,7	6,8	6,9	6,10
						7,7	7,8	7,9	7,10
							8,8	8,9	8,10
								9,9	9,10
									10,10

When analyzing DO, all pristine streams were found to be statistically from the same population. When comparing urban streams, it was determined that Streams 6 and 7 also were from the same population as all five pristine streams. Therefore, for DO, the first group contains Streams 1, 2, 3, 4, 5, 6, and 7. Stream 8 has significant difference from all the streams in Group 1; however, it was found to be from the same population as Streams 9 and 10. Consequently, Group 2 consists of Stream 8, 9, and 10. While turbidity saw numerous differences between streams (Table 4.6) all steam could be grouped into two distinct groups. All five pristine streams were deemed to be from the same population. Streams 9 and 10 matched the pristine streams; however, they differed from the other urban streams (Streams 6,7, and 8). Thus, Group 1 contains Streams 1, 2, 3, 4, 5, 9, and 10 while Group 2 consists of Stream 6, 7, and 8.

Table 4.6. Matrix Displaying Differences Between Streams for Turbidity

1,1	1,2	1,3	1,4	1,5	1,6	1,7	1,8	1,9	1,10
	2,2	2,3	2,4	2,5	2,6	2,7	2,8	2,9	2,10
		3,3	3,4	3,5	3,6	3,7	3,8	3,9	3,10
			4,4	4,5	4,6	4,7	4,8	4,9	4,10
				5,5	5,6	5,7	5,8	5,9	5,10
					6,6	6,7	6,8	6,9	6,10
						7,7	7,8	7,9	7,10
							8,8	8,9	8,10
								9,9	9,10
									10,10

When analyzing nitrate concentrations, the pristine streams were looked at first. Similar to EC, Stream 3 was determined to be statistically different from Stream 1,2 and 5 with respect to nitrate. Therefore, Streams 1, 2, 4, 5 were made up Group 1 while Stream 3 remained in its very own group (Group 2). Next urban streams were analyzed, and it was determined that Streams 8 and 10 differed from the other urban streams (Table 4.7), but matched Streams 1, 2, 4, and 5. So, Streams 8 and 10 were included into group 1 with Streams 1, 2, 4, and 5. Group 3 consists of Streams 6, 7, and 9.

Table 4.7. Matrix Displaying Differences Between Streams for Nitrate

1,1	1,2	1,3	1,4	1,5	1,6	1,7	1,8	1,9	1,10
	2,2	2,3	2,4	2,5	2,6	2,7	2,8	2,9	2,10
		3,3	3,4	3,5	3,6	3,7	3,8	3,9	3,10
			4,4	4,5	4,6	4,7	4,8	4,9	4,10
				5,5	5,6	5,7	5,8	5,9	5,10
					6,6	6,7	6,8	6,9	6,10
						7,7	7,8	7,9	7,10
							8,8	8,9	8,10
								9,9	9,10
									10,10

While examining phosphate concentrations, it is important to note that Streams 8 and 10 were not considered due to the fact that the public database where urban stream parameters were acquired did not have values for these streams. Therefore, these streams have been excluded from the matrix. Only Streams 1, 2, 3, 4, 5, 6, 7, and 9 were analyzed and placed into statistically significant groups. Streams 6 and 7 both displayed differences with all five pristine streams (Table 4.8) and therefore were grouped together to make Group 1. Stream 9; however, showed no differences with any of the pristine streams so Group 2 contains Streams 1, 2, 3, 4, 5 and 9.

Table 4.8. Matrix Displaying Differences Between Streams for Phosphate

1,1	1,2	1,3	1,4	1,5	1,6	1,7		1,9	
	2,2	2,3	2,4	2,5	2,6	2,7		2,9	
		3,3	3,4	3,5	3,6	3,7		3,9	
			4,4	4,5	4,6	4,7		4,9	
				5,5	5,6	5,7		5,9	
					6,6	6,7		6,9	
						7,7		7,9	
								8,9	
								9,9	

When analyzing chloride, pristine Streams 1, 2, 4, and 5 were all found to be from the same population and thus make up Group 1. Stream 3 showed differences (Table 4.9) with the other four pristine streams as well as with Streams 6, 7, and 8. Stream 3 was found to be from the same population as only Stream 9 and 10, making up Group 2. Lastly, Streams 6, 7, and 8 are statistically form the same population so they make up Group 3. With respect to stream discharge, all pristine and urban streams are deemed to be from the same statistically significant population since there were no differences observed between any of the streams.

Several conclusions can be determined from the ANOVA results summarized above. The first deduction is that most of the time the five pristine streams (1, 2, 3, 4 and 5) are from the same population. Stream 3 occasionally is found to be from another population such as with EC, nitrate, and chloride. It is expected that the pristine streams would be from the same population since they have similar LULC, soil type, slope, and geology. When out in the field sampling, it was noted that Stream 3 did appear to have more human impact than the other four pristine streams. Tire tracks and trash were often found in Stream 3, indicating that people frequently explored the area. This increased human activity in and around the stream could help to explain why it varied from the rest of the pristine streams occasionally.

Table 4.9. Matrix Displaying Differences Between Streams for Chloride

1,1	1,2	1,3	1,4	1,5	1,6	1,7	1,8	1,9	1,10
	2,2	2,3	2,4	2,5	2,6	2,7	2,8	2,9	2,10
		3,3	3,4	3,5	3,6	3,7	3,8	3,9	3,10
			4,4	4,5	4,6	4,7	4,8	4,9	4,10
				5,5	5,6	5,7	5,8	5,9	5,10
					6,6	6,7	6,8	6,9	6,10
						7,7	7,8	7,9	7,10
							8,8	8,9	8,10
								9,9	9,10
									10,10

The next conclusion is that location affects water quality. In this study the LULC was deemed to be the greatest factor influencing stream health. Overall the pristine streams showed higher water quality with respect to water chemistry indicators as well as benthic macroinvertebrate surveys. On the other hand, the five urban sites overall showed lower water quality by comparison. For several water chemistry parameters (EC and chloride notably), there were significant differences between the urban streams. Higher EC and chloride values at Streams 6, 7, and 8 compared to 9 and 10 most likely was a result of the location of the urban streams. Streams 6, 7, and 8 were all located near St. Louis, Missouri, while Streams 9 and 10 were located near Columbia, Missouri. The subbasins near Columbia, while still very urban, had significant agricultural activity. St.

Louis, however, had little to no agricultural activity and those subbasins were mostly urban. The differences in LULC certainly impact the water quality found at the five urban streams. The last conclusion notes that activities within the urban watersheds greatly impact water quality. While Streams 6, 7, and 8 were all located near St. Louis and were all predominantly urban, Stream 8 seemed to have higher levels of water chemistry parameters that are results of human activity. Streams 6 and 7 were located in areas where people were near, but not necessarily in the stream due to fences and barriers. However, the sampling point for Stream 8 was in the middle of a public park. People and their pets played directly in the stream and consistently used the area for recreation. Therefore, Stream 8 can likely attribute impaired water chemistry values to greater human activity.

4.3.5. Biological Monitoring. Table 4.10 compares the water quality rating, percent EPT and HFBI for all 10 streams, pristine and urban. Rogers Creek and Middle West Fork have the highest water quality ratings, falling in the excellent category for each sampling campaign. The streams with the next highest values are Mill Creek, Bee Fork, and Ottery Creek, all having either excellent or good water quality ratings. Hominy Creek and Grindstone Creek both have overall water quality ratings of fair, with Hominy Creek climbing into the good category in Fall 2018. The worst water quality ratings are at Grand Glaize Creek, Glaize Creek, and Sugar Creek, where the values are typically poor. Overall, according to the Missouri Department of Natural Resources invertebrate collection method, the pristine streams have water quality that is much better than that of the urban streams. In these pristine streams, a higher diversity of taxa was found from the pollution sensitive, somewhat pollution sensitive, and pollution tolerant groups. The urban streams, however, had either a lack of diversity of taxa, or simply low overall invertebrate counts due to stream drying.

Percent EPT classifies water quality into three different groups which have each been assigned a different color on Table 4.10 Green represents good water quality, yellow represents moderate water quality, and red represents poor water quality. The five pristine streams all have the highest water quality according to percent EPT, always falling into the good and moderate categories. The urban streams, on the other hand, typically have water quality classified as moderate and poor. However, during the fall of 2018 both Grand Glaize Creek and Grindstone Creek reached the good water quality. Grand Glaize Creek, Glaize Creek, and Sugar Creek at one point all had a percent EPT of 0 which means that during that sampling campaign there were no taxa present (mayflies, stoneflies, or caddisflies) which are tolerant to higher levels of pollution.

Rogers Creek, Middle West Fork, Bee Creek, and Ottery Creek have the best water quality when using the Hilsenhoff Family Biotic Index (HFBI). These streams have water quality that is classified as good or very good, meaning these streams have little to no pollution. Mill Creek and Hominy Creek have the next highest water quality, being categorized as good or fair. Good water quality implies that there is some pollution possible while fair water quality suggests that streams have fairly substantial pollution. Grand Glaize Creek, Glaize Creek, Sugar Creek, and Grindstone Creek have the lowest water quality overall. Glaize Creek and Grindstone Creek typically have fair or fairly poor water quality indicating very substantial pollution. Grand Glaize Creek and Sugar Creek display mostly fairly poor and very poor water quality, suggesting that the stream potentially has substantial or severe pollution.

Table 4.10. Summary of WQR, percent EPT, and HFBI for all Streams

Site	Stream	Season	Water Quality Rating		% EPT	HFBI	
1	Rogers Creek	Fall 2017	34	Excellent	31.28	4.42	Good
		Spring 2018	24	Excellent	56.06	4.17	Very Good
		Fall 2018	32	Excellent	33.33	4.12	Very Good
2	Mill Creek	Fall 2017	40	Excellent	28.26	5.25	Fair
		Spring 2018	22	Good	60	4.28	Good
		Fall 2018	37	Excellent	29.81	4.52	Good
3	Middle West Fork	Fall 2017	33	Excellent	74.74	4.19	Very Good
		Spring 2018	31	Excellent	66.28	4.29	Good
		Fall 2018	30	Excellent	46.67	4.88	Good
4	Bee Fork	Fall 2017	37	Excellent	74.51	4.58	Good
		Spring 2018	18	Good	80	4.23	Very Good
		Fall 2018	35	Excellent	56.96	4.77	Good
5	Ottery Creek	Fall 2017	33	Excellent	72	4.42	Good
		Spring 2018	21	Good	77.78	4.04	Very Good
		Fall 2018	28	Good	44.44	4.7	Good
6	Grand Glaize Creek	Fall 2017	1	Poor	0	10	Very Poor
		Spring 2018	2	Poor	0	6	Fairly Poor
		Fall 2018	5	Poor	75	4.75	Good
7	Glaize Creek	Fall 2017	11	Poor	0	6.24	Fairly Poor
		Spring 2018	21	Good	31.03	5.45	Fair
		Fall 2018	8	Poor	25	6	Fairly Poor
8	Sugar Creek	Fall 2017	8	Poor	0	8	Very Poor
		Spring 2018	11	Poor	0	6.33	Fairly Poor
		Fall 2018	27	Excellent	42.42	5.94	Fairly Poor
9	Hominy Creek	Fall 2017	14	Fair	9.82	5.54	Fair
		Spring 2018	12	Fair	37.5	4.5	Good
		Fall 2018	19	Good	35.29	4.71	Good
10	Grindstone Creek	Fall 2017	15	Fair	46.15	6.08	Fairly Poor
		Spring 2018	14	Fair	36.36	4.91	Good
		Fall 2018	13	Fair	54.17	5.08	Fair

4.3.6. Comparison of BI and Water Chemistry Parameters. In order to determine which macroinvertebrate analyses best correlates to different chemical water quality indicators, plots were made displaying how the WQR, percent EPT, and HFBI correlate to each water chemistry parameter. This technique was used to determine whether benthic macroinvertebrate communities can be used to predict specific water quality parameters. In the plots, only data from Spring 2018 and Fall 2018 were analyzed. To determine the seasonal water chemistry parameters, the average of all measurements was used so each parameter had one value to compare to each biotic index value. A total of 18 plots were made and during analysis it was noted that a vast majority of the R^2 values were low, indicating weak or little correlation between the different biotic indices and the water quality parameters. To more accurately analyze the plots, all relationships between parameters were examined to determine which correlations were in fact statistically significant. Significant F values were calculated in Excel and the values that were ≤ 0.05 were deemed significant. Only the plots having significant relationships ($F \leq 0.05$) will be shown here. Some plots appeared to have moderate R^2 values between water chemistry parameters and each biotic index; however, they were determined to not be statistically significant.

During Spring 2018 the only significant correlations are seen with chloride, EC, and turbidity as seen in Figures 4.20 to 4.22 Chloride has a statistically significant correlation with the WQR, percent EPT, and the HFBI with F values of 0.036004, 0.00175, and 0.00329. Slope trends follow what is expected, with WQR and percent EPT having a negative slope and HFBI having a positive slope. This indicates that water quality decreases with increasing chloride concentrations. The strongest correlation is between chloride and the HFBI with an R^2 value of 0.868. Chloride also shows a strong correlation with the percent EPT with an R^2 value of 0.7979. While chloride has significant correlation with the WQR the correlation is deemed moderate due to the R^2

value being 0.5197. EC displayed correlations with both the percent EPT and the HFBI having significant F values of 0.008309 and 0.00980. The slope for percent EPT was negative while the slope for HFBI was positive, indicating that water quality decreases as a stream has more dissolved inorganic material (higher EC). The correlations between EC and percent EPT and HFBI were both moderately strong with R^2 values being 0.6517 and 0.6638 respectively. The last significant correlation is seen with turbidity between percent EPT and HFBI with F values of 0.044714 and 0.05. The slope for percent EPT is negative while the slope for HFBI is positive. This trend is expected since increased turbidity is associated with poorer stream health. The correlations with turbidity between percent EPT and the HFBI are both classified as moderated since R^2 values are 0.4168 and 0.4312.

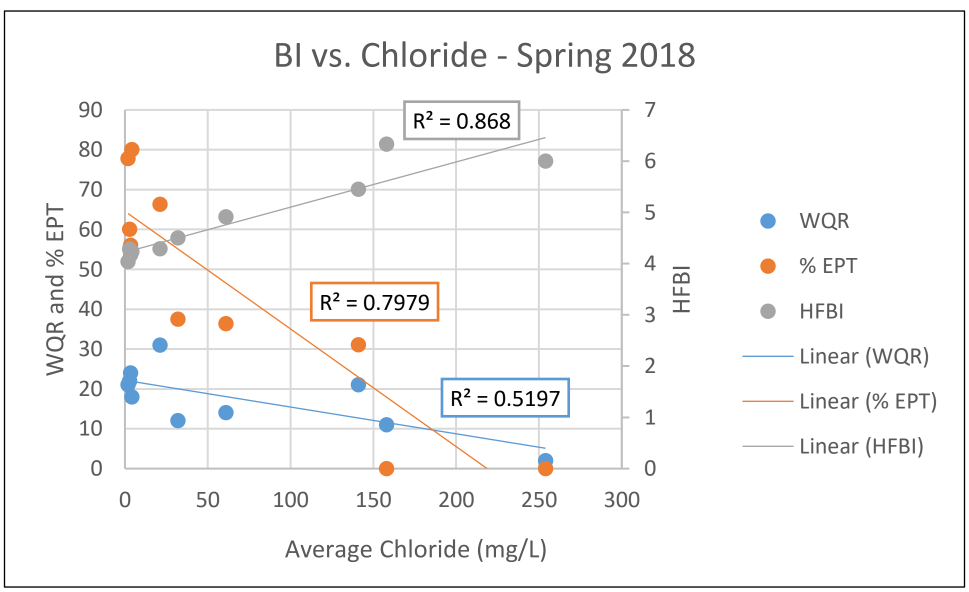

Figure 4.20. Biotic Index vs. Chloride for Spring 2018

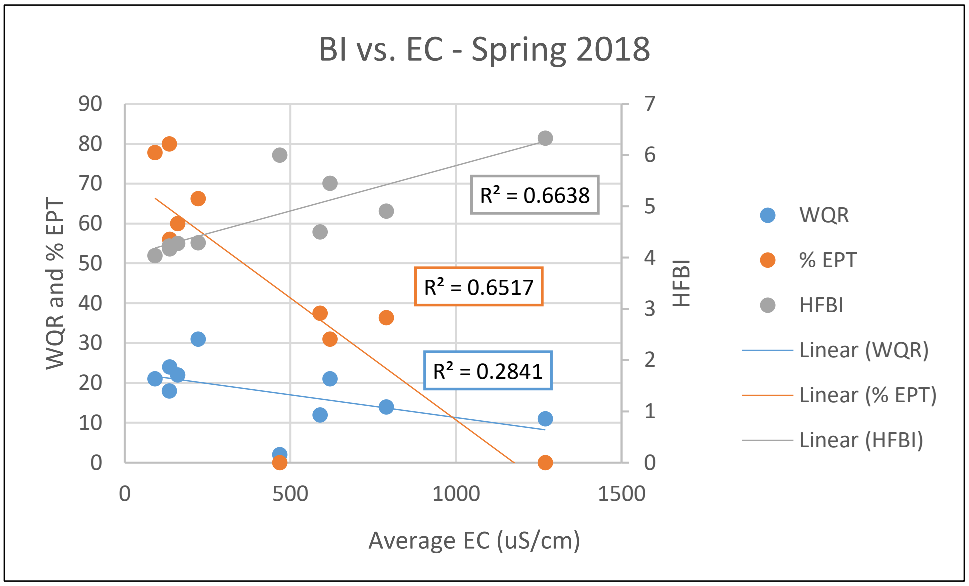

Figure 4.21. Biotic Index vs. EC for Spring 2018

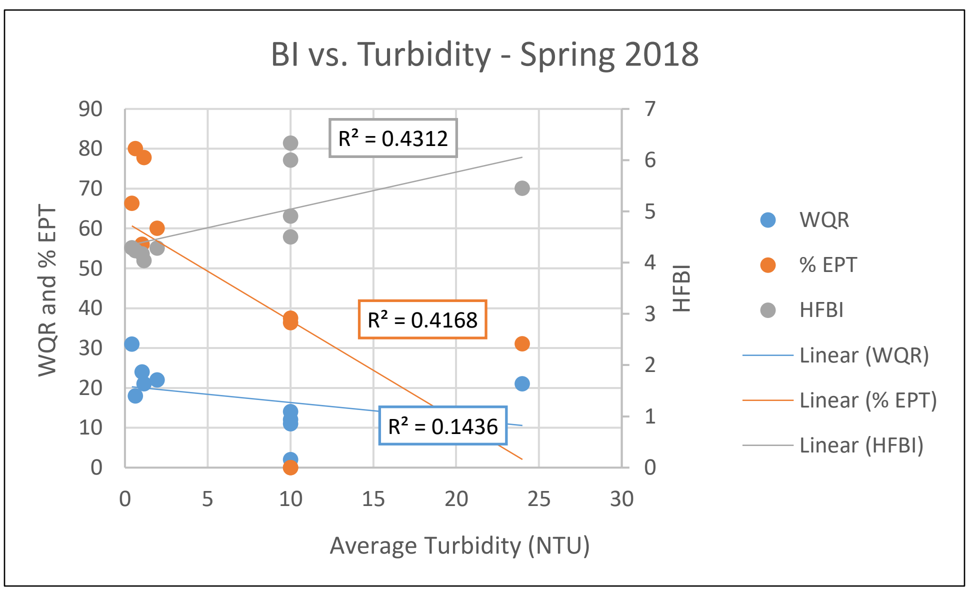

Figure 4.22. Biotic Index vs. Turbidity for Spring 2018

During Fall 2018, significant correlations were observed in chloride, turbidity, and temperature as seen in Figures 4.23 to 4.25 Chloride's only significant correlation was with the HFBI (F=0.034576). The positive trend indicates that as chloride concentration increases, the HFBI value increases, suggesting lower water quality in streams. The R^2 value between chloride and the HFBI is 0.5376 and is classified as a moderate correlation. Turbidity had one significant correlation with the WQR (F=0.02458). The negative slope trend seen in the plot is expected since increased turbidity lowers the WQR value as water quality decreases. The correlation between the WQR and turbidity is moderately strong since R^2 equals 0.5694. The last significant correlation is seen between temperature and the WQR (F=0.02624). The R^2 value is 0.5434 which is categorized as a moderate strong correlation. While this observed correlation is statistically significant, the slope trend is not what would be expected. There is a positive slope in the plot between temperature and the WQR which means that as temperature increases the biotic index increases, suggesting higher water quality. Normally, higher water quality (and a higher WQR) would be seen in colder water which would be seen by a positive slope trend.

Overall, chloride impacts invertebrate health the most by correlating with all three methods used to determine the biotic index in the spring and with the HFBI in the fall. The R^2 values for all the correlations between chloride and the three biologic indices are high enough that it is concluded that biological monitoring could predict chloride impact on streams. EC seems to have the second greatest impact; however, if a step-wise regression was performed the EC values would most likely be overshadowed by the stronger chloride correlations. Turbidity correlates with the percent EPT and HFBI in the spring and the WQR in the fall making it the physiochemical parameter next impacting invertebrate health the most. While a correlation between temperature and the WQR was observed in the fall, its impact on water quality is most likely spurious.

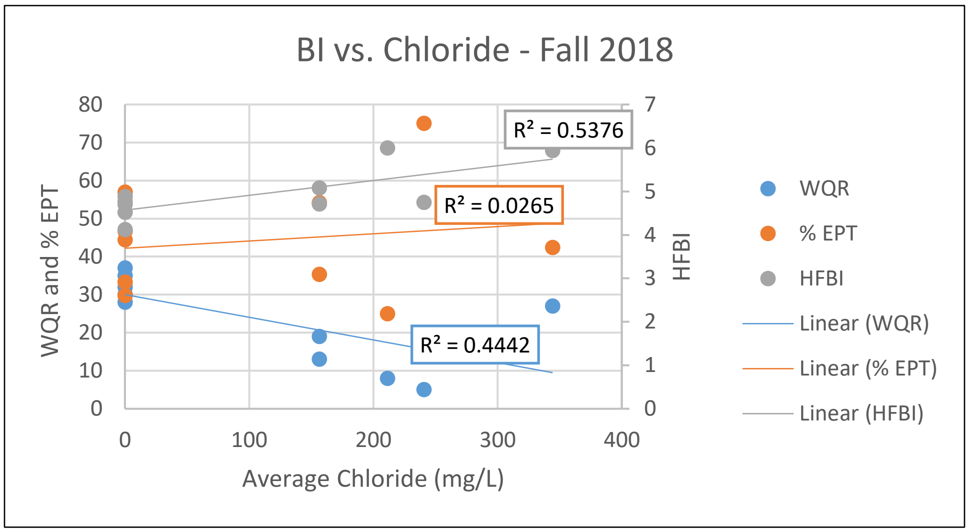

Figure 4.23. Biotic Index vs. Chloride for Fall 2018

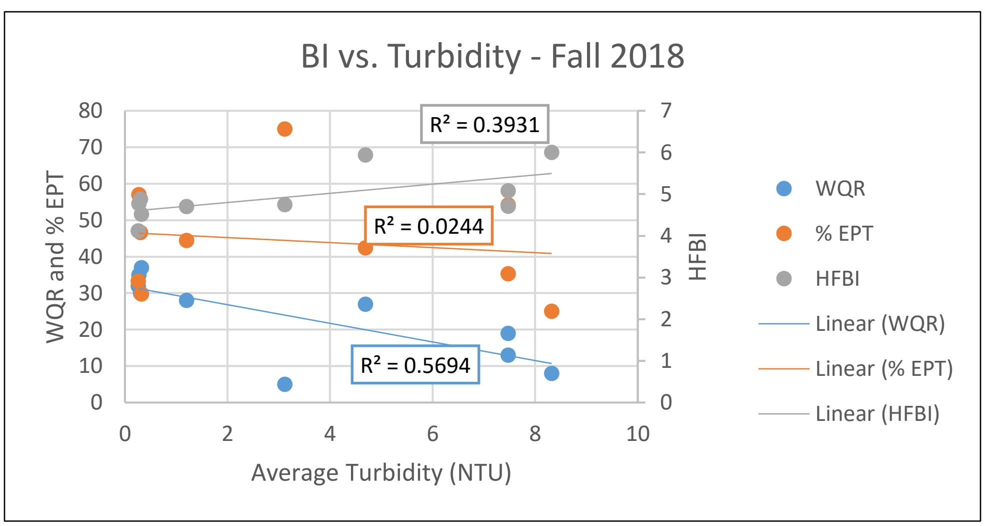

Figure 4.24. Biotic Index vs. Turbidity for Fall 2018

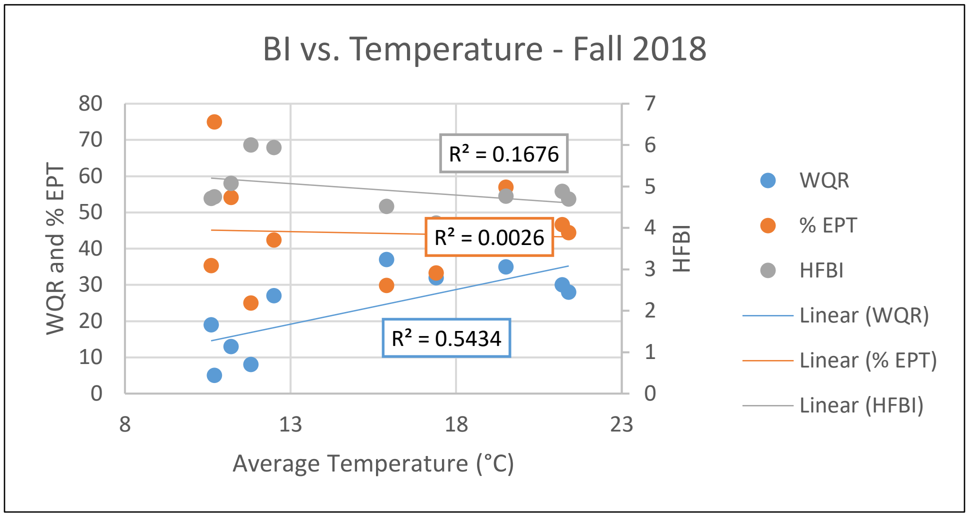

Figure 4.25. Biotic Index vs. Temperature for Fall 2018

5. CONCLUSIONS

5.1. LIMITATIONS

One of the biggest limitations of this study comes from using a public database to obtain water chemistry and benthic macroinvertebrate data for all urban streams. While the database was used to find urban sites with past available data for all seasons, the data was collected during various years. This resulted in different temporal resolution for this study. To help account for this, water chemistry data was averaged between seasons to try and limit variation as much as possible. The assumption was made that parameters are nearly the same every year and do not differ significantly. By using a public database, site location choice also was limited for the urban streams. Only previously monitored streams could be chosen and exact sampling locations were predetermined. This resulted in some of the urban sub-basins near Columbia, MO, to have slightly lower urban land use than desired.

5.2. FUTURE WORK

If this study is continued, it is recommended that sampling be performed concurrently at both the pristine and urban sites so that the data can be collected over the same time frame and a public database would not need to be used to obtain urban water quality data. This would allow stronger comparisons and patterns to be achieved more accurately. Collecting data at the same times and more frequently for both streams over longer periods of time would additionally provide strong correlations and would help to better determine temporal fluctuations.

If benthic macroinvertebrate data was collected alongside water chemistry parameters over the same time scale, the water quality index (WQI) could be calculated to better understand stream health. The WQI is scaled from 0 (very poor water quality) to 100 (excellent water quality) and is based on sub-index measurements of water chemistry parameters such as pH, DO, total dissolved solids, nitrate, and phosphate. One could plot the BI against the WQI for both pristine and urban streams during different months to determine which months are indicative of longer-term conditions.

Five watersheds with significant agricultural impact could also be sampled from to determine how strong agricultural activity such as row crops and animal operations affect Missouri water quality. The results from such data could be compared against data from the pristine and urban watersheds from this study to determine new correlations and seasonal patterns in water quality.

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