DISTRIBUTION OF *E. COLI* LEVELS AND RECREATION USE AS FACTOR OF STREAM ORDER IN THE CENTRAL GREAT PLAINS, CENTRAL OKLAHOMA/TEXAS PLAINS, AND SOUTH CENTRAL PLAINS ECOREGIONS

A Thesis

by

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ABSTRACT

This study examines the relationship of *E. coli* concentrations and recreational use to both stream order and watershed size. To determine possible ecoregion effects, the *E. coli* data used in this study were obtained from monitoring stations located on freshwater streams located in three ecoregions in Texas and Oklahoma – the Central Great Plains, Central Oklahoma/Texas Plains and South Central Plains (Ecoregions 27, 29, and 35, respectively). Median *E. coli* concentrations from the monitoring stations were analyzed for correlation with respect to the stream order of each monitoring site as well as the watershed size of each monitoring station. Geospatial analysis was used to determine stream order and watershed size and to identify un-impacted/least impacted streams in each ecoregion. Stream order was classified based on the traditional stream order classification method by Horton (1945) and Strahler (1957) and stream link magnitude analysis method by Shreve (1966). The analysis of two stream order systems and watershed size for each monitoring site with respect to median *E. coli* showed no significant relationship between *E. coli* and stream orders/watershed size of unimpacted watersheds. The watersheds with wastewater outfalls and urban areas exceeding 10% of land use showed a statistically significant, yet a weak negative relationship between *E. coli* and stream order/watershed size i.e. *E. coli* decreased with increase in stream order/watershed size.

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NOMENCLATURE

EPA	United States Environmental Protection Agency
IRNR	Institute of Natural and Renewable Resources – Texas A&M University
OCC	Oklahoma Conservation Commission
RUAA	Recreational Use Attainability Analysis
TCEQ	Texas Commission on Environmental Quality
USDA	United States Department of Agriculture

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CHAPTER I

INTRODUCTION

Need for Study

Pathogen contamination in freshwater streams is a significant water quality concern in the United States. Pathogen contamination is often assessed by determining the amount of an indicator bacteria such as *Escherichia coli (E. coli)* in freshwater systems whereas enterococcus is used as an indicator in marine waters (Halliday and Gast 2011).

Pathogen contamination is by far the largest cause for water quality impairment with approximately 11,000 instances reported to date. Over 80 percent of the pathogen impairment is caused by excessive fecal coliform bacteria which include *E. coli* (USEPA 2014a). Diarrhea, gastrointestinal illness, and diseases such as cholera can be attributed to waterborne pathogens along with approximately 900 deaths and 900,000 illnesses each year (Arnone and Walling, 2007). This makes setting appropriate recreational use standards for water bodies very important. In Texas, the recreational use standard for a water body is assumed primary body contact unless determined otherwise after a Recreational Use Attainability Analysis (RUAA) has been conducted. The RUAA is a site-specific study to determine what uses a stream can and does support based on its current and historic use and it’s physical and flow characteristics (TCEQ 2014). An appropriate recreational standard for a water body can then be set for the water body based on the findings of RUAA and approval by EPA.

The Texas Commission on Environmental Quality (TCEQ) is responsible for development and implementation of the Texas Water Quality Standards including the recreational use standards as specified in the Texas Administrative Code (TAC). The contact recreation standards with respect to *E. coli* concentrations as listed in the TAC regulations are as follows (TCEQ 2010):

I. Primary contact recreation – activities involving significant risk of water ingestion (e.g. swimming, diving, surfing, etc.). The geometric mean criterion for *E. coli* is 126 per 100 ml. In addition, the single sample criterion for *E. coli* is 399 per 100 ml.

II. Secondary contact recreation 1 – commonly occurring activities with limited body contact incidental to shoreline activity (e.g. fishing, kayaking, rafting, etc.). The geometric mean criterion for *E. coli* is 630 per 100 ml.

III. Secondary contact recreation 2 – activities with limited body contact incidental to shoreline activities with less significant risk of water ingestion than secondary contact recreation 1. The geometric mean criterion for *E. coli* is 1,030 per 100 ml.

IV. Noncontact recreation – activities with no significant risk of ingestion. The geometric mean criterion for *E. coli* is 2,060 per 100 ml.

As previously stated, in Texas, all fresh water bodies are classified as primary contact recreation unless a RUAA study determines a change in the classification is required (TCEQ 2010). The applicability of these standards has been questioned especially related to scale. Regime based standards, which account for temperature, sediment, and stream order of the water bodies have been proposed as possible alternatives (Poole et al., 2004). A central Texas study conducted by Harmel et. al. (2010) established a trend of decreasing *E. coli* concentrations when aggregating sampled segments from upstream to downstream. The trend was visible in both the "impacted" (land use of mixed rural with known point sources of pollution such as dairies, waste water treatment plants (WWTP), small communities, etc.) and "unimpacted" (land use of mixed rural excluding point sources of pollution) streams of the study area. The study also found that with increasing watershed scale the median *E. coli* concentrations decreased. Furthermore, the study found "unimpacted streams to be in violation of TAC regulations even though the anthropogenic impact was insignificant.

A study conducted by Lyautey et. al. (2010) in the South Nation River Drainage basin of Canada compared *E. coli* concentrations using Shreve (1966) stream link. The study found Shreve stream link to be inversely correlated to *E. coli* concentrations with strongest correlations seen during summer. Another Canadian study by Edge et al. (2012) used Horton (1945) and Strahler (1957) stream order and found that streams with order 3 and lower had higher *E. coli* concentrations compared to the streams of order greater than 3. Another study (Lyautey et. al. 2011) explained, "the persistence factor is less important than dilution (i.e. stream order) in describing *E. coli* densities, followed by factors that influence the loading of *E. coli* into watersheds."

Conversely, some studies dispute the inverse correlation between stream order and *E. coli* concentrations and suggest other factors (i.e. nonpoint source inputs) as more vital. A study conducted by the Virginia Department of Environmental Quality (VDEQ) found no correlation to stream order or ecoregions (level III) when investigating fecal coliform bacteria. The report found highest fecal coliform concentrations at the stream orders 3 and 4. The report suggested use of *E. coli* in future study as a better indicator for pathogen contamination (VDEQ 2003). Another study by Byappanahalli et. al. (2003) found *E. coli* increasing steadily downstream in the Dunes Creek watershed in Indiana. The study found a relation between excessive ditching and consequent increase in non-point source input responsible for increase in *E. coli*.

Lyautey et. al. (2010, 2011) attribute the inverse relationship of *E. coli* and stream order to the dilution in the water body as smaller stream orders combine to make larger streams. However, other studies hypothesize an influence of physical stream processes such as turbidity/sediment process and flow rates, on the *E. coli* concentrations, (Bai and Lung 2005; Brettar and Hofle 1992; Craig et. al. 2004; Oliver et. al. 2007) which may be processes dependent on stream order as well. This study investigates the relationships between stream order and *E. coli* concentrations of unimpacted and impacted watersheds in the Texas and Oklahoma ecoregions 27 (Central Great Plains), 29 (Central Oklahoma/Texas Plains), and 35 (South Central Plains).

Stream Order Theory

The relative position of a stream within a stream system can provide valuable information regarding the stream segment and help to explain similarities and differences within a stream network. Stream order, a measure of the relative size of streams, can be easily associated to the drainage area, stream size, and other physical properties of the streams. Classifying a stream according to stream order provides an important measure for the size and characteristics of specific segments within the stream network, which is essential for water management. Classifying stream order can also help in studying the amount of sediment in an area and facilitate a more effective use of waterways as natural resources. Ichim (1987) noted a strong relationship of decreasing sediment due to runoff with increasing stream order in easily erodible rocks and soils whereas a more moderate relationship was seen when the sediment source was less erodible. In lower order streams, the erosion of soils is usually more dominant than the deposition, therefore making stream order a factor in the sampling of the sediment (Otteson & Theobald 1994).

Gravelius (1914) as quoted by Horton (1945) was one of the first scientists to propose stream ordering. According to Gravelius, the source to mouth segment of the river (i.e. the segment that does not become a tributary of another stream and drain into a larger water body) has the first order since it is the greatest collector of the water. The direct tributaries of the first order stream have the order of two; the direct tributaries of the second order have the stream order of three; and so forth. The Gravelius method did not account for the length, catchment area, or flow of the streams and was subjective in nature.

Horton (1945) modified Gravelius subjective and somewhat difficult method and presented a more objective and simplified method of stream ordering in a dimensionless way. Horton reversed Gravelius ordering method and assigned the stream order of one to the stream with the initial concentrated flow. Meeting of two first order streams leads to a second order stream and the order of the stream will stay two as long as all of its tributaries have an order of one. Similarly meeting of the two second order streams results in a 3rd order stream and it will stay a 3rd order stream as long as all of its tributaries have an order of two or less. This ordering of streams will continue until the mouth of the stream is reached.

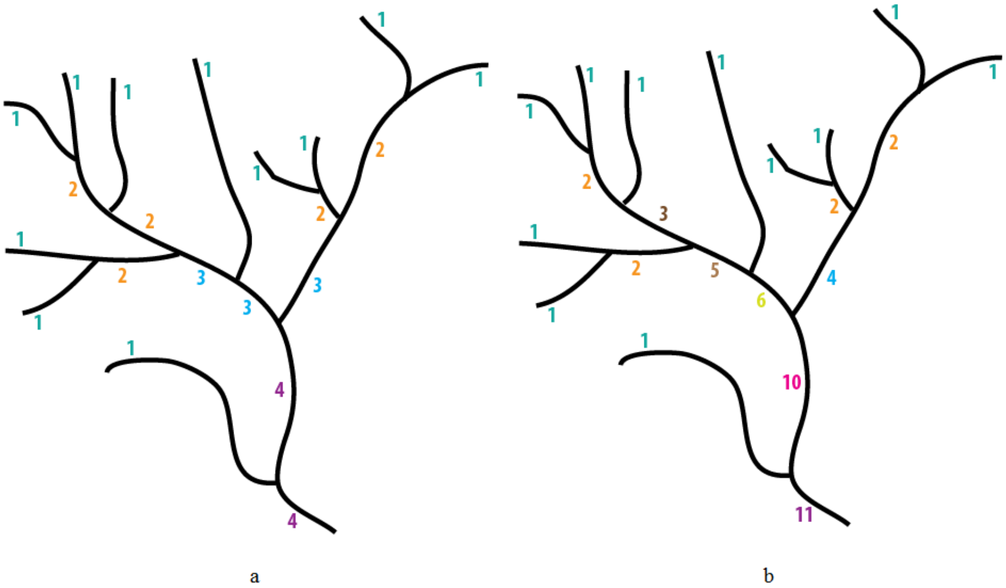


Figure 1. Strahler stream ordering (a) and Shreve stream ordering (b).

In 1952, Strahler created his own system based on Horton’s theory. The Strahler classification system (Strahler 1952) assigned a stream order of one to the basic water course and increased the order as theorized by Horton (1945) where the streams order does not change if a stream receives a lower order tributary (Figure 1). Zăvoianu (1985) noted the similarity in catchment area, average length of the water network, average slope, average flow rates, and other characteristics of streams when they had the same stream orders.

The Shreve classification system (Shreve 1966) provides a more representative depiction of the basin size by taking into account all tributaries of a stream system. In this method, all of the streams are accounted for as links in a network. Similar to the Strahler method, all of the basic watercourses considered exterior links are assigned an order of one. For order of all the other interior links, the orders of the links intersecting to make that interior are added together. This means if an interior link consists of the intersection of two first order streams links than it will have an order of two. Similarly, if a stream link of order 2 intersects with a stream link of order 5 the resulting link will have an order of 7. Due to the additive nature of the Shreve classification method, the stream numbers are sometimes referred to as magnitude instead of orders (Tarboton et. al. 1991). Therefore, the magnitude of a stream link in the Shreve ordering system is the number of all the links upstream to it.

Microbial Transport in Surface Waters

There are many factors that affect microbial transport in surface waters. Microbes may be found in the water column or within the sediments associated with the surface waters. These microbes may be unattached, attached, or resuspended within the water body. The unattached microbes do not get absorbed in the sediment and flow with velocities similar to the water since they are osmotically similar. Due to their electro-potential attractions, these unattached microbes bond easily with other particles that decrease their buoyancy while increasing their settling rates. On the other hand, attached microbes may be surficial in nature with a weak bond or may become completely absorbed into another particle. Detaching microbes from the absorbed particles is very difficult and often requires sediment removal for complete decontamination (Berry 1991).

The unattached microbes are less likely to deposit in the streambed and become part of the sediment whereas the attached microbes will deposit at the same rate as their host particle. The attached microbes deposited in the sediment bed may resuspend due to natural processes such as high flows due to flooding. This resuspension may cause the microbes to dislodge from host particles and flow unattached or attach to another particle and resettle once the resuspension event is over (Pachepsky and Shelton 2011). The attachment rates for different microbes vary significantly. A study done by Krometis et. al. (2007) found attachment rates of approximately 40% for the fecal coliforms, *E. coli*, and Enterococci. Krometis also concluded that these attachment rates were the same through the storm events and the resuspension rates of attached particles were higher during the storm events.

Objectives

The overall goal of this research is to better understand and predict the relationships between stream orders, *E. coli* concentrations, and recreational use and determine whether current recreational use standards are suitable.

The specific objectives are to:

1. Assess stream order and watershed size of all sites used in the study using GIS. Various stream order classification systems will be evaluated for suitability including traditional stream order classification (Horton 1945; Strahler 1957) and stream link magnitude analysis (Shreve 1966).

2. Evaluate correlations between concentrations and stream order (plus watershed size) as impacted by ecoregion
3. Evaluate correlation between recreation standard and stream order/watershed size.

CHAPTER II

STUDY AREA, DATA COLLECTION, AND GIS METHODS

Introduction

The purpose of this chapter is to provide a brief description of the study area as well as to detail the data sources used in this study. GIS steps undertaken such as delineation of streams, calculation of stream orders and watershed area, and determination of unimpacted watersheds are also described. Study area chosen for this study includes parts of ecoregions 27 (Central Great Plains), 29 (Central Oklahoma/Texas Plains also known as Cross Timbers), and 35 (South Central Plains) located within the states of Texas and Oklahoma. With the exception of some large population urban areas such as Dallas and Fort Worth in Texas and Oklahoma City in Oklahoma most of the study area is of rural to semi-rural in nature. The large rural area allowed for a greater selection of unimpacted streams and watersheds within the study area. Selecting the study area at ecoregional scale also allowed for a larger number of monitoring stations for the study. This was important since a significant number of the rural monitoring sites do not collect *E. coli* data regularly and would not be included in the analysis due to lack of *E. coli* data. A smaller number of monitoring sites would have therefore curtailed the *E. coli* data required for analysis in this study. Collecting data at ecoregion scale also provides an opportunity to compare the *E. coli* relationships in these ecoregions as well. Many state resource management agencies including Texas agencies have used ecoregions when setting up water quality standards and non-point pollution management goals (Omernik and Bailey 1997). The ecoregional comparison will also help in understanding *E. coli* relationships in the selected ecoregions and their comparison to the state standards.

Description of Study Area

The three ecoregions selected for this study are shown in Figure 2. Ecoregion 27 is the most westward of the three ecoregions. The average temperatures in the region range from a low of minus eight and high of 13 °C during January and a low of 19 and high of 37 °C during July. The temperature increases moving southwards in the ecoregion. The vegetation in the ecoregions was once a mostly mixed-grass prairie but has evolved into mostly cropland today. Range and grassland are mostly located in more rugged areas (Griffith et. al. 2004). The terrain of the region mainly consists of irregular plains and broad alluvial valleys. The hydrology of the area includes mostly intermittent streams with some perennial streams and a few natural lakes. There are some large rivers such as Red and Brazos River, which pass through the region as well. The larger rivers have braided sandy channels with mostly turbid flows. After heavy rains, the streams have stronger flows with large amounts of suspended sediments. The streams draining from rangeland contain less sediment load than the streams downstream of croplands. Many streams in the region have been channelized and/or impounded leading to unnatural flow regimes, higher erosion, and loss of the riparian forests. The precipitation in the area ranges from 560 to 965 mean annual millimeters (mm) and increases from west to east and the region is mostly semi-arid in nature. The wildlife in region mostly consists of white-tailed deer, mule deer, pronghorn, coyote, and jackrabbit. The land use in the region is dominated by dryland and irrigated cropland with some pasture and rangeland also occurring. Grazing for cattle, sheep and goat along with oil and gas production is also a common land use. (Griffith et. al. 2004; Woods et. al. 2005).

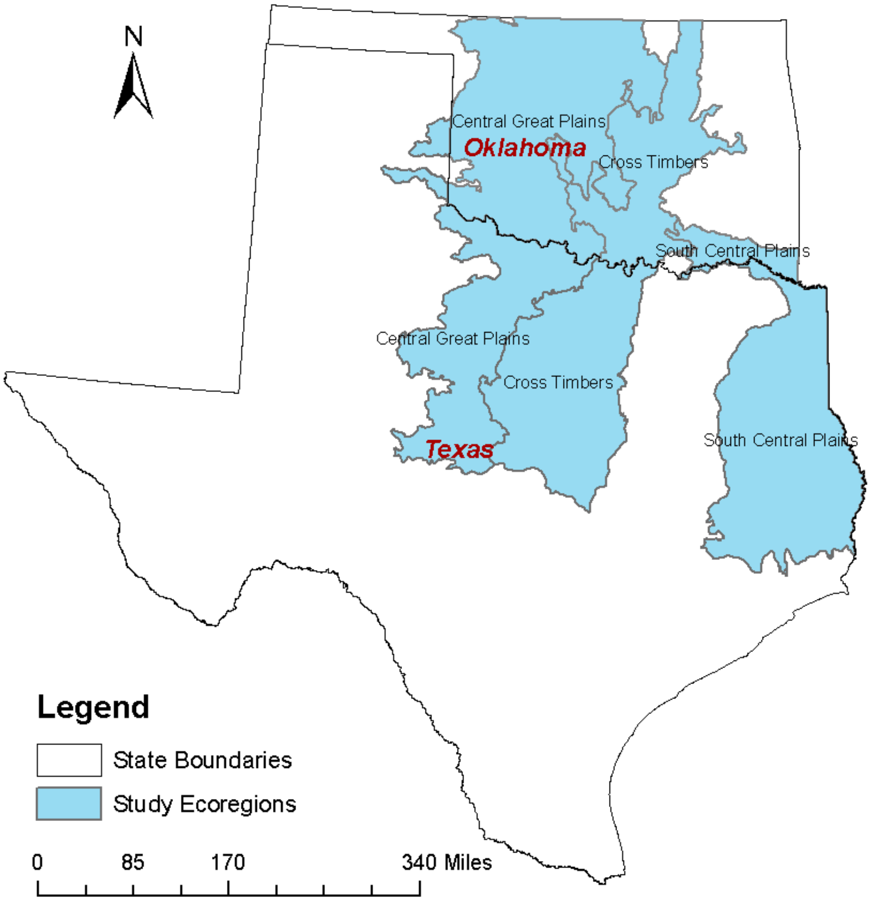


Figure 2. Study area comprising of ecoregions 27, 29, and 35 in Texas and Oklahoma.

Ecoregion 29 occurs in the north-central Texas, central Oklahoma, and southeastern Kansas and is located between ecoregions 27 and 35 the other two ecoregions included in this study. The region is transitional area between winter wheat growing areas in the ecoregion 27 and the forested low mountains of eastern Oklahoma.

Vegetation is mainly comprised of forest, woodland, savannah, and prairie. Cropland is not very common and is restricted to valleys near channelized streams. Both intermittent and perennial streams can be found in the region, which have a low to moderate gradient due to the rolling plains type terrain found in the region. Several large rivers cross the ecoregions along with some large reservoirs and lakes to serve the urban areas located in the region. The temperature in the region ranges from a low of minus six and high of 14 °C during January and a low of 21 and high of 36 °C during July. The precipitation in the region ranges from 780 to 1170 mean annual mm with higher precipitation occurring on the eastern side of the region. Wildlife in the region mainly consists of white-tailed deer, gray fox, bobcat, black-tailed jackrabbit, and prairie chicken etc. Main landuse in the area is pastureland and rangeland. Areas of woodland are also dominant, along with some cropland. Oil and gas production is a major landuse and the region also includes some major urban centers such as Dallas, Fort Worth, and Arlington in Texas. (Griffith et. al. 2004; Woods et. al. 2005).

Ecoregion 35 is the eastern region in the study area. The region comprises mainly a temperate coniferous forest with several species of pine along with hardwoods such as hickory and oak. Approximately one sixth of the region consists of cropland mainly within the Red River floodplain. Perennial streams are most common in the region but the flow can become limited during the summer months. The streams located in the forested regions normally have lower concentrations of suspended solids whereas the Red River the largest river flowing through the region is mostly turbid. The region lacks natural lakes but some reservoirs have been built within the region to account for inconsistent flow. The average temperatures range from a low of minus two and high of 16 °C during January and a low of 21 and high of 34 °C during July. The precipitation ranges between 1066 to 1422 mean annual mm with higher rainfall towards the east. The wildlife mainly consists of white-tailed deer, coyote, beaver, raccoon, muskrats, and rabbits. Major land uses include commercial pine plantations, timber production, livestock grazing, and oil and gas production. (Griffith et. al. 2004; Woods et. al. 2005).

The three ecoregions have very different characteristics as detailed above. Major differences include the amounts of precipitations and temperatures as well as the hydrology of each region. The type of vegetation changes as we move eastward from dryland and irrigated cropland in ecoregion 27 to a predominantly coniferous forest in ecoregion 35. The analysis of *E. coli* relationship with stream orders and watershed area by ecoregion will be an important part of this study to assess if these differences in characteristics have any bearing on the *E. coli* relationships.

Data Sources

Where available the data for the study ecoregions was collected for each monitoring station location in the study area. All of the *E. coli* and monitoring sites data for this study was collected from TCEQ (TCEQ 2014, updated daily) for the Texas portion of the ecoregions and the OCC for the Oklahoma portion of the ecoregions. The *E. coli* reported by TCEQ was in the units of MPN/100ml (most probable number/100 milliliter). TCEQ data was collected from the years of 2006 to 2014. Similarly, the OCC data from 2006 to 2013 was used. The most recent 7 years data was used in the study to ensure that the data is representative of the current hydrologic and environmental condition. Hydrologic regimes, precipitation patterns, and land use practices all change over time and can have a big impact on both the *E. coli* concentrations and its transport through the streams. Use of recent data allows for investigation of current conditions and ensures that historic conditions do not affect the results. The *E. coli* observations for each monitoring site ranged from five to 112. On average, there were 28 observations per monitoring site and median number of observations was 22. Out of the final 742 monitoring sites selected for this study, 74 sites had less than 10 *E. coli* observations available while 245 monitoring sites had more than 28 observations.

TCEQ provided the ID's of monitoring sites along with the *E. coli* data. These site ID's were matched with the TCEQ GIS layer for monitoring sites (TCEQ 2014a) to conduct the GIS steps of this study. OCC provided the longitude and latitudes for each of their monitoring sites, which were used to determine the geographic location of the sites for the GIS steps. Figure 3 shows the geographic location of the monitoring sites and wastewater outfalls in the study area. The ecoregion boundaries were obtained from the EPA website (USEPA 2014b). The flow direction and flow accumulation raster's based on 30-m digital elevation models (DEMs), which are provided in the NHDPlus Version 2, were obtained from Horizons Systems Corporation (Horizon 2014). The land cover/land use data for the watersheds was obtained from the USDA National Agricultural Statistics Service (NASS) 2013 Cropland Data Layer from USDA CropScape (NASS 2014). Wastewater outfall locations were obtained from TCEQ (TCEQ 2014a) for Texas regions and from EPA (USEPA 2014c) for Oklahoma regions.

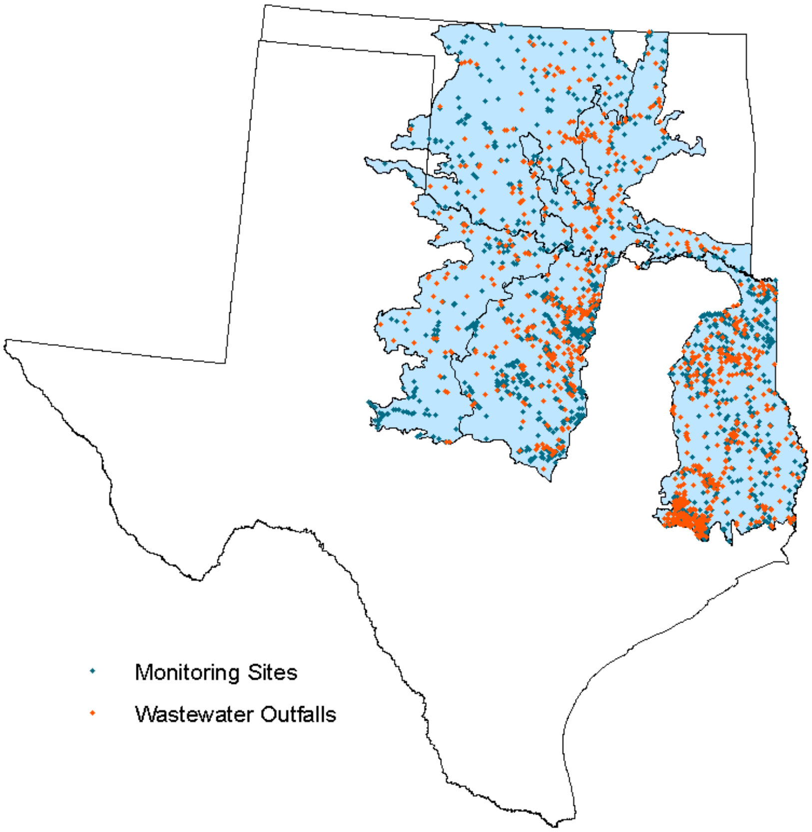


Figure 3. Location of monitoring sites and wastewater outfalls in the study area.

GIS Methods

Stream delineation and stream order calculation

Streams were delineated using the ArcHydro add-in for ArcMap. The flow direction and flow accumulation raster's obtained from Horizon were used as inputs in the ArcHydro stream definition tool. The stream threshold was selected at 5000 30 x 30 m cells (an area of 4.5 km2) for catchment definition, which is based on the USGS Elevation Derivatives for National Applications (EDNA 2014). The threshold determines the density of the stream network. For example, a smaller threshold will result in a denser stream network and a larger threshold will result in a less dense stream network. The stream raster is then used in the stream segmentation and drainage line processing tools to get the stream vectors. The monitoring stations locations are added from TCEQ for Texas regions and from the latitude and longitude provided by OCC in ArcMap. The Monitoring stations are snapped using the Snap Pour Point tool to the stream segments if they are within 200 meter of the stream segment. This slightly moved the monitoring location site so that stream order could be extracted. Not all monitoring sites were snapped to streams using a 200-meter distance and those sites were excluded from statistical analysis.

Watershed and urban area calculation

Using the watershed tool in the Spatial Analyst toolset in ArcMap, the watershed raster's are delineated for each monitoring station point. The flow direction raster and the snap pour points are used as inputs in the watershed tool to get the watershed area. Once the watershed raster's are calculated, the Raster to Polygon conversion tool in ArcMap is used to get vector polygons for each watershed.

Urban area polygons are determined from the cropland GIS layer (NASS 2014) and are delineated for each watershed using the clip tool in ArcMap. The percentage of each watershed's urban area is then determined by dividing the area of the urban area within each watershed by the total watershed area and added to the attributes of the monitoring station layer. The wastewater outfall layers obtained from TCEQ and EPA were added to ArcMap at this point. The count of wastewater outfalls in each watershed was added to the attributes of the monitoring station shapefile. The attribute table of the monitoring station layer, which includes urban area percentages, watershed area in square meters (m), wastewater outfall counts, and the Shreve and Strahler stream orders, is exported to MS Excel for further analysis.

The stream order is obtained using the spatial analyst toolset in the ArcMap. Stream order tool in the spatial analyst menu is applied to determine the stream order of each flow line segment in the stream raster using flow accumulation and flow direction raster's. The tool allows calculation of a stream order raster based on both Strahler and Shreve stream order systems. Once the stream order raster is calculated, the Raster to Poly line tool is used to convert the stream order raster into a stream vector shapefile. This shapefile contains the stream order for each segment in its attributes. Stream vector is then joined with the monitoring stations to get the stream order of each monitoring site.

Unimpacted watersheds

The criterion for unimpacted watersheds was based on the percentage of urban areas and the number of wastewater outfall. Since the volume discharge of each wastewater outfall was not readily available from EPA, only the watersheds that did not have a wastewater outfall were selected as unimpacted. This lead to 278 monitoring sites with no wastewater outfall in their watersheds. To identify the rural watersheds, only the watersheds that had a maximum of 10 percent urban area were selected in the unimpacted classification. This classification was made after considering other thresholds of 50, 25, and 5 percent urban areas and conducting the statistical tests discussed in Chapter III. The statistical tests showed that urban area percentage did not have a major impact on the significance of the results therefore the 10 percent threshold was chosen as representative of below which an area would be considered rural. This classification lead to a final data with 252 monitoring sites which also was not a significant reduction from the 278 sites that did not have a wastewater outfall in the their watersheds.

CHAPTER III

STATISTICAL ANALYSIS

Materials and Methods

This chapter will discuss the statistical methods undertaken to evaluate relationships between *E. coli* and the two stream order methods and *E. coli* and watershed areas, which were obtained from the GIS methods in Chapter II. *E. coli* data was collected by TCEQ and OCC over the 7-year study period at irregular intervals and at different flow condition for many of the monitoring sites. This can affect the *E. coli* concentrations measured in the stream since very low and very high concentrations can be result of specific conditions such as floods or droughts in the area or landuse practices that are time sensitive. Median *E. coli* would be representative of the normal conditions of the stream eliminating the too low or too high concentrations, which may occur due to other than normal conditions and was therefore used as the metric in this study. Average *E. coli* measurements were also considered but not used as many of the sites in the study had less than 10 observations and a small number of high or low observations could easily skew the average, which would not be representative of the normal stream conditions. Another measure that would be appropriate for similar analysis but was not considered for this study is the geometric mean, which is the nth root of the product of n numbers. Geometric mean also reduces the effect of very low and very high numbers and could be an appropriate measure for a future study of similar nature.

For calculation of median *E. coli*, monitoring stations data was first sorted based on the *E. coli*'s date of observation. Only the monitoring stations that had minimum of five *E. coli* observations during the study period of January 2006 to May 2014 were included in the analysis. Cursory analysis of sites that had less than five observations showed that those observations were collected either too sporadically or in a short frame of time. These sites were also impacted by high and low concentrations more severely, which made the use of median inappropriate. Therefore, a cutoff was made at five *E. coli* observations per site to calculate median *E. coli*, which would be better representative of the normal stream conditions. Using the median function in MS Excel, the median for each monitoring station was obtained. The data obtained from GIS methods, which included stream orders (both Strahler and Shreve), watershed area, urban area percentages, ecoregion number, and the wastewater outfall count for each monitoring site, was joined with *E. coli* data. The monitoring stations, which did not snap to 200 meters of a stream segment or lacked sufficient *E. coli* data, were eliminated from analysis. This resulted in a final database of 742 monitoring stations for the study regions.

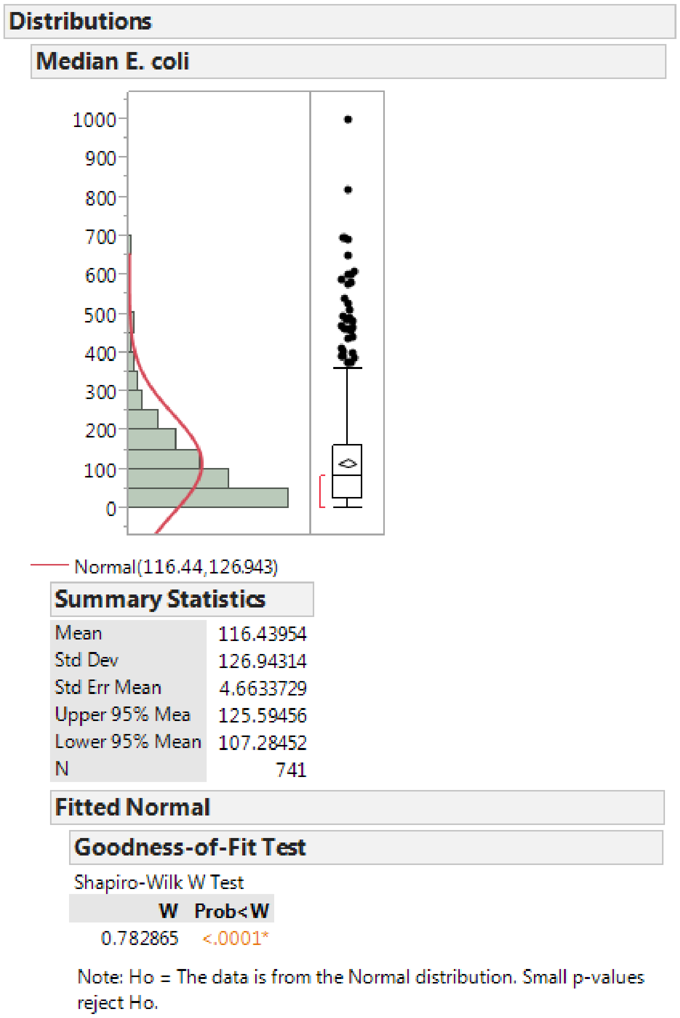
The data included a maximum median *E. coli* concentration of 2400 MPN/100ml with next largest value of 1000 MPN/100ml. The maximum *E. coli* concentration occurred at monitoring station number 10786 in ecoregion 29. The monitoring station had six *E. coli* observation all made between 11/22/2011 to 05/22/2012 with four observations that had concentrations of 2400 MPN/100ml or more. Since all of the *E. coli* observations were made within 7 months and had high concentration values it was decided to eliminate this monitoring site from analysis as the period when observations were collected was too small and concentrations measured may not have been representative of the true conditions at the site.

From this database, two subsets were extracted. For the first subset only those monitoring sites were selected where the urban area percentage was 10 percent or less. In the second subset, monitoring sites with wastewater outfalls in their watersheds were also removed in addition to limiting the urban area at less than 10 percent, which is the unimpacted watersheds definition for this study. The following three datasets were then analyzed using statistical techniques:

a) All sites with at least five observations;
b) Sites with 10 percent or less urban area in their watersheds;
c) Unimpacted watershed sites with 10 percent or less urban area and no wastewater outfalls in their watersheds.

The first two datasets are referred to as impacted with wastewater outfall (WWTF) datasets in this study. All three data sets were analyzed in the JMP statistical software (JMP®, Version *11*) according to procedures described by Helsel and Hirsch (2002) and Haan (2002).

The *E. coli* data was first analyzed in its entirety without the consideration of impacted or unimpacted monitoring sites to determine the suitability of the tests. The analysis of *E. coli* showed that it was not normally distributed as shown in Figure 4 below. The p-value for the Shapiro Wilk test for the goodness of a normal fit was less than 0.0001, which signifies that the data is not normally distributed. The box plot analysis in Figure 4 shows most of the *E. coli* observations over 400 MPN/100ml are plotted as outliers. Similarly lognormal and exponential distribution goodness of fit tests showed p-values of 0.01, which signified a lack of fit for these distributions as well. Based on these results the non-parametric tests are most suitable for this dataset.



Summary Statistics

Mean	116.43954
Std Dev	126.94314
Std Err Mean	4.6633729
Upper 95% Mea	125.59456
Lower 95% Mean	107.28452
N	741

Fitted Normal

Goodness-of-Fit Test

Shapiro-Wilk W Test

W	Prob<W
0.782865	<.0001*

Note: Ho = The data is from the Normal distribution. Small p-values reject Ho.

Figure 4. Goodness of fit normality test for *E. coli* distribution.

The non-parametric analysis of the data was conducted using Wilcoxon/Kruskall-Wallis tests for Strahler and modified Shreve stream orders with a null hypothesis of mean of each monitoring sites median *E. coli* being equal at each stream order. The alpha threshold of 5 percent was used to determine the significance of the probability values obtained throughout the analysis. The same analysis for each stream order method was conducted at ecoregion scale as well. Further, each stream order method was tested for correlation between stream order and *E. coli* concentration using Kendall τ and Spearman's ρ.

Continuous nonlinear tests were used to evaluate if a nonlinear distribution would fit the *E. coli* data with respect to Shreve order and watershed size since they represent continuous variables. Data distributions modeled using nonlinear fit allow testing of the correlation using the parametric Pearson's r coefficient. Non-parametric Kendall τ and Spearman's ρ tests were used to determine correlation between *E. coli* and watershed size/Shreve stream order when nonlinear models were not applicable. Kendall τ and Spearman's ρ are monotonic correlation tests and apply well to the data similar to this study. These tests are also resistant to the effect of outliers. The Kendall's τ approaches a normal distribution more rapidly than ρ, as N, the sample size, increases. τ is also a rank based test and is therefore more resistant to small number of unusual values (Helsel and Hirsch 2002).

The correlation coefficient values range from -1 to +1. The values closer to ±1 indicate that the variables are highly correlated and the values closer to zero indicate they are not correlated. Negative correlation signifies that the variables are inversely correlated such that one variable decreases when the other increases. Conversely, a positive coefficient means the variables are moving in the same direction. The tests also provide a probability value for the correlation co-efficient, which is compared at the 5% alpha threshold. If the p-value is less than 0.05 then the test is significant and there is correlation present between the x and y variables.

Results and Discussion

Analysis of median E. coli *by Strahler stream order*

Data distribution

The Strahler stream order for the study areas ranged from one to seven for all data. However, when only unimpacted watersheds are considered, the range is reduced to orders one to five due to reduction in the sampled data.

Table 1 summarizes the number of monitoring site at each stream order for the three datasets analyzed in this study and the median *E. coli* concentration for those sites. The table shows a consistent *E. coli* distribution for all three datasets with increasing *E. coli* for the first three orders and a drop in the concentrations for later orders. Study conducted by Edge et. al. (2012) had found similar relationships between stream order and *E. coli* concentrations where the concentrations increased for the first three orders and then decreased. Another study by VDEQ (2003) had found the highest fecal coliform concentration at orders 3 and 4, which can also be seen in the table below for datasets with limited urban areas. For unimpacted watersheds, it is significant to note that the median *E. coli* concentration increases initially and then remains constant. The median *E. coli* for each of the three datasets stays below the geometric mean standard of 126 cfu/100ml required for primary contact recreation in Texas but approaches the standard limit at stream order 3.

Table 1. Summary of *E. coli* by Strahler stream order for a) all monitoring sites, b) sites with less than 10% urban area, and c) unimpacted sites.

	a) All moitoring sites		b) <10% Urban area		c) Unimapcted watersheds	
Strahler stream order	Number of monitoring sites	Median *E. coli*	Number of monitoring sites	Median *E. coli*	Number of monitoring sites	Median *E. coli*
1	34	91.75	22	65.25	22	65.25
2	144	95.00	113	80.00	82	80.00
3	214	106.50	194	105.00	99	100.00
4	175	93.00	171	90.00	39	100.00
5	106	62.00	106	62.00	10	102.25
6	30	28.00	27	26.00		
7	38	21.25	37	20.50		
Total	741	81.00	670	78.00	252	93.75

Analysis of *E. coli* at ecoregion scales reveals differences in the *E. coli* concentration in each ecoregion. Table 2, 3, and 4 provide a breakdown of the distribution for each ecoregion. Ecoregion 27 shows consistently higher median *E. coli* concentrations than the other two ecoregions. The differences in median *E. coli* between ecoregions 29 and 35 are not as significant but at higher stream orders ecoregion 35 has lower *E. coli* than the other two ecoregions. Ecoregion 27 is also the only ecoregion that shows a consistently decreasing trend in *E. coli* concentrations as stream order increases for all three datasets. This trend is similar to what has been found by Harmel et. al. (2010) and Lyautey et. al. (2010) in their studies. The median *E. coli* concentrations in ecoregion 27 are also higher than the geometric mean standard of 126 cfu/100ml required for primary contact recreation for the first four stream orders in all three datasets.

Ecoregion 29 has a decreasing trend for median *E. coli* concentration for all data similar to ecoregion 27. However, for less than 10 percent urban area and unimpacted datasets there is increasing *E. coli* concentrations for first few orders and then decreasing concentrations. Ecoregion 35 shows an increasing trend at first and then a decreasing one for all datasets. Both ecoregion 29 and 35 have median *E. coli* values much lower than the 126 cfu/100ml required for primary contact recreation.

Table 2. Median *E. coli* concentrations by Strahler stream order and ecoregions for all monitoring sites.

Strahler stream order	Number of monitoring sites			Median *E. coli*		
	Ecoregion 27	Ecoregion 29	Ecoregion 35	Ecoregion 27	Ecoregion 29	Ecoregion 35
1	2	22	10	293.75	92.50	66.50
2	16	65	63	192.50	79.50	104.00
3	59	66	89	155.00	80.75	110.00
4	54	59	62	135.00	61.00	89.00
5	31	45	30	70.00	62.00	34.50
6	19	1	10	30.50	130.00	25.00
7	5	27	6	37.00	20.00	21.25

Table 3. Median *E. coli* concentrations by Strahler stream order and ecoregions for sites with <10% urban area.

Strahler stream order	Number of monitoring sites			Median *E. coli*		
	Ecoregion 27	Ecoregion 29	Ecoregion 35	Ecoregion 27	Ecoregion 29	Ecoregion 35
1	2	12	8	293.75	65.25	66.50
2	16	53	44	192.50	79.00	82.75
3	58	56	80	152.50	76.00	104.00
4	55	58	58	140.00	58.50	87.00
5	30	46	30	69.50	64.50	34.50
6	20	1	6	30.75	130.00	17.50
7	4	27	6	23.50	20.00	21.25

Table 4. Median *E. coli* concentrations by Strahler stream order and ecoregions for unimpacted watershed sites.

Strahler stream order	Number of monitoring sites			Median *E. coli*		
	Ecoregion 27	Ecoregion 29	Ecoregion 35	Ecoregion 27	Ecoregion 29	Ecoregion 35
1	2	12	8	293.75	65.25	66.50
2	14	39	29	192.50	75.00	92.50
3	40	25	34	120.00	104.00	76.00
4	23	9	7	185.00	50.00	45.00
5	10			102.25		

Figure 5 shows a graphic comparison of *E. coli* at different stream orders for all datasets by ecoregions. The figure shows an increasing *E. coli* trend for ecoregions 29 and 35 in the first three stream orders and then a decreasing trend for remaining orders. Ecoregion 27 shows a decreasing trend only from lower to higher stream orders. For ecoregion 27, there is an increase in *E. coli* at order 6, which can be attributed to a lack of observations, as there is only one monitoring station available with *E. coli* data.

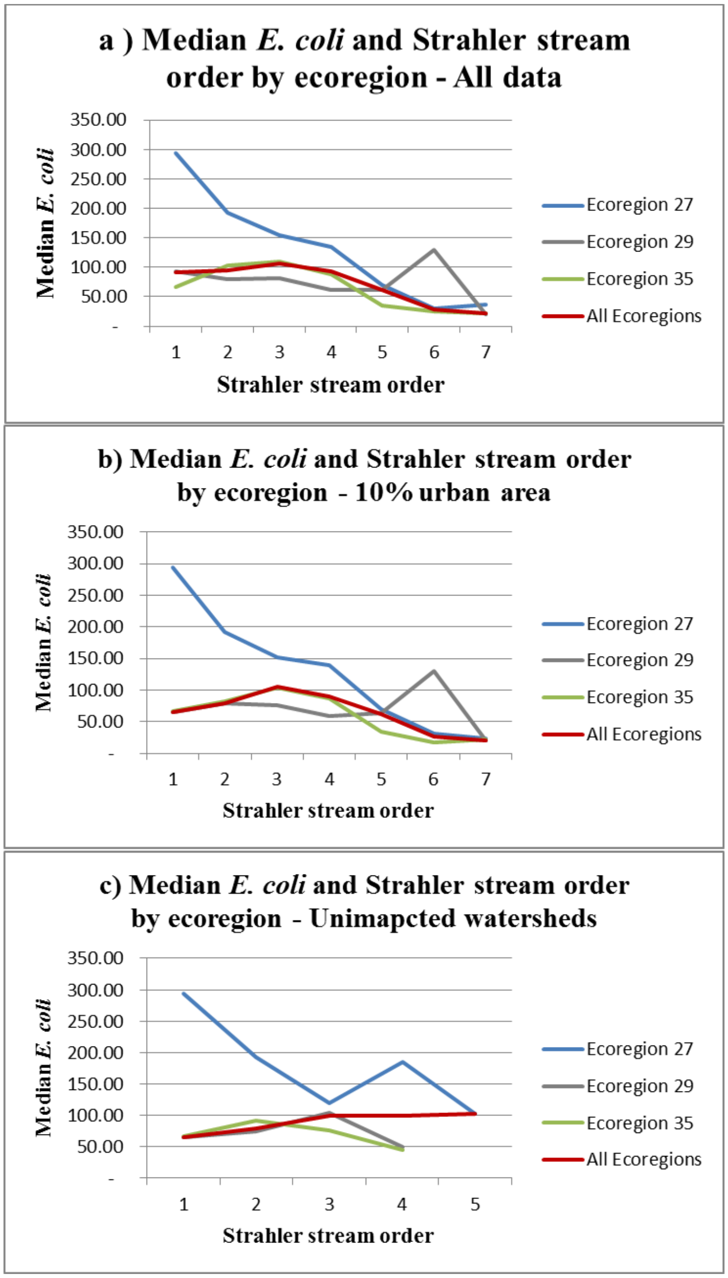


Figure 5. Comparison of median *E. coli* concentrations by Strahler stream order and ecoregions.

Wilcoxon/Kruskall-Wallis test

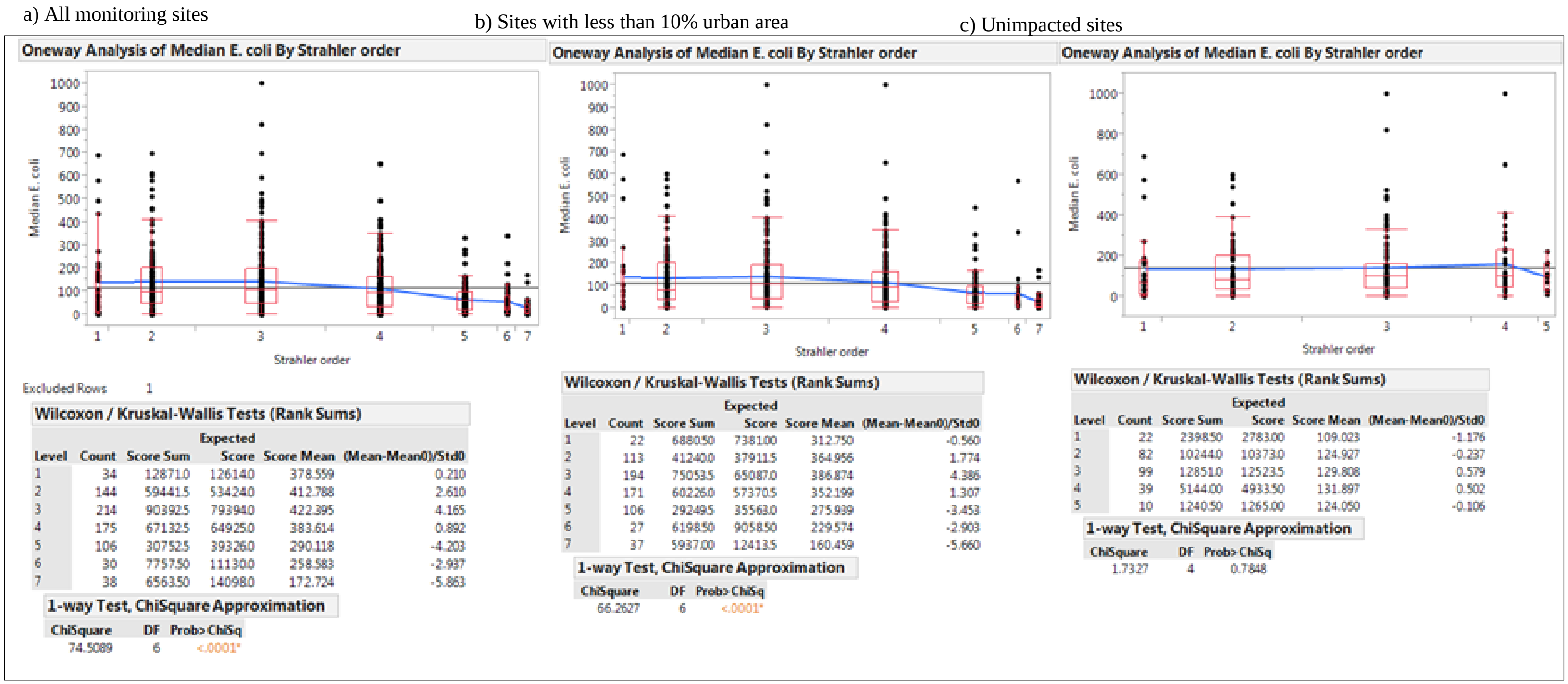
Strahler stream order follows discrete variable characteristics and along with non-normality of the *E. coli* data, the Wilcoxon/Kruskall-Wallis test is the most suitable nonparametric test for this method. Figure 6 shows the results of the tests for the three datasets below. The tests were run using JMP software ((JMP®, Version *11*) using JMP software procedures outlined by Schlotzhauer (2007).

The Wilcoxon/Kruskall-Wallis method tests the null hypothesis that the mean ranks of observations at each stream order are equal i.e.:

- Null hypothesis (H_0): μ(stream order 1) = μ(stream order 2) = … = μ(stream order 7)
- Alternate hypothesis (H_a): at least two means are different.

In this study, the observations are the median *E. coli* calculated for each monitoring site to which Wilcoxon/Kruskall-Wallis method will be applied. For example in Figure 6a the level column represents the stream order, the count column represent the number of monitoring sites with median *E. coli*, score sum and expected score columns are computed based on the test formula using the median *E. coli*, and score mean is score sum divided by the count. This score mean is the statistics that is compared by the test to calculate the significance probabilities. The test results are deemed significant, i.e. at least two score means are different, if the Chi Square p-value is less than $\alpha = 0.05$. The p-value for the WWTF impacted datasets are less than <0.0001 from which it can be concluded that there are at least two stream orders with different means. Whereas the p-value of 0.7848 for unimpacted watersheds is not significant and it can be concluded that, the score means for all stream orders are equal. The plots in Figure 6 show the median *E. coli* plotted on the Y-axis and Strahler stream order on X-axis. The median *E. coli* for each monitoring site in the analysis is plotted as dots in the figure. The straight grey line horizontal to the x-axis is the grand mean of each dataset and the blue line is connecting the mean of each stream order's median *E. coli*.

Further analysis for each pair of stream orders was conducted using nonparametric comparisons for each pair using the Wilcoxon method. The comparison of each pair showed that most of the pairs of stream orders for impacted datasets had different score means at 5% alpha as seen in Table 5. Whereas all pairs for unimpacted sites showed score means were statistically equal using the 5% alpha threshold.



a) All monitoring sites

Oneway Analysis of Median E. coli By Strahler order

Excluded Rows 1

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
1	34	12871.0	12614.0	378.559	0.210
2	144	59441.5	53424.0	412.788	2.610
3	214	90392.5	79394.0	422.395	4.165
4	175	67132.5	64925.0	383.614	0.892
5	106	30752.5	39326.0	290.118	-4.203
6	30	7757.50	11130.0	258.583	-2.937
7	38	6563.50	14098.0	172.724	-5.863

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
74.5089	6	<.0001*

b) Sites with less than 10% urban area

Oneway Analysis of Median E. coli By Strahler order

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
1	22	6880.50	7381.00	312.750	-0.560
2	113	41240.0	37911.5	364.956	1.774
3	194	75053.5	65087.0	386.874	4.386
4	171	60226.0	57370.5	352.199	1.307
5	106	29249.5	35563.0	275.939	-3.453
6	27	6198.50	9058.50	229.574	-2.903
7	37	5937.00	12413.5	160.459	-5.660

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
66.2627	6	<.0001*

c) Unimpacted sites

Oneway Analysis of Median E. coli By Strahler order

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
1	22	2398.50	2783.00	109.023	-1.176
2	82	10244.0	10373.0	124.927	-0.237
3	99	12851.0	12523.5	129.808	0.579
4	39	5144.00	4933.50	131.897	0.502
5	10	1240.50	1265.00	124.050	-0.106

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
1.7327	4	0.7848

Figure 6. Wilcoxon/Kruskall-Wallis test of *E. coli* by Strahler order at a) all monitoring sites, b) sites with up to 10% urban area, and c) unimpacted monitoring sites.

Table 5. Strahler stream order pairs with equal score means using Wilcoxon each pair test.

a) All monitoring sites			b) <10% Urban sites			c) Unimpacted sites		
Stream order	Stream order	p-Value	Stream order	Stream order	p-Value	Stream order	Stream order	p-Value
1	2	0.4750	1	2	0.3211	1	2	0.3177
1	3	0.3921	1	3	0.1625	1	3	0.2476
1	4	0.9913	1	4	0.4421	1	4	0.2998
1	5	0.0669	1	5	0.6698	1	5	0.5150
1	6	0.1048	1	6	0.4269			
1	7	0.0011	1	7	0.0322			
2	3	0.6830	2	3	0.3660	2	3	0.6240
2	4	0.2095	2	4	0.5420	2	4	0.6314
2	5	<.0001	2	5	0.0007	2	5	0.9600
2	6	0.0004	2	6	0.0016			
2	7	<.0001	2	7	<.0001			
3	4	0.0613	3	4	0.0757	3	4	0.8629
3	5	<.0001	3	5	<.0001	3	5	0.8419
3	6	0.0001	3	6	0.0001			
3	7	<.0001	3	7	<.0001			
4	5	0.0002	4	5	0.0007	4	5	0.6822
4	6	0.0026	4	6	0.0020			
4	7	<.0001	4	7	<.0001			
5	6	0.2263	5	6	0.1000			
5	7	<.0001	5	7	<.0001			
6	7	0.0391	6	7	0.0694			

The Wilcoxon/Kruskall-Wallis test for each of the ecoregions showed similar results to the ones for entire study area. The main challenge arising for the test at the ecoregion level was the lack of observations at certain stream orders. As seen in Table 2, 3, and 4, stream order 6 in ecoregion 29 has only one monitoring site and a few others have less than 10 sites. This causes problems comparing score means of these low observation orders with better populated orders. The score means of the lower count stream orders will be skewed and the comparisons may provide us with incorrect significance values for alpha comparison. However, even considering this issue, the Wilcoxon test results were very similar, significance wise, for the three datasets. The impacted datasets had significance probabilities of less than 0.05, which meant that at least two of the stream order score means were different in those datasets. The unimpacted sites data set had probabilities of greater than 0.05 for all three ecoregions signifying that the score means of the stream orders were equal for all stream orders. Table 6 summarizes the Chi square probabilities for each test.

Table 6. Wilcoxon/Kruskall-Wallis test probabilities by ecoregion.

Ecoregion	Probability > Chi Square		
	All monitoring sites	<10% Urban sites	Unimpacted sites
27	<0.0001	<0.0001	0.2446
29	<0.0001	0.0002	0.4076
35	0.0085	0.0144	0.5716

Correlation tests

Nonparametric Kendall τ and Spearman's ρ correlation tests were applied to the three datasets with the results shown in Table 7. The WWTF impacted datasets showed significant correlation probabilities but the correlation co-efficient for both were closer to 0 to than -1. This meant that the test did show there was a statistically significant negative correlation between Strahler order and median *E. coli* but it was a weak correlation. The correlation test for unimpacted sites resulted in a non-significant probability. The correlation co-efficient was also close to zero which means there was no correlation between stream order and median *E. coli* of unimpacted monitoring sites.

Table 7. Correlation values between *E. coli* and Strahler stream order.

Test	All monitoring sites		<10% Urban sites		Unimpacted sites	
	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.2491	<0.0001	-0.2346	<0.0001	0.0622	0.3254
Kendall's τ	-0.1882	<0.0001	-0.1786	<0.0001	0.0483	0.3128

The correlation tests at the ecoregion level revealed similar results to the analysis of all data. Table 8 shows the correlation co-efficient and the probability associated with them. Ecoregion 27 showed much stronger correlation between *E. coli* and stream order for the impacted datasets compared to the other two ecoregions but it was still lower than -0.50 for both Kendall τ and Spearman’s ρ which would be the criterion for a strong correlation. Ecoregion 29 and 35 showed very similar correlation coefficients and stayed with the trend where the *E. coli* concentrations in the unimpacted monitoring sites were not correlated with the Strahler stream order.

Another observation was the reduction in correlation coefficient values as the data moved eastward i.e. ecoregion 27 had the highest coefficients followed by 29 and lastly ecoregion 35. This signified that *E. coli* became less correlated to stream orders moving eastward.

Table 8. Correlation values between median *E. coli* and Strahler stream order at ecoregion level.

Ecoregion 27						
	All monitoring sites		<10% Urban sites		Unimpacted sites	
Test	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.4795	<0.0001	-0.4476	<0.0001	-0.1444	0.1768
Kendall's τ	-0.3696	<0.0001	-0.3447	<0.0001	-0.116	0.153
Ecoregion 29						
	All monitoring sites		<10% Urban sites		Unimpacted sites	
Test	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.2785	<0.0001	-0.2431	<0.0001	0.0673	0.5405
Kendall's τ	-0.2051	<0.0001	-0.1778	<0.0001	0.0592	0.4814
Ecoregion 35						
	All monitoring sites		<10% Urban sites		Unimpacted sites	
Test	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.1787	0.0032	-0.1627	0.0131	-0.154	0.1784
Kendall's τ	-0.1424	0.0017	-0.1359	0.0055	-0.1177	0.1846

Analysis of median E. coli *by modified Shreve stream order*

Data distribution

Since Shreve orders are distributed from one to 7,447 for the 742 monitoring sites in the data, the stream orders were grouped to apply the non-parametric Wilcoxon/Kruskall-Wallis test. The GIS calculated stream order were grouped into 9 categories with the first three orders being kept the same as the original order, and remaining observations distributed fairly equally in groups 4 to 9 as seen in Table 9. This was an important step since large numbers of orders can invalidate the

Wilcoxon/Kruskall-Wallis test. In addition, small numbers of observations i.e. 10 or less for each stream order may also produce less reliable statistics.

Table 9. Modified Shreve stream orders for Wilcoxon/Kruskall-Wallis test (all monitoring sites).

Shreve modified	Shreve order	No. of monitoring sites
1	1	34
2	2	36
3	3	31
4	4-8	109
5	9-16	108
6	17-33	108
7	34-97	108
8	98-374	108
9	375-7,447	100

Similar to the Strahler order the modified Shreve order showed that the median *E. coli* for each stream order increased initially and decreased for higher stream orders. The analysis of the *E. coli* at the ecoregion scale also had a similar pattern to the Strahler order where Ecoregion 27 had consistently higher median *E. coli* compared to ecoregions 29 and 35. Lack of observations at ecoregion scale was another issue that was common with a few orders having less than 10 monitoring sites.

Table 10. Summary of *E. coli* by modified Shreve stream order for all monitoring sites.

All monitoring sites

Modified Shreve stream order	Number of monitoring sites	Median *E. coli*
1	34	91.75
2	36	88.50
3	31	95.00
4	109	96.00
5	107	120.00
6	108	112.50
7	108	100.00
8	108	56.00
9	100	26.00
Total	741	81.00

Table 11. Median *E. coli* concentrations by Shreve stream order and ecoregion for all monitoring sites.

Modified Shreve stream order	Number of monitoring sites			Median *E. coli*		
	Ecoregion 27	Ecoregion 29	Ecoregion 35	Ecoregion 27	Ecoregion 29	Ecoregion 35
1	2	22	10	293.75	92.50	66.50
2	2	21	13	355.00	75.00	104.00
3	2	14	15	58.75	87.00	130.00
4	18	38	53	295.00	80.00	97.00
5	30	34	43	172.50	100.50	110.00
6	36	35	37	150.50	93.00	103.00
7	36	35	37	111.25	73.50	120.00
8	27	48	33	90.00	42.00	55.50
9	33	38	29	37.00	23.75	25.00

Table 12. Median *E. coli* concentrations by Shreve stream order and ecoregion for <10% urban area.

Modified Shreve stream order	Number of monitoring sites			Median *E. coli*		
	Ecoregion 27	Ecoregion 29	Ecoregion 35	Ecoregion 27	Ecoregion 29	Ecoregion 35
1	4	28	16	353.75	72.00	54.25
2	4	18	19	60.00	78.00	70.00
3	9	16	19	185.00	79.50	90.00
4	8	13	17	295.00	80.00	90.00
5	23	15	29	200.00	96.00	117.50
6	36	37	37	122.50	75.00	97.00
7	39	35	35	140.00	95.00	110.00
8	28	52	29	88.75	47.50	49.00
9	34	39	31	39.00	24.00	25.00

Table 13. Median *E. coli* concentrations by Shreve stream order and ecoregion for unimpacted watershed sites.

Modified Shreve stream order	Number of monitoring sites			Median *E. coli*		
	Ecoregion 27	Ecoregion 29	Ecoregion 35	Ecoregion 27	Ecoregion 29	Ecoregion 35
1	3	26	13	577.50	69.00	100.00
2	8	19	18	127.50	77.00	59.75
3	10	11	13	180.00	80.00	90.00
4	13	6	12	160.00	105.00	108.75
5	9	11	11	80.00	120.00	88.00
6	20	4	7	117.50	84.00	4.00
7	26	8	4	128.75	77.50	34.00

Figure **7** shows a graphic comparison of median *E. coli* of each stream order and the trend of decreasing *E. coli* at higher stream orders can be clearly seen for the impacted datasets. On the other hand, the unimpacted watershed dataset has a minimal difference at higher stream orders and median *E. coli* stays flat throughout.

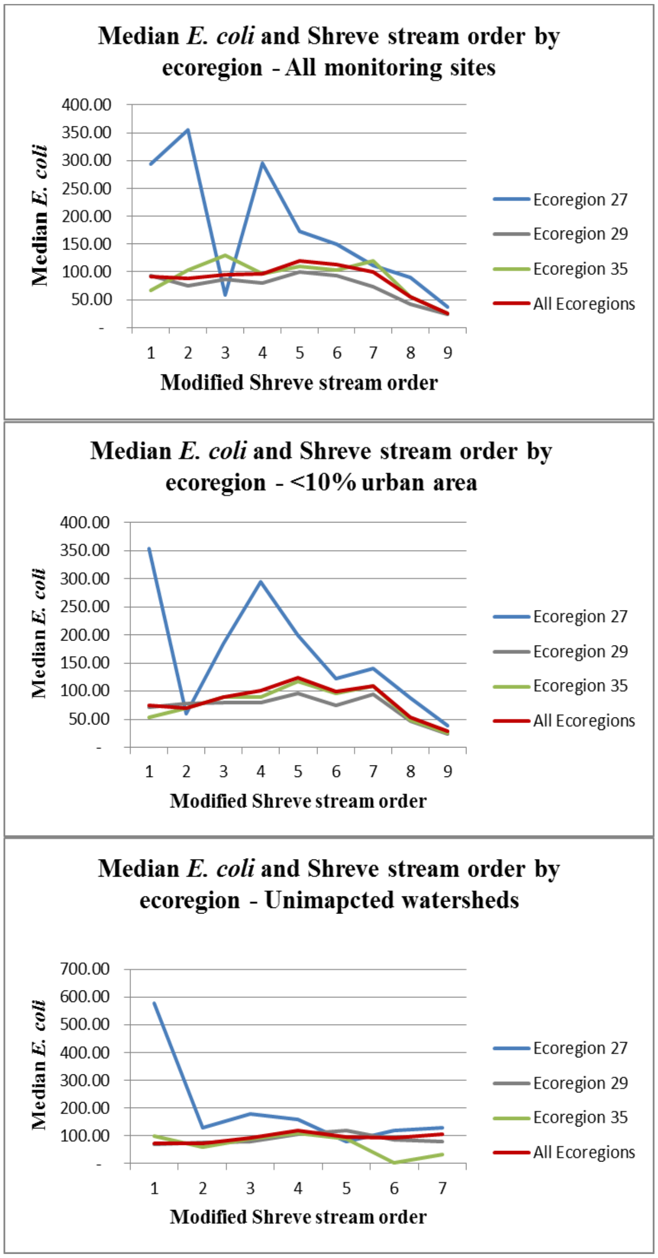
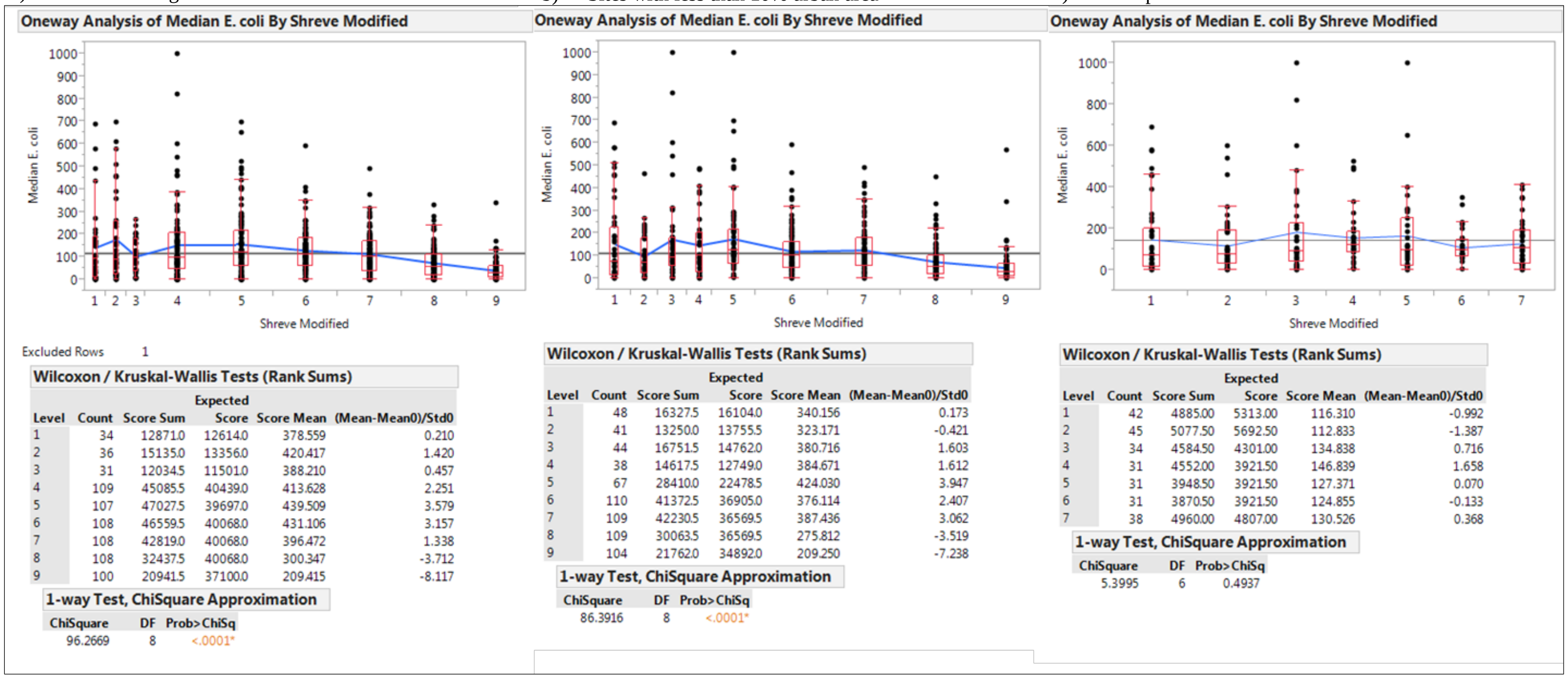


Figure 7. Comparison of median *E. coli* by modified Shreve order at ecoregion scale.

Wilcoxon/Kruskall-Wallis test

The modified Shreve stream order also has discrete characteristics therefore the Wilcoxon/Kruskall-Wallis test was applied to the data. The Chi Square p-value of datasets with wastewater outfalls (i.e. impacted sites) returned significant probabilities of less than 0.0001. This meant there are at least two stream orders that have different score means for median *E. coli*. On the other hand, similar to Strahler stream order results for the Chi Square probability for unimpacted watershed sites was not significant as the p-value of 0.4937 was higher than the alpha limit of 0.05 as seen in Figure 8.

The analysis of each stream order pair showed that there were pairs of streams orders that had similar medians for datasets with WWTF outfalls, but the majority of the pairs differed as seen in Table 14. For the unimpacted dataset, all of the stream order pairs showed a probability greater than 5% significance threshold.



a) All monitoring sites

Oneway Analysis of Median E. coli By Shreve Modified

Excluded Rows 1

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
1	34	12871.0	12614.0	378.559	0.210
2	36	15135.0	13356.0	420.417	1.420
3	31	12034.5	11501.0	388.210	0.457
4	109	45085.5	40439.0	413.628	2.251
5	107	47027.5	39697.0	439.509	3.579
6	108	46559.5	40068.0	431.106	3.157
7	108	42819.0	40068.0	396.472	1.338
8	108	32437.5	40068.0	300.347	-3.712
9	100	20941.5	37100.0	209.415	-8.117

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
96.2669	8	<.0001*

b) Sites with less than 10% urban area

Oneway Analysis of Median E. coli By Shreve Modified

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
1	48	16327.5	16104.0	340.156	0.173
2	41	13250.0	13755.5	323.171	-0.421
3	44	16751.5	14762.0	380.716	1.603
4	38	14617.5	12749.0	384.671	1.612
5	67	28410.0	22478.5	424.030	3.947
6	110	41372.5	36905.0	376.114	2.407
7	109	42230.5	36569.5	387.436	3.062
8	109	30063.5	36569.5	275.812	-3.519
9	104	21762.0	34892.0	209.250	-7.238

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
86.3916	8	<.0001*

c) Unimpacted sites

Oneway Analysis of Median E. coli By Shreve Modified

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
1	42	4885.00	5313.00	116.310	-0.992
2	45	5077.50	5692.50	112.833	-1.387
3	34	4584.50	4301.00	134.838	0.716
4	31	4552.00	3921.50	146.839	1.658
5	31	3948.50	3921.50	127.371	0.070
6	31	3870.50	3921.50	124.855	-0.133
7	38	4960.00	4807.00	130.526	0.368

1-way Test, ChiSquare Approximation

ChiSquare	DF	Prob>ChiSq
5.3995	6	0.4937

Figure 8. Wilcoxon/Kruskall-Wallis test of median *E. coli* by modified Shreve order.

Table 14: Modified Shreve stream order pairs with equal score means using Wilcoxon each pair test.

a) All monitoring sites			b) <10% Urban sites			c) Unimpacted sites		
Stream order	Stream order	p-Value	Stream order	Stream order	p-Value	Stream order	Stream order	p-Value
1	2	0.4072	1	2	0.6775	1	2	0.9661
1	3	0.9215	1	3	0.3169	1	3	0.2315
1	4	0.4782	1	4	0.4623	1	4	0.1234
1	5	0.2483	1	5	0.0686	1	5	0.6153
1	6	0.3790	1	6	0.4206	1	6	0.5282
1	7	0.8018	1	7	0.2973	1	7	0.4580
1	8	0.1065	1	8	0.1159			
1	9	0.0014	1	9	0.0019			
2	3	0.4812	2	3	0.1769	2	3	0.1626
2	4	0.7853	2	4	0.1679	2	4	0.0429
2	5	0.7819	2	5	0.0065	2	5	0.4527
2	6	0.9430	2	6	0.1396	2	6	0.3255
2	7	0.5079	2	7	0.0808	2	7	0.3124
2	8	0.0070	2	8	0.2035			
2	9	<.0001	2	9	0.0012			
3	4	0.4713	3	4	0.8561	3	4	0.4422
3	5	0.1731	3	5	0.2232	3	5	0.6504
3	6	0.3090	3	6	0.9061	3	6	0.6316
3	7	0.8238	3	7	0.7917	3	7	0.8790
3	8	0.0319	3	8	0.0021			
3	9	<.0001	3	9	<.0001			
4	5	0.3831	4	5	0.3591	4	5	0.4140
4	6	0.6971	4	6	0.6152	4	6	0.1038
4	7	0.4569	4	7	0.8595	4	7	0.4258
4	8	0.0002	4	8	0.0041			
4	9	<.0001	4	9	<.0001			
5	6	0.5234	5	6	0.0548	5	6	0.8769
5	7	0.0978	5	7	0.1551	5	7	0.9279
5	8	<.0001	5	8	<.0001			
5	9	<.0001	5	9	<.0001			
6	7	0.2222	6	7	0.5813	6	7	0.6596
6	8	<.0001	6	8	<.0001			
6	9	<.0001	6	9	<.0001			
7	8	0.0004	7	8	<.0001			
7	9	<.0001	7	9	<.0001			
8	9	0.0007	8	9	0.0062			

Similar to the Strahler order, the WWTF impacted datasets had significance probabilities of less than 0.05 which means that at least two of the stream order score means were different in those sets. The unimpacted sites data set had probabilities of greater than 0.05 for all three ecoregions signifying that the score means of the stream orders were equal for all stream orders. Table 15 summarizes the Chi square probabilities for each dataset.

Table 15. Wilcoxon/Kruskall-Wallis test probabilities by ecoregion.

	Probability > Chi Square		
Ecoregion	All monitoring sites	<10% Urban sites	Unimpacted sites
27	<0.0001	<0.0001	0.647
29	<0.0001	0.0015	0.8269
35	0.0004	0.0025	0.459

Correlation tests

The Kendall τ and Spearman's ρ correlation tests were significant for the impacted datasets with included the wastewater outfalls but the correlation coefficients as shown in Table 16 were not very high which meant that the correlation between the modified Shreve order and *E. coli* for the monitoring sites was not very strong. Similar to the Strahler analysis, the unimpacted watershed sites did not show any correlation between the modified Shreve order and *E. coli*.

Table 16. Correlation values between *E. coli* and modified Shreve stream order.

Test	All monitoring sites		<10% Urban sites		Unimpacted sites	
	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.2716	<0.0001	-0.2484	<0.0001	0.0735	0.2449
Kendall's τ	-0.2	<0.0001	-0.1826	<0.0001	0.0551	0.2244

The analysis of correlations at the ecoregion level had similar results to the Strahler analysis with ecoregion 27 showing the highest correlation coefficients for the datasets with wastewater outfalls. The only difference from Strahler analysis here was that the unimpacted sites stream orders also showed a significant but weak negative correlation with *E. coli* in ecoregion 27. The correlation coefficients also decreased when going eastward, i.e. ecoregion 27 had the highest correlation followed by ecoregion 29 and ecoregion 35 similar to Strahler method.

Table 17. Correlation values between median *E. coli* and modified Shreve stream order at ecoregion level.

Ecoregion 27						
Test	All monitoring sites		<10% Urban sites		Unimpacted sites	
	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.4855	<0.0001	-0.4596	<0.0001	-0.2176	0.0405
Kendall's τ	-0.3701	<0.0001	-0.3472	<0.0001	-0.166	0.0319
Ecoregion 29						
Test	All monitoring sites		<10% Urban sites		Unimpacted sites	
	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.303	<0.0001	-0.2642	<0.0001	0.1114	0.3102
Kendall's τ	-0.2189	<0.0001	-0.1877	<0.0001	0.0969	0.2098
Ecoregion 35						
Test	All monitoring sites		<10% Urban sites		Unimpacted sites	
	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.2082	0.0006	-0.1678	0.0105	-0.1321	0.249
Kendall's τ	-0.158	0.0003	-0.1329	0.0046	-0.0894	0.2664

Analysis of median E. coli *by Shreve stream order and watershed area*

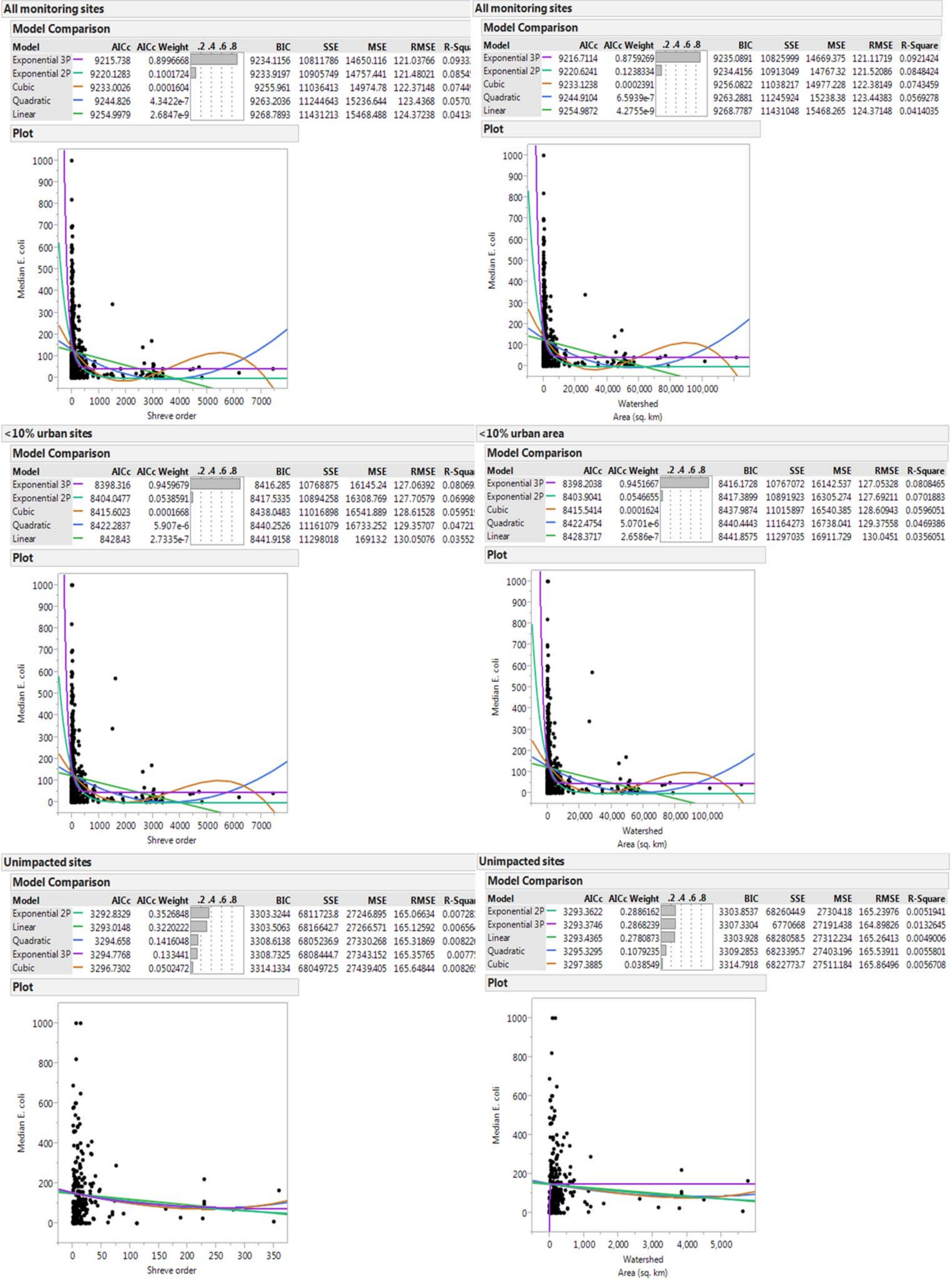
Application of nonlinear models

The *E. coli* data for both the Shreve order and the watershed area plots very similarly and in a nonlinear fashion. At low orders and smaller watershed areas, i.e. orders less than 15 and watershed areas less than 10,000 square kilometers (km), there is higher density of monitoring stations along with higher *E. coli* concentrations. As the stream order and watershed area increase, *E. coli* concentrations start to get smaller and plots closer to the x-axis. This behavior of the data follows a decreasing exponential curve and application of nonlinear exponential models will show if those are a fit for this data.

Figure 9 shows the application of the three polynomial distribution fits and the two and three parameter exponential distribution fits using the procedures described by Walsh (2013). The impacted datasets with wastewater outfalls had the best fit with the two and three parameter exponential distributions for both Shreve order and watershed size. Based on the lowest AICc value, the three parameter (3P) exponential distribution was the best fit. Akaike information criterion (AIC) is an estimator of the quality of statistical model. AIC computes an estimate of the information lost in certain statistical model as compared to other models and assigns a value based on that. The lower AIC values mean a better model fit (Helsel and Hirsch 2002). Even though the 3P exponential distribution showed a very good fit based on AIC, there were monitoring stations which plotted far from the curve, which means if this fit was adopted those stations would not be represented. Figure 9 also shows how similar the Shreve order and watershed area *E. coli* distributions are. Both datasets plot very similarly and the nonlinear fits for them are alike as well. From this, it can be concluded that Shreve order and watershed area depict the same characteristic for the *E. coli* distributions as noted in the stream order theory chapter earlier. Another observation that can be gleaned from Figure 9 is that most of the higher *E. coli* concentrations plot at lower stream order and watershed sizes. At higher orders and watershed sizes, *E. coli* concentrations stay lower than 150 MPN/100ml. Therefore, it is recommended that future studies should be focused on the lower Shreve stream orders and watershed sizes. These results also compare well with the study conducted by Harmel et. al. (2010) where it was found that *E. coli* concentrations decrease with increase watershed scales. Correlation for these two data would be tested by applying the non-parametric test since all the models showed existence of outliers. The parametric Pearson’s r test result after applying the 3P exponential model will also be presented for comparison purposes only. For the unimpacted watersheds, none of the nonlinear distributions provided a good fit; therefore, only non-parametric correlation tests are applicable.

Shreve stream order

Watershed area (sq. km)



All monitoring sites (Shreve stream order)

Model Comparison

Model		AICc	AICc Weight	.2 .4 .6 .8	BIC	SSE	MSE	RMSE	R-Squa
Exponential 3P	—	9215.738	0.8996668		9234.1156	10811786	14650.116	121.03766	0.0933
Exponential 2P	—	9220.1283	0.1001724		9233.9197	10905749	14757.441	121.48021	0.0854
Cubic	—	9233.0026	0.0001604		9255.961	11036413	14974.78	122.37148	0.0744
Quadratic	—	9244.826	4.3422e-7		9263.2036	11244643	15236.644	123.4368	0.0570
Linear	—	9254.9979	2.6847e-9		9268.7893	11431213	15468.488	124.37238	0.0413

Plot

All monitoring sites (Watershed area)

Model Comparison

Model		AICc	AICc Weight	.2 .4 .6 .8	BIC	SSE	MSE	RMSE	R-Square
Exponential 3P	—	9216.7114	0.8759269		9235.0891	10825999	14669.375	121.11719	0.0921424
Exponential 2P	—	9220.6241	0.1238334		9234.4156	10913049	14767.32	121.52086	0.0848424
Cubic	—	9233.1238	0.0002391		9256.0822	11038217	14977.228	122.38149	0.0743459
Quadratic	—	9244.9104	6.5939e-7		9263.2881	11245924	15238.38	123.44383	0.0569278
Linear	—	9254.9872	4.2755e-9		9268.7787	11431048	15468.265	124.37148	0.0414035

Plot

<10% urban sites

Model Comparison

Model		AICc	AICc Weight	.2 .4 .6 .8	BIC	SSE	MSE	RMSE	R-Squa
Exponential 3P	—	8398.316	0.9459679		8416.285	10768875	16145.24	127.06392	0.08069
Exponential 2P	—	8404.0477	0.0538591		8417.5335	10894258	16308.769	127.70579	0.06998
Cubic	—	8415.6023	0.0001668		8438.0483	11016898	16541.889	128.61528	0.05951
Quadratic	—	8422.2837	5.907e-6		8440.2526	11161079	16733.252	129.35707	0.04721
Linear	—	8428.43	2.7335e-7		8441.9158	11298018	16913.2	130.05076	0.03552

Plot

<10% urban area

Model Comparison

Model		AICc	AICc Weight	.2 .4 .6 .8	BIC	SSE	MSE	RMSE	R-Square
Exponential 3P	—	8398.2038	0.9451667		8416.1728	10767072	16142.537	127.05328	0.0808465
Exponential 2P	—	8403.9041	0.0546655		8417.3899	10891923	16305.274	127.69211	0.0701883
Cubic	—	8415.5414	0.0001624		8437.9874	11015897	16540.385	128.60943	0.0596051
Quadratic	—	8422.4754	5.0701e-6		8440.4443	11164273	16738.041	129.37558	0.0469386
Linear	—	8428.3717	2.6586e-7		8441.8575	11297035	16911.729	130.0451	0.0356051

Plot

Unimpacted sites (Shreve stream order)

Model Comparison

Model		AICc	AICc Weight	.2 .4 .6 .8	BIC	SSE	MSE	RMSE	R-Squa
Exponential 2P	—	3292.8329	0.3526848		3303.3244	6811723.8	27246.895	165.06634	0.00728
Linear	—	3293.0148	0.3220222		3303.5063	6816642.7	27266.571	165.12592	0.00656
Quadratic	—	3294.658	0.1416048		3308.6138	6805236.9	27330.268	165.31869	0.00822
Exponential 3P	—	3294.7768	0.133441		3308.7325	6808444.7	27343.152	165.35765	0.0077
Cubic	—	3296.7302	0.0502472		3314.1334	6804972.5	27439.405	165.64844	0.00826

Plot

Unimpacted sites (Watershed area)

Model Comparison

Model		AICc	AICc Weight	.2 .4 .6 .8	BIC	SSE	MSE	RMSE	R-Square
Exponential 2P	—	3293.3622	0.2886162		3303.8537	6826044.9	27304.18	165.23976	0.0051941
Exponential 3P	—	3293.3746	0.2868239		3307.3304	6770668	27191.438	164.89826	0.0132645
Linear	—	3293.4365	0.2780873		3303.928	6828058.5	27312.234	165.26413	0.0049006
Quadratic	—	3295.3295	0.1079235		3309.2853	6823395.7	27403.196	165.53911	0.0055801
Cubic	—	3297.3885	0.038549		3314.7918	6822773.7	27511.184	165.86496	0.0056708

Plot

Figure 9. Nonlinear model fits for *E. coli* by Shreve stream order and watershed area.

Correlation tests

The correlation values for the *E. coli* by Shreve stream order and watershed area are very similar to earlier tests conducted on Strahler and modified Shreve stream order methods. The impacted datasets show weak yet significant negative correlations between *E. coli* and Shreve stream order (Table 18) and between *E. coli* and watershed size (Table 19). On the other hand, the unimpacted datasets do not show any correlation between Shreve order and *E. coli*. Spearman's ρ test does show some correlation between *E. coli* and watershed size for unimpacted dataset but the Kendall's τ does not. The correlation co-efficient of 0.1292 in this case is small enough to ignore the Spearman's ρ significance probability of 0.405. The Pearson’s r test, done on the modeled distributions for the data with wastewater outfalls, gives correlation co-efficient of -0.60 for both the Shreve order and the watershed size. This is a strong correlation with *E. coli* but at the same time, the model ignores the effect of outliers. The exponential model calculates a fitted curve based on the median *E. coli* of each monitoring site. As seen in Figure 9 the fitted curve (purple line) for 3P exponential model goes through many of actual data points but there are some outlier points with high *E. coli* concentrations and higher stream order/watershed sizes that are not well represented by the curve. In addition, a large number of data points below the curve and near the origin point of zero are also not close to the curve but that may be less of a concern since the curve at that point is below the TAC limit of 126 cfu/100ml.

Table 18. Correlation values between *E. coli* and Shreve stream order.

Test	All monitoring sites		<10% Urban sites		Unimpacted sites	
	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.2714	<0.0001	-0.2571	<0.0001	0.0733	0.2463
Kendall's τ	-0.1914	<0.0001	-0.1824	<0.0001	0.0509	0.24
Pearson's r^*	-0.5963		-0.5939			

* Using the three parameter exponential model

Table 19. Correlation values between *E. coli* and watershed area.

Test	All monitoring sites		<10% Urban sites		Unimpacted sites	
	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.2572	<0.0001	-0.2441	<0.0001	0.1292	0.0405
Kendall's τ	-0.183	<0.0001	-0.1754	<0.0001	0.0819	0.0539
Pearson's r^*	-0.6014		-0.5967			

* Using the three parameter exponential model

The correlation analysis at the ecoregion scale also had similar results to the Strahler method. The impacted datasets showed a weak but significant negative correlation for both Shreve stream order (Table 20) and watershed size (Table 21) with *E. coli* for each ecoregion. The unimpacted datasets for Shreve order showed a weak negative correlation with *E. coli* in ecoregion 27 but no correlation in the other two ecoregions. The unimpacted dataset for watershed size at ecoregion 29 showed a weak positive relationship between *E.coli* and watershed size i.e. *E. coli* increased as the watershed size increased. The other two ecoregions showed no correlation between watershed size and *E. coli* for unimpacted data.

Similar to findings from the Strahler method, the correlation coefficients for both Shreve order and watershed size were highest for ecoregion 27 and lowest for ecoregion 35 showing a decreasing trend going eastward.

Table 20. Correlation values between *E. coli* and Shreve stream order at ecoregion level.

Ecoregion 27						
	All monitoring sites		<10% Urban sites		Unimpacted sites	
Test	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.4874	<0.0001	-0.4626	<0.0001	-0.2108	0.0474
Kendall's τ	-0.3519	<0.0001	-0.3340	<0.0001	-0.1508	0.3900
Pearson's r^{*}	-0.6218		-0.6297			
Ecoregion 29						
	All monitoring sites		<10% Urban sites		Unimpacted sites	
Test	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.3036	<0.0001	-0.2692	<0.0001	0.1074	0.3278
Kendall's τ	-0.2089	<0.0001	-0.1832	<0.0001	0.0935	0.2231
Pearson's r^{*}	-0.6569		-0.6476			
Ecoregion 35						
	All monitoring sites		<10% Urban sites		Unimpacted sites	
Test	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|	Correlation co-efficient	Prob>|ρ|
Spearman's ρ	-0.2025	0.0008	-0.1869	0.0043	-0.1405	0.2198
Kendall's τ	-0.1490	0.0003	-0.1415	0.0015	-0.0966	0.2239
Pearson's r^{*}	-0.5162		-0.5200			

* Using the three parameter exponential model

Table 21. Correlation values between *E. coli* and watershed area at ecoregion level.

<table>
<tr><th colspan="7">Ecoregion 27</th></tr>
<tr><th rowspan="2">Test</th><th colspan="2">All monitoring sites</th><th colspan="2"><10% Urban sites</th><th colspan="2">Unimpacted sites</th></tr>
<tr><th>Correlation co-efficient</th><th>Prob>|ρ|</th><th>Correlation co-efficient</th><th>Prob>|ρ|</th><th>Correlation co-efficient</th><th>Prob>|ρ|</th></tr>
<tr><td>Spearman's ρ</td><td>-0.4708</td><td><0.0001</td><td>-0.4586</td><td><0.0001</td><td>-0.1907</td><td>0.0735</td></tr>
<tr><td>Kendall's τ</td><td>-0.341</td><td><0.0001</td><td>-0.3318</td><td><0.0001</td><td>-0.1379</td><td>0.0565</td></tr>
<tr><td>Pearson's r*</td><td>-0.622</td><td></td><td>-0.626</td><td></td><td></td><td></td></tr>
<tr><td colspan="7"></td></tr>
<tr><th colspan="7">Ecoregion 29</th></tr>
<tr><th rowspan="2">Test</th><th colspan="2">All monitoring sites</th><th colspan="2"><10% Urban sites</th><th colspan="2">Unimpacted sites</th></tr>
<tr><th>Correlation co-efficient</th><th>Prob>|ρ|</th><th>Correlation co-efficient</th><th>Prob>|ρ|</th><th>Correlation co-efficient</th><th>Prob>|ρ|</th></tr>
<tr><td>Spearman's ρ</td><td>-0.2671</td><td><0.0001</td><td>-0.2414</td><td><0.0001</td><td>0.2376</td><td>0.0285</td></tr>
<tr><td>Kendall's τ</td><td>-0.1859</td><td><0.0001</td><td>-0.1668</td><td><0.0001</td><td>0.1667</td><td>0.0248</td></tr>
<tr><td>Pearson's r*</td><td>-0.6669</td><td></td><td>-0.6516</td><td></td><td></td><td></td></tr>
<tr><td colspan="7"></td></tr>
<tr><th colspan="7">Ecoregion 35</th></tr>
<tr><th rowspan="2">Test</th><th colspan="2">All monitoring sites</th><th colspan="2"><10% Urban sites</th><th colspan="2">Unimpacted sites</th></tr>
<tr><th>Correlation co-efficient</th><th>Prob>|ρ|</th><th>Correlation co-efficient</th><th>Prob>|ρ|</th><th>Correlation co-efficient</th><th>Prob>|ρ|</th></tr>
<tr><td>Spearman's ρ</td><td>-0.2011</td><td>0.0009</td><td>-0.168</td><td>0.0104</td><td>-0.0712</td><td>0.5355</td></tr>
<tr><td>Kendall's τ</td><td>-0.1498</td><td>0.0003</td><td>-0.1325</td><td>0.0028</td><td>-0.0564</td><td>0.4682</td></tr>
<tr><td>Pearson's r*</td><td>-0.5237</td><td></td><td>-0.5233</td><td></td><td></td><td></td></tr>
</table>

* Using the three parameter exponential model

Discussion of results

Statistical analysis of the data collected was done by didviding it into three datasets – 1) all monitoring sites; 2) sites which had 10 percent or less urban area in their watersheds; and 3) unimpacted watersheds sites (i.e. those without WWTFs and <10% urban area). The datasets were analyzed for *E. coli* relationships by using nonparametric Wilcoxon/Kruskall-Wallis test on Strahler stream order and modified Shreve stream order. Since Shreve order and watershed area were continuously distributed, nonlinear exponential distributions were applied to find a best fit model to test the *E. coli* relationship with them parametrically.

The Strahler and modified Shreve stream order Wilcoxon tests showed very similar results for the two impacted datasets. Both stream orders showed a Chi square probability of less than 0.0001 which means there are at least two stream orders that have different score means for median *E. coli* concentrations of datasets impacted with wastewater outfalls. The unimpacted dataset show that the median *E. coli* for all the stream orders is statistically same due to the insignificant Chi square probabilities of 0.7848 and 0.4937 for Strahler and Shreve stream orders respectively. These results differ somewhat from the Harmel et. al (2010) where a decreasing trend in both impacted and unimpacted datasets was seen. The notable differences between Harmel study and this one include the smaller study area and inclusion of site specific impacted identifiers such as dairy operations etc. in addition to the WWTF in the Harmel study. This study though found similarities with the Edge et. al. (2012) where it was found that streams with order less than 3 had higher *E. coli* concentrations when compared to orders greater than 3. This study found that order 4 and less have higher *E. coli* concentration when compared to higher stream orders. Again the major difference in the two studies is the study area scale with Edge et. al. study area being much smaller than used in this study.

The correlation tests on Strahler and modified Shreve order also had very similar results with a weak but significant negative correlation present between *E. coli* and stream orders for the two WWTF impacted datasets i.e. the *E. coli* decreases as the stream order increases. Both stream orders had p-values of less than 0.0001 for the WWTF impacted datasets. But when looking at the unimpacted dataset there is no correlation between *E. coli* and the two stream order methods. The p-values for both methods were greater than 0.22 which are insignificant at 0.05 alpha level.

The tests on Shreve stream order and watershed area also had very similar results and their distributions with *E. coli* plotted in the same way as well which reinforces the theory of Shreve order being more representative of basin size as stated by Tarboton et. al (1991). A 3P exponential model was able to fit very well for the impacted datasets, but it did leave some monitoring stations as outliers. It may be of value to apply this fit to the WWTF impacted monitoring sites excluding the outliers. The outlier sites can than be indivdually assessed and studied to identify the sources of higher *E. coli* since they did not fall along the curve. For this study, it was decided to use nonparametric correlation tests on the WWTF impacted datasets as preferred tests with all sites included. None of the nonlinear models was a good fit for the unimpacted datasets therefore they could be only tested for nonparametric correlations. The nonparametric correlations tests revealed very similar results to the Strahler method where the WWTF impacted datasets showing weak but statistically significant negative *E. coli* relationship with Shreve order and watershed size and the unimpacted datset showing no correlation of *E. coli* with Shreve order and watershed size. The WWTF impacted results compared favorably with the Lyautey et. al (2010) study where it was found that shreve orders are inversely related to the *E. coli* concentrations.

The modeled exponential distributions for the impacted datasets did show a relatively strong negative correlation of *E. coli* with Shreve order and watershed size. This strong correlation means that the 3P exponential curve calculated in the study can be very useful tool in identifying the outlier sites that have high *E. coli* concentrations at higher orders and larger watersheds. Those sites can then be individually studied to understand the reasons behind higher *E. coli* concentrations. At lower order and smaller watershed sizes the curve may not be as suitable since the range of *E. coli* concentrations is too large and higher concentrations are having a large impact on the curve. The Pearson's r correlation coefficient for both datasets was approximately -0.60 but as mentioned earlier the modeled distribution did not account for all of the monitoring sites therefore this correlation result was not preferred for comparison with Strahler analysis.

The analysis of the datasets by ecoregion had very similar results for each distribution as well. Ecoregion 27 consistenly showed higher *E. coli* concentrations than the other 2 regions. The correlation tests at the ecoregion scale also had very similar outcomes to the entire study region where the impacted datasets showed weak negative correlation between *E. coli* and stream orders/watershed size but the unimpacted data had no correlation. Ecoregion 27 showed a stronger correlation for the impacted data between *E. coli* and stream orders/watershed size compared to the other two ecoregions. The correlation at the ecoregion scale decreased as the data moved eastward similar to the over all *E. coli* values. As noted in the study area description that the vegetation types, precipitation amount and temperatures are different in the three ecoregion. Ecoregion 27 the dryest of the three region in terms of preciptation and temperatures, showed higher *E. coli* concentrations. Ecoregion 27 also had more dryland and irrigated cropland compared to the other two ecoregion. Ecoregion 35 which had the most rainfall and where streams had the lower concentrations of suspendid solids also showed the lowest amounts of *E. coli* concentrations. These variables were not specifically tested in this study but can be important part of future studies done on this subject.

Another important result seen was that the median *E. coli* for stream orders remained below the the geometric mean standard of 126 cfu/100ml for unimpacted datasets. This differed from the Harmel et. al. (2010) observation where the unimpacted streams violated the TAC regulation. Harmel et. al. looked at six sites in the Leon river watershed which showed this trend. It is possible when looking at much larger areas and numbers of monitoring sites, the finding by Harmel et. al. and other similar watersheds were smoothed with the over all trend at the ecoregion level. Based on this, a recommendation can be made to conduct more site specific studies to understand the dynamics of smaller watersheds.

CHAPTER IV

RUAA ANALYSIS

The RUAA data was collected from TCEQ. The RUAA analysis have been completed on and approved by TCEQ on 94 stream segments. From these 94 segments, 46 monitoring stations were selected for the study due to the *E. coli* data availability from TCEQ and their sites snapping within 200 meters of a stream segment during the GIS steps. Out of those initially selected, one station was deemed an outlier due to a very high *E. coli* measurement of 240,000 MPN/1000ml. Of the remaining selected, 36 stations had been assigned the Primary Contact Recreation (PCR) 1 status, two were assigned Secondary Contact Recreation (SCR) 1 status, and seven stations were assigned SCR2 status. Based on this limited data no significant statistical tests could be conducted.

Table 22 shows the distribution of median *E. coli* by Strahler stream order. The SCR1 and SCR2 statuses are not available for all the monitoring stations and as the table and Figure 10 below show, *E. coli* for available stations does not have any discernable pattern.

Table 22. RUAA status of *E. coli* by Strahler stream order.

Strahler stream order	Number of monitoring stations			Median *E. coli*		
	PCR1	SCR1	SCR2	PCR1	SCR1	SCR2
1	4			340.00		
2	17		4	190.00		310.75
3	9	1	3	244.00	120.00	96.00
4	6	1		185.00	89.50	

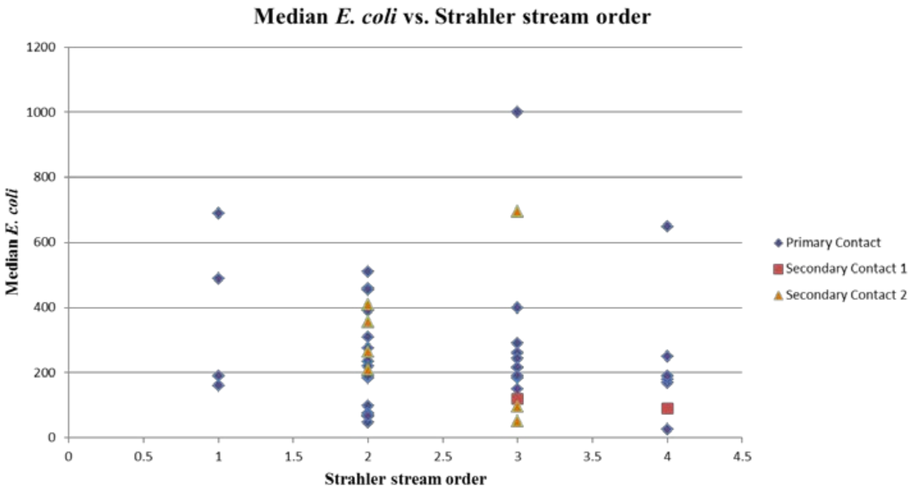


Figure 10. RUAA status of *E. coli* by Strahler stream order.

Similarly Shreve stream order and watershed area do not provide any significant information. The *E. coli* concentrations decrease as the Shreve stream order and watershed area grow larger as seen in Figures 11 and 12. With a larger dataset these relationship may be expanded.

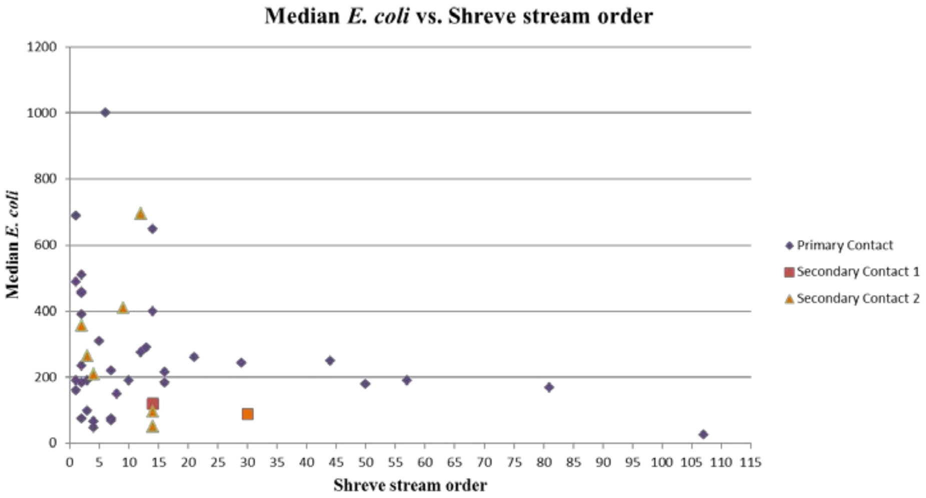


Figure 11. RUAA status of *E. coli* by Shreve stream order.

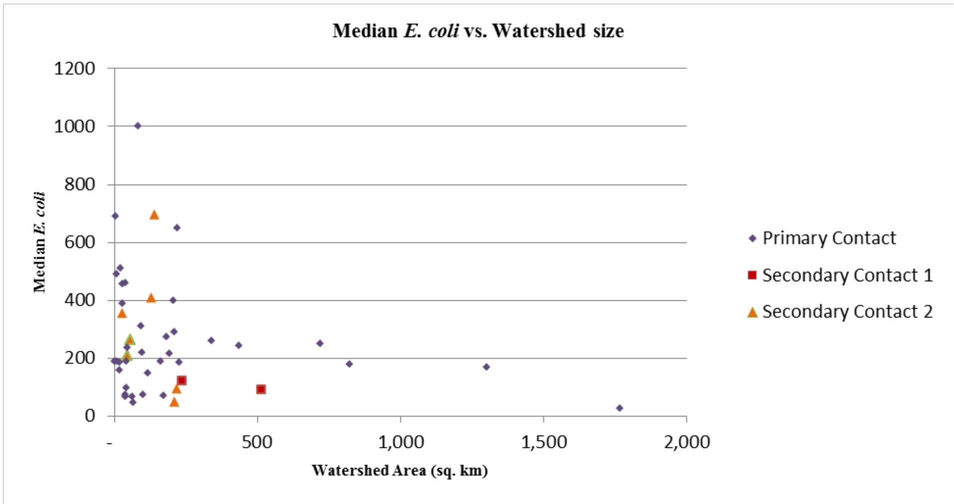


Figure 12. RUAA status of *E. coli* by watershed area.

CHAPTER V

CONCLUSIONS

The overall goal of this research was to understand and predict a relationship between stream order and *E. coli* and evaluate the application of that relationship to the current recreational use standards. The data analysis of impacted monitoring sites with wastewater outfalls showed a weak but significant negative correlation of *E. coli* concentrations with Shreve and Strahler orders and watershed area. Analysis of the data showed that at larger watershed sizes and stream orders, *E. coli* concentrations were significantly lower. At smaller stream orders and watersheds, the *E. coli* concentrations ranged from very low to very high. It can be argued that the wider range of *E. coli* concentrations at smaller stream orders and watershed sizes was a driving factor in making the correlation coefficients weak. The *E. coli* concentrations did become lower as the stream orders and watersheds became larger which points to the dilution factor as suggested by Lyautey et. al. (2010, 2011). But site specific studies may be a better way to understand *E. coli* relationships at smaller watershed and stream order scales where *E. coli* concentration range from very low to very high.

The data analysis of unimpacted sites showed that there was no correlation between *E. coli* concentrations and Shreve and Strahler orders as well as watershed area. Another significant observation for the unimpacted dataset was that in the Strahler and Modified Shreve stream order studies the median *E. coli* stayed statistically the same through all the stream orders which means that median *E. coli* concentration did not change and stayed within TAC limits as stream length and wateshed area increased. This leads to the conclusion that in the absence of anthropogenic factors median *E. coli* will stay the same as watershed area and stream order increase and dilution does not impact the concentrations. The limitations for this conclusion include lack of monitoring stations available at higher stream orders. Many of the monitoring sites which had higher stream orders, e.g. sites at orders 6 and 7 for Strahler order, did not qualify as unimpacted watersheds due the presence of wastewater outfalls. With availability of more data such as discharge volume or hydrologic distance of the wastewater outfalls to the monitoring site, the criteria for exclusion of wastewater outfalls may be modified to include more monitoring sites. This would allow for a better definition of unimpacted watersheds and a decreasing *E .coli* trend may be seen in the unimpacted sites as well at higher stream orders similar to impacted watersheds.

The wastewater outfall impact on the streams was another significant finding of this study. The datasets that included wastewater outfalls, showed a weak but statistically significant negative correlation with *E. coli*, but when the outfalls were removed, there was no relationship. This suggests that wastewater outfalls are a big influence on stream *E. coli* and further research on just the sites that are impacted by these outfalls may provide more valuable information on *E. coli* concentrations in the streams. Further studies with the types and volume of discharges made by wastewater outfalls may also help to assess the impact of wastewater outfalls on the water bodies.

The comparison of the three ecoregions also revealed that the median *E. coli* concentrations and their correlation with both stream order methods and watershed area decreased moving eastward. Ecoregion 27, which is the western most ecoregion in this study and has a more arid to semi-arid environment compared to the other ecoregions showed the highest *E. coli* concentration as well as correlations. Ecoregion 35, the eastern most region and with wettest climate of the three regions, has the lowest *E. coli* concentrations and correlations. This finding can be a good starting point for a future study that can include additional environmental factors such as precipitation, temperatures, types of streams (seasonal or perennial) etc., which make up each region and can contribute to the hydrology of these areas and its impact on the *E. coli* transport.

The study utilized Strahler and Shreve stream orders and watershed area as the variables to which the *E. coli* was correlated. Shreve stream order and watershed area provide similar information with respect to basin size and results based on them are very similar, it is recommended for future studies only one of the two variables should be used. The Strahler stream order method produces a smaller number of stream orders that helps in conducting the statistical analysis when comparing each stream order. This method is a good tool for comparing individual stream order numbers at ecoregion or river basin scales i.e. compare stream order 1 in ecoregion 27 with stream order 1 in ecoregion 29. Out of the three methods watershed area provides the most relevant information with respect to the scale of the basin but Strahler stream order can be the preferred method when comparing streams with similar characteristics.

For future research, inclusion of other variables such as flow volume at the time of the observation or a classification of the flow, such as low, normal, or high, which could identify if the sample was collected after a storm or in drought or normal conditions, may be beneficial as flow can influence *E. coli* concentrations. Other variables such as temperature at the time of sample collection, and time of collection in terms of seasonality, such as summer, spring etc., may by valuable in understanding *E. coli* relationships. A larger number of *E. coli* observations at monitoring sites at higher stream orders may also help provide a better and clearer understanding of the dilution affect. Further, a study on the major contributors, fate, and transport of *E. coli* in each ecoregion may also help understand the decreasing *E. coli* and correlation trends when moving eastward.

Lack of completed RUAA studies did not allow for correlation tests between *E. coli* recreation standards based on the stream orders. The available data showed a decreasing trend in *E. coli* with increase in stream order and watershed size but due to lack of monitoring sites it is recommended that site specific studies continue to be conducted. In the future, there may be cause for reassessing this information when there are more RUAA studies completed.

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