EFFECTS OF ENVIRONMENTAL AND ANTHROPOGENIC FACTORS ON WATER QUALITY IN THE ROCK CREEK WATERSHED

by

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ABSTRACT

Effects of Environmental and Anthropogenic Factors on Water Quality in the Rock Creek Watershed:

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The need to understand the spatial and temporal distribution of pollutants within urban aquatic systems has increased in importance as surface water quality continues to degrade. Rock Creek, a tributary of the Potomac River, spans 33 miles originating in the agricultural and suburban areas of Maryland and continuing through the more urbanized District of Columbia, ultimately running into the Chesapeake Bay. The purpose of this study is to investigate environmental and anthropogenic factors that impact surface water quality in the Rock Creek watershed. Water quality samples were collected weekly from 15 sites along Rock Creek for approximately four months. The samples were analyzed for physical and chemical parameters including: turbidity, nitrogen, and phosphorus. Additionally, concentrations of *E. coli* and total coliforms were quantitatively assessed. Additional samples were collected following significant rain events, in order to assess the impact of precipitation events on the water quality.

Spatial and temporal data analysis using geographic information systems software and nonparametric statistical analysis determined that water quality variation is not uniform along the creek. No significant correlations were found between anthropogenic factors studied and mean enteric bacteria concentrations. The distribution and intensity of anthropogenic factors is clearly not the sole basis that determines the bacterial quality of Rock Creek. There are also temporal influences, in terms of watershed practices and hydrological behavior as well as spatial influences (sources of pollution and recreational activity), that ultimately influence the dynamics of fecal indicator bacteria contamination over the watershed. Rain, temperature and discharge were positively correlated with mean enteric bacteria concentration. Turbidity values varied with rain event characteristics. The effect of rainfall on turbidity (increase or decrease) was found to be dependent on the temporal pattern of rainfall versus the quantity. Sites with increased turbidity were found to have increased total coliform. No other associations were found between bacteria, nutrients and turbidity, possibly resulting from limitations of the study or the variety of other influences within the watershed that contribute to the abundance of these parameters. These data may ultimately assist decision makers in understanding the relationship between water quality of Rock Creek, the factors studied, and the potential health hazards resulting from precipitation events.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

STATEMENT OF PURPOSE

The purpose of this research is to assess how environmental and anthropogenic factors (e.g. precipitation, temperature, sewer characteristics, impervious surfaces, and land use) may affect spatial and temporal variations in surface water quality in an urban environment. This was accomplished through the monitoring of fecal indicator bacteria (FIB) and physical water quality parameters in the Rock Creek Watershed (RCW) in Maryland and the District of Columbia. As most environmental data are associated with a time and location, methods using Geographic Information Systems (GIS) were used to visualize and analyze these data according to spatial and temporal features. The use of GIS mapping and spatial analysis can provide information to facilitate the decision-making and recommended course of action among public health officials and other invested stakeholders to improve the protection of public health.

BACKGROUND AND SIGNIFICANCE

Over the last ten years, the need to better understand spatial and temporal variability of pollutants within aquatic systems has grown exponentially as the degradation of surface water quality continues to present increasingly significant issues for human society and natural ecosystems (2, 47). Surface waters have a variety of uses such as recreation (swimming, fishing and other public uses), irrigation, and cooling electricity-generating equipment utilized by the thermoelectric-power industry. Although surface waters make up only a small percentage of the available freshwater supply on earth, the advantage of accessibility makes it a prime choice for selection as a source for drinking water.

The operational paradigm for military operations is constantly adapting global changes affecting the spread and concentration of troops. Although traditional phase-line and linear combat operational strategies are still an important planning tenet for military operations; today's environment focuses on asymmetric battlefield engagement (15). The current focus, which tends to demand the highest concentration of troops in urban and population-dense areas of operation creates very specific force-health protection needs in order to support the primary war-fighting functions.

From a commander's perspective, the classes of supply that most directly affect mission readiness are; Class I (food, rations, and water), Class V (Ammunition), and Class VIII (Medical Supplies). Of these, Class I, and particularly water, represent a very difficult logistical challenge in terms of delivery and sustainment in a steady-state operation posture within an asymmetric battlefield. The modular military of today employs smaller units specifically tailored to a particular environment. These modular units are often smaller and leaner in terms of personnel and equipment with the expectation that these "agile" components will be highly mobile, globally deployable, and able to self-sustain for a finite amount of time instead of depending on a more traditional support and re-supply concept (16).

Among the many changes that modular units adapt to their operational paradigm is the ability to utilize locally available resources in order to sustain their war-fighting functions or warfighter support functions. Of particular importance to this end is the unit's ability to provide itself with water which is often available in the form of surface water (lakes, rivers, estuaries, etc.). In conjunction with medical intelligence available to the commander prior to and during the deployment period, surface water sources are identified and evaluated for viability of use. Units are then provided with equipment and personnel to treat, collect, process, and distribute fresh water instead of relying on a supply train which may be non-conducive for operations. This is particularly true for remotely-located units or isolated units where ground re-supply is restricted due to a non-permissive environments (17).

Surface water quality monitoring, as a means to supporting the war-fighting function is rapidly becoming a top priority for military operations and currently being incorporated in the military decision making process for execution. Thus, it is important to investigate the effects of multiple environmental and anthropogenic factors on surface water quality to adequately identify and characterize viable water sources which enable commanders to establish steady-state operations within both austere and urban environments.

Geographic Information Systems

Public health threats present unique challenges, which require the public health professional to have a thorough understanding of the associations among the numerous factors affecting health. With the development of public health informatics, public health professionals are realizing the benefits of applying GIS to public health problems (74). GIS are spatial data management systems, additionally applicable to multiple facets such as health services, telecommunications, geology, and public utilities. It allows public health professionals to combine risk factors, demographics, and changes in environmental variables into a common operating picture in order to facilitate the visualization of public health problems and exercise improved and more robust decision-making capabilities based on analyzed data.

The increasing use and evolution of GIS over the last decade have facilitated various research studies in areas such as human effects from soil contamination, investigation of epidemics, and accessibility to drinking water and served as both a decision-making and problem-solving tool that enhances the ability for policy makers and health professionals to work more effectively (25). Ricketts (59) described GIS as, "a simple extension of statistical analyses that join epidemiological, sociological, clinical, and economic data with reference to space." The influences which affect surface water quality are numerous, complex, and differ in various regions and spatial scales, therefore, one must use a multi-faceted approach to examine them both within the water body and the watershed as a whole. GIS is one of the many tools available used to assist in this endeavor. The system allows a user to input data from a plethora of sources, integrate, manipulate, and then transform the data to produce maps, which allow the visualization and understanding of spatial and temporal relationships within the hydrologic system.

Surface Water Vulnerability

Surface waters are extremely vulnerable to contaminants from natural (aerial deposition, soil erosion, or leaching of contaminants through soil) as well as anthropogenic sources (water runoff from urban areas, point sources, or combined sewer overflows (CSOs). As water flows through a watershed, it encounters areas such as forests, agricultural lands, and urban areas, collecting pollutants along its path. Pollutants can include nutrients, petroleum products, road salt, pesticides, sediment, and pathogens.

Numerous surface water studies have been conducted globally, aimed at evaluating spatial and temporal variations in physical and chemical water quality parameters. There is no standard method for conducting such evaluations; therefore, each study has its own set of selected parameters, correlations, applied statistics, and various outcomes. Adeogun et al. (2) studied the spatio-temporal variations in water and sediment quality within an urban watershed to determine major sources of pollution on the Ona River, Nigeria. The multivariate analysis applied to the data determined that sediments were the major determinant of water quality, with seasonal factors playing an augmenting role. The authors found a seasonal relationship with temperature, pH, and dissolved oxygen (DO). Additionally, they discovered that over the study period, biological oxygen demand (BOD) and chemical oxygen demand (COD) increased and heavy metals (Cu, Pb, Mn, Cd, and Cr) accumulated significantly within the sediment and the water (2).

Akkaraboyina et al. (3) examined the predicted versus actual measured observations of water quality parameters in River Godavari located in one of the largest mixed-use watersheds in India using time series forecasting. In this three-year study, pH, temperature, and DO were analyzed and forecasted, and the researchers found that the proposed models were performing satisfactorily. The forecasted observations for the study period as well as the future three year period demonstrated a seasonal trend pattern which indicated that the quality of the river water is influenced by seasonal inflows (3), a result also observed by Eneji et al. (31). This finding supported the researcher's recommendation that time series forecasting methods with allowable error, can be used to assess future water quality.

Augustijn et al. (5) examined the spatio-temporal variation of total suspended and dissolved solids (TSS and TDS) in Manoa Stream, Hawaii located within a coastal watershed. The researchers found that TSS and TDS had irregular temporal and spatial variations and no relation with discharge. TSS and TDS concentrations increased from upstream to downstream, suggesting that forested soils generate less TSS and TDS than urbanized areas downstream.

Several authors used multivariate analysis such as principal component analysis and factor analysis to explore seasonal variations of water quality parameters and determine which are most important in assessing water quality. In a three year study of the lower St. Johns River, Florida, Ouyang et al. (55) found that water quality variables that play important roles in influencing water quality in one season may not be important in another, with the exception of Dissolved Organic Carbon (DOC) and Electrical Conductivity (EC), which were always the most important contributing parameters for all seasons. The authors observed large seasonal variations in correlations between organic-related parameters and BOD as well as DO. In the spring, their first principal component (PC) contributed to 56.8% of the total variance, positively and mostly contributed by organic related parameters such as Total Kjeldahl Nitrogen (TKN), the total concentration of nitrogen and ammonia, and physical parameters such as DO, and negatively affected by mineral-related parameters and inorganic nutrients. Their second PC accounted for 26.8% of the total variance, positively and primarily contributed by temperature, turbidity and anthropogenic inputs such as total phosphorus (TP), and negatively affected by natural inputs such as pH. Although results reflected similar patterns in the summer and winter, opposite results were found in the fall (55).

Similarly, Najafpour et al. (49) found variations with water quality parameters attributed to seasonal changes and various anthropogenic inputs around samples sites in their investigation of the Shiroud River, Iran located within a coastal watershed. Their first significant component accounted for 25.76% of the total variance, positively and highly contributed by EC, TDS, total hardness, calcium ion, and water temperature. The second component accounted for 13.99% of the total variance, positively and highly contributed by silicate, DO, and pH. The third factor, accounting for 10.72% of the total variance, was positively and highly contributed by TP and orthophosphate. The additional three components accounted for a variance of less than 10%. In their investigation of variations in water quality between the sites, mean river depth (87.6%) and DO, EC, and ammonium (6.1%) accounted for 93.7% of the total variance, demonstrating that there were significant differences between each site. Unlike Ouyang et al. (55), the researchers also found that the most important water quality parameters to discriminate between temporal variations are calcium ion, TDS, silicate, water temperature, total hardness, and BOD_5 (49).

Distinct from both aforementioned studies, Shrestha et al. (63) concluded that the most important water quality parameters to distinguish between temporal variations in the mixed-use Fuji River watershed located in Japan were discharge, temperature, DO, BOD, EC, and nitrate. To distinguish spatial variations, the most important parameters were the same as for temporal variations with the exception of DO, and the addition of pH and ammonical nitrogen. In this study, of the total variance for the three groups of sites identified (low, medium and high pollution), the first component accounted for 71.3%, the second component accounted for 77.61%, and the third component accounted for 65.39% (63).

Though the researchers did not always agree on the observation of spatial or temporal variations, nor on which water quality parameters are most significant for the detection of those differences, they do demonstrate the complexity of various waters systems and support that "one size does not fit all." Overall, the studies share the common theme that anthropogenic factors are the greatest contributor to surface water pollution. This can frequently lead to the entry of concentrated amounts of microbial pathogens into the water, which unlike inorganic contaminants; originate from a specific biological source such as sewage or animal feces.

Fecal Indicator Bacteria

An example of a prominent pathogen found in surface water is *Escherichia coli* (*E. coli*), a type of fecal coliform bacteria commonly found in the intestines of humans and animals. The presence of *E. coli* in water is a strong indicator of contamination from animal waste or human waste from sewers or septic systems, which can contain numerous pathogens and lead to adverse health effects (39, 43, 61, 81). Numerous studies have shown that potential health risks to humans, particularly vulnerable populations, have resulted from the exposure to human and animal feces in recreational waters (14, 42, 66). The extent of the presence of microorganisms in water that pose a health threat to humans determines the overall microbiological quality of the water. Waterborne pathogenic organisms are widespread and include viruses, protozoa, and bacteria; they are too numerous to measure, therefore it is common practice to measure select fecal indicator bacteria (FIB) (14, 81). For decades, FIB have been used worldwide to recognize the potential for adverse human health effects resulting from recreational activities in surface waters contaminated by fecal pollution. In 2012, in accordance with Section 304(a) of the Clean Water Act (CWA), the United States Environmental Protection Agency (USEPA) (66) updated its' science-based Recreational Water Quality Criteria (RWQC) aimed at protecting human health from microbial organisms in water bodies such as rivers. In this document, the USEPA recommends using *E. coli* as the indicator bacteria of fecal contamination for fresh waters, and culture-based methods for detecting their presence.

There exists a growing body of knowledge investigating the occurrence of microbial pathogens in both surface water and ground water systems within urban, rural and mixed-use watersheds. While certain studies focus on microbial source tracking and modeling of FIB, others aim to test FIB viability over time, or the variation of FIB abundance over both time and location. Mallin and colleagues (40-42) conducted several studies focused on analyzing the abundance and distribution of FIB throughout various watersheds in North Carolina, within both coastal and non-tidal creeks (40). The researchers investigated the relationship between FIB and demographic and land use factors, water quality parameters, as well as beach and shellfish closures. In "Wading in Waste" (39), Mallin states that 85% of closures and advisories result from excessive counts of fecal bacteria in the beach waters. He attributes these increased numbers to improperly planned development and storm water runoff from increased impervious surfaces near the shoreline (39). These findings are supported by results of the four-year study which analyzed five estuarine watersheds, each of varying level of development, for the abundance and distribution of fecal coliform and *E. coli* (42). In this study, Mallin and colleagues made several findings to include that although fecal coliform abundance was significantly correlated with watershed population and strongly correlated with the percentage of developed land in the watershed, the percentage of impervious surface coverage in the watershed was the most important human-related factor associated with the abundance of fecal coliform (42).

In 1998, Mallin and colleagues incorporated hydrological data in their study and examined the effect of rainfall and stormwater runoff on the water quality of 11 coastal streams within rural watersheds in North Carolina over a 6-month period. Here, they identified significant correlations between fecal coliforms and turbidity, rain and fecal coliform counts, and rain and turbidity (40). These findings support the common idea that fecal coliforms combine with sediment in the water, which can serve as a transportation mechanism for the bacteria, as well as protection from UV radiation.

Seker et al. (62) examined the bacteriological water quality and abundance of enteric bacteria of coastal surface water in southwestern Istanbul, Turkey using GIS, the multiple tube fermentation method, and the Dominant, Abundant, Frequent, Occasional, Rare (DAFOR) scale. Multiple sources of data were inputted into GIS, converted to the same Universal Transverse Mercator (UTM) coordinate system and scale, and used to create spatial maps displaying the distribution of enteric bacteria over the yearlong study. GIS also enabled the authors to calculate the values of the monthly average density of enteric bacteria. The researchers found the most abundant enteric bacteria to be *E. coli* and observed an expected seasonal variation of enteric bacteria (lower in the spring and summer), and both ambient and water temperature. In this study, enteric bacteria were higher at locations receiving Waste Water Treatment Plant (WWTP) effluent in every season except the winter. Untreated wastewater and anthropogenic activities on the coastal line were credited to the elevated levels of enteric bacteria at three other locations (62).

The two types of fecal indicator bacteria most frequently examined in surface water are *E. coli* and total coliform. Common sources of fecal coliform in an urban environment include pets and wildlife, illegal sanitary sewer connections to the storm drain, leaky sewer lines, failed septic tanks to the storm drain, or CSO. A combined sewer is one in which wastewater from sanitary sewage and stormwater are conveyed into one piping system. When the capacity of this combined sewer is exceeded, normally resulting from significant rainfall, excess flow is discharged directly into surface water. This excess flow is called, "combined sewer overflow" (76).

Many methods exist for analyzing FIB in surface water, the most common two being those in which the organisms are cultured (67), and those in which the deoxyribonucleic acid (DNA) is extracted from an environmental sample and quantified using real-time polymerase chain reaction (PCR) techniques (14, 81). The IDEXX Colilert Quanti-tray enumeration method is a culture-based method which produces *E. coli* and total coliform bacteria concentrations using a most probable number (MPN) method based on statistical probability (35). Factors that attract researchers to this method over others include EPA approval, cost-effectiveness, speed, and accuracy.

Nutrients

In 1990, the USEPA found eutrophication to be the most prevalent impairment of surface waters (10). Eutrophication, the over-enrichment of receiving waters with mineral nutrients (primarily nitrogen and phosphorous), results in a surplus of autotrophs such as algae and cyanobacteria. Subsequently, this leads to excessive bacterial populations and elevated respiration rates, which can cause hypoxia or anoxia in bottom waters or surface waters (6, 10). A review by Carpenter and associates (10) support the USEPA's findings and outlines an additional five findings regarding the pollution of surface waters with phosphorus and nitrogen. The authors found that:

1) a major source of nitrogen and phosphorus to surface waters in the U.S. is nonpoint pollution resulting from agriculture and urbanization

2) the contribution nitrogen and phosphorus from the application of fertilizer to agriculture is greater than outputs in produce across the globe

3) there is a direct correlation between nutrient flows to waterways and animal stocking densities; more manure is produced than what is needed to fertilize local crops when this density is large

4) excess amounts of phosphorus accumulate in soil as a result of over-fertilization and manure production which can then find its way to aquatic ecosystems

5) excess amounts of nitrogen from over-fertilization and manure production on agricultural lands can mobilize in various soil types, leach into aquatic ecosystems or volatilize into the atmosphere (10).

Gächter et al. (26) investigated the nutrient transfer from soil to surface water by examining agricultural drainage systems from three rivers in Switzerland which drained primarily grassland and cropland. The researchers found an inverse relationship between phosphate and nitrate with respect to their relationship to discharge, as well as their prevalence in the surface waters. This study documented a positive correlation between discharge and phosphate and a negative correlation with nitrate. They also found average concentrations of nitrate to exceed that of phosphate by 27 and up to 680 times dependent on the river. The differences in mobility and behavior of the two nutrients were attributed to the affinity for phosphate to bind to the soil, and the preferred flow of rainwater across the soil column (26).

Nitrate

Nitrogen is an essential nutrient needed for the successful development of plant life and can be deposited into the water and soil through both natural (i.e. precipitation, decomposition of organic matter) and anthropogenic processes such as agriculture (36). Nitrogen comprises of approximately 80% of the Earth's atmosphere, however, in this gaseous state as N_2, it is inaccessible to most organisms (6). Nitrogen must therefore, be transformed into a useable form for living things through a process called, "the nitrogen cycle." The primary transformations of nitrogen are dependent on the various activities of a multitude of microorganisms and include nitrogen fixation, nitrification, denitrification, anammox, and ammonification (Figure 1) (6). One form of nitrogen typically found in the environment is nitrate (NO_3^-), which finds its way into surface water through transportation resulting from erosion or leaching from the soil. Once in the water, it can return to the atmosphere by way of de-nitrification or volatilization. Common applications of nitrate to the soil include animal manure and fertilizer (10). In urban areas, households, lawn companies, or golf course managers most frequently apply fertilizer for the development of grass and/or shrubs, while in agricultural areas its application to the land augments naturally existing nitrate in the soil for crop growth (10). Another potential source of nitrate to surface water is from leaking sewer lines, septic system leachate, runoff from failed septic systems, and CSOs (10, 53, 76). Leaking sewer lines and septic systems can additionally affect the groundwater quality, which can subsequently affect surface water through hydrogeological interactions (53, 79). Excess nitrogen can pose a direct threat to humans and other mammals and can have detrimental effects on aquatic systems (6, 26, 36). An excessive growth of algae can be seen in waters with a concentration of nitrate greater than 0.3mg/L, which in turn may affect the health of aquatic life (36).

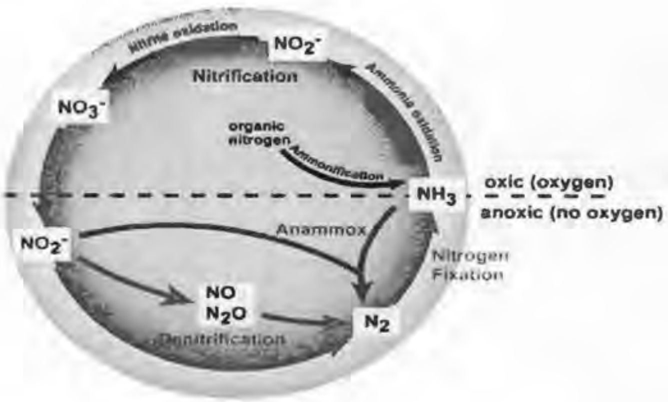

Figure 1.The major transformations in the nitrogen cycle (6).

Phosphate

Phosphorus is another essential nutrient needed for the successful development of life and is found naturally occurring in rocks and other mineral deposits. Phosphates (PO_4^{3-}) are formed during the natural process of weathering, and exist in three forms, orthophosphate, metaphosphate, and organically bound phosphate (54). The only form which can be assimilated by bacteria, algae and plants is orthophosphate. Although the presence of phosphorus in water is not considered directly toxic to animals and humans, indirect effects are well documented within the body of scientific knowledge (10, 13, 44).

The rapid growth of the algal population as a result of eutrophication can cause death and decay of vegetation and aquatic life, and impede the use of the water for activities such as recreation, agriculture, fisheries, and drinking (10). Additionally, blooms of cyanobacteria can produce toxic chemicals which can kill livestock, fish or shellfish and pose human health problems varying from skin irritation, liver damage, or death (10, 54).

Research has shown that phosphorus is commonly the limiting nutrient (the lesser available nutrient which limits the amount of plant matter that can be produced) in freshwater aquatic systems (44). Contributions to phosphorus in surface water are often from nonpoint sources such as failing septic systems, developed land, nursery or crop and point sources such as concentrated animal feeding operations (CAFO), National Pollutant Discharge Elimination System (NPDES) regulated stormwater, and regulated process water (69). In contrast to nitrogen, phosphate is retained in the soil by an intricate system of biological uptake, absorption, and mineralization (54). Consequently, most of the phosphorus inputs occur in surface water versus groundwater, except in cases where watersheds are volcanic in origin or where soils are anoxic and saturated (13). Though there is currently not a common standard for the concentration of total phosphorus acceptable for surface water, Correll (13) found that 100 μg/L was "unacceptably high" for most streams, lakes, reservoirs and estuaries, and that concentrations of 20 μg/L are commonly problematic.

Stormwater Runoff

In urban areas, precipitation generates surface runoff that typically travels from impervious surfaces such as rooftops and driveways, and collects on hard-surfaced streets or parking lots. The contaminants mobilized through surface water runoff have the potential to migrate to wetlands, rivers, and larger bodies of water, thus impacting human and ecological receptors in those environments (46). Rivers are frequently at the terminal end of this runoff migration from land surfaces as well as from external sources such as tributaries, storm-water discharges, CSOs, or permitted discharge facilities. The primary routes for contaminants to enter the river are through surface water inputs (movement within the river from upstream or direct inputs from external sources), groundwater, or through sediment transport from upstream (12). Once in the river, contaminants have the potential to transfer from surface water to groundwater and vice versa. Continuous or repeated exposure to these influences can degrade river water quality and the aquatic habitat for fish and other biological communities, and pose a health threat to humans. High water quality is associated with natural forests and wetland resources, as well as the use of best practices associated with stormwater management, conservation easements, stream buffer protection, and agricultural uses (46).

Impacts of Urbanization

Urbanization changes the hydrologic cycle and affects water quality, which ultimately affects not only the health of aquatic life, but also the safety of water for human consumption and other anthropogenic activities. The impacts of urbanization on surface waters has been greatly studied, and it is of no surprise that as populations and population density increase, the demand on water supplies and stress on aquifer systems will proportionally increase as well (9, 58, 75). From 1970 to 2000, the population of Maryland increased 35%, and is projected by the Maryland Department of Planning (MDP) to increase an additional 27% between 2000 and 2030 (80). Maryland Department of the Environment (MDE) estimates the total population in the Rock Creek Watershed to be 307,000 people based on the 2000 U.S. Census (22, 44). In Montgomery County alone, the most recent 30 year projection estimates a population increase of 22% (24).

Hand-in-hand with the increase in population comes an increase in developed land, transitioning areas previously forest, agricultural, or of other rural uses into urban areas. This subsequently increases the amount of impervious surface coverage in the land, resulting in an impaired ability for the earth to naturally filter contaminants prior to their return to ground or surface water (19, 21, 58). The quantity and velocity of water entering a stream following a storm is affected by a variety of factors; the time it takes water to travel over developed land surfaces is decreased, and as a result of development, water efficiently flows from roads and parking lots into storm drains, quickly reaching the stream, resulting in stream channel erosion and flashiness (9, 19). Ultimately, if left unmanaged, urbanization paves the way for stream impairment, water quality degradation, and recharge reduction.

Several researchers have found the leading cause of pollution to fresh and brackish receiving waters to be urban stormwater (32, 41). Investigations on the environmental damage caused by impervious surface coverage date back to the late 1980s, and later its effects on the fecal bacterial concentration in water (39). Mallin and colleagues (40, 42) conducted several studies researching such phenomena and found that there exists a strong positive correlation between mean estuarine fecal coliform bacterial abundance and impervious surface coverage. During a watershed scale analysis, Mallin and colleagues (42) developed and tested a regression model that predicts the overall geometric mean fecal coliform count for a tidal creek based on percent watershed impervious surface coverage. The model provided results within 6% of the predicted value of fecal coliform counts, and results showed that the watersheds with less than 10% impervious surface coverage had generally good microbial water quality, watersheds with 10-20% impervious surface coverage were impaired, while watersheds with over 20% impervious surface coverage were severely polluted, which is in agreement with previous studies (40, 41).

Widmer et al. (78) studied *E. coli* in surface water across Vietnam, Indonesia, Cambodia, and Thailand and found similar associations with *E. coli* and land development. They observed significantly higher concentrations in urban surface waters than in agricultural or rural waters, supporting that urban areas have a larger fecal contaminant load in surface waters that receive runoff. The researchers also noted a seasonal difference in the overall mean values of *E. coli* for all land use types, with the dry season having almost 1.5 log higher counts than the wet season, attributed to the dilution effect of monsoon-driven rainfall events in the wet season. This observation is considered typical for Southeast Asian surface waters. Widmer and associates also discovered the presence of pathogenic genes in 3.9% of the *E. coli* isolates analyzed, many of which were Shiga toxin producing genes *E. coli*, as well as enteropathogenic *E. coli*, and enterotoxigenic *E. coli*, all thought to have entered the surface water through urban runoff. Although this percentage is relatively low, the continuous exposure of a person to such a contaminated waterway harboring pathogenic strains of *E. coli* or the use of this water for irrigation purposes could pose a significant public health threat (78).

Liu et al. (37) investigated water pollution mitigation by exploring surface water quality in relation to land-use within a watershed in Wisconsin, USA. GIS-based spatial analysis was used to further examine the water quality variables found significantly related to land use. Out of 21 variables, 14 were found to be significantly and positively related to urban and agricultural lands and significantly and negatively correlated with forest and wetland land-use. A strong positive correlation between EC related water quality variables and urban and agricultural lands was also observed (37).

Study Area

The Rock Creek Watershed is approximately 76 square miles with nearly 80% of the drainage area in Maryland and 20% in Washington, District of Columbia (D.C.) (Figure 2) (43). Rock Creek spans 33 miles and is a tributary of the Potomac River, which runs into the Chesapeake Bay approximately 108 miles after the confluence with Rock Creek. The headwaters of Rock Creek are in Laytonsville, Maryland from which the river flows for 21 miles before entering D.C., where it continues through Rock Creek National Park (RCNP) (7, 12). From RCNP it continues to flow south, becoming tidally influenced in the last quarter mile prior to entering the Potomac River (7, 43).

The Maryland Route 28 corridor splits the Rock Creek Watershed by distinct geographic characteristics, separating the Upper Rock Creek and Lower Rock Creek Watersheds. The watershed spreads over two physiographic provinces separated by what is known as the "Fall Line;" in Maryland, the Piedmont Province, made up of crystalline bedrock, and in D.C., the Coastal Plain Province, containing unconsolidated sedimentary deposits (11, 24). The dominant land use in the watershed was classified in 2000 by the MDP as primarily residential followed by commercial and forest land use (43). South of Maryland Route 28, the watershed becomes very developed and heavily populated, with a variety of industrial, commercial, and residential land uses. A large portion of the development in this area was constructed during the 1960s, when stormwater management was not typically practiced and public gravity sewer lines were commonly installed, many of which are in need of repair today (7).

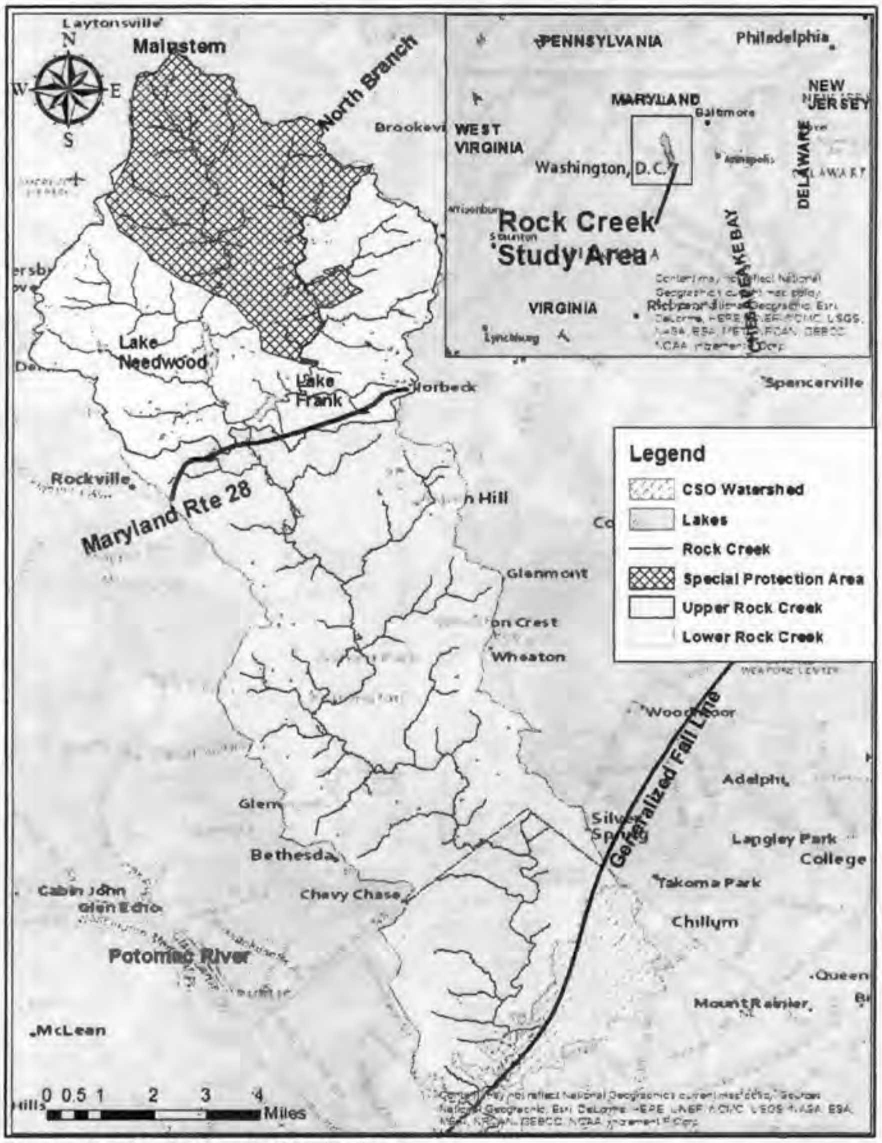

Figure 2. Rock Creek Study Area

Three major drainage areas form the Rock Creek Watershed: the mainstem of Rock Creek (on the northwestern side), the North Branch (on the northeastern side), and the tidal drainage area (7). The North Branch begins at Mount Zion, Maryland until Rockville, MD where it discharges into Rock Creek. To control sediment from upstream, and minimize downstream flooding, the mainstem and North Branch were dammed before they join, forming recreational Lakes Needwood and Frank located within Rock Creek Regional Park (RCRP) (47). Here, visitors can participate in a variety of activities such as hiking, boating, and year-round fishing. This portion of the watershed is densely forested, comprising of approximately 5,000 acres, with about two-thirds of the total forest covered by the Upper Rock Creek Planning Area, located in the middle of the Upper Rock Creek Watershed (47). The 1985 Upper Rock Creek Master Plan, amended in 2004, minimized zoning densities above Muncaster Mill Road, which combined with the protection of natural resources, resulted in a significant reduction to the impact on the water quality in this area (47). Additionally, the Master Plan of 2004 designated the Upper Rock Creek Watershed within the planning area North of Muncaster Mill Road as a Special Protection Area (SPA) in order to preserve water quality in the tributaries of the North Branch directly affected by significant development projects within the SPA (Figure 2). Although the creek is home to a variety of warm-water fish communities, certain species, like the brown trout, have become scarce (46). Due to the extensive impacts on Lower and Middle Rock Creek from urbanization, the overall resource condition is classified by MCDEP as fair to poor (7, 46).

The Montgomery County Department of Environmental Protection (MCDEP) recognizes Lower Rock Creek as having one of the first county stream valley park systems (46). Over the years, the watershed as a whole, and in particular the lower section has felt the greatest pressure of development, as many residents of D.C. built summer homes in the area or transitioned their residence or employment to the suburbs. Lower Rock Creek directly connects to the RCNP, operated by the National Park Service

(NPS) in D.C. There are over 217 stormwater drains or combined sewer outfalls that empty into Rock Creek (11). The RCNP hiker-biker trail system provides a vegetative buffer along Rock Creek and is one of the most heavily used and esteemed recreation corridors, receiving an average of 300,000 visitors per year (12, 46). Activities in the park include running, walking, biking, or skating along its paths, or on Beach Drive, whose sections through RCNP are closed to vehicles on the weekends. Other activities in the park include hiking, horseback riding, exercising dogs, golfing, or visiting historic Pierce Mill or the Nature Center (51).

Sewer Service

In Maryland, the public water and sewerage system is mainly operated and maintained by the Washington Suburban Sanitary Commission (WSSC), and much smaller portions by the City of Rockville, and the Town of Poolesville (46). According to the 2004 Upper Rock Creek Area Master Plan, "trunk sewer lines" run parallel to the mainstem and North Branch of Rock Creek (47). The North Branch additionally receives sewage from two wastewater pumping stations, and ultimately joins the mainstem trunk sewer (47). The trunk lines flow into the D.C. sewer system and end their course at the Blue Plains Advanced Wastewater Treatment Facility (Blue Plains), as there are no wastewater treatment plants within the watershed (47). In addition to the pumping stations, the Maryland-National Capital Park and Planning Commission (M-NCPPC) operates a wastewater storage facility along Rock Creek trunk sewer during major storm events to minimize the potential of sanitary sewer overflows downstream. A few areas in the Maryland portion of the watershed receive service through the Great Seneca Creek sewerage system via individual pump systems. The only combined sewer systems within the Rock Creek Watershed are located in the District (Figure 2), which since 1960, has been working on a long term plan to separate sanitary and stormwater sewer systems. In the meantime, a long-term control plan (LTCP), implemented in July 2002, was developed by the D.C. Water and Sewer Authority (WASA) to control CSOs (19).

Old wastewater infrastructure negatively affects Lower Rock Creek, which periodically results in its contamination from potentially leaking sewer lines along the banks or at stream crossings, or from sewer lines that leak into the shallow, unconfined groundwater around the creek. During large precipitation events, Lower Rock Creek is also subject to receiving sanitary sewer discharge resulting from overwhelmed combined sewer systems, a prime source for *E. coli* contamination.

Existing State of Water Quality in Rock Creek

Water quality in the Rock Creek Watershed has been monitored for over 25 years, during which time there have been numerous exceedances of established water quality standards (7, 12, 43, 44). Per the Code of Maryland Regulations (COMAR) 26.08.02.08 "Stream Segment Designations," MDE defines the Surface Water Use Designation for the Rock Creek Watershed to include uses listed in Table 1 (20, 29, 47). Per the D.C. Municipal Regulations (DCMR), Rock Creek's designated categories of beneficial use include uses listed in Table 2, which apply to Rock Creek and its tributaries, however, current use is limited to Category B-E.

Table 1. Stream Segment Designations Defined by COMAR 26.08.02.08

Category	Definition	Applicability
I-P	Water Contact Recreation, and Protection of Aquatic Life	Rock Creek and all tributaries except those designated as Use III or IV
III	Natural Trout Waters	Rock Creek and tributaries above Muncaster Mill Road
IV	Recreational Trout Waters	Rock Creek and tributaries from Maryland Route 28 to Muncaster Mill Road

Table 2. Designated Categories of Beneficial Use for the D.C. portion of Rock Creek

Category	Definition
A	Primary Contact Recreation
B	Secondary Contact Recreation
C	Protection and propagation of fish, shellfish, and wildlife
D	Protection of human health related to consumption of fish and shellfish
E	Navigation

The 2012 RWQC apply to all U.S. waters designated for primary contact recreation: "activities where immersion and ingestion are likely and there is a high degree of bodily contact with the water, such as swimming, bathing, surfing, water skiing, tubing, skin diving, water play by children, or similar water-contact activities" (67). Under the CWA, and 40CFR 130.7, Total Maximum Daily Loads (TMDLs) must be developed for impaired waters and a list of these waters must be maintained by the state, territory or authorized tribes. The TMDL for fecal bacteria establishes that a steady state geometric mean of criteria listed in Table 3 be calculated with available data, where there are a minimum of five representative sampling events during steady state conditions between Memorial and Labor Day (43).

Table 3. USEPA approved TMDL requirements for fecal bacteria in MD and D.C.

Fecal Indicator Bacteria	TMDL (MPN of CFU/100mL)
Enterococci	33
E. coli	126

Studies have found that the greatest contributor to the impairment of Rock Creek are pathogens, metals and organics transported through uncontrolled, untreated stormwater, amplified by the amount of impervious surface cover in the watershed (12, 19). Over the years, MDE has identified the waters of the Rock Creek Watershed as impaired by nutrients, specifically total phosphorus (1996), sediments (1996), fecal bacteria (2002), and impacts to biological communities (2002) (22). The most recent Maryland Integrated Report (65) identifies the North Branch of Rock Creek as impaired by water temperature (exceeding the 90th percentile temperature criteria of 20°C outside the mixing zone determined in accordance with COMAR 26.08.03.03-.05) (65). EPA approved TMDLs in Maryland exist for fecal bacteria (2007), sediments (2011), and nutrients (2013).

In 1994, a Public Health Advisory (fish consumption advisory) which is still in place today, was issued by the D.C. Commissioner of Public Health (22). Four years later, the D.C. portion of Rock Creek was listed as impaired for fecal coliform bacteria counts in excess of Water Quality Standards, and the corresponding TMDL was revised in 2014 (8). In 2003, it was additionally listed as being impaired for metals and organics (19). In the most recent D.C. Integrated Report (2014), Lower Rock Creek was listed as impaired by Total Suspended Solids, and within six tributaries by *E. coli* (22). The Integrated Report also emphasized that swimming in Rock Creek is prohibited "until all the parameters used to determine use support are being consistently attained" (22).

Public Health Relevance

It is of utmost importance to ensure the health and safety of both recreational users and aquatic life within the Rock Creek watershed. The utilization of surface waters such as Rock Creek for recreational activities present exposure routes through both dermal contact and/or accidental ingestion of contaminated water. Aquatic wildlife can also be exposed to contaminated surface water runoff through ingestion, leading to bioaccumulation. Subsequently, fish from polluted habitats pose a potential health risk, if consumed by humans. This research will facilitate this endeavor by examining how environmental and anthropogenic factors influence spatial and temporal variations in surface water quality.

Utility of the Study

Although many studies have examined the general relationships between water quality, anthropogenic factors, and environmental factors, there is still much to learn. The highly complex relationship between the hydrological dynamics of a watershed, the variety of influences on it, and the surface water quality have led to mixed results in the body of knowledge. For example, there exist uncertainties regarding whether urban land use or agricultural land use is more important in influencing water quality, or which parameter is the most important indicator of water quality variation (18). Few studies have evaluated the distribution of enteric indicator microbes, their relationship with physical and chemical water quality parameters, and the effects of anthropogenic and environmental factors in surface water. This study examines such relationships in the Rock Creek Watershed and aid in the understanding of variations and identification of potential threats in its surface water quality. Ultimately, this research will assist decision makers in understanding the existing microbial water quality of Rock Creek and in the mitigation of potential health hazards resulting from the transport of pollutants through precipitation events and discharges.

HYPOTHESIS AND SPECIFIC AIMS

In this study, the spatial and temporal variability of land use, precipitation, and sewer characteristics on water quality will be investigated to address the following questions:

1) What are the impacts of land use and sewer characteristics on the water quality in Rock Creek?

2) What are the impacts of temperature and precipitation events on the water quality between the sampling sites and over the sampling period?

3) How do nutrients and turbidity in Rock Creek temporally and spatially vary in relationship with fecal indicator bacteria concentration?

The below Hypotheses and Specific Aims outline the strategy used to answer the aforementioned questions.

Hypothesis #1: Anthropogenic factors (land use and sewer age) impact enteric bacteria concentration in Rock Creek.

<u>Specific Aim #1.1:</u> Examine the influences of land use (percent impervious cover, land use classification) on enteric bacteria concentrations in Rock Creek.

<u>Specific Aim #1.2:</u> Examine the influence of sewer characteristics (age and type) on enteric bacteria and dissolved oxygen concentrations in Rock Creek.

<u>Specific Aim #1.3:</u> Analyze the spatial and temporal distribution and abundance of enteric bacteria along Rock Creek based on land use classification.

Hypothesis #2: Environmental factors (temperature and precipitation) impact enteric bacteria abundance and dissolved oxygen in Rock Creek.

<u>Specific Aim #2.1:</u> Investigate the variation of water quality and temperature between sample sites.

Specific Aim #2.2: Examine the relationship between environmental factors (temperature and precipitation) and water quality parameters over time at sampling sites with gaging stations.

Specific Aim #2.3: Examine the influence of precipitation and impervious surface cover on discharge.

Hypothesis #3: An increase in turbidity, nitrate and phosphate concentrations correspond with an increase in enteric bacteria abundance in Rock Creek.

Specific Aim #3.1: Examine the variation of water quality (turbidity, nitrates and phosphates) and enteric bacteria at each sample site over the sampling period.

CHAPTER 2: MATERIALS AND METHODS

OVERVIEW

This quantitative study, conducted in the Rock Creek Watershed, aimed to evaluate the effects of anthropogenic and environmental factors on the temporal and spatial variations in water quality of Rock Creek. Collection of water samples occurred weekly at 15 locations along Rock Creek between July and October 2015 (Figure 3 and Table 4). The study analyzed approximately 295 water samples: 255 routine, 30 quality control, and 10 precipitation samples at 80% power with an alpha=0.05 level.

Figure 3. Rock Creek Sampling Locations.

Table 4. Sampling Locations

Site	Description	Latitude (°N)	Longitude (°W)	Prominent Land Use
A	Northernmost point, within Agricultural Farm Park and the location least suspected to be contaminated	39.160556	77.131111	Low-density residential
B	South of Intercounty Connector 200	39.134809	77.129902	
C	South of confluence with Southlawn Branch (traverses a historic industrial area that includes cement-mixing facilities and vehicle scrap metal yards with little stormwater runoff controls)	39.105278	77.125278	Recreational
D	South of Baltimore Rd Bridge	39.090000	77.115278	
E	South of Parklawn Park Entrance, within Rock Creek Stream Valley Park	39.065010	77.099556	Medium-Density Residential
F*	On Turkey Branch, along Matthew-Henson Trail	39.068333	77.081389	
G	South of confluence with Turkey Branch at Winding Creek Park	39.057949	77.093088	
H	South of E-W Highway and confluence with Rock Creek Stream and the southernmost sampling location in Maryland	38.991694	77.061590	
I	North of MD/D.C. line and Boundary Bridge, off of Valley Trail; the last site prior to entering the Rock Creek National Park	38.987060	77.052540	
J	South of confluence with Fenwick Branch and Wise Rd Bridge	38.982800	77.041610	Medium-High Rise
K	North of Sherrill Drive, off of Area 9 Parking Lot	38.973840	77.039860	Recreational
L*	South of Joyce Rd Bridge	38.960070	77.041060	Medium-Density Residential
M	South of the confluence with Broad Branch and Soapstone Branch	38.942800	77.050350	Recreational
N**	South of confluence with Piney Branch, North of Kringle Rd Bridge	38.933860	77.049410	
O**	Southernmost point, within National Zoo, which lies within the combined sewer system watershed.	38.928150	77.044490	

*denotes sampling sites with a USGS Gage, **denotes sampling sites influenced by Combined Sewer Overflows, thick horizontal line separates MD and DC sites with Rock Creek National Park

SELECTION OF SAMPLE LOCATION

Sampling locations were selected based on map and ground reconnaissance, anthropogenic activities that occur close to or along the river course, and suspected areas of contamination or cleanliness identified by previous studies (4, 45, 47, 57). In order to have samples representative of the entire watershed and multiple land-use areas, sample collection activities extended between Maryland and D.C. For efficiency, they began at the farthest site and ended at the nearest site to the Uniformed Services University of Health Sciences (USUHS) Analytical Water Laboratory. In the Northern Sampling Area, sample collection activities began at Sampling Point A and ended at Sampling Point H while in the Southern Sampling Area, sample collection began at Sampling Point O and ended at Sampling Point I (Figure 3-4 and Table 4). Due to logistical limitations, it took one day to sample each area. A gap exists between sites G and H due to little variation in land use, accessibility, and logistical constraints.

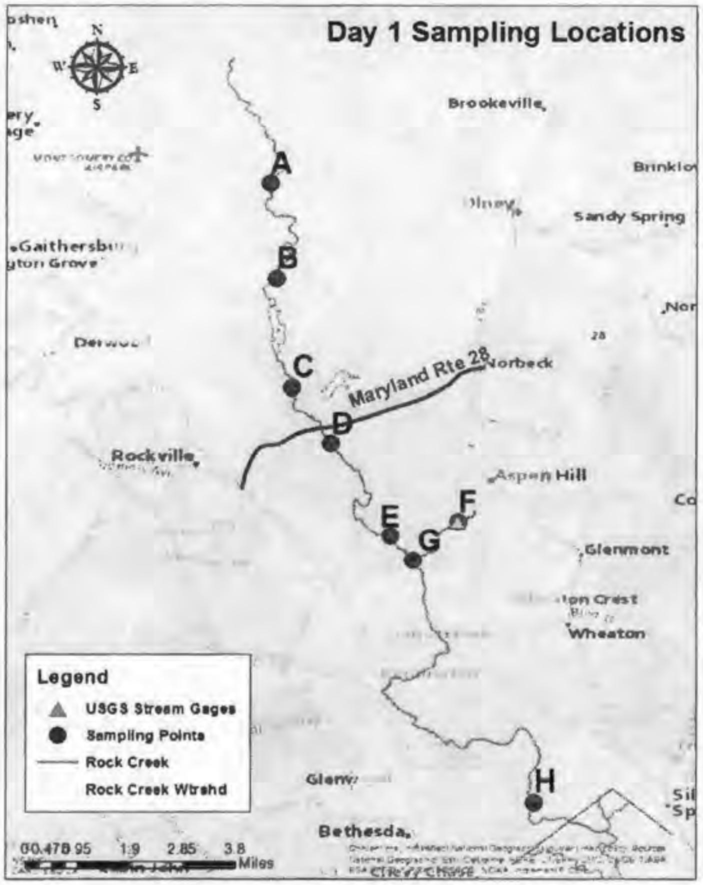

Figure 4. Northern Sampling Sites (Day 1).

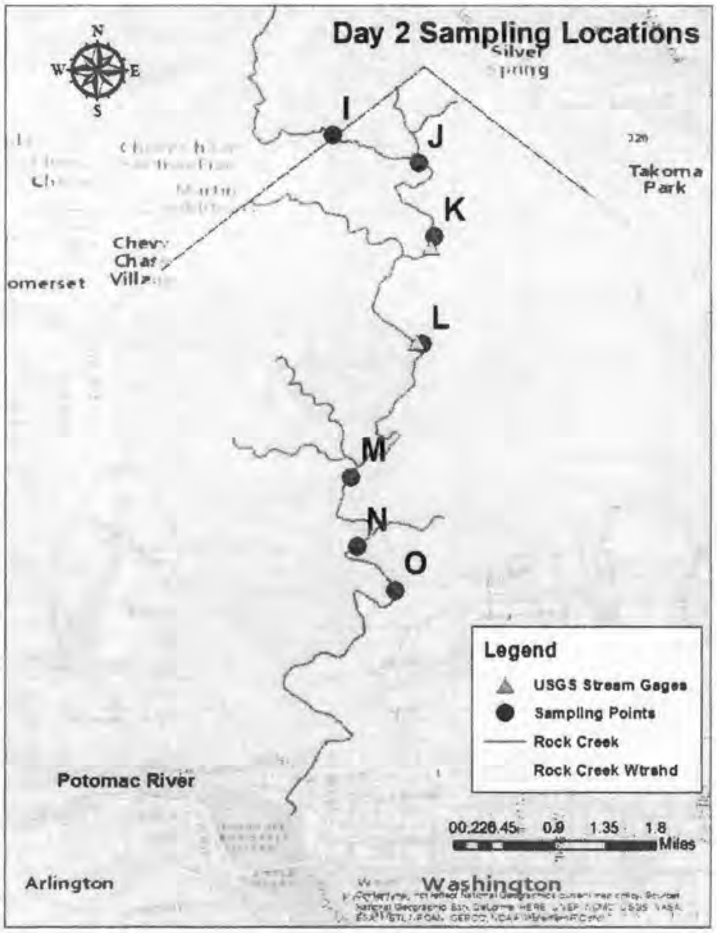

Figure 5. Southern Sampling Sites (Day 2).

Within the Rock Creek Watershed, streamflow gages providing streamflow data since 1929 in some instances, are monitored by MCDEP and USGS and located at Turkey Branch (Sampling Point F), Sherrill Drive (downstream from Sampling Point K) and Joyce Road (Sampling Point L). In addition to scheduled sampling, sampling occurred at Sampling Points F and L within 24h following a significant precipitation event. Sampling Point F is the only location on a tributary of Rock Creek, *Turkey Branch*, chosen so that there would be rain event data associated with USGS gages in both MD and DC. In 2001, MCDEP classified Turkey Branch tributary to be in "poor condition with severe erosion" due to three exposed sewer pipes and two stormwater outfalls releasing water into the stream from the surrounding residential area.

MCDEP defines a significant precipitation event as one resulting in accumulated rainfall greater than one half of an inch over a 24hr period, in accordance with the guidance provided. Precipitation events above this threshold can overburden the capacity of the pipelines to retain stormwater and/or sanitary sewer wastewater, leading to overflow events. To determine when a significant rain event had occurred, the National Climatic Data Center (NCDC) Climate Data Online (CDO) website of the National Oceanic and Atmospheric Administration (NOAA) (50) and the local National Weather Station were consulted.

COLLECTION OF WATER SAMPLES

Guidelines from the USGS Field Sampling Manual and USEPA Region 4 Surface Water Sampling Standard Operating Procedures were used as a basis for sample collection (68, 73). Prior to sample collection for turbidity measurement, a triple rinse of the glass vials with stream water occurred to flush out any contaminants that might be present. The rinsate was disposed downstream of the collection point. Collection of laboratory samples utilized 500mL High Density Polyethylene (HDPE) bottles using the grab sample technique described below.

Grab Sample Technique

Grab samples were typically collected directly into the sample container while wading, facing upstream, and midway through the stream at mid-depth for homogeneity (73). Sample collection followed methods described by Myers, et al. (5) and required inverting the bottle open end down, and lowering to half the water column depth, taking care not to disturb any sediments on the stream bottom. The sample bottle was then turned so that it was parallel to the streambed, allowing the air to escape and the bottle to fill. The bottle was either recapped under water during sampling or inverted again and capped above the surface, to prevent surface contaminants from being sampled (48). If the stream was too deep or the current too strong to wade, the sample was collected by decanting the water from a telescopic dipper with sterile ladle. To prevent contamination during sample collection, if transferring the sample from the ladle, care was taken that the device not come into contact with the sample containers. Samples were immediately chilled in a cooler with ice at 1-4°C until prior to analysis.

Quality Control (QC)

Many factors influence data quality to include planning, awareness of potential sources of sample contamination, and proper sample collection and handling. The study minimized bias and variability associated with environmental data through the quality assurance plan which included the execution of accuracy checks, pre-planned sampling events, quality control samples, rehearsed post-sampling analysis, and periodic equipment maintenance and calibration. Collecting and analyzing samples in the same manner, at approximately the same time, and by sampling within one day of each other also minimized sampling variability. Duplicate samples are a set of similar samples collected from the same site, at about the same time, and analyzed in the same manner (68). Duplicate samples provide basic quality control data for surface-water sampling and integrate the total variability introduced from collection, processing, and transporting the sample, the variability intrinsic in the stream across a short spatial and temporal distance, and the variability inherent in the laboratory handling and analysis of the samples (72).

Due to the nature of the sampling frequency, weekly sampling over a two-day period at less than 10 sites per day, weeks 7 and 17 were chosen in order to capture the full sampling period and have more than one quality control sample per site. One duplicate for every 10 samples per site was collected at each sampling location during weeks 7 and 17 of the sampling period, resulting in 30 total duplicate samples collected.

SAMPLE ANALYSIS

Physical and Chemical Analysis

Table 5 lists the lab and field parameters utilized in this study. Field measurements for the physical/chemical parameters listed characterize the surface water taken prior to the sample collection and following the equilibration of the meter probe. On-site analysis of these parameters involved the use of an Oakton Meter Kit 450, Hach HQ30d Portable Meter with Rugged LDO101 Luminescent/Optical Dissolved Oxygen (LDO) Probe, and Hach 2100Q Portable Turbidimeter.

Table 5. Sampling Parameters

Field		**Lab**
pH	Total Dissolved Solids (TDS)	*Escherichia coli* (*E.coli*)
Temperature	Dissolved Oxygen (DO)	Total Coliforms
Electrical Conductivity (EC)		Nitrate (NO_3-N)
Turbidity		Phosphate (PO_4^{3-})

Analysis of nitrate and phosphate concentrations took place in the USUHS laboratory within 24hrs post-sample collection using either a HACH DR 890 or DR 900 colorimeter. Nitrate analysis entailed the use of Nitra Ver5 powder pillows in the Hach Method 8171 and Phosphate analysis entailed the use of Phos Ver3 powder pillows in the Hach Method 8048 (equivalent to USEPA Method 365.2 and Standard Method 4500-P-

E) (28). Since shaking time and technique influence color development in the sample, for each lot of reagent used, an accuracy check was performed using the HACH Standard Additions Method (28).

Prior to analysis, samples were brought to room temperature per the method procedures. For Hach Methods 8171 and 8048, two sample cells were prepared by pipetting 10mL of sample water into each 25 mL glass sample cell (Hach 25x95 mm). One cell served as the blank, while the other sample was mixed with the respective reagent (NitraVer5 or PhosVer3) for a length of time dictated by the method, using Scientific Industries' Vortex-Genie 2 at a speed of position 8.5. Following the completion of respective reaction times, zeroing of the blank occurred and lastly, the reading of the reacted sample.

Bacteriological Analysis

Analysis of samples for two fecal indicator bacteria (total coliform and *E. coli*) occurred at the USUHS Analytical Water Laboratory using the IDEXX Colilert reagent and Quanti-Tray enumeration procedure for MPN analysis. Colilert-18/Quanti-Tray is the International Organization for Standardization (ISO) worldwide standard for detecting coliforms and *E. coli* in water (35). Colilert is used for the simultaneous detection and confirmation of total coliforms and *E. coli* in fresh waters.

The Colilert system utilizes prepackaged reagents, which include additives to support the growth of coliform bacteria in addition to particular compounds that react with coliforms in general and *E. coli* specifically. When total coliforms metabolize Colilert's nutrient indicator reagent, O-Nitrophenyl-β-d-galacotpyranoside (ONPG), the reaction produces an easily recognized yellow color (35). When *E. coli* metabolizes

Colilert nutrient-indicator, 4-Methumbelliferyl-β-glucuronide (MUG), the sample fluoresces (35). Non-coliform bacteria that also have these enzymes are suppressed, for the incubation period, by other reagents in the media. Colilert can simultaneously detect these bacteria at one colony forming units (CFU)/100mL within 18 or 24h. Per IDEXX laboratories, the test is effective and free of interference in waters with population densities of other heterotrophic bacteria up to 2,000,000 CFU/100mL present.

High CFU counts were expected during storm run-off events, high recreational periods, or downstream of known fecal pollution sources. If the results were expected to be greater than 2,419 CFU/100mL, dilutions of 100X, 1000X, or 10,000X were prepared with distilled water as needed, in order to accurately quantify the bacteria. If more than one dilution for a sample was analyzed, the most reliable estimate was reported; this was lowest dilution that could be enumerated (48).

Samples analysis occurred as soon as possible, typically within six hours of collection. The first few weeks of sampling revealed the constant need to dilute the sample x100 in order to enumerate total coliform concentrations. Subsequent to this discovery, an x100 dilution accompanied the sample for analysis. When results could still not be enumerated, however, an additional dilution was performed and the sample was re-analyzed within the 24hr hold period (48). Samples were first decanted or pipetted from field collection bottles into a sterile 120mL bottle using an Eppendorf Pipet or Hach TenSette Pipet, (0.1-1.0 mL or 1.0-10mL) while wearing nitrile gloves. The sample was brought to room temperature, then thoroughly mixed with Colilert, poured into a Quanti-Tray, and run through the IDEXX Quanti-Tray Sealer PLUS. The sample was then placed in the incubator at 35 ± 0.5°C for 18-22hrs (Colilert-18) or 24-28hrs (Colilert)

(35). Wells were observed for presence/absence of total coliforms and *E. coli* and interpreted using the IDEXX Comparator and below chart (Table 6). A 6-watt, 365nm black light placed in a UV viewing cabinet enabled the testing of fluorescence within 5 inches of the sample. Counting and recording the number of large and small yellow, fluorescing wells yielded the sample results. Once these numbers were obtained, the resulting MPN was recorded from the MPN table. For diluted samples, the MPN was multiplied by the dilution factor prior to recording.

Table 6. Bacteriological result interpretation table for Presence/Absence procedure

APPEARANCE	RESULT
Colorless or slight tinge	Negative for total coliforms and *E. coli*
Yellow equal to or greater than the comparator	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator	Positive for *E. coli*

EQUIPMENT MAINTENANCE

Field meter calibration occurred monthly and prior to sampling in accordance with manufacturer specifications. Equipment probes were rinsed with distilled water before and after sample collection. Maintenance of water testing equipment was conducted every three months in accordance with manufacturer specifications. During week 12, the Dissolved Oxygen Meter probe faulted due to a wiring issue, and was replaced by the manufacturer.

DATA ANALYSIS

Geographic Information Systems

Sources of GIS data for this study include MCDEP, M-NCPPC, WSSC, D.C. Water, and open-use data listed in Table 7 (27, 70).

Table 7. GIS Data Sources and File Types

Data Type	Geographic Coordinate System	Description	Source
Feature Class	GCS_WGS_1984	USGS Stream Gages, Special Protection Areas	ArcGIS Online
Shapefile	GCS_WGS_1984	D.C. Impervious Surface Coverage 2010	DC Office of Planning
Shapefile	GCS_North_American_1983	Combined Sewershed, Sanitary Sewershed, Gravity Sewerlines	DC Water
Shapefile	GCS_WGS_1984	MD Rivers and Streams, Waterbodies, 12-Digit Watersheds, Maryland 2010 Land Use	Maryland Geographic Information Office
Feature Class	GCS_North_American_1983	Rock Creek (MD) Impervious Surface Coverage 2014 derived by Water Quality Protection Charge and supplemented with Rockville data	MCDEP
Feature Class	GCS_North_American_1983	Stormlines (MD): combination of MCDEP, DPS, Rockville, and State Highway Administration data from 2003-2012	MCDEP
Raster	GCS_North_American_1983	National Elevation Dataset, 1/9 arc sec Digital Elevation Map, n39x25_w077x25_md_washingtondc_2008	U.S. Geological Survey
Feature Class	GCS_North_American_1983	NHD Flowline	U.S. Geological Survey
Shapefile	GCS_North_American_1983	Sanitary Sewer Overflows	WSSC
Feature Class	GCS_North_American_1983	Gravity Sewerlines	WSSC

ArcGIS Desktop 10.3 allowed the integration of various data such as land use coverage, sewer conditions and sampling locations to visualize and analyze data using tools such as Spatial Analyst and Hydrology in order to examine the influences of these factors on the water quality of Rock Creek. Reports of Sanitary Sewer Overflows (SSOs) were downloaded or provided by WSSC and DC Water. Locations of SSOs were imported into ArcMap using the *Geocode Addresses* tool and examined for their proximity to Rock Creek. Drainage basin areas were created for each sampling point and GIS tools were used to determine the variation in impervious surfaces, sewer pipes, and percentage of land cover with the greatest influence on the sampling point.

Watershed Delineation

ArcMap facilitated the delineation of watersheds in preparation for spatial analysis of sample sites in the Rock Creek Watershed. The first step was to create a depressionless Digital Elevation Model (DEM) in order to prevent areas of internal drainage from causing problems later in the watershed delineation process. Using the *Flow Direction* and *Flow Accumulation* tools, a DEM-generated drainage network was created. Sample points were then added to the map as "pour points" and edited to ensure the points lie on a high flow pathway. Everything upstream of these points would then define a single watershed. Prior to using the *Watershed* tool, the point features required conversion to raster cell format. To do this, the *Point to Raster* tool was used. The *Watershed* tool was then executed using the flow direction grid as the input flow direction raster, and the raster version of the pour points as the input raster.

Land Use Data

Land Use codes for MD and D.C. land use data were not equivalent, thus, the first step in preparing these data for analysis was to determine the best suited to categorize the overall land use of the Rock Creek Watershed. Nine primary land use categories in D.C. and their respective land use codes were compared to the seven in Maryland and adjustments were made in ArcMap using tools such as *Split*, *Erase*, *Dissolve*, and *Merge* to re-categorize them into the twelve categories listed below (Table 8 and Appendix D).

Table 8. Final List of Combined Land Use Categories for MD and D.C.

Land Use Categories
Agriculture
Commercial
High-density residential
Industrial
Institutional
Low-density residential
Medium High-rise
Medium Mid-rise
Medium-density residential
Recreational
Transportation
Water

Calculating Areas of Land Use Categories

Using the created watersheds as zones, and the Land Use Union map, the *Zonal Statistics* tool was used to calculate the area of each respective category within a watershed. Each watershed file (.dbf) was then imported into a worksheet in Microsoft Excel. A new worksheet was then created to summarize the values by land use categories and watershed in order to calculate total area (m^2) as well as the percentage of watershed covered by each land use.

Sewer Data

GIS density analysis allows visualization of a phenomena across a landscape by creating a raster layer (or surface) based on a quantity that is measured at each location and the spatial relationship of the locations of the measured quantities (23). To analyze and visualize the concentration of sewer pipeline (storm, sanitary, and combined) in the

Rock Creek Watershed, the *Kernel Density* tool in ArcGIS was used. Kernel density analysis indicates where point features are concentrated; estimations are based on probability "kernels", which are regions around a point location which contain some likelihood of point presence (23). The tool creates a "magnitude per unit area from point or polyline features using a kernel function to fit a smoothly tapered surface to each point or polyline" (23). Thus, higher value output cells have more points around them than lower value cells.

Pipe Density and Sewer Age Data

The sewer data was first *Projected* onto NAD 1983 (2011) UTM Zone 18N. ArcGIS *Merge* and *Clip* tools then facilitated the combining of stormwater and sanitary sewer pipeline data with the Rock Creek Watershed. Next, a geodatabase was created, and the merged sewer layers were combined with the sub-watersheds using the *Intersect* tool. The intersection of the files in a geodatabase provided the calculation of the total length of the sewer pipelines by attribute. Exporting the data tables for each layer into excel enabled the calculation and examination of the mean sanitary sewer age and sewer pipeline length (m) including both storm and sanitary sewers, within each sub-watershed. Sewer pipeline density was subsequently derived by dividing the total length of sewer pipeline in a sub-watershed by the area of the sub-watershed.

Impervious Surface Coverage

Similar to the sewer data, the impervious surface data was processed using the *Project, Merge* and *Clip* tools described above. The new layers were then *Intersected* with the sub-watersheds. Next, the *Calculate Areas* tool was used, and the exporting of the data table into excel enabled the calculation and examination of the total area of impervious surface coverage per sub-watershed. The percentage of impervious surface was subsequently calculated by dividing the area of impervious surface within each sub-watershed by its respective total area (Appendix D).

Hydrological Data

Daily rainfall data were requested for three stations within the Rock Creek Watershed, listed in Table 9, from the NCDC CDO website of the NOAA (50). These stations are managed by the Community Collaborative Rain, Hail and Snow (CoCoRaHS) Network.

Table 9. National Weather Stations used for gaging significant precipitation event

Station:	**Location**	**Latitude (°N)**	**Longitude (°W)**
GHCND:US1MDMG0074	Rockville, MD	39.074	77.146
GHCND:US1MDMG0086	Silver Spring, MD	39.058	77.091
GHCND:US1DCDC0014	Washington, D.C.	38.958	77.082

The range of days which defined a "rain event" for precipitation analysis was determined by analyzing the rain and discharge data at sampling sites with stream gages (Sites F and L). Site K stream gage was not used due to the proximity to Site L (~1.6km). To analyze hydrographs for sampled rain events, the USGS National Water Information System web (NWIS) interface provided the appropriate gaging station (Joyce Road:01648010, Turkey Branch: 01647850), gage height, and discharge information (71). The hydrographs were downloaded for the sampling period, July through October (Figure 6). From these hydrographs, the time to reach peak discharge rate and the time to return to baseline was recorded for all events that caused a significant deviation above the average baseline value. Subsequent calculations determined the average time to peak and average time to baseline for both sites.

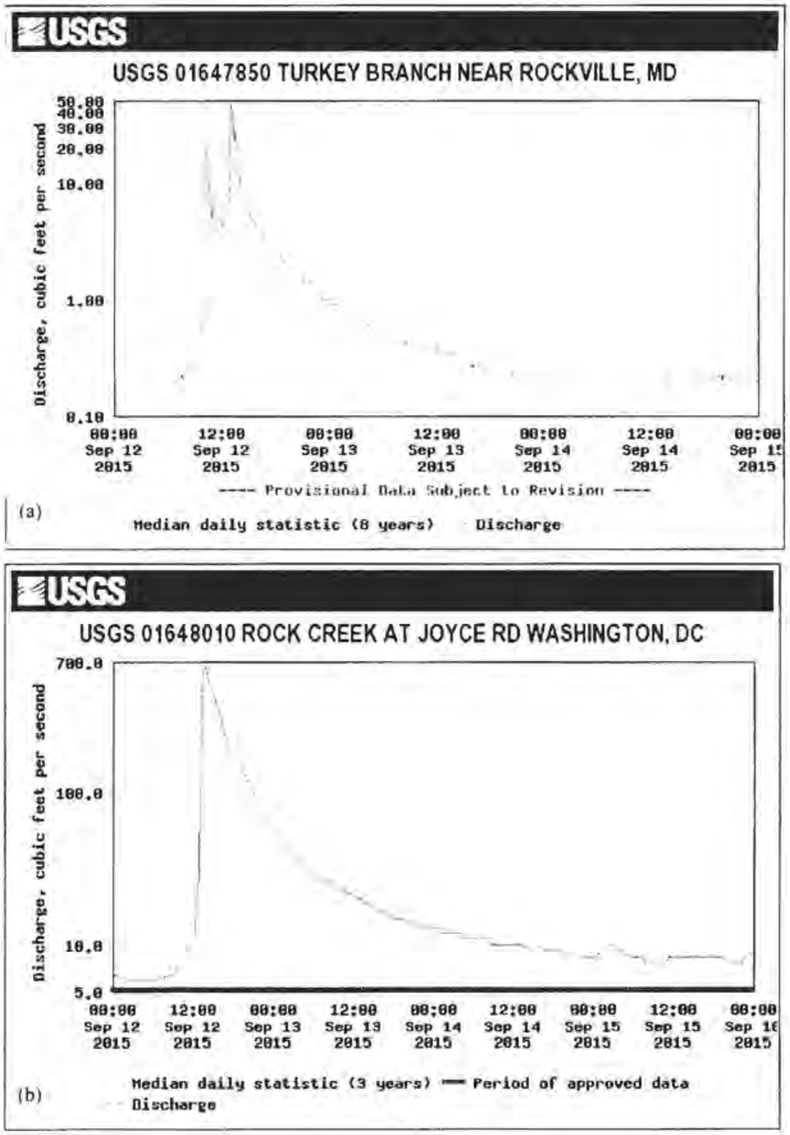

Figure 6. USGS Hydrographs depicting the discharge rate for a significant rain event during week 11 of the sampling period at stream gages in sub-watersheds (a) F and (b) L.

The average time to baseline plus two standard deviations was found to be approximately 91h. Since rain data are available for 24h periods, the 96h time period was used to define a rain event. The sum of rainfall for the 96h prior to the sampling event was then calculated and used for precipitation analysis in order to capture the full impact of significant rain events on Rock Creek. Through comparison with the discharge data for Sites F and L, it was determined that rain data from the Washington, D.C. rain station best captured the precipitation events which impacted Rock Creek and was thus used for analysis.

When comparing Sites F, L and O (sites where significant precipitation event sampling occurred), the data used included all weeks in which the rain event impacted the discharge (significant rain event data plus weekly data of which rain affected the discharge). For Sites L and O, this included weekly sampling data for weeks 1, 4, 6, 11 and 13. For Site F, this included weekly sampling data for weeks 4, 6, 11 and 15.

In the temporal analysis of the effects of temperature and precipitation on water quality, the mean of the 96h sum values for the two sampling days of the week was used to define a rain event. In the spatial analysis of the effects of precipitation on water quality, weekly sampling data were used to compare weeks with rain events to non-rain weeks. Weeks with rain events in this case, were defined as those which had greater than 13 mm of rainfall for the 96h sum for both sampling days one and two (weeks 4,6, and 11), and non-rain weeks were defined as all other weeks (weeks 1,2,3,5,7-10, and 12-17).

Statistical Analysis

Previous studies found correlations with water quality parameters of 0.35 with DO and 0.4 to 0.6 with bacterial concentrations, therefore, the required total sample size (sites x weeks) is at least 61 for correlations (42, 55). A sample size of 193 has 80% power to detect a correlation of 0.2 based on Fisher's Z test for the Pearson correlation coefficient with 5%, two-sided significance level. For correlations with water quality variables, this study had a sample size of 255 (15 sites x 17 weeks). For correlations with spatial variables, however, a sample size of 15 can only detect correlations of 0.66 (moderate).

The Statistical Package for the Social Sciences (SPSS) analysis software was used to conduct statistical analysis on data collected at a significance level of ≤ 0.05. The distribution of the original water quality data was inspected using descriptive statistics, the Shapiro-Wilk test, and graphical representations (normal Q-Q plot, histogram). All parameters with the exception of dissolved oxygen were not normally distributed therefore, the statistical analyses were confined to the nonparametric statistical test of Spearman's rank-order correlations. Due to the variability of the distribution of enteric bacterial indicators in surface, they are commonly represented as a log10 normal distribution. The log10 values were therefore, used for all statistical analysis of *E. coli* and total coliform.

A number of previous studies used multivariate statistics such as principal component analysis and discriminant analysis to investigate temporal and spatial variations in water quality. These studies however, varied between 1-9 years in duration, comparing 12-14 water quality parameters (not including FIB), and found that this type of analysis was better suited for reducing large data sets, as well as discriminating between seasons (49, 63, 64). Studies investigating water quality including FIB primarily used correlation or regression analysis (40, 42, 61). As the timeframe of this study was limited to 17 weeks, seasonality was not analyzed, the data set was relatively small in comparison to aforementioned studies, therefore, multivariate statistics was not used. To test for anthropogenic influences, correlation analyses were performed among water quality parameters and the respective sub-watershed land use percentages, percentage of impervious surface coverage, mean sewer age, and sewer pipeline density. To test for the environmental influences, additional correlation analyses were run among water quality parameters, temperature, discharge and rainfall events.

CHAPTER 3: RESULTS AND DISCUSSION

WATER SAMPLING RESULTS

Water sampling was conducted typically between the hours of 7:45 am - 2:00 pm, for two consecutive days (Wednesday/Thursday) for the first 7 weeks of the sample period, and then due to scheduling conflicts, every other day (Monday/Wednesday) for the last 10 weeks, with the exception of week 13, which had 3 days between sampling (Monday/Friday). The telescopic dipper was used on three days (October 2nd, 3rd, and 29th) at sites L and O, when the current prevented wading to the center of the creek. Listed in Table 10 are the descriptive statistics for measured water quality parameters. Boxplots are shown for each parameter in Appendix A, and descriptive statistics by sampling site is available in the supplemental data cd. The mean values for all water quality parameters for the samples collected were within the applicable permissible or recommended standard limits for Maryland surface waters with the exception of *E. coli*. This finding supports the existing classification of Rock Creek as impaired for fecal coliform bacteria. Additionally, fecal indicator bacteria abundance was irregularly distributed within the Rock Creek watershed.

Table 10. Descriptive Statistics of Water Quality Parameters during the study period

Descriptive Statistics for Water Quality Parameters											
	Depth	**Temp**	**pH**	**EC**	**TDS**	**DO**	**Turb**	**N**	**P**	***E.coli***	**TC**
UNITS	(cm)	(°C)	$-\log([H^+])$	(µS/cm)	(mg/L)	(mg/L)	(NTU)	(mg/L)	(mg/L)	(CFU/100mL)	(CFU/100mL)
MIN	19.21	7.00	6.95	158.10	99.10	5.19	0.53	0.10	0.02	20.3	980.0
MAX	114.30	25.90	8.09	875.60	487.00	11.45	59.60	3.70	0.58	51720.0	686700.0
MEAN	61.31	19.69	7.60	506.45	299.10	7.84	4.69	0.88	0.15	930.7	26055.8
STDEV	21.32	4.62	0.23	199.39	108.47	1.25	6.03	0.66	0.07	3861.2	71499.4
STND	N/A	<32.2	6.5-8.5	<600	<1000	>5	150	<2	N/A	126*	N/A

Notes: n= 255. Temp= water temperature, EC= electrical conductivity, TDS= total dissolved solids, DO= dissolved oxygen, Turb= turbidity, N= nitrate (NO_3^-), P= phosphate (PO_4^{3-}), TC= total coliforms, STND= standard. Standards are based on the Code of Maryland Regulations, or recommended values from published studies. NA indicates no data available. * indicates that this value is based on a steady state geometric mean where there are at least 5 sampling events

The depth of Rock Creek varied across the sites, averaging approximately 61cm and was found to be lowest at site C, and deepest at site O. As expected, temperature showed seasonal variation, decreasing as the sampling period transitioned from summer to fall (26-7°C). On average, temperatures slightly increased from upstream (17°C, site A) to downstream (20°C, site O). The mean value of pH was 7.6, ranging between 6.95-8.09 and was found to increase slightly over the sampling period as well as from upstream to downstream (Appendix A and B). The mean value for conductivity (EC) and total dissolved solids (TDS) were approximately 506 µS/cm and 299 mg/L, respectively. Both parameters showed the same relationship, increasing over time and the sampling period (Figure 7). On average, both parameters at sites A and B were 50% of those at the other sampling sites.

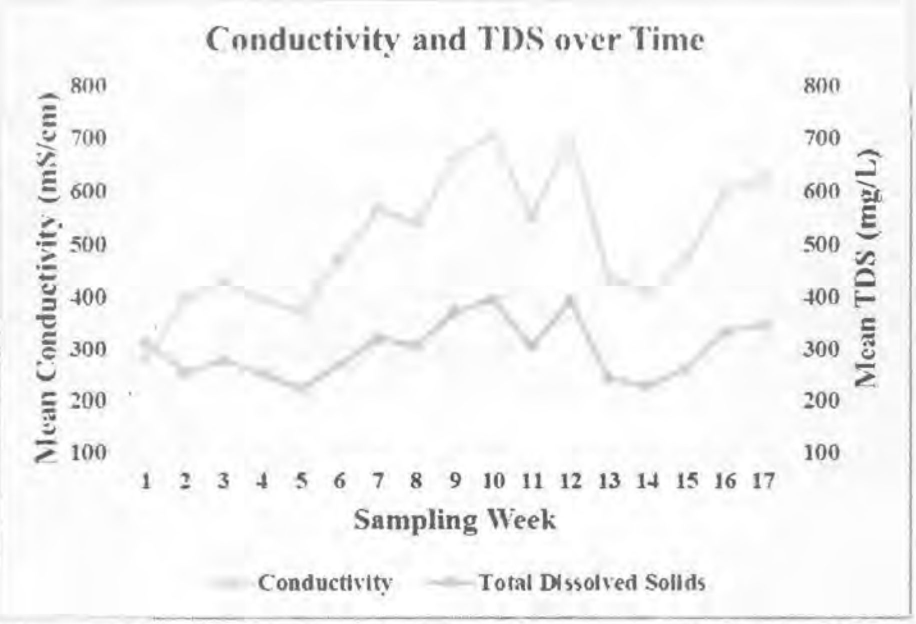

Figure 7. Mean electrical conductivity and mean total dissolved solid concentration over the sampling period

As expected, dissolved oxygen (DO) showed seasonal variation, increasing as the sampling period transitioned from summer to fall and water temperature declined. The mean DO concentration was found to be approximately 7.8mg/L. On average, DO concentrations fluctuated around 8mg/L at sites A-C, dropped at site D (mean of 6.4mg/L), and increased from sites E-O (7.4-8.5mg/L). DO values for week 12 sites B-H were not taken due to an equipment malfunction. The mean value for turbidity was 4.7 NTU. Turbidity was consistently higher at Site A. Values were highly variable from upstream to downstream and across the sampling period. The minimum value was taken at site C and maximum value at site I.

The mean nitrate (NO_3^-) concentration was 0.88 mg/L. Nitrate concentrations were consistently highest at sites A and B, as high as 3.7mg/L, compared to an average of approximately 0.6-0.9 mg/L across the other sampling sites (Figure 8). On average, nitrate concentrations slightly decreased from upstream to downstream and across the sampling period. Nitrate values for significant rain event sampling in week 17 were not reportable due to an interference with the detection method. The river water had a black discoloration on that day as a result from the rain event. The mean phosphate (PO_4^{3-}) concentration was 0.15 mg/L. On average, phosphate concentrations slightly increased from upstream to downstream (0.11-0.16 mg/L) but fluctuated over the sampling period without a clear pattern. The maximum value was found at site K and minimum value at site D. Similar to nitrate, on week 17, a discoloration in the river water prevented the analysis of phosphate following significant rain event sampling.

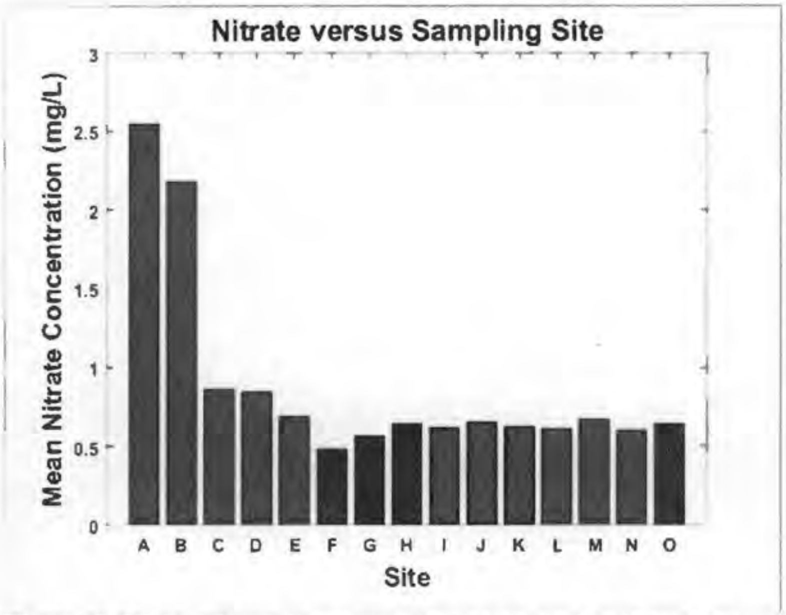

Figure 8. Mean nitrate by sampling site depicting increased concentrations at sampling sites A and B

The geometric mean for *E. coli* (CFU/100mL) was calculated for each month in the study and found to be 345 (July), 323 (August), 245 (September), and 231 (October). These values are well above the Code of Maryland Regulation standard of 126 CFU/100mL for freshwater. Mean *E. coli* and total coliform concentration (TC) was found to be approximately 931 CFU/100mL and 26,056 CFU/100mL, respectively. On average, fecal indicator bacteria concentrations were lowest at site C and highest at site F, with the greatest standard deviation found at site F (Appendix C).

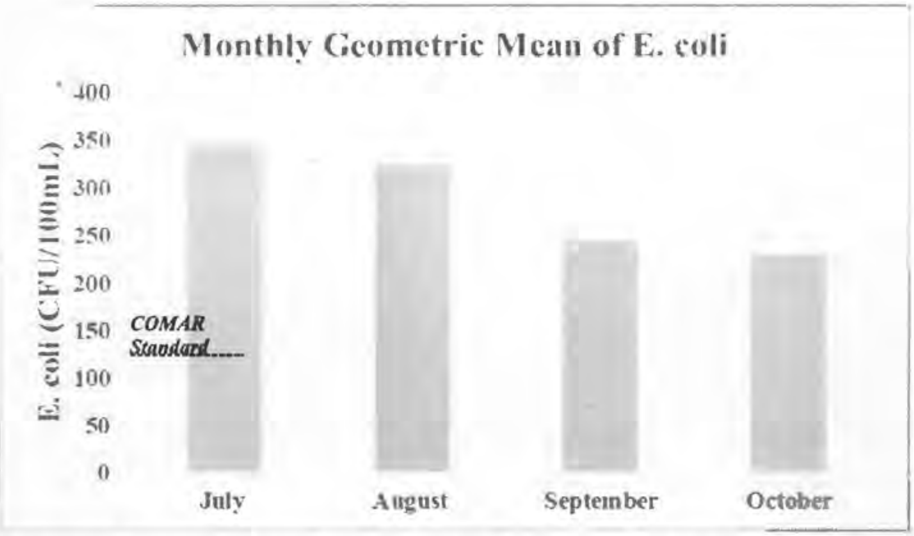

Figure 9. Geometric mean for *E. coli* (CFU/100mL) for each month in the sampling period. Highlighted is the Code of Maryland Regulation Standard for E. coli in surface waters.

THE INFLUENCE OF ANTHROPOGENIC FACTORS ON THE CONCENTRATIONS OF ENTERIC BACTERIA INDICATORS IN ROCK CREEK

Land Use

Upper Rock Creek is dominated by rural areas and as the creek flows south, the land use becomes more developed and densely populated (Figure 10). Sub-watershed A and F are the only sub-watersheds not influenced by others. Taking into account that each sub-watershed influences the one below it, the cumulative areas of land use categories were added for each respective sub-watershed to produce the "influenced land use" map (Figure 11). The dominant land use changed slightly, relocating the transition from rural to urbanized areas along the watershed downstream, to the top of sub-watersheds F and H. The land use category of water was not used in analysis due to the fact that the Maryland land use data did not include Rock Creek, while the DC land use data did so it was not appropriate to compare the two.

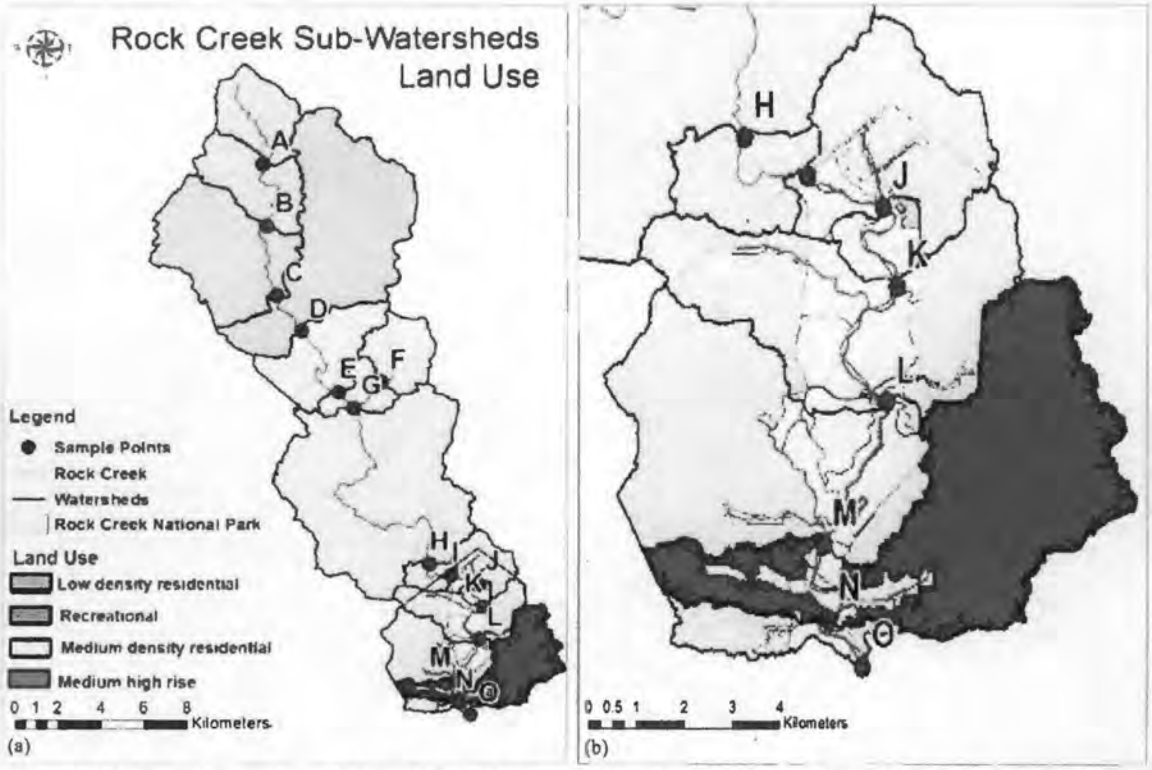

Figure 10. Dominant Land Use within the Rock Creek Watershed by (a) Sub-watershed, and (b) zoomed figure of the DC sub-watersheds highlighting Rock Creek National Park.

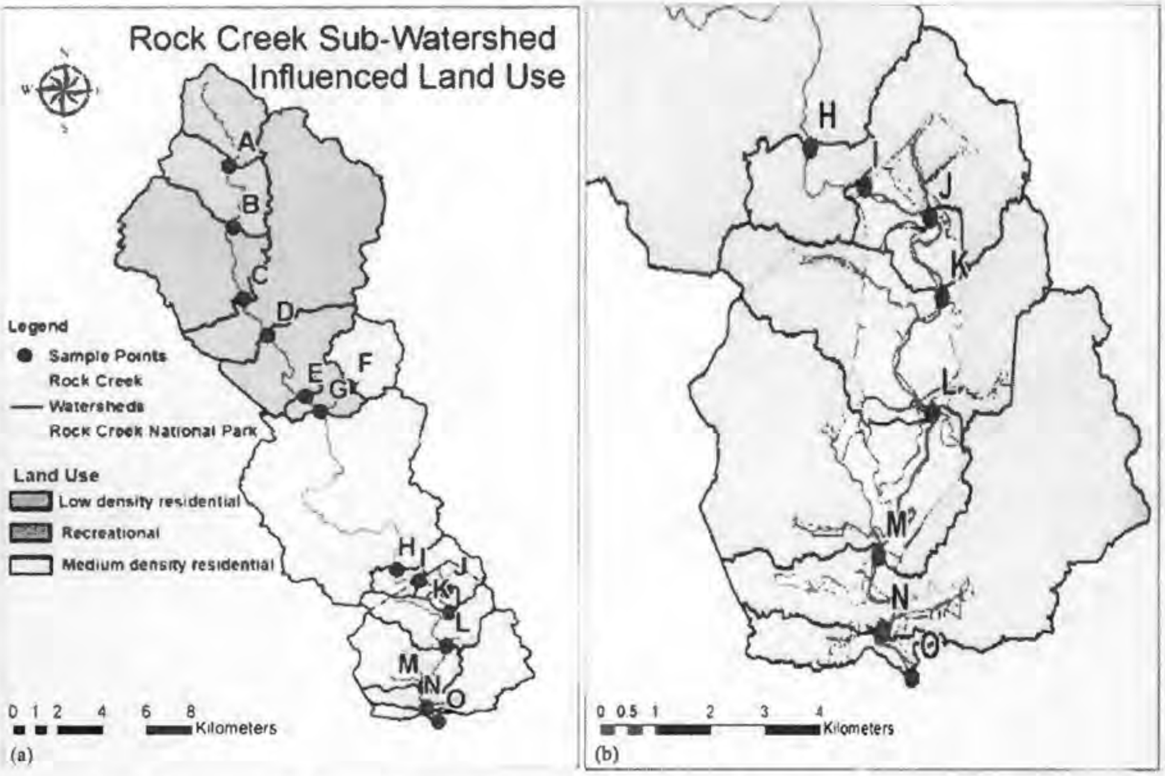

Figure 11. Dominant Influenced Land Use within the Rock Creek Watershed by Sub-watershed, and zoomed figure of the DC sub-watersheds highlighting Rock Creek National Park.

Spearman's rank correlation analysis yielded no significant correlations between the dominant land use coverage and mean enteric bacteria concentration within a sub-watershed. As shown in Figure 12, Sub-watersheds A and B with increased agricultural land use, had increased mean nitrate (NO_3^-) concentrations. Although the land use coverage in sub-watersheds A and B is predominantly low density residential, they contain the greatest percentage of agricultural land use coverage in the watershed. Agricultural land use in sub-watersheds A and B include cropland and pasture, of which the former is most abundant. Dependent on weather and growing conditions, fertilizer is typically applied to cropland based on soil testing results in March for wheat, April for corn and/or May for soybeans (J. Fetzer & N.M. Cintron, personal communications). Although the specific type of fertilizer used is not known, the data suggest it is less likely a manure source, in which case an increase in both nutrients and enteric bacteria indicators would be expected. Legumes such as soybeans are also known for increasing the nitrogen content of the soil as a result of the nitrogen fixation process carried out by certain bacteria such as *Rhizobium*, which are attracted to the root exudates (Bernhard, 2010). The significant decrease in nitrate concentration between sampling sites B and C is likely attributed to the presence of Lake Needwood, which acts as a flood control and has served as a sediment and nutrient trap. Nitrate concentrations continue to decrease through predominantly recreational areas (Sites C-E, and G) and then level out through the medium density residential areas.

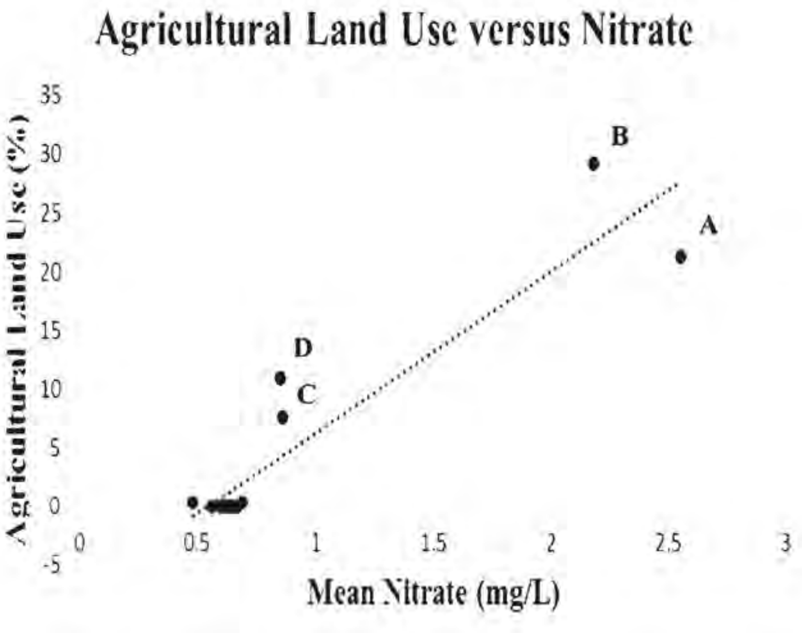

Figure 12. Correlation between agricultural land use and mean nitrate (NO_3^-) concentration highlighting increased values at Sites A and B

Increased agricultural land use coverage also related to decreased conductivity and phosphate. Site A was found to have much higher mean turbidity levels (~9.5 NTU) than the rest of the sampling sites. Water quality in watersheds with increased agricultural use have been found to be greatly affected by soil erosion, resulting in high suspended loads (37). This could explain the increased turbidity levels found at site A. Although sub-watershed B also has increased agricultural use, site B differs from A in that aside from the forest buffer around the creek, it is surrounded by a residential area and the Intercounty Connector/ MD 200 highway. The colder waters upstream are likely due to the proximity to the headwaters of Rock Creek which originate from a cold-water spring. Conductivity is affected by temperature: the lower the temperature, the lower the conductivity (30). The lack of positive correlation with phosphate may attributed to its affinity to bind with the soil, or the application of nitrogen rich fertilizer. An inverse relationship between nitrate and phosphate was also shown by Gächter et al. (26) and King et al. (33). Gächter et al. studied three agricultural drainage systems which drained grassland and cropland. They also found a greater abundance of nitrate in the surface water, attributed to the high affinity of phosphate for the soil and the increased ability for the soil/crop system to retain phosphate more than nitrate. Gächter et al. postulated that the concentrations of these nutrients are highly variable in the topsoil, dependent on the "timing of the last manure application, the intensity of biogeochemical reactions, and the recent nutrient uptake by crop plants (26)." King et al. studied these nutrients within subsurface drainage from managed turfgrass. Unlike what the data shown in this study, King et al. found that phosphate was more of a significant threat to the ground and surface water than nitrate (33). Studies such as these speak to the complexity of the effects of land use on river water quality (37, 41, 60).

Sewer Characteristics

Figure 13 was created in GIS to visualize and analyze the concentration of sewer pipelines (storm, sanitary, and combined) in the Rock Creek Watershed. The total length of sewer pipeline (4,531 km), ranged between 6.2 km (site K) and 1801 km (site H). As shown in Figure 10(a), sewer pipeline density ranged between .001% (site A) and .042% (Site I). Sub-watershed A is the most rural sub-watershed, located in a designated special protection area which minimizes the development of infrastructure, therefore, it only contains storm and septic sewers. Septic sewer data was not available and therefore, not analyzed in this study. Sub-watershed I is the last sub-watershed prior to entering Rock Creek National Park in D.C. The mean sanitary sewer age (approximate year of construction or emplacement) in the watershed (1955) was calculated using data tables exported from GIS and values ranged between 1926 and 1989. The sanitary sewer data provided by WSSC and DC Water took into account refurbishments and/or repairs made to the sewer lines and were current as of 11 September (DC Water) and 31 October (WSSC) of 2015.

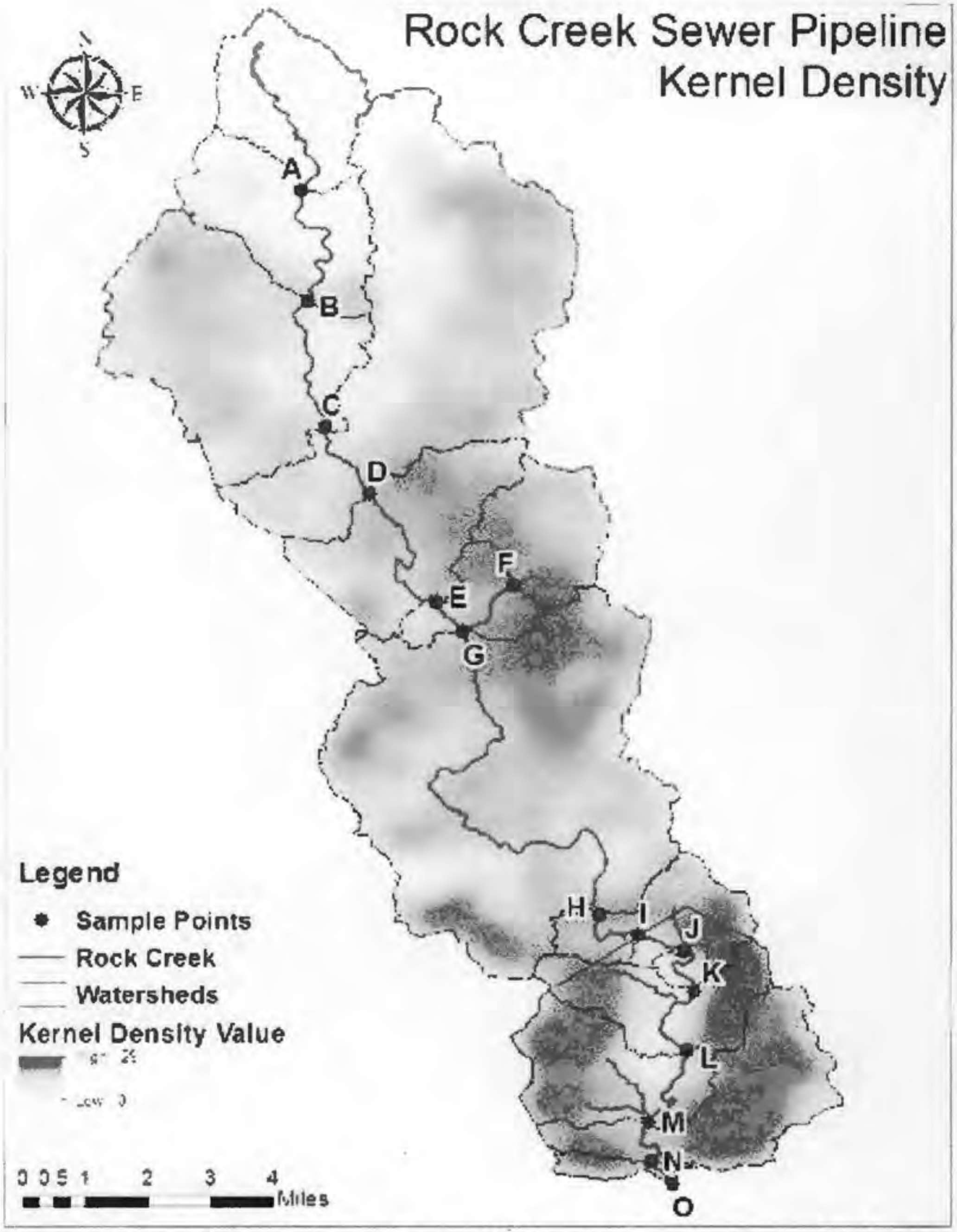

Figure 13. Spatial visualization of sewer pipeline density by sub-watershed using GIS Kernel Density Tool. Planimetric information shown is based in part on copyrighted GIS Data from M-NCPPC and may not be copied or reproduced without express written permission from M-NCPPC.

No significant correlations were found between mean sewer age or density and mean enteric bacteria indicator concentration within the sub-watersheds. There were no reported sanitary sewer or combined sewer overflows into Rock Creek during the sampling period, which would have provided this strong correlation. Although leaking sewers could have also shown this correlation, the high variability of additional sources of bacteria may have masked this relationship. Sub-watersheds with a greater density of sewer pipeline (Figure14(a)) had higher mean temperatures and decreased mean nitrate concentrations. This could be attributed in part to spatial variations in Rock Creek. Sewer densities are greater in the urbanized areas of the watershed, which increase as you move closer to D.C. As aforementioned, temperature and nitrate concentrations were found to increase from upstream to downstream. Additionally, a greater density of sewers results in more outfall discharges into Rock Creek, and stormwater input is known to increase the temperature.

Statistical analysis found that mean sanitary sewer age increased downstream through the watershed (Figure 14(b)). This reflects the migration of early settlers to the area. Residents first settled in Washington, D.C. and as it became more populated and urbanized, they began moving away from the city and thus the sewer infrastructure expanded northward. Sub-watersheds with older sewers (Figure 14(b)) had increased mean conductivity and phosphate concentrations. There are 18 minor industrial facilities and one minor municipal waste water treatment plant permitted as point sources to discharge phosphorus into the Rock Creek watershed. Older sewers have a greater risk of leaking due to exposures from erosion, or breakdown from weathering. Although indicators of structural repairs or replacement to sewer lines or their structural components were observed during the sampling period, accurate data regarding the location, timeframe, and nature of the work was not available to this study. During the process of such improvements or repairs, the water quality of Rock Creek may have been temporarily affected by increased turbidity levels or the leakage of sewage water. Leaking sewage water is known to have increased temperatures, bacteria, and nutrients among other constituents (21, 45, 76). Future research is needed in the areas of sewer outfall monitoring and microbial source tracking in order to rule out whether sewers are the primary source of bacterial or nutrient contamination to the watershed.

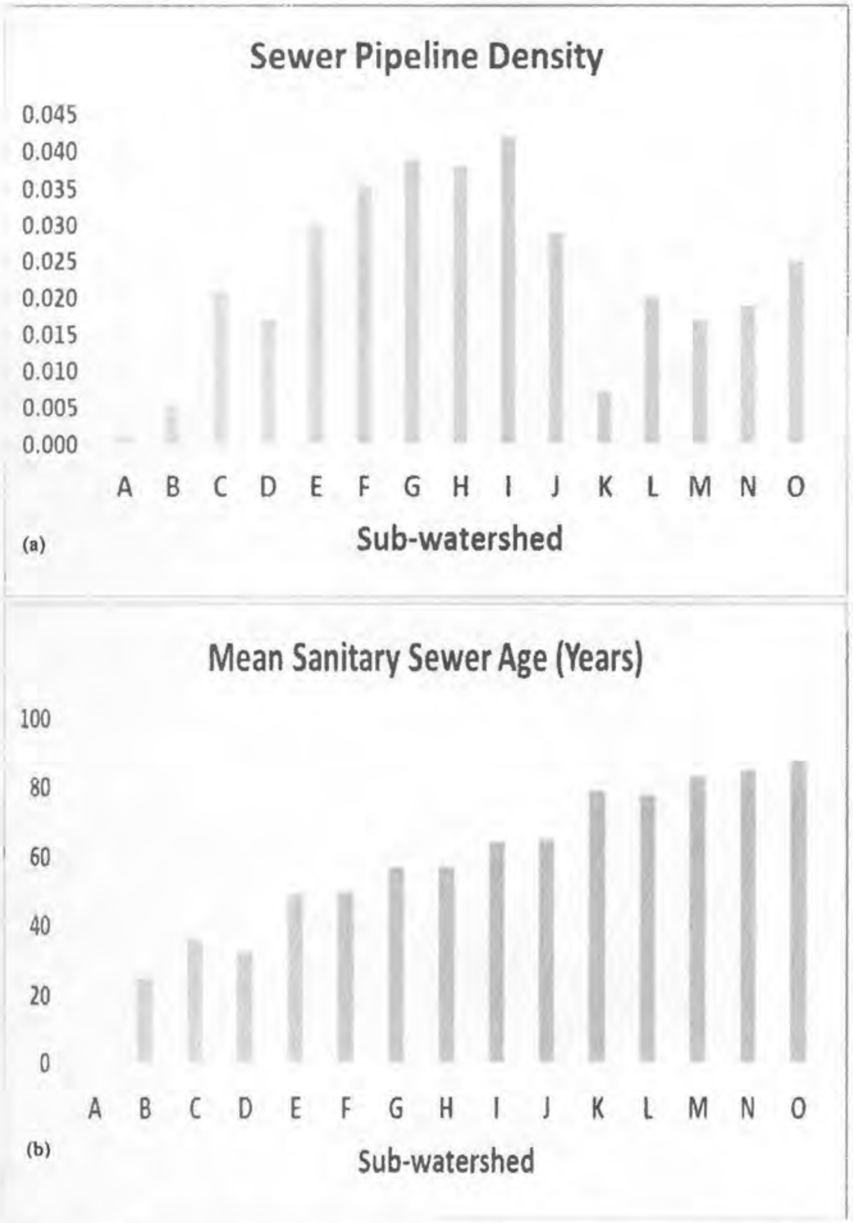

Figure 14. Bar graph depicting sewer characteristics in the Rock Creek Watershed. (a) Sewer Pipeline Density (storm, sanitary, and combined) and (b) Mean Sanitary Sewer Age (approximate year of construction/ emplacement)

Impervious Surface Coverage

The percentage of impervious surface within a sub-watershed was examined using GIS and Spearman's rank correlation analysis (Figure 15-17). A greater percentage of impervious surface coverage was not significantly correlated with enteric fecal indicator abundance, however, was positively correlated with mean conductivity and negatively correlated with mean nitrate concentration. The data suggests a stronger correlation with conductivity above 25% impervious surface coverage (Figure 15). Contaminants are known to collect on impervious surfaces such as rooftops, driveways and streets, and be mobilized through stormwater runoff into wetlands, rivers, and larger bodies of water, thereby increasing conductivity. Impervious surface coverage increased downstream, and as aforementioned, nitrate concentrations decrease. Mallin et. al (77) did not find that the percentage of watershed impervious surface coverage correlated with these parameters, rather, found a strong positive correlation with five-day biological oxygen demand (BOD_5), phosphate, and surfactant concentrations and negative correlation with total organic carbon. Although BOD_5 was not assessed in this study, DO was, yet it was not found to be significantly correlated with percentage impervious surface coverage.

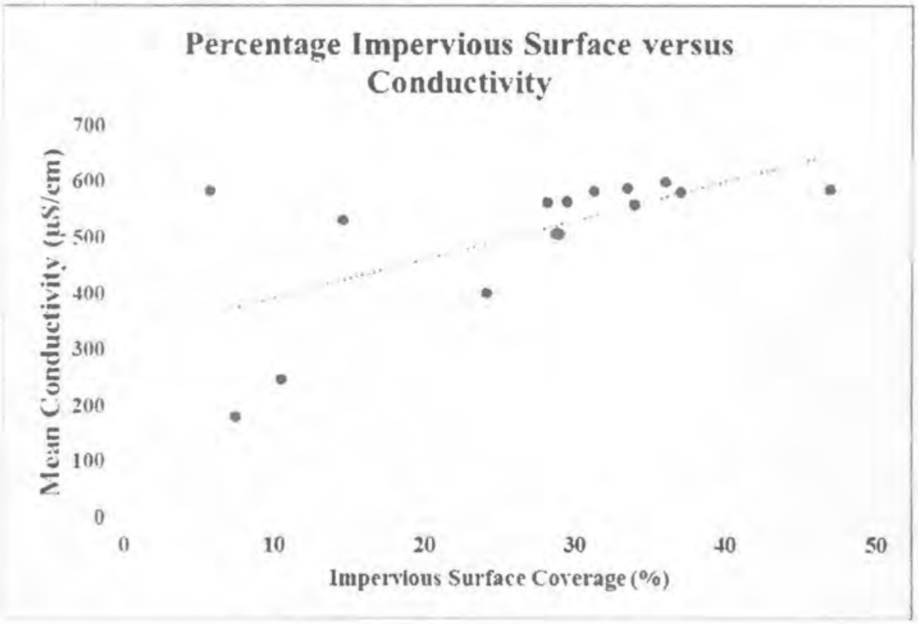

Figure 15. Correlation of the percentage of impervious surface coverage per sub-watershed and mean conductivity

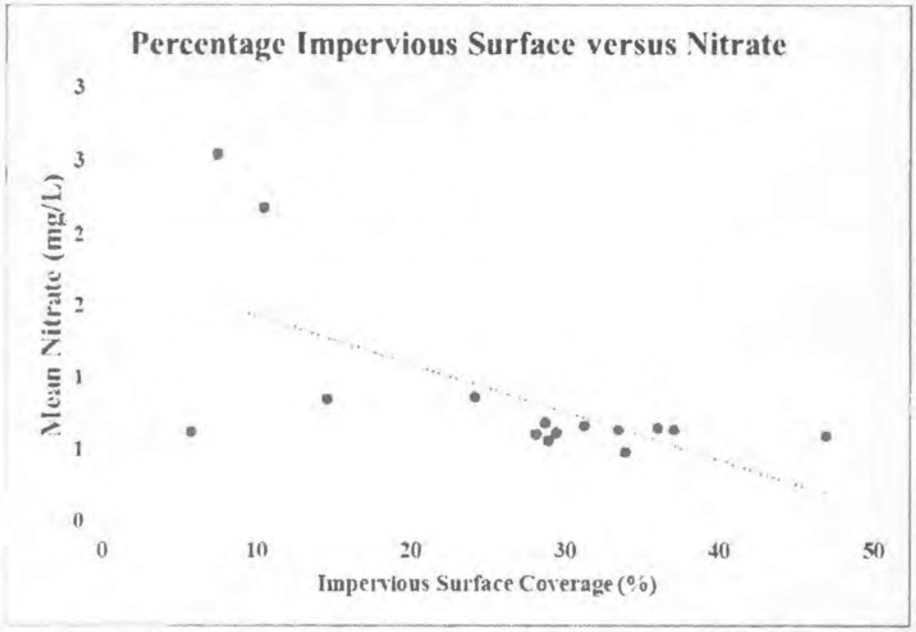

Figure 16. Correlation of the percentage of impervious surface coverage per sub-watershed and mean nitrate

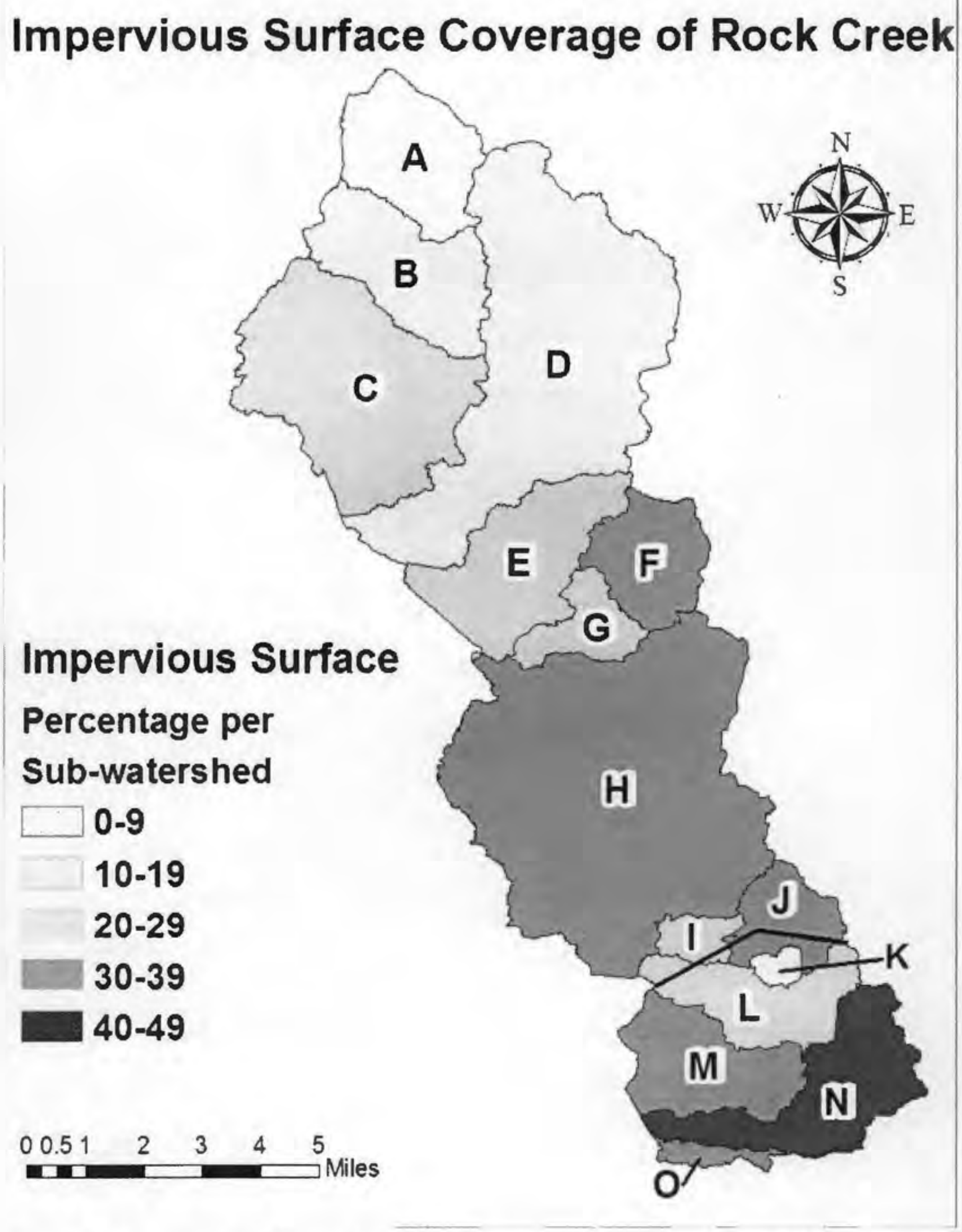

Figure 17. Percentage of impervious surface coverage visualized using GIS

THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE CONCENTRATION OF ENTERIC BACTERIA INDICATORS AND DISSOLVED OXYGEN IN ROCK CREEK

Precipitation and Discharge

During four significant rain events (>13 mm), samples were taken before or after the peak discharge level, implying that contamination during rising flows is more severe than what can be estimated from the data available in this study. The discharge rate for both locations with stream gages (F and L) were affected the same day of a significant rain event, however when comparing site F to site L, several factors that affect the behavior of the hydrograph (Figure 6) at each location must be considered. Sub-watershed F (7.1 km^2) is only slightly smaller than sub-watershed L (7.8 km^2), but it is also not affected by other sub-watersheds because it is on a Turkey Branch, a tributary of Rock Creek. Sub-watershed L is affected by all upstream sub-watersheds A-L (166.5km^2) due to the flow direction and topography of Rock Creek. The Turkey Branch tributary is also much narrower and has a smaller vegetative buffer surrounding the stream than the main stem of Rock Creek flowing through sub-watershed L. Sub-watershed F was found to have approximately 34% impervious surface coverage while sub-watershed L had 28% (Figure 17). A slightly greater percentage of impervious surface coverage (6%) related to decreased time for the river to reach peak discharge as the water flows directly over these surfaces and has less time to be absorbed by the land prior to reaching the river. More significantly, however, is the impact of the total drainage basin size on each site. The drainage basin affecting site L is 23 times greater than the drainage basin of site F. The effect on discharge rate between the two locations was highlighted when comparing the mean time to peak and baseline for each location, approximately 1.5hrs and 28hrs (site F) versus 5hrs and 43hrs (site L). Between July and October, the highest discharge rate observed at stream gage F occurred when it had rained significantly (18 mm) 96hrs prior and lightly (approx. 3 mm) for three days prior to July 4th. In contrast, at stream gage L, the highest discharge rate occurred when it had not rained 96hrs prior, but then rained significantly (36 mm) across the watershed within 24hrs of September 30th. Therefore, dependent on the soil saturation conditions, as little as 3 mm of rainfall was able to significantly impact discharge rate at both locations.

Statistical analysis showed that increased discharge rate correlated to increased concentrations of total coliform and *E. coli* (Figure 18(a)). Increased discharge rate at these sites also correlated to decreased conductivity. Additionally, at Site L, increased discharge was correlated to increased turbidity (Figure 18(b)). Once again, this is attributed to the time it takes a rain event to affect each site, as well as the location of site L and its upstream influences. Increased flow has the ability to carry particles and larger sediment in the creek, and can increase the resuspension of the bottom sediment, resulting in the elevated turbidity values. These relationships were also found in previous studies (41, 61).

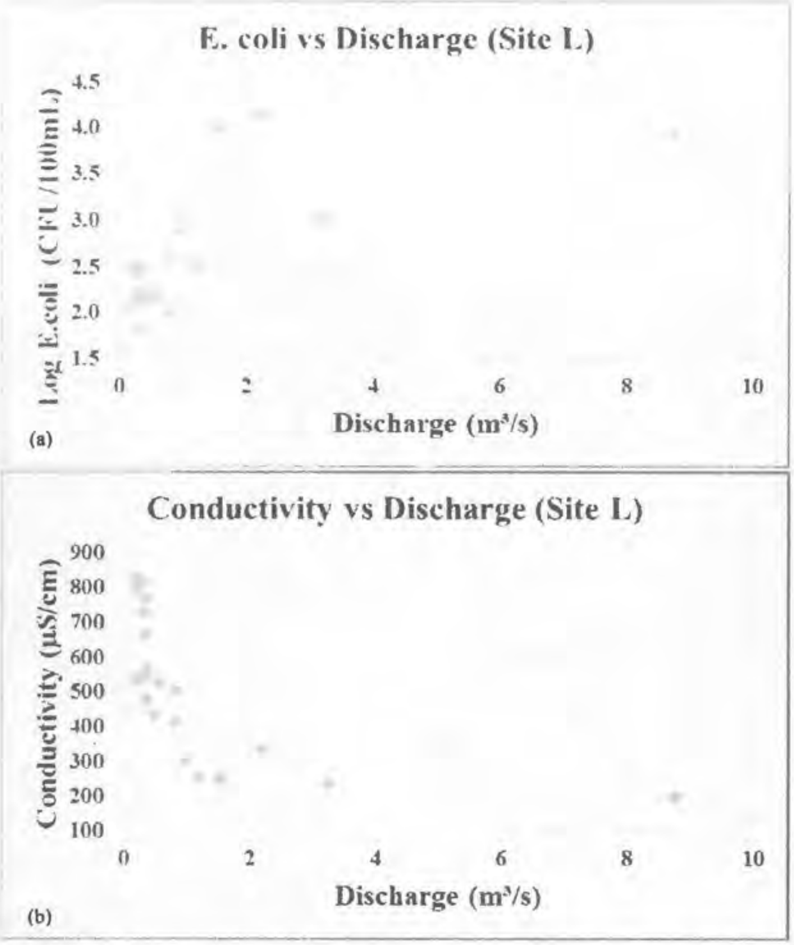

Figure 18. Scatter plots showing the (a) correlation at Site L between *E. coli* and discharge and (b) correlation between electrical conductivity and discharge

Precipitation and Sites F, L, and O

The relationship between precipitation, nutrients and *E. coli* at sampling sites where significant rain event sampling occurred (F, L, and O) is shown in Figure 20. When comparing Sites F, L and O, the data used included all weeks in which the rain event impacted the discharge (significant rain event data plus relevant weekly data). At site F, this included weekly sampling data for weeks 4, 6, 11 and 15. At sites L and O, this included weekly sampling data for weeks 1, 4, 6, 11 and 13. At site F (Figures 3&4), mean nitrate concentrations showed a dilution effect, while phosphate and *E. coli* concentrations showed an accumulation effect during rain periods. This relationship was also seen in previous studies (38, 41). During periods of dry weather, the creek receives only groundwater, however, during rain events, there is the additional input of stormwater which enters through outfalls, as well as that which quickly flows over the topsoil. A natural vegetative buffer surrounds Rock Creek throughout the watershed which serves as a passive filter for contaminants. The resulting river quality is therefore dependent on the amount and concentration of both input sources.

Gächter et al. (26) explain that the majority of the phosphate applied to the soil accumulates in the topsoil versus the groundwater and attribute the sustainment of elevated concentrations in the pore water of the topsoil to desorption (26). They further explain, that this holds true as long as the rate of desorption is equal to or higher than the dilution of the soil pore-water by the rain. Thus, while hydrograph is rising, the dilution rate of the soil pores is high, offering little time for rainwater to spend in the topsoil, resulting in lower phosphate concentration in the runoff water. Once the rain and the dilution of the topsoil pore-water stops, the phosphate concentration accumulates in the runoff water. As a result, the phosphate concentration in the creek quickly rises and then slowly decreases with recession of the river discharge. Unlike phosphate, nitrate does not have an affinity to the soil matrix, therefore, the topsoil cannot retain elevated nitrate concentrations when its pores are diluted with rainwater (low in nitrate) (26).

Consequently, as long as the runoff is drained through to topsoil, nitrate concentrations should be inversely related to the total discharge of the river. The data suggest that the source of increased phosphate and *E. coli* at Site F during rain events is a result of stormwater runoff and potentially a leaking exposed concrete sewer pipe located approximately 2m upstream of the site (Figure 19). Sampling Site F is located in a residential area and alongside the Matthew-Henson Trail, a prime location for human/pet recreational activities. Pet feces, lawn fertilizers and detergents from car-washing activities are additional potential sources of increased *E. coli* and phosphate levels accumulated into the creek during rain events.

Figure 19. Picture at Site F depicting the exposed concrete sewer pipe (bottom center)

At Site L, rain events have a dilution effect on nitrate and an accumulation effect on *E. coli*. For phosphate, this accumulation effect is observed within the first 24hrs of a rain event, but by return to baseflow conditions, a dilution effect is predominant. Site L is located within Rock Creek National Park, and downstream of various stormwater outfalls. The data suggest that soon after a rain event, accumulation of *E. coli* and phosphate are likely attributed to stormwater runoff from outfalls as well as the surrounding recreational land. Once the water from upstream has had time to reach Site L, it then dilutes the concentration of phosphate. *E. coli* likely remains increased as it is not only washed into the river from the surrounding area but the bacteria present in the stream sediment are also mobilized as a result of increased upstream discharge (34, 38, 45).

Site O, located within the Washington D.C. National Zoo grounds (Figures 3&5), is the southernmost sampling site and influenced by combined sewers outfalls. At Site O, a dilution effect was observed for nitrate, phosphate and *E. coli* during the first 24hrs of a rain event. Upon the return to baseflow conditions, *E. coli* and phosphate concentrations increased. The data suggest that after 96hrs, the creek had returned to baseline conditions, and contaminant concentrations returned to normal.

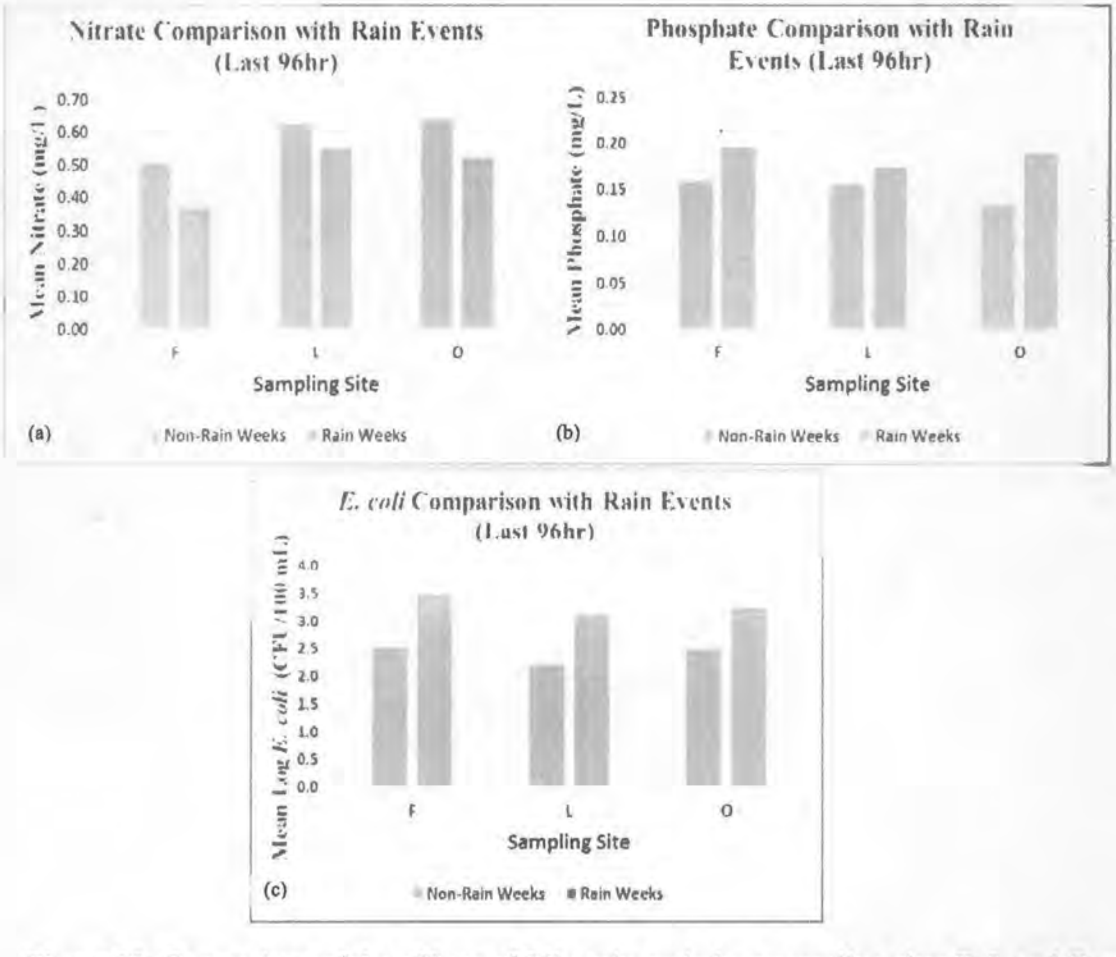

Figure 20. Comparison of the effects of 96hr rain periods at sampling sites F, L and O on (a) nitrate, (b) phosphate, and (c) *E. coli* concentrations in the Rock Creek Watershed

Precipitation events and water quality parameters were examined using Spearman's Rank Correlation analysis over the entire sampling period at Sites F, L, and O. As expected, precipitation events and enteric bacteria concentrations were positively correlated at all three sites. Precipitation events were negatively correlated with conductivity concentrations at all three sites. Conductivity was expected to decrease with rainfall because rainwater has low conductivity and an increase in the water volume dilutes mineral concentrations. Additionally, at Sites L and O, precipitation was positively correlated with turbidity. This is attributed to the fact that a rain event takes a much shorter time to affect Site F which is influenced by its own drainage basin, while at Sites L and O, the size of the total drainage area from the upstream sites (166.5km^2 and 188.4 km^2, respectively) influence the water at this location.

Temperature and Precipitation

Temporal and spatial analysis of the effects of temperature and precipitation on water quality was examined in Rock Creek using correlation analysis. The same positive correlation between precipitation events and enteric bacteria concentration and negative correlation between precipitation events and conductivity was found. Conductivity is decreased with each rain event with the exception of week 6, possibly due to the intensity of the rain in week 4. Although the conductivity rose on this occasion, it likely didn't rise as fast as it would have, had it stopped raining. The data showed that small rain events less than 38 mm had 1/3 of the average *E. coli* concentration (931 CFU/100mL), except in instances of dry weather conditions followed by a significant rain event within 24h of sampling, which increased *E. coli* concentrations 4 times above average. Rain events greater than 51 mm increased *E. coli* concentration 5 times above the average. Large rain events greater than 38 mm, such as those during weeks 4 and 13 dilute conductivity but accumulate enteric bacteria into the river (Figure 21).

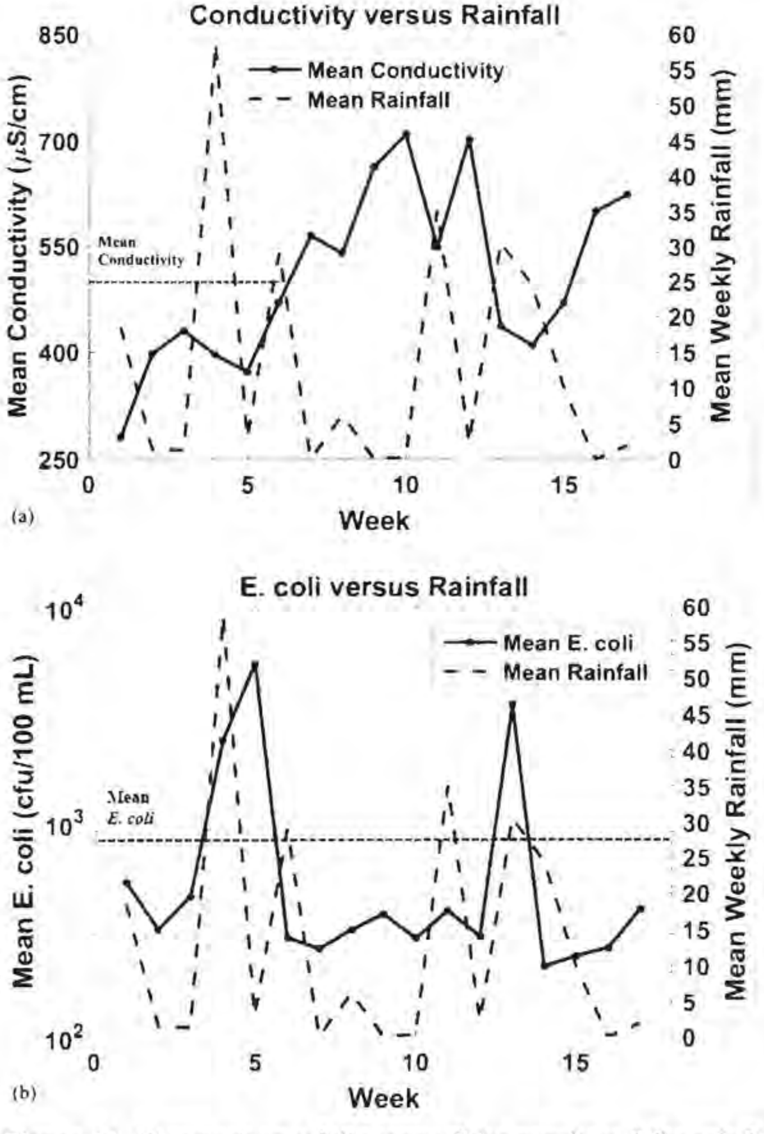

Figure 21. Comparison of the sum of the previous 96hr rainfall and water quality parameter over the sampling period. (a) weekly mean conductivity and (b) weekly mean *E. coli*

Water temperatures showed a characteristic seasonal cycle, with higher values during the summer and lower values in the fall. Temperature is known to be affected by a variety of factors such as the sun, inputs to the water (stormwater runoff, precipitation, groundwater, upstream water), heat exchanges with air, and heat gained or lost through condensation or evaporation. As expected, temperature was positively correlated with *E. coli*, and total coliform concentrations and negatively correlated with DO. Temperature was found to increase as the water flows downstream. The waters upstream originate from underground cold water springs, and as the water flows through the watershed it is influenced by various land uses and inputs which increase the temperature. These findings support the body of knowledge which state that temperature affects factors such as the solubility of oxygen and chemicals as well as biological processes such as metabolism, growth and reproduction (45, 56, 71).

In the spatial analysis of the effects of precipitation on water quality, weeks with rain events were defined as those which had greater than 13 mm of rainfall (96hr sum) for both sampling days (weeks 4,6, and 11), and non-rain weeks were defined as all other weeks. The data suggest that rain events in the rural part of the watershed tend to have a dilution effect on phosphate concentrations, and as the watershed becomes more urbanized, it has an accumulation effect (Figure 22). A similar relationship between urban versus rural areas having higher phosphate concentrations was found in a previous study (41). At some sampling locations, rain events had a dilution effect on turbidity while in other locations it had an accumulation effect (Figure 23). The data is limited in that only three precipitation events are captured in this comparison. Additionally, it should be taken into account that the summer months are characterized by convective thunderstorms, which are localized and random in time and location, making it difficult to interpret how the rainfall is affecting the stream at sampling sites without stream gages. Further investigation is needed to thoroughly analyze the spatial analysis of precipitation and water quality parameters.

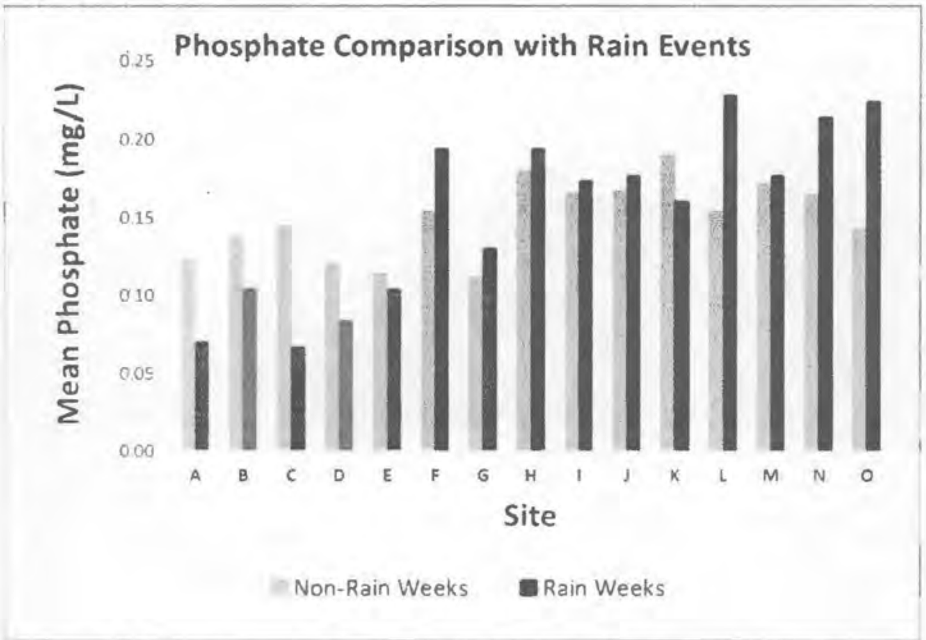

Figure 22. Comparison of mean phosphate (PO_4^{3-}) concentrations at each sampling site on weeks of no-rain and rain events.

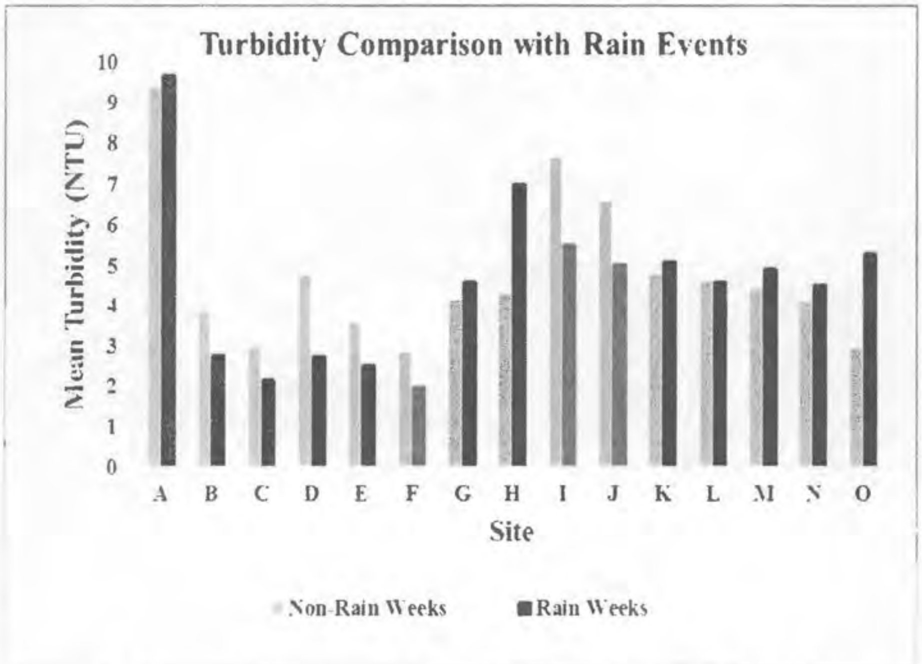

Figure 23. Comparison of mean turbidity at each sampling site on weeks of no-rain and rain events

In the temporal analysis of the effects of precipitation on turbidity (Figure 24), the mean of the 96hr sum for both sampling days of the week was used for the rain value. Unlike previous studies, however, a significant correlation was not always found between turbidity and precipitation events (40, 41). Week 4 had the greatest rainfall during the sampling period (>51 mm). Week 4 had significant rainfall for several days prior to sampling, and mean turbidity levels increased almost twice the average value (~5 NTU). Though week 13 did not have rain the week prior to the first day of weekly sampling, it subsequently rained significantly the 24hrs prior to the second day of sampling and prior to significant rain event sampling, raising the turbidity values over three times the mean value. Although both weeks observed an increase in turbidity, the latter rain event had a greater impact. In contrast, weeks 6 and 11, which also received 25-25mm of rainfall, resulted in a slight deviation of turbidity levels (~2 NTU less) from the mean value. During week 6 it rained significantly 72hrs prior to sampling and slightly the 48hrs prior to sampling. During week 11 it had not rained the week prior and then significantly rained 48hrs prior to the first day of sampling. Although week 4 shared a similar rainfall pattern with week 6, and week 11 with week 13, they had opposite effects on turbidity.

With respect to precipitation events, the data suggest that the effect of rainfall on turbidity (increase versus decrease) is dependent on the temporal pattern of rainfall, and not necessarily the quantity. Rainfall greater than 30 mm increases turbidity values when previous weather conditions 1) are dry followed by significant rain within 24h of sampling or 2) include consecutive significant rain events for several days prior to sampling. Similarly, the data suggest that the magnitude of an increase in turbidity is also most influenced by the temporal pattern (highest following previously dry conditions and subsequent significant rainfall (>30 mm) within 24hrs prior to sampling).

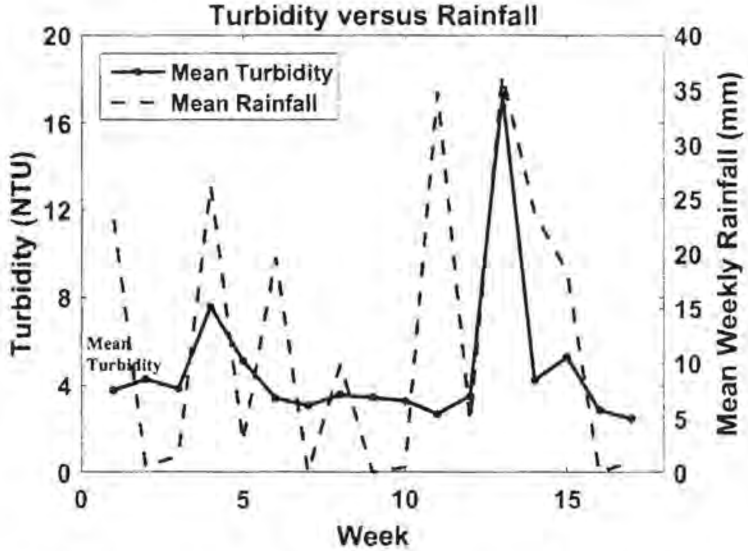

Figure 24. Comparison of the sum of the previous 96hr rainfall and mean turbidity over the sampling period

THE INFLUENCE OF INCREASED TURBIDITY, NITRATE, AND PHOSPHATE CONCENTRATIONS ON ENTERIC BACTERIA INDICATORS IN ROCK CREEK

Spatial and Temporal Variation of All Water Quality Parameters

Upon spatial and temporal assessment of water quality parameters, it was found that conductivity levels increased (Figure 25), while nitrate concentration decreased as water flowed downstream as well as over the sampling period. Upon further spatial assessment, downstream sites also had increased phosphate. Phosphate can originate from fertilizer, pet or wildlife manure, cleaning agents, or leaky sewers. Conductivity measures the ability of water to pass an electrical current and is therefore affected by the presence of inorganic dissolved solids such as phosphate ions. Previous studies found phosphate to be higher in urbanized areas which is supported by these findings (5, 41).

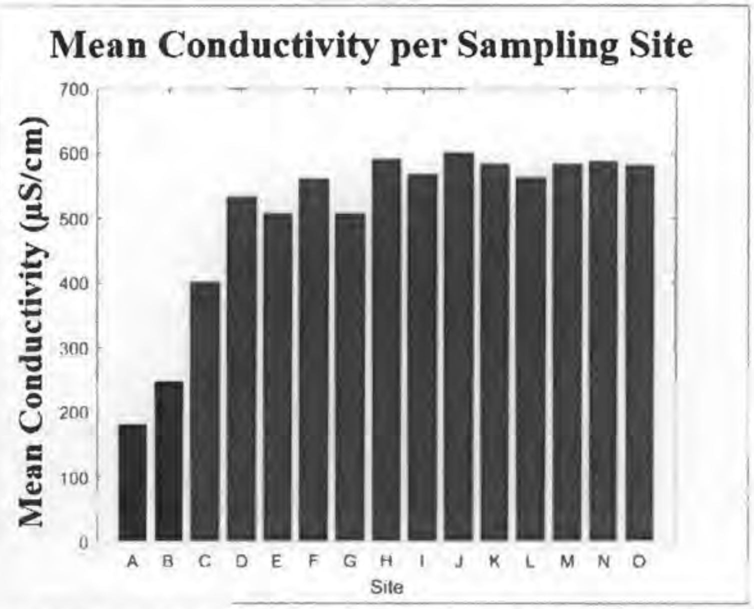

Figure 25. Mean conductivity by sampling site depicting increased concentrations as you move downstream

Upon further temporal assessment, an increase in turbidity correlated with a decrease in conductivity. Turbidity measures the clarity of the water, therefore if the water is opaque, there are more suspended solids present. Turbidity levels could rise as a result of numerous factors such as soil erosion, resuspension of or added sediment, decomposition of plant matter or sewage effluent (1, 71). This finding is surprising, as the majority of sources would correlate with an increase in nutrients or minerals which would also increase conductivity, resulting from the addition of dissolved ions to the surface water. Increased DO correlated with decreased *E. coli*. As aforementioned, the water temperature in Rock Creek decreased due to seasonal changes. The solubility of O2 increases as water temperature decreases. Decreased temperatures, typically reflect a decrease in bacterial growth and reproduction. Additionally, however, DO can be reduced through the respiration of microorganisms which oxidatively decompose organic compounds for food.

The Relationship between Turbidity, Nutrients, and Enteric Bacteria Indicators

Fecal indicator bacterial abundance was irregularly distributed within the Rock Creek watershed, and was intensified following rainfall. Sites with increased turbidity had increased total coliforms. Elevated concentrations of particulate matter affect not only sunlight penetration, but can provide food, shelter, and transport for pollutants such as bacteria. Other studies also found this correlation (38, 40). The transport of nonpoint sources such as excretions from pets and wildlife, stormwater runoff, agricultural runoff, and leaking sewers to the river is also influenced by the soil type, land use, and topography of the watershed. Additional inputs of enteric bacterial indicators into Rock Creek include permitted stormwater and combined sewer discharges. The majority of Rock Creek is surrounded by a natural vegetative buffer which includes both Rock Creek Regional and National Parks. Additionally, the local governments of Montgomery County, MD and D.C. have several ongoing projects which include the emplacement of man-made vegetative passive runoff treatment systems. The flow of the water through these pervious areas allows constituents to settle out, supports the uptake of nutrients such as nitrate and phosphate, facilitates denitrification within the soil, and naturally filters out contaminants. The results of this study supports findings from the 2001 Fecal Coliform study performed by Montgomery County which determined that each sampling point is affected by separate influences which differ the extent and make-up of bacterial abundance from one point to the next (12). No other associations were found between bacteria, nutrients and turbidity possibly resulting from limitations of the study or the variety of influences within the watershed which contribute to the abundance of these parameters.

CHAPTER 4: CONCLUSIONS

River hydrology and water quality have both seasonal and geographic trends which emphasize the importance of identifying spatial-temporal variations of water quality at the watershed level. The goal of this study was to assess how environmental and anthropogenic factors may impact the spatial and temporal variations in surface water quality in an urban environment. This was accomplished through the monitoring of fecal indicator bacteria and physical water quality parameters in the Rock Creek Watershed within Maryland and the District of Columbia. The below hypotheses outline the strategy used to reach this goal.

Hypothesis #1: Anthropogenic factors (land use and sewer age) will impact enteric bacteria concentration in Rock Creek.

Statistical analysis yielded no significant correlations between anthropogenic factors studied and mean enteric bacteria concentration. Areas primarily consisting of agricultural land use had increased mean nitrate (NO3-) concentrations and decreased conductivity and phosphate concentrations. Increased nitrate concentration and conductivity were likely a reflection of the headwaters of Rock Creek, which originate from a groundwater-fed cold-water spring. Land use and bedrock type are two factors known to drive the variance of nitrate concentrations in ground water which can persist for decades (36, 52). Nitrogen from septic systems may contribute to ground water concentrations. Additional influences of nitrogen include the product of nitrogen fixation related to planted crops, and the application of nitrogen-rich fertilizer (10, 26). Erosion and leaching then transport nitrogen from agricultural fields to both surface water and ground water. The lack of positive correlation with phosphate was attributed to its affinity to bind with the soil, which has been observed in previous studies (26). Turbidity values were also found elevated, attributed to soil erosion resulting from agricultural land use (37).

Sub-watersheds with older sanitary sewers had increased mean conductivity, and phosphate concentrations. Older sewers have a greater risk of leaking due to exposures from erosion, or breakdown from weathering, and sewage effluent is known to have increased temperature, bacteria, and nutrients among other constituents (21, 45, 76). Sub-watersheds with a greater density of sewer pipeline (storm, sanitary and/or combined) had higher mean temperatures and decreased mean nitrate concentration potentially resulting from spatial variations in Rock Creek. Sewer densities were greater in the urbanized areas which increase downstream through the watershed. Similarly, temperature was found to increase and nitrate concentrations decrease further downstream. Dependent on the temperature of sewage effluent in relation to the creek, sewage effluent may decrease water temperatures in the summer and increase water temperatures in the winter. This study however, was unable to analyze seasonal variations due to limitations of the timeframe.

Impervious surface coverage was positively correlated with conductivity and negatively correlated with nitrate concentration. Although increased discharge rates had a dilution effect on existing conductivity values, the mobilization of contaminants which collect on impervious surfaces through stormwater runoff was found to increase mean conductivity (1, 30).

The distribution and intensity of anthropogenic factors is clearly not the sole basis which determines the bacterial quality of Rock Creek. There are also temporal influences, in terms of watershed practices and hydrological behavior as well as spatial influences (sources of pollution and recreational activity) that ultimately influence the dynamics of fecal indicator bacteria contamination over the watershed.

Hypothesis #2: Environmental factors (temperature and precipitation) will impact enteric bacteria abundance and dissolved oxygen in Rock Creek.

Rain, temperature and discharge were positively correlated with mean enteric bacteria concentration. Rain events (96h sum) greater than 51mm increased *E. coli* concentrations 5 times above the average. Water temperature showed a characteristic seasonal cycle, with higher values during the summer and lower values in the fall. Mean nitrate concentrations were diluted, while phosphate and *E. coli* concentrations were accumulated following rain events. This inverse relationship between nitrate and phosphate was again attributed to the affinity of phosphate to bind to the soil, as well as the likelihood of rainwater diluting existing nitrate concentrations within the creek (26).

Unlike previous studies, a significant correlation was not always found between turbidity and precipitation events (40, 41). The data suggest that the effect of rainfall on turbidity (increase versus decrease) is dependent on the temporal pattern of rainfall, and not necessarily the quantity. Rainfall greater than 30 mm increases turbidity values when previous weather conditions 1) are dry followed by significant rain within 24h of sampling or 2) include consecutive significant rain events for several days prior to sampling. Similarly, the data suggest that the magnitude of an increase in turbidity is also most influenced by the temporal pattern (highest following previously dry conditions and subsequent significant rainfall (>30 mm) within 24h of sampling).

Results from precipitation event analysis suggest that contamination of Rock Creek during rising flows is more severe than what can be estimated from the data available in this study. In order to thoroughly investigate the effects of precipitation on water quality, it is important to consider several factors, including the quantity and intensity of rainfall, the temporal pattern and frequency of rainfall, and the seasonal differences in rain events.

Hypothesis #3: An increase in turbidity, nitrate and phosphate concentrations will correspond with an increase in enteric bacteria abundance in Rock Creek.

The mean values for all water quality parameters for the samples collected were within applicable permissible or recommended standard limits with the exception of *E. coli*. The geometric mean for each month in the study was calculated and found to be above the standard of 126 CFU/100mL (20). This finding supports the existing classification of Rock Creek as impaired for fecal coliform (8, 43). Fecal indicator bacteria abundance was irregularly distributed within the Rock Creek watershed.

Sites with increased turbidity, known to provide food, shelter and transport for pollutants such as bacteria, were found to have increased total coliform bacteria (38, 40). Additionally, an increase in turbidity correlated with a decrease in electrical conductivity, a surprising finding taking into account that turbidity values are affected by several factors such as soil erosion, resuspension of or added sediment, or sewage effluent, all of which would be expected to increase conductivity. No other associations were found between bacteria, nutrients and turbidity possibly resulting from limitations of the study or the variety of influences within the watershed which contribute to the abundance of these parameters.

Significance

The results of this study help inform ongoing stream restoration efforts by documenting variations in water quality throughout the Rock Creek Watershed. Continued efforts by both Montgomery County and Washington, D.C. to impact land management practices and human behavior will likely reduce the nutrient and fecal indicator bacteria abundance within Rock Creek. Better understanding of spatial and temporal variations in fecal indicator bacteria and nutrient transport in Rock Creek is essential for developing effective strategies to reduce concentrations for protection of water supplies and primary contact recreation opportunities. Urban streams such as Rock Creek can be affected by multiple contaminant sources, which may ultimately affect the safety of water for human consumption or other anthropogenic activities and aquatic ecosystems. Results of this research may aid in the understanding of similar causes of impairment in other urban streams and enable decision-makers to maximize the protection of public health and aquatic life.

As military operations are constantly adapting to global changes affecting the spread and concentration of troops, what remains constant is the need to protect the health of military personnel. As such, the ability to adequately identify and assess locally available water sources (lakes, rivers, estuaries, etc.) in order to maintain sustainable operations is paramount. Results of this study support this effort by assisting commanders in understanding the complexity of the hydrological environment and identifying factors that degrade water quality. Adaptation to a contemporary war-fighting environment requires a multi-disciplinary approach that considers a myriad of functional areas. Combat power, combat support, and ancillary services all play a direct role in sustaining the force, and steady-state operations. The active monitoring of surface water quality, as part of the force health protection strategy, sustainment efforts, force generation, and medical intelligence focus, directly affects the end-state of units of all sizes from Special Operations units to a brigade combat team to. In addition, surface water quality monitoring and the impact on the surrounding environment serves an important role in civil-military operations and the domestic emergency management response strategy.

LIMITATIONS

Although this study investigated the effects of precipitation on the water quality of Rock Creek, it was limited due to the timeframe of the study. The month of June had frequent rain events that were not captured in this study. There were only three rain weeks of data applicable for spatial examination, therefore, the effects of rain on spatial variations in water quality were not adequately analyzed. Additionally, significant rain sampling was unable to catch the peak of a rain event, instead reflecting a time where the discharge rate was either rising or receding. Seasonality is a factor that has been incorporated into previous studies, but was not thoroughly assessed. Extending the sampling period would be beneficial both to the sample size as well as the ability to discriminate water quality factors by season. A larger sampling size of which multivariate statistical analysis is better suited may better clarify the complexities of land use and water quality and the variation of fecal indicator bacteria throughout this mixed use watershed.

FUTURE RESEARCH

Further research is needed to better understand the variations in turbidity, fecal indicator bacteria and nutrient abundance and transport within Rock Creek and to further examine the complex relationships with environmental and anthropogenic factors. Potential areas of investigation include:

1) ground-surface water interactions. Although a large portion of water flowing into rivers comes from precipitation runoff, much of it also comes from the outflow of groundwater into the streambed. In other locations and/or at other times of the year, the water leaves the riverbed and enters the groundwater. Further investigation into these interactions can assist in understanding the impact they have on the loss or gain of pollutants into Rock Creek.

2) sewer outfall monitoring. In order to better understand the extent to which point sources such as stormwater and sanitary sewer outfalls have on the water quality of Rock Creek, monitoring of these sources is necessary.

3) sediment transport. It is difficult to determine the comparative contribution of streambed versus watershed surface stores from only surface water samples as they both act as reservoirs for fecal indicator bacteria and other contaminants. Further research into the mechanisms of sediment transport within Rock Creek would aid in this endeavor and would clarify whether sediments act as a source or sink for pollutants.

4) microbial source tracking. Knowing the distribution of sources of microbial water quality of Rock Creek such as humans, pet, birds, and other wildlife can assist in defining the extent to which point or nonpoint sources are influencing the water quality.

5) effectiveness of passive treatments. A better understanding of the effectiveness of passive treatments such as the vegetative buffers along Rock Creek in reducing

nutrient and bacterial loads would aid in understanding the variations in water quality found in this study.

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APPENDIX A: Boxplots of Water Quality Parameters

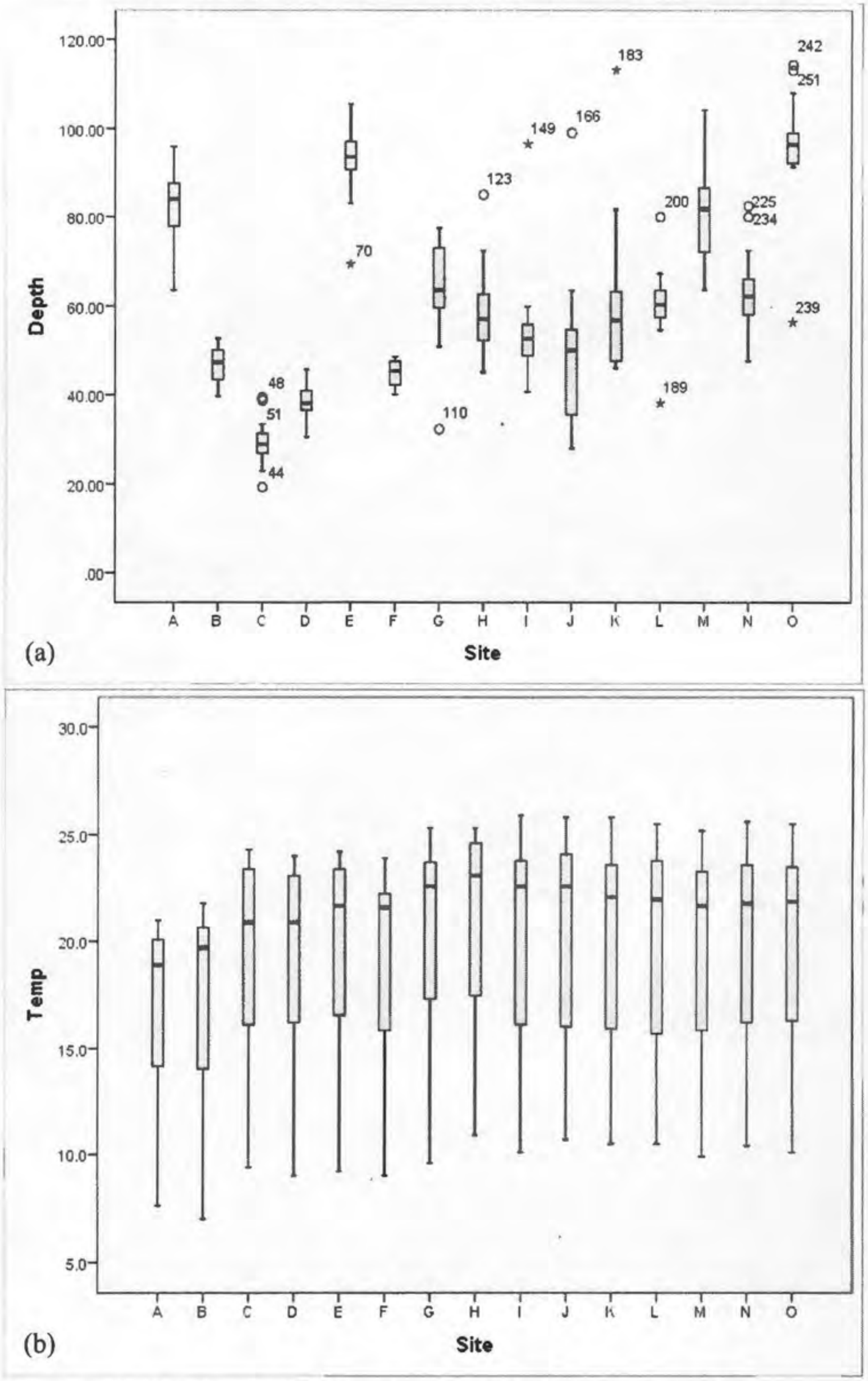

Figure A-1. Boxplots for (a) Depth (cm) and (b) Temperature (°C)

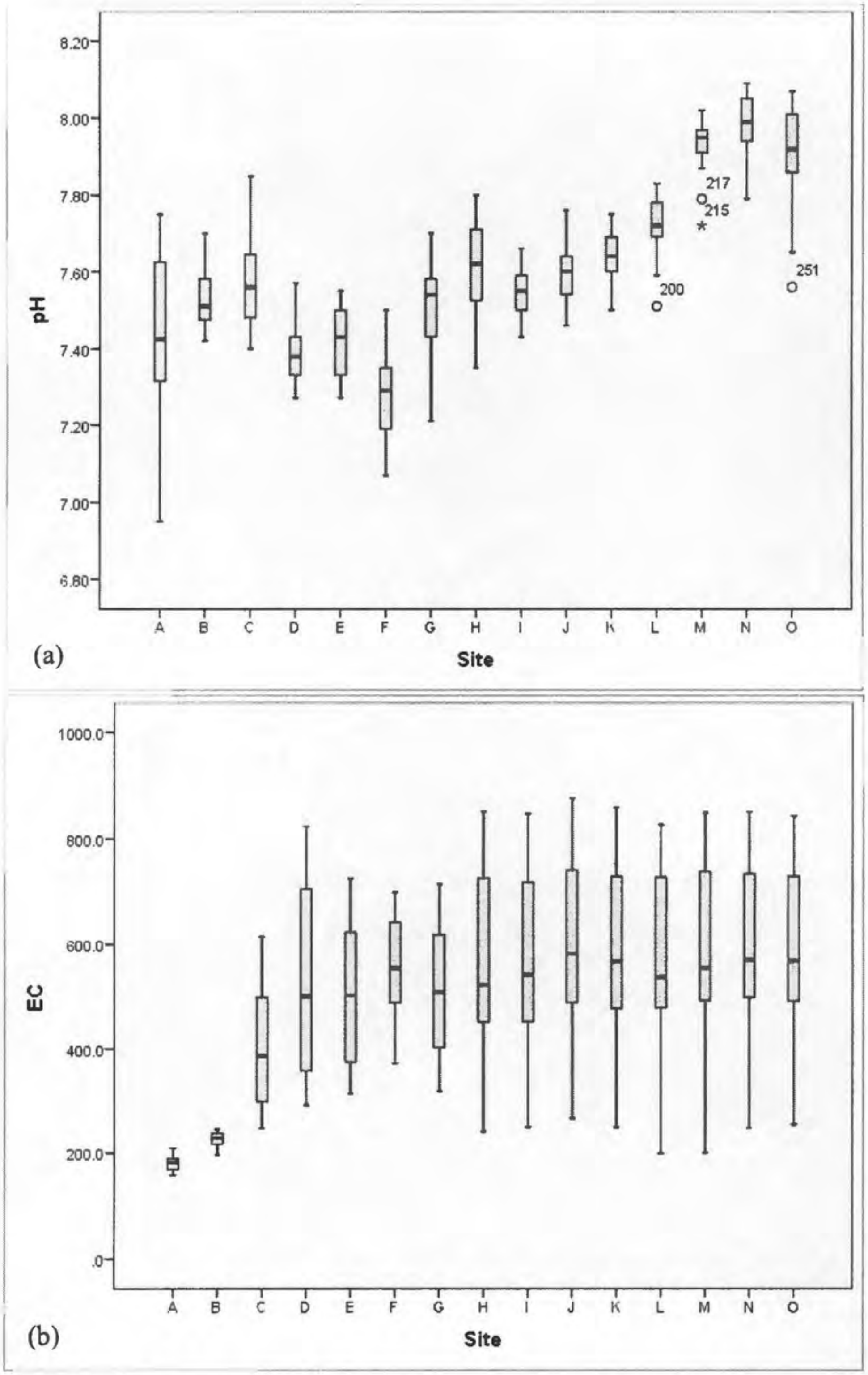

Figure A-2. Boxplots for (a) pH and (b) Electrical conductivity (μS/cm)

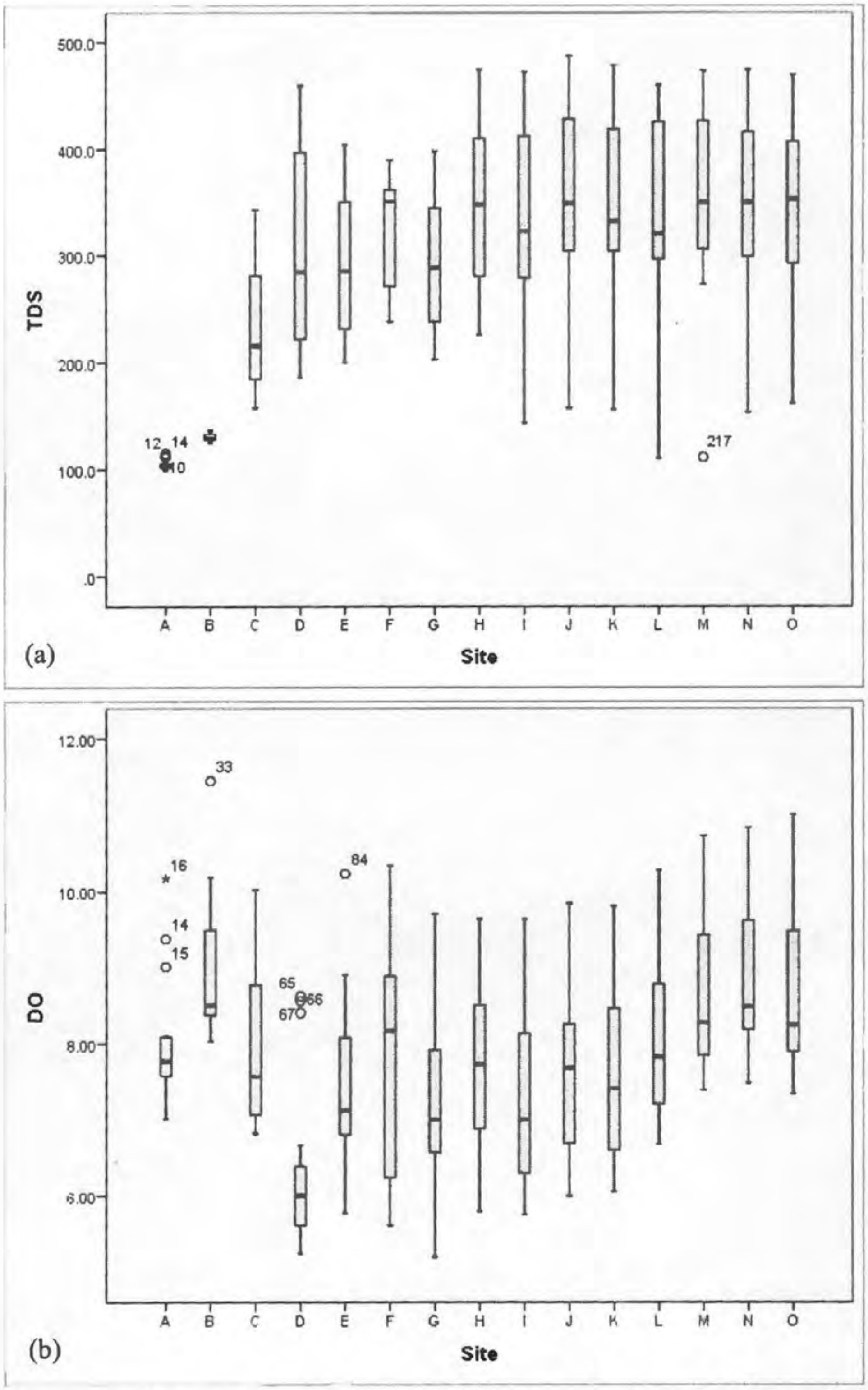

Figure A-3. Boxplots for (a) Total dissolved solids (mg/L) and (b) Dissolved oxygen (mg/L)

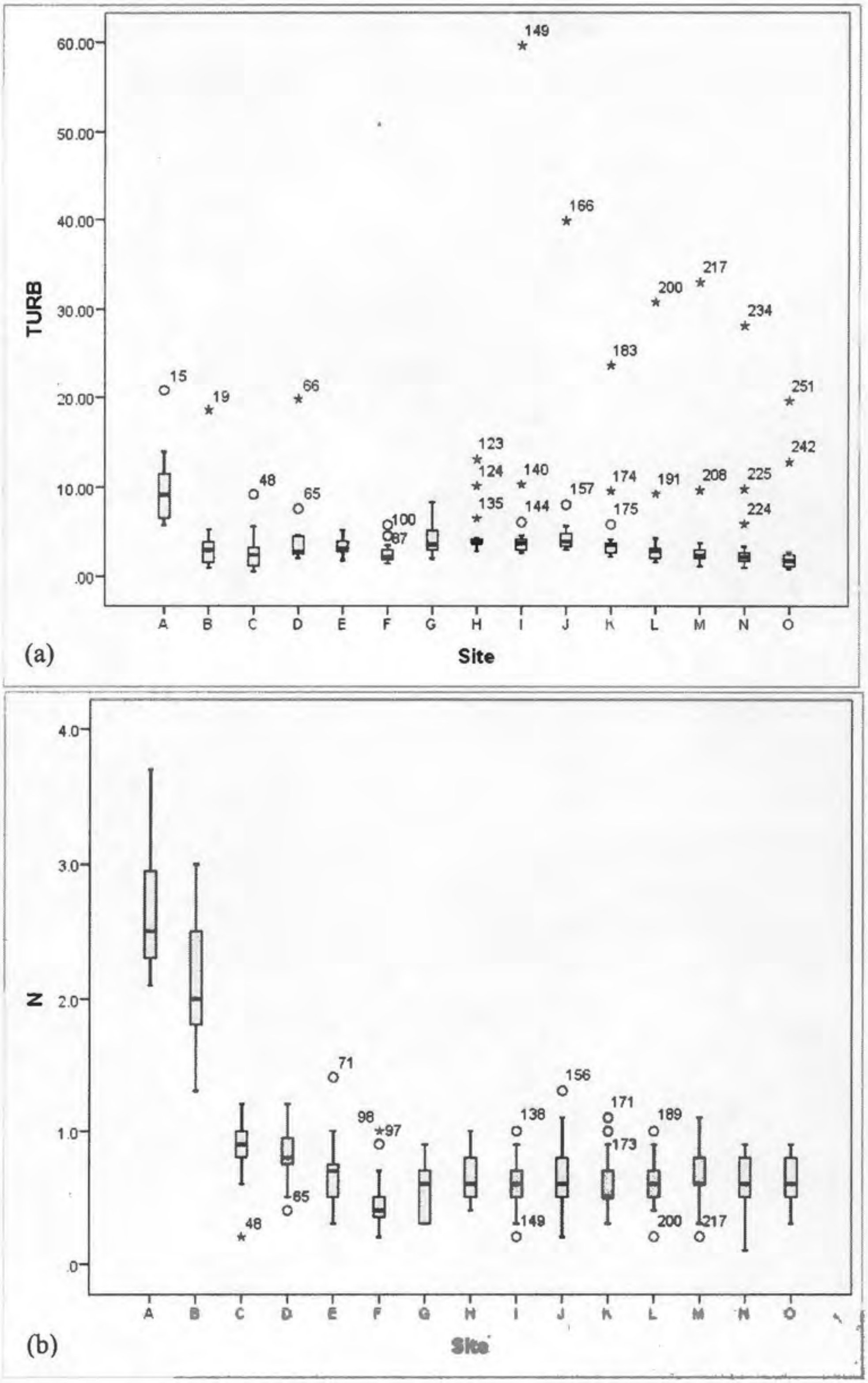

Figure A-4. Boxplots for (a) Turbidity (NTU) and (b) Nitrate (mg/L)

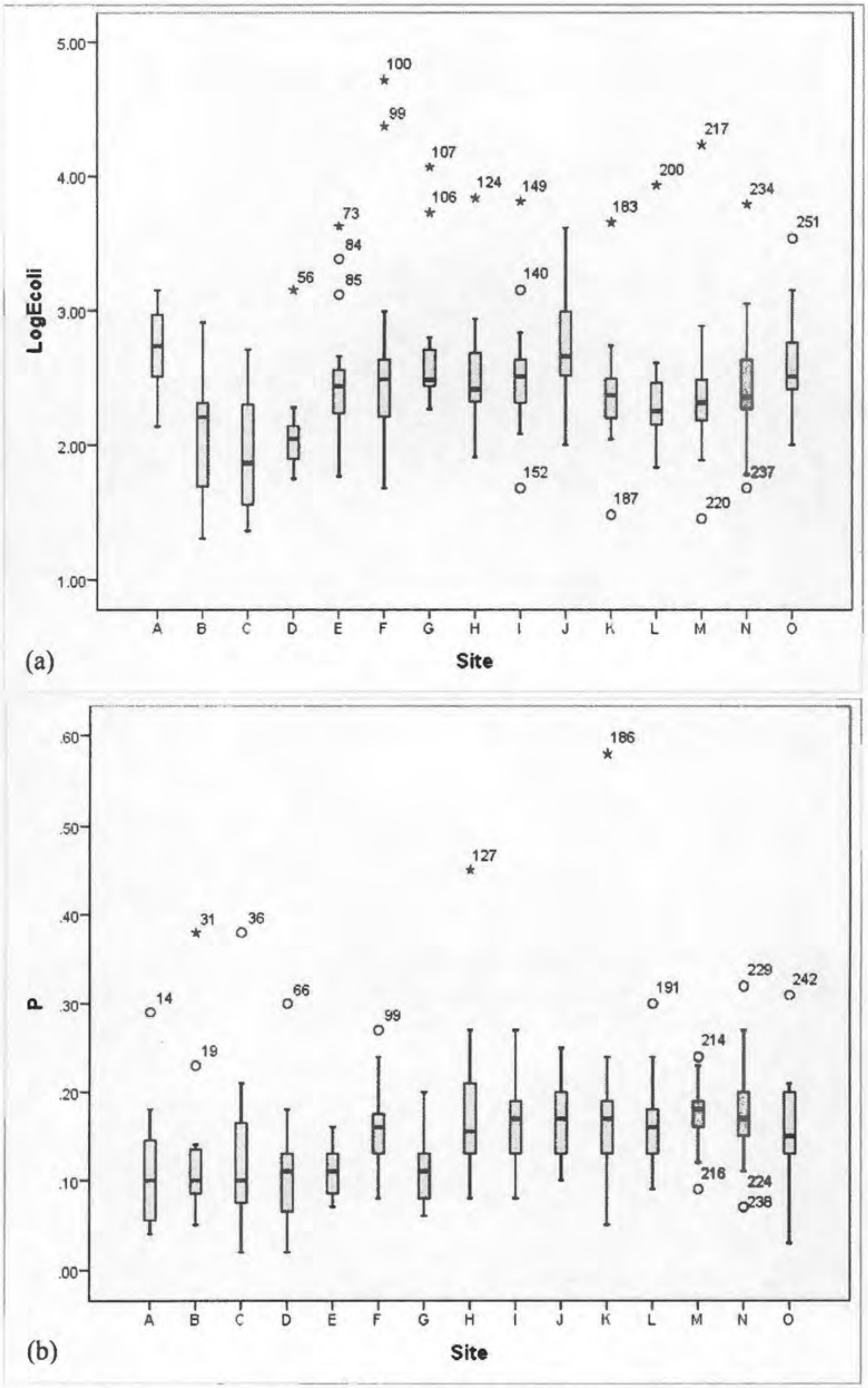

Figure A-5. Boxplots for (a) Phosphate (mg/L) and (b) $\log_{10}$ *E. coli* bacteria (CFU/100mL)

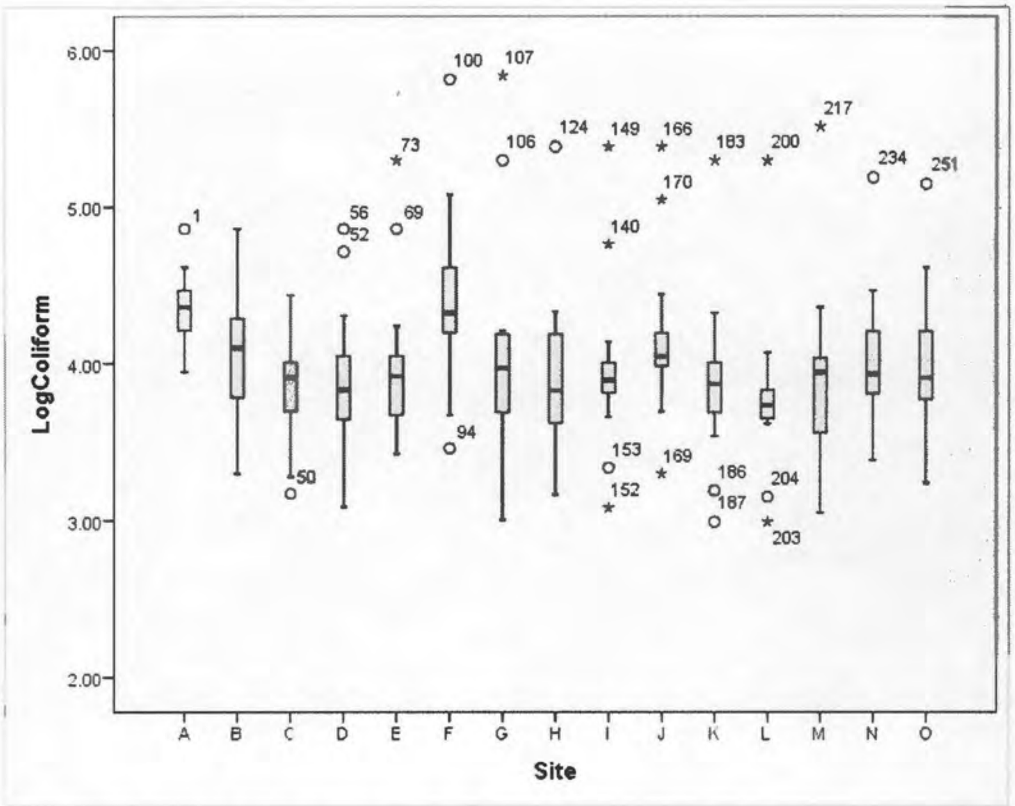

Figure A-6. Boxplot for Log_{10} Total Coliform bacteria (CFU/100mL)

APPENDIX B: Water Quality Parameters Averaged over Time

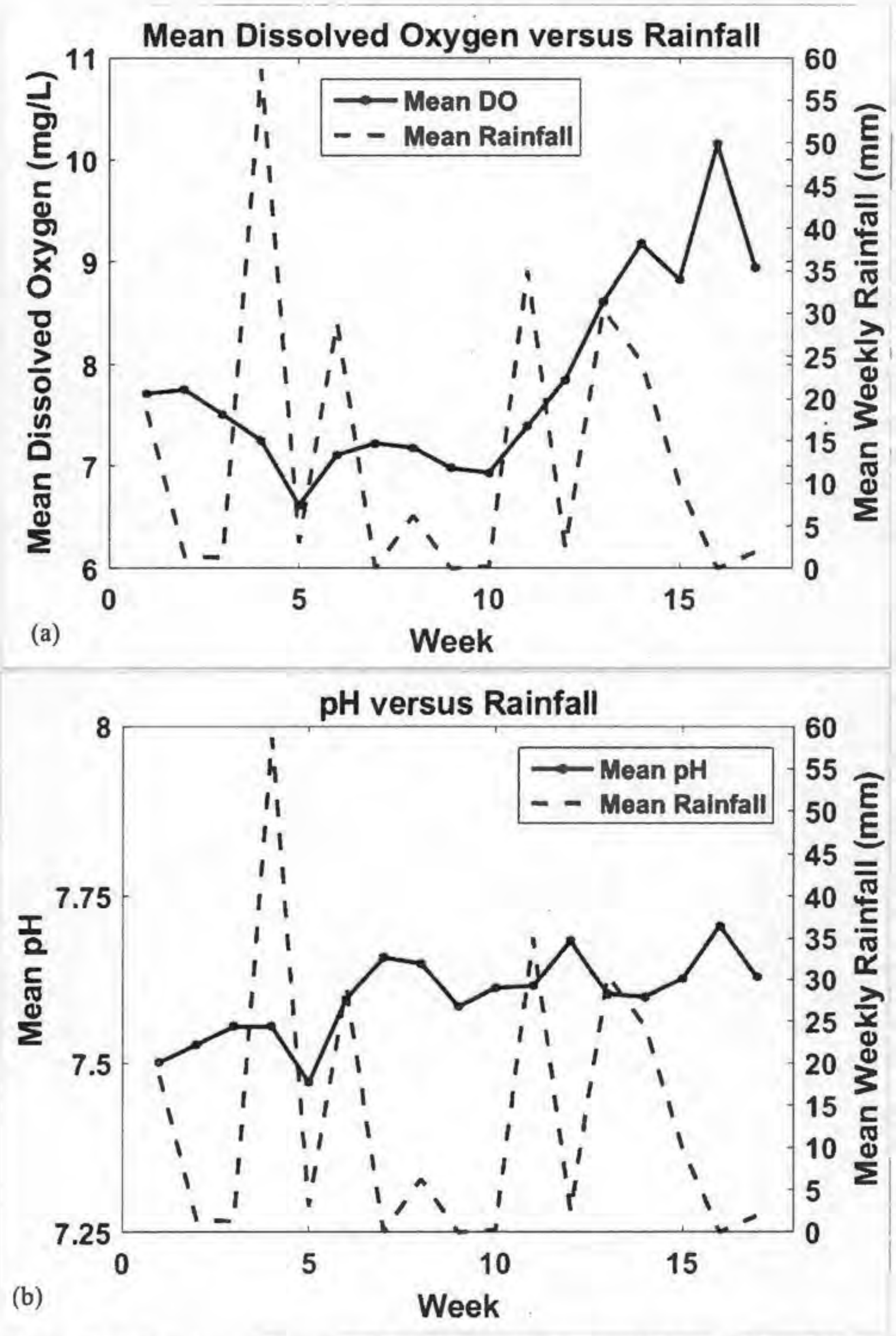

Figure B-1. Previous 96h sum of rainfall and water quality over time. (a) mean DO versus sampling week and (b) mean pH versus sampling week

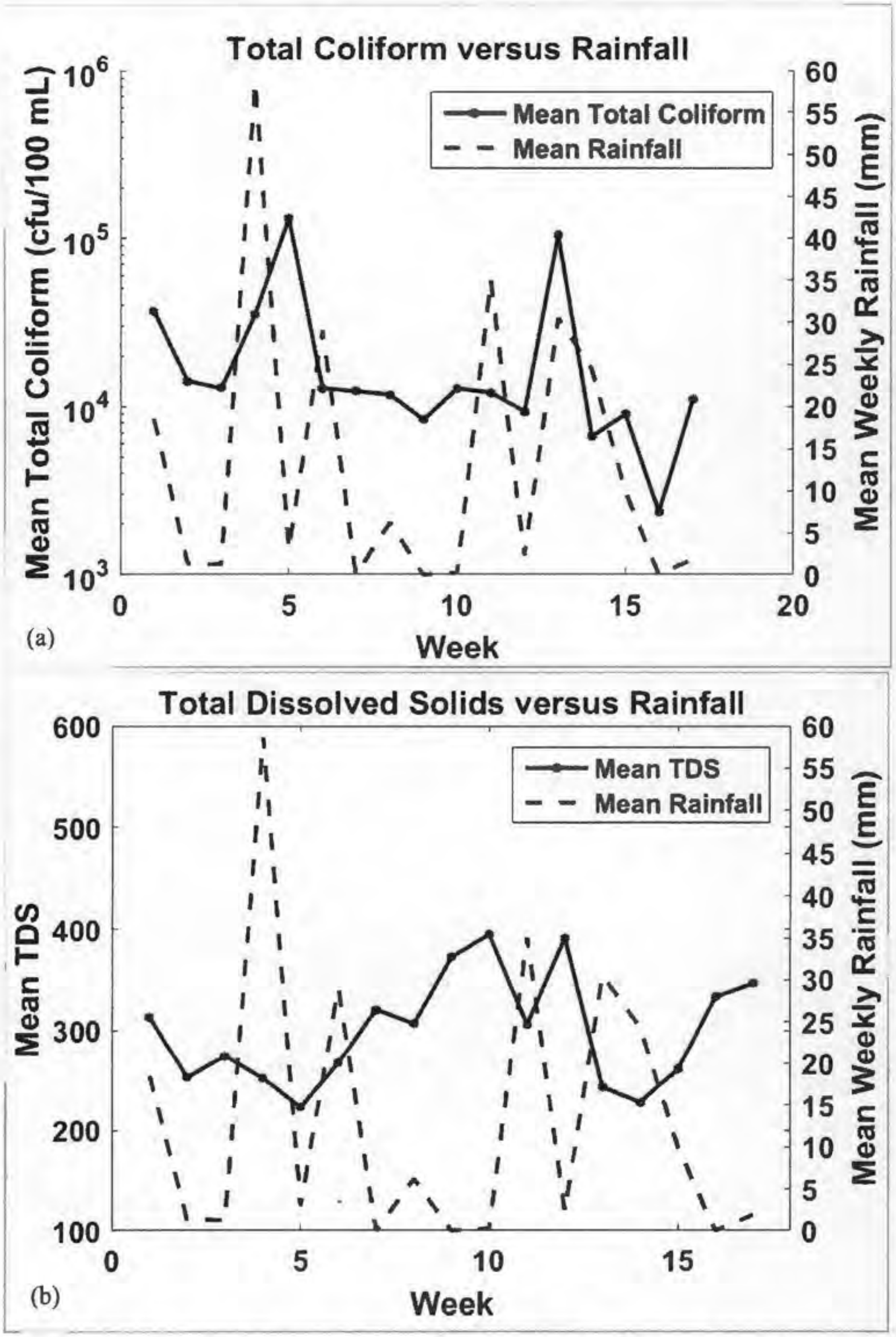

Figure B-2. Previous 96h sum of rainfall and water quality over time. (a) mean Total coliform (CFU/100mL) versus sampling week and (b) mean TDS (mg/L) versus sampling week

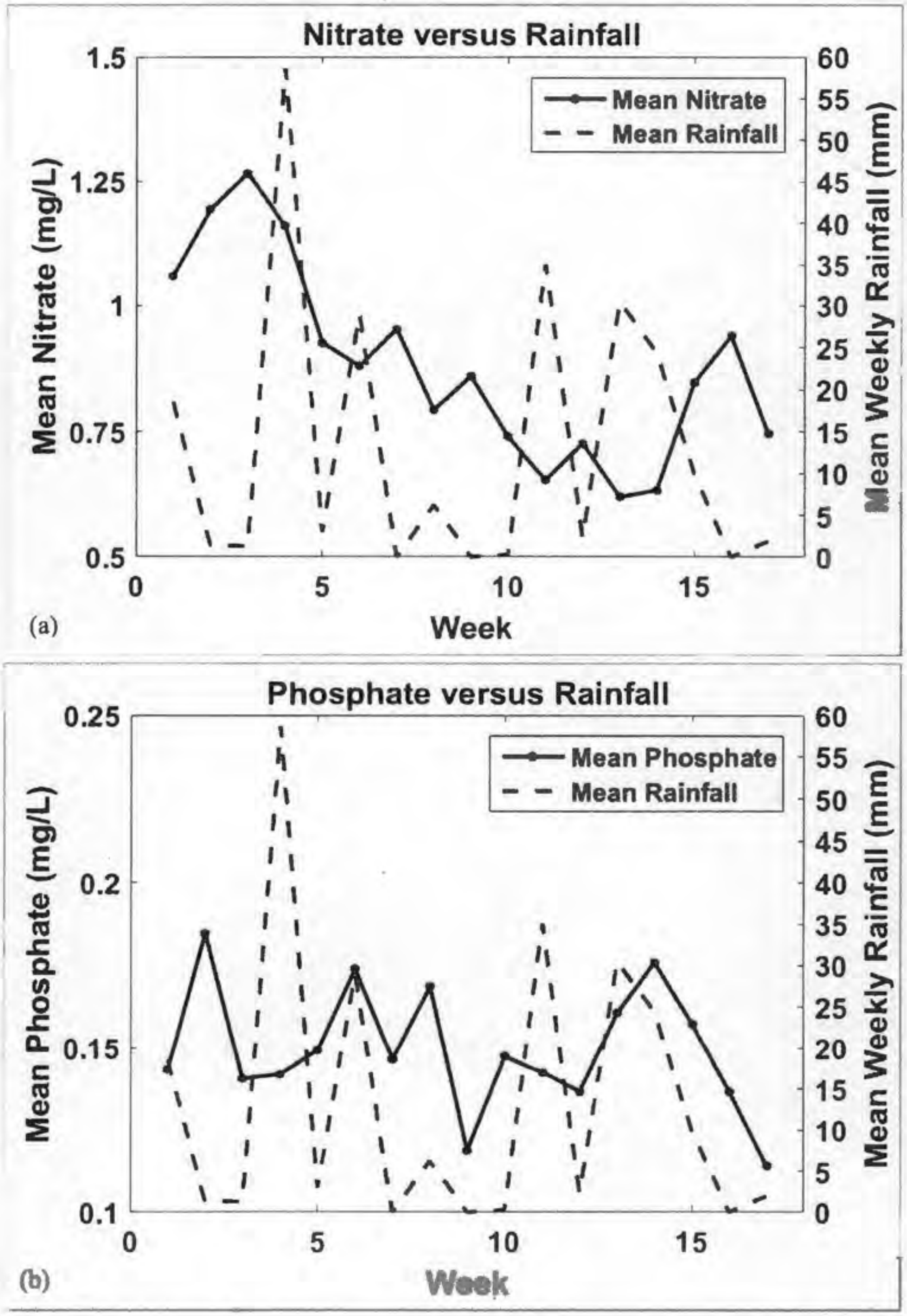

Figure B-3. Previous 96h sum of rainfall and water quality over time. (a) mean nitrate (mg/L) versus sampling week and (b) mean phosphate (mg/L) versus sampling week

APPENDIX C: Water Quality Parameters Averaged over Sampling Site

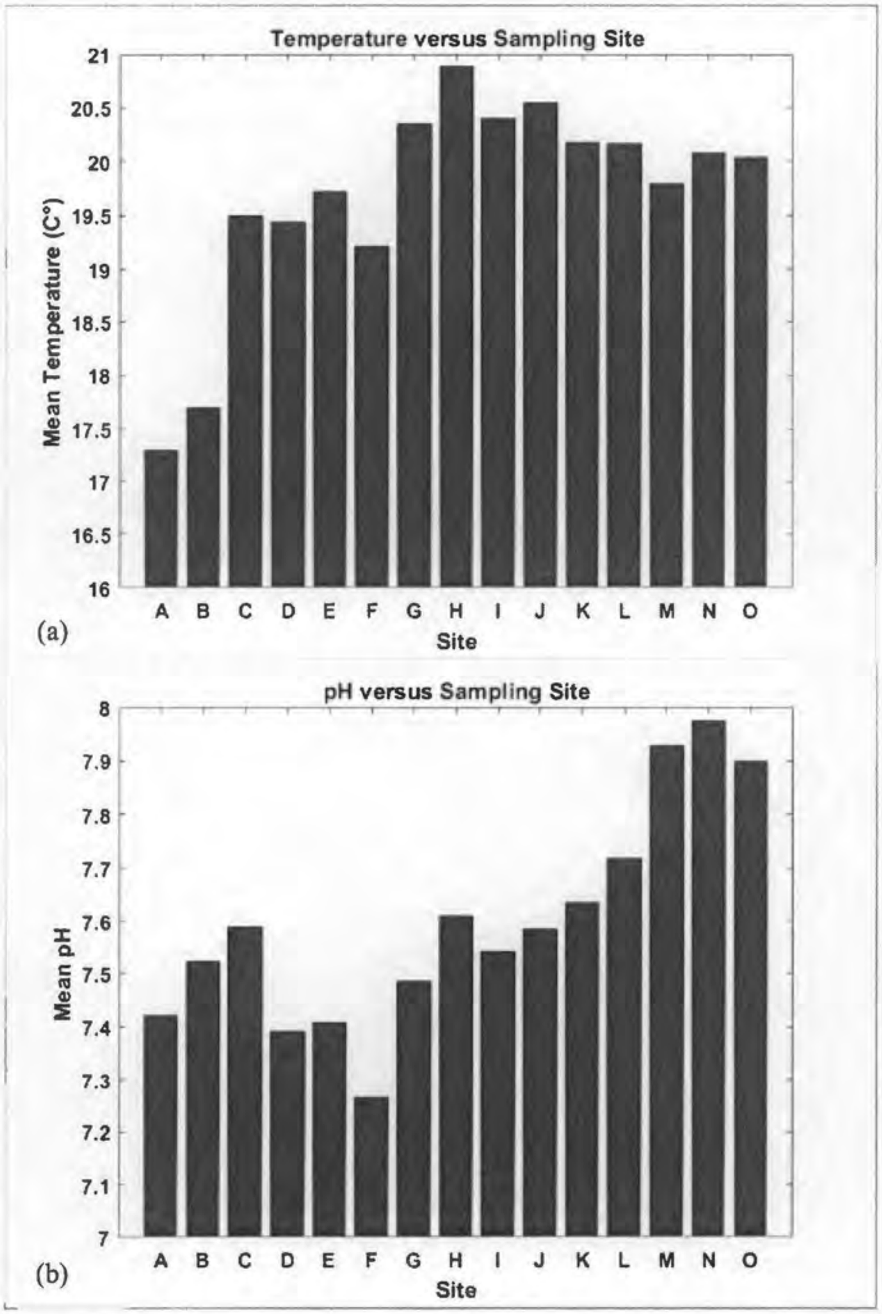

Figure C-1. Previous 96h sum of rainfall and water quality over sampling site (a) mean temperature versus sampling site and (b) mean pH versus sampling site

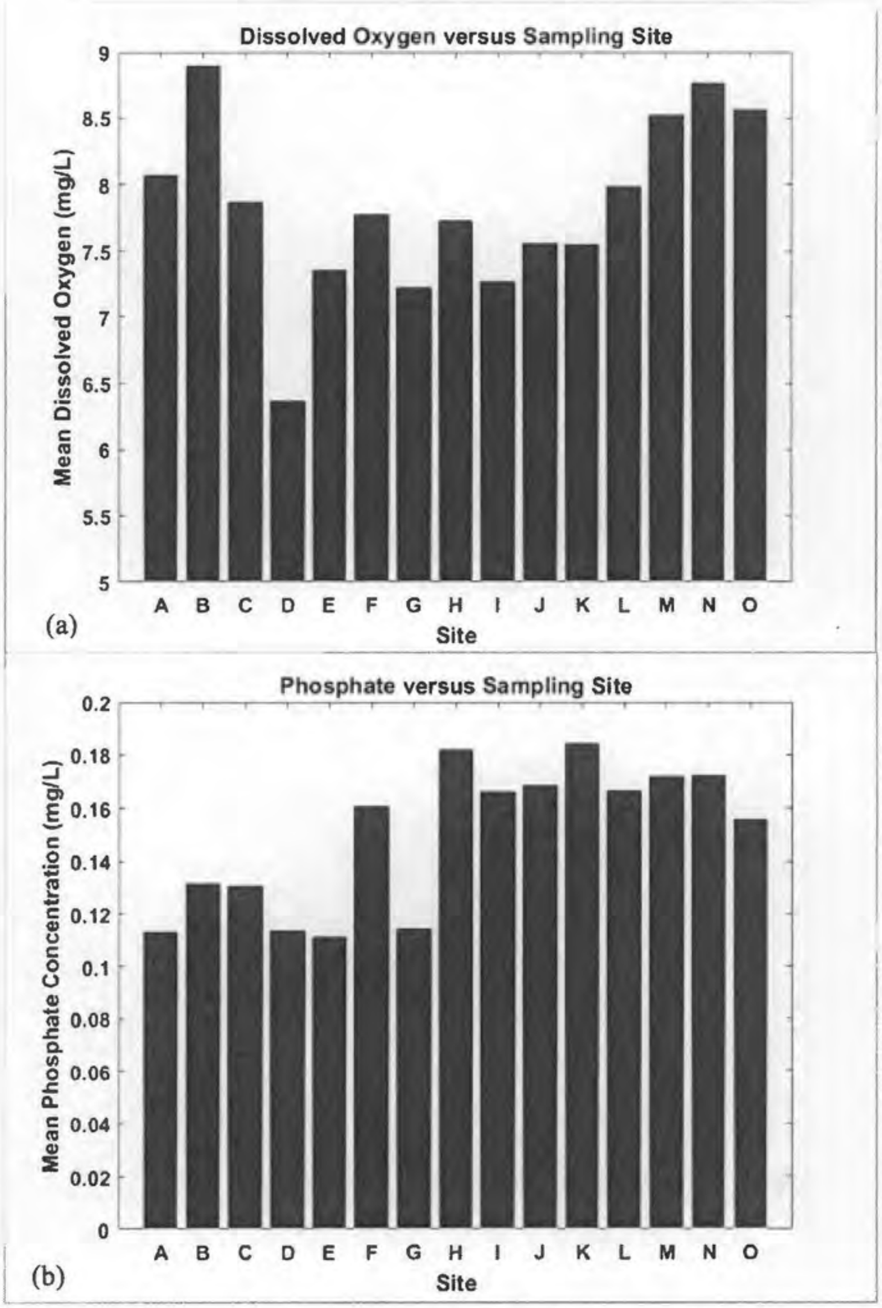

Figure C-2. Previous 96h sum of rainfall and water quality over sampling site (a) mean DO versus sampling site and (b) mean phosphate versus sampling site

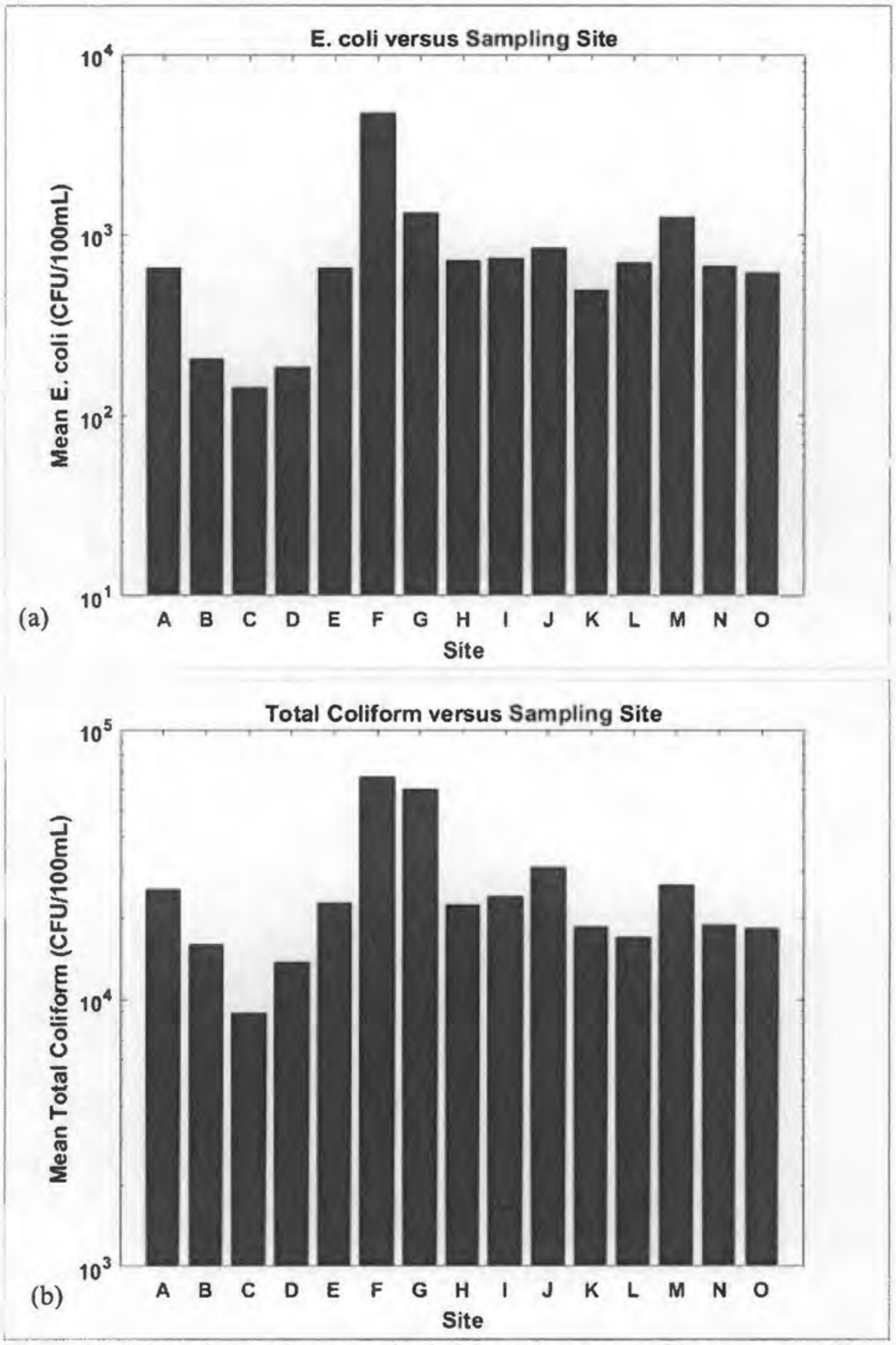

Figure C-3. Previous 96h sum of rainfall and water quality over sampling site (a) mean *E. coli* versus sampling site and (b) mean total coliform versus sampling site

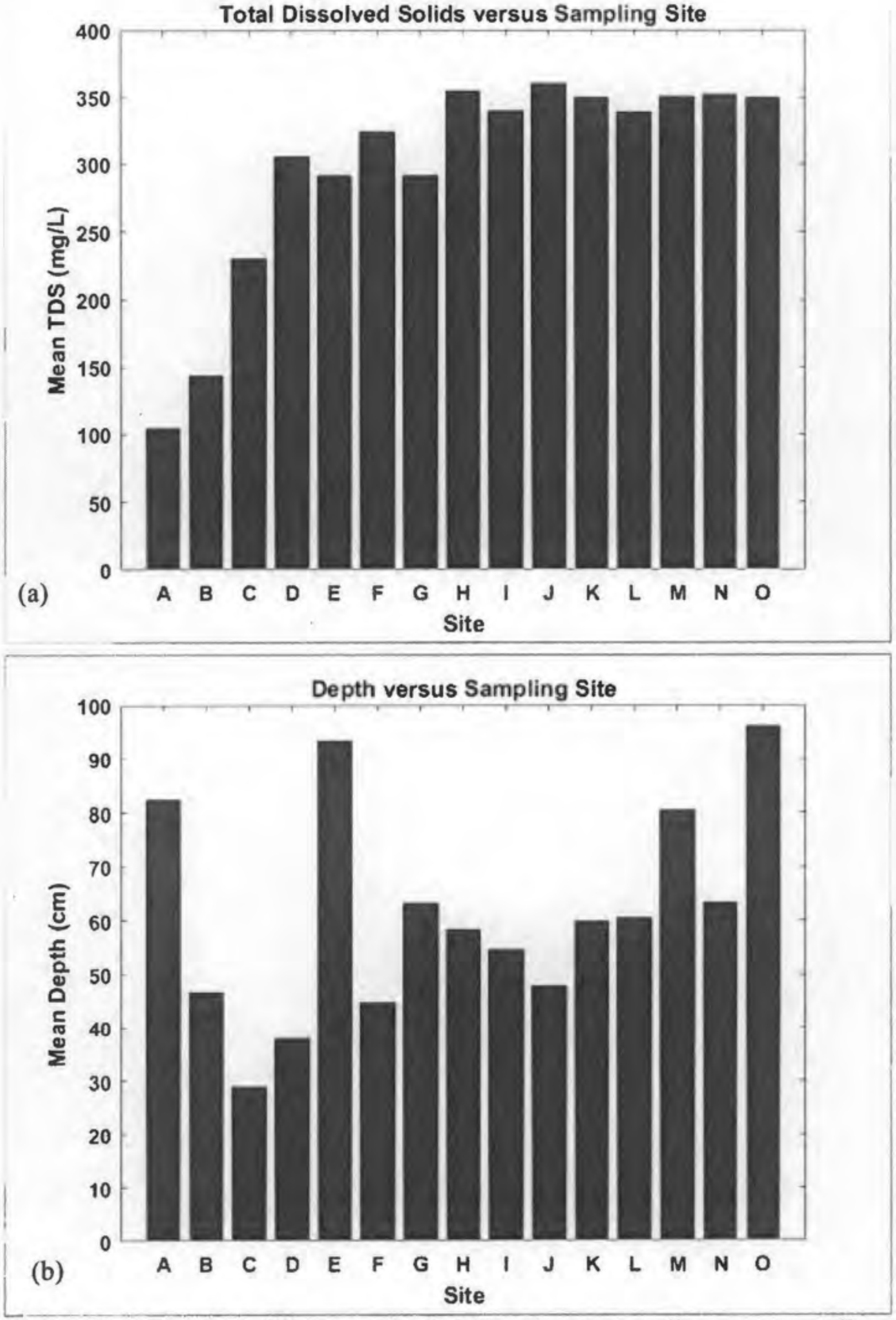

Figure C-4. Previous 96h sum of rainfall and water quality over sampling site (a) mean total dissolved solids versus sampling site and (b) mean depth versus sampling site

APPENDIX D: Hydrological Data

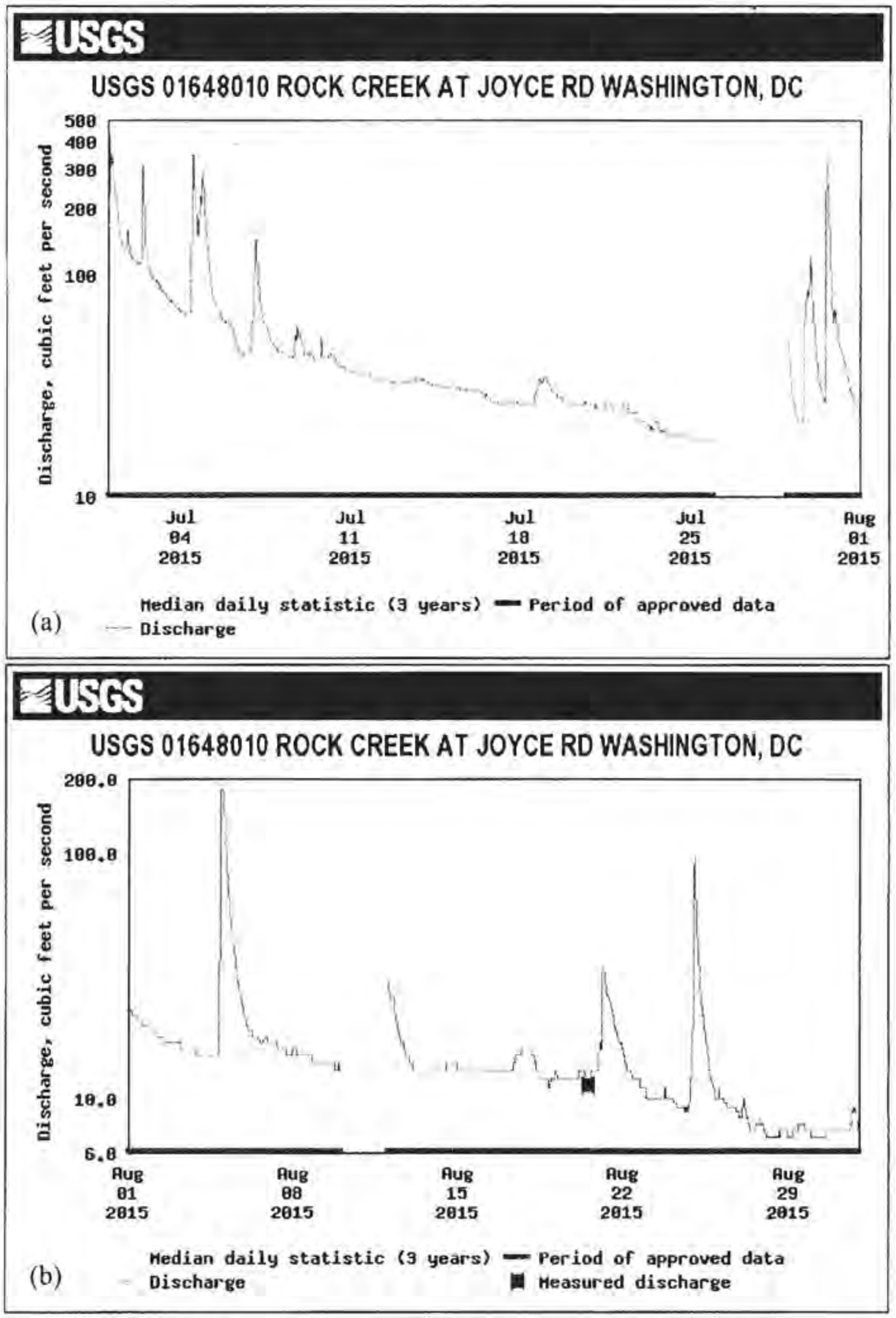

Figure D-1. USGS Hydrographs depicting the discharge rate for a significant rain event during week 4 of the sampling period at stream gages in sub-watersheds (a) F and (b) L.

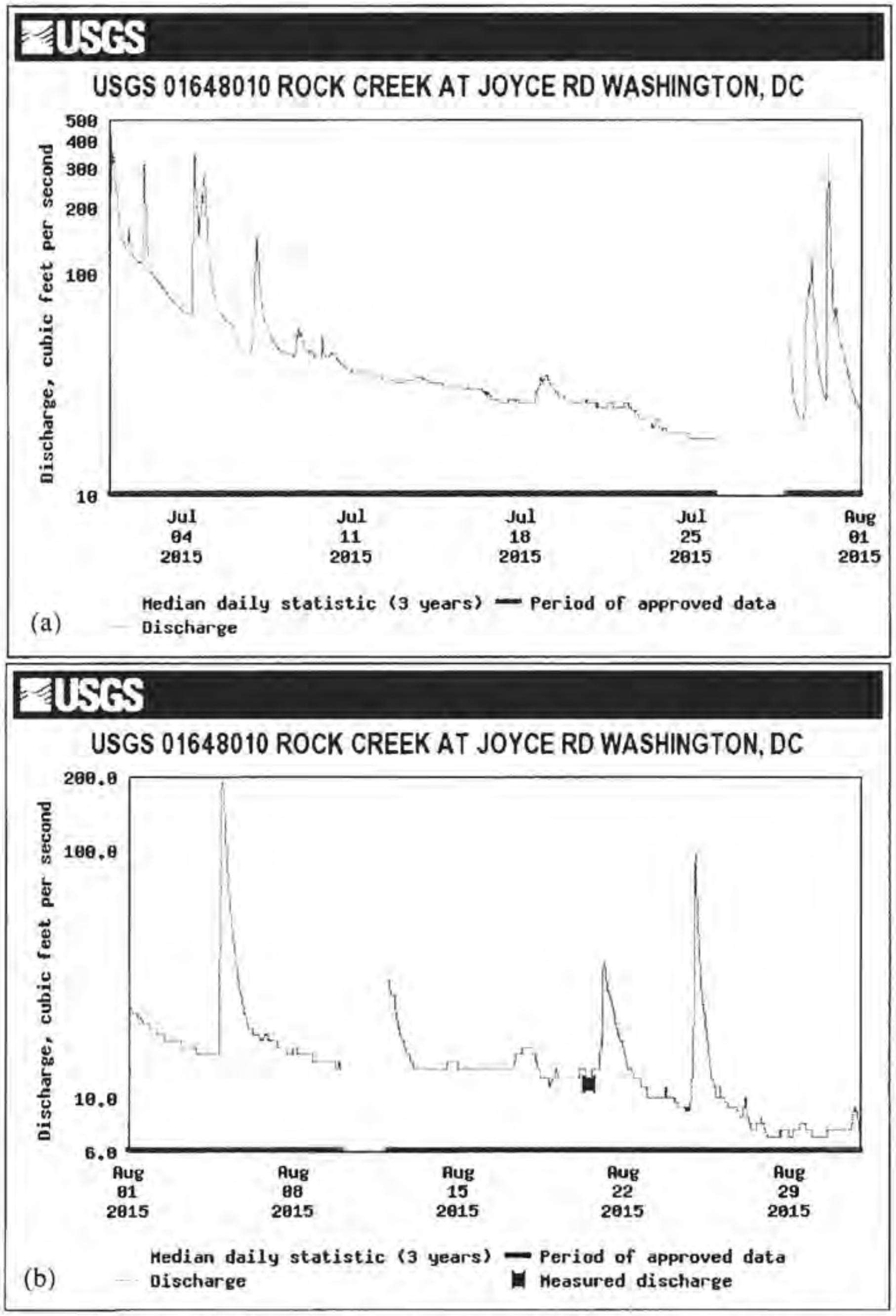

Figure D-2. USGS Hydrographs depicting the discharge rate for a significant rain event during week 6 of the sampling period at stream gages in sub-watersheds (a) F and (b) L.

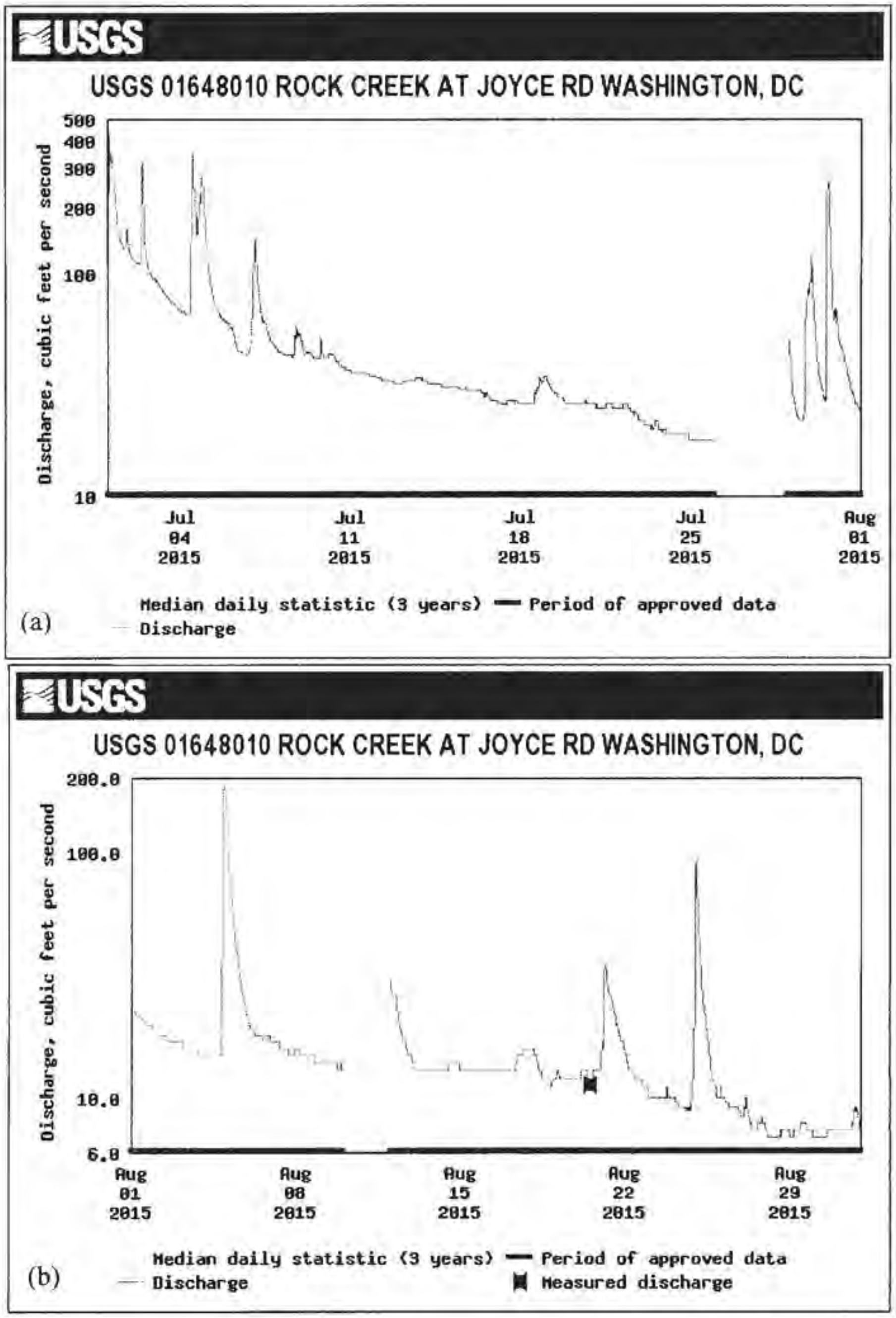

Figure D-3. USGS Hydrographs depicting the discharge rate for a significant rain event during week 13 of the sampling period at stream gages in sub-watersheds (a) F and (b) L.

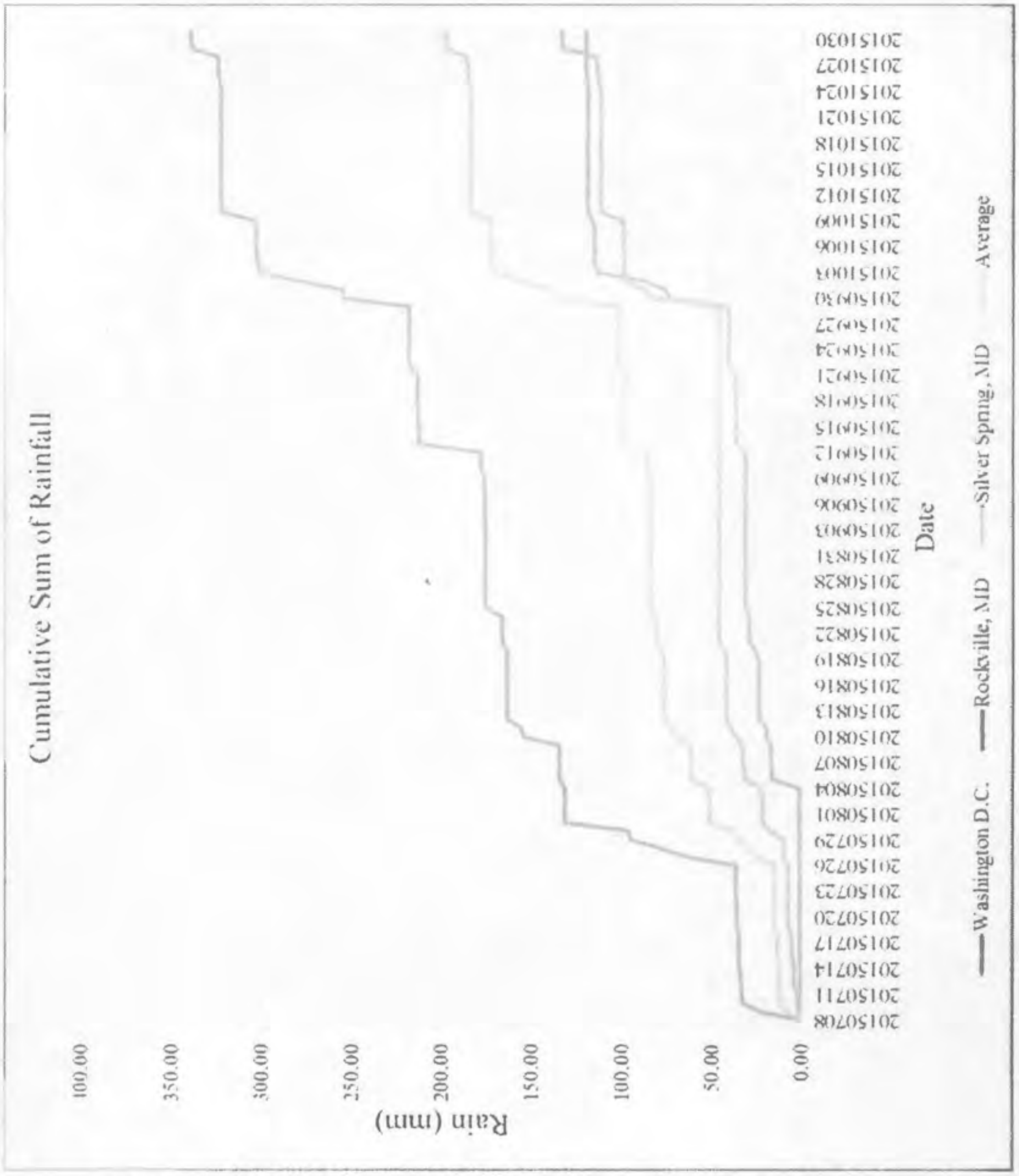

Figure D-4. Cumulative rainfall data in the Rock Creek Watershed throughout the sampling period. Depicted are the reported rain values for three CoCoRaHs Stations in Rockville and Silver Spring, MD and Washington, D.C. as well as the average of the three stations.

APPENDIX E: Geographic Information System Figures

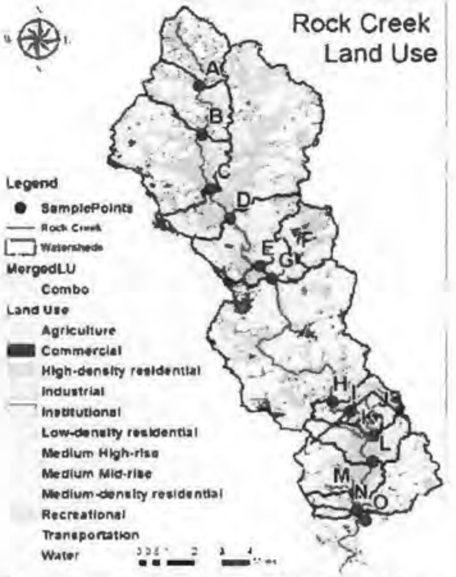

Figure E-1. MD and DC Land Use Union Map

Table E-1. Total area of sub-watersheds

SUBWTRSHD	GRIDCODE	AREA (m²)
A	0	9495781
B	1	10641825
C	2	22741661
D	3	37330192
E	4	12180838
F	5	7062593
G	6	3992208
H	7	47314801
I	8	2056711
J	9	4888994
K	10	921729
L	11	7829019
M	12	9140595
N	13	11479060
O	14	1277794
Total		**188353801**

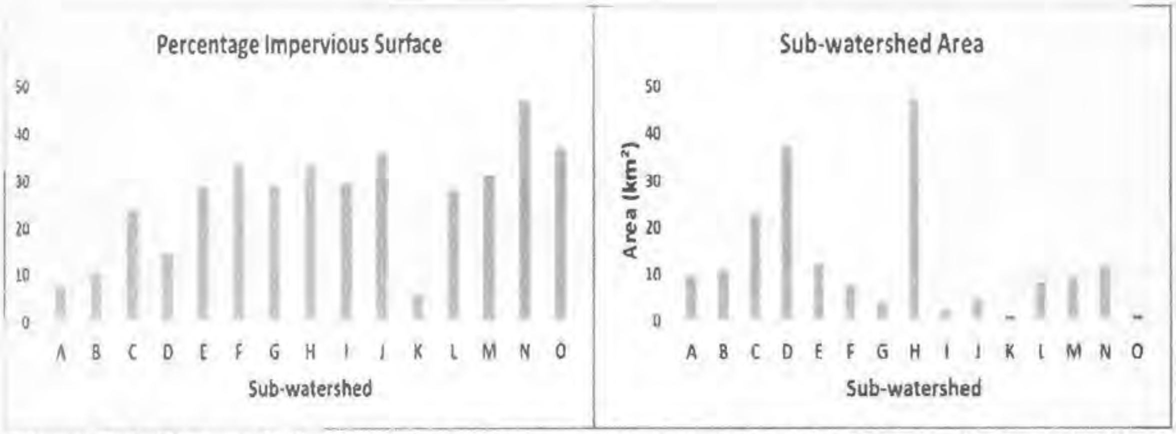

Figure E-2. Bar graph depicting impervious surface area and total area per sub-watershed in Rock Creek (a) Percentage of impervious surface coverage (b) total sub-watershed area (km²)

Table E-2. Nearest Distance (m) from Sampling Point to Sewer pipeline (sanitary, storm or combined)

SITE	GRIDCODE	NEAR_DIST
A	0	393.3
B	1	24.3
C	2	21.1
D	3	76.6
E	4	13.5
F	5	3.7
G	6	78.8
H	7	57.7
I	8	34.5
J	9	53.8
K	10	60.2
L	11	41.7
M	12	44.3
N	13	21.7
O	14	16.1

Table E-3. Summary table of calculations of sewer characteristics exported from GIS. Includes the total length (m) of sewer pipeline per sub-watershed (sanitary, storm, and/or combined), sewer pipeline density, average year of construction/emplacement, and total sub-watershed area (m^2)

SUBWTRSHD	GRIDCODE	LENGTH (m)	DENSITY	AVE_AGE	WTRSHDAREA (m^2)
A	0	7893.35	0.001		9495781.3
B	1	55797.95	0.005	1989	10641825.3
C	2	466503.87	0.021	1978	22741660.8
D	3	637571.02	0.017	1981	37330192.0
E	4	365184.68	0.030	1965	12180838.0
F	5	244233.83	0.035	1964	7062593.2
G	6	154652.11	0.039	1957	3992207.9
H	7	1800931.66	0.038	1957	47314801.4
I	8	87258.45	0.042	1950	2056711.1
J	9	142748.91	0.029	1949	4888993.8
K	10	6234.36	0.007	1935	921729.4
L	11	157598.31	0.020	1936	7829018.5
M	12	151536.32	0.017	1931	9140595.2
N	13	221117.67	0.019	1929	11479059.7
O	14	31418.29	0.025	1926	1277793.6
Grand Total		**4530680.78**	**0.344**	**1955**	**188353801.3**

APPENDIX F: Abbreviations

BOD	Biological Oxygen Demand
CFU	Colony Forming Units
COD	Chemical Oxygen Demand
CSO	Combined Sewer Overflow
D.C.	District of Colombia
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
E. coli	*Escherichia coli*
EC	Electrical Conductivity
FIB	Fecal Indicator Bacteria
GIS	Geographic Information System
ICPRB	Interstate Commission on the Potomac River Basin
MCDEP	Montgomery County Department of Environmental Protection
MCNCPPC	Maryland National Capital Parks and Planning Commission
MD	Maryland
MDDNR	Maryland Department of Natural Resources
MDE	Maryland Department of the Environment
MPN	Most Probable Number
MS4	Municipal Separate Storm Sewer System
NAWQA	National Water-Quality Assessment Program (USGS)
NPDES	National Pollution Discharge Elimination System
NWIS	National Water Information System
PC	Principal Component
RCNP	Rock Creek National Park (D.C.)
RCP	Rock Creek Park (MD)
RCW	Rock Creek Watershed
SSO	Sanitary Sewer Overflow
TC	Total Coliform
TDS	Total Dissolved Solids
TKN	Total Kjeldahl Nitrogen
TMDL	Total Maximum Daily Loads
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WRAP	Watershed restoration action plan