

UNDERSTANDING *ESCHERICHIA COLI* AND WATER QUALITY IN STORMWATER
RETENTION PONDS AND DETENTION BASINS IN FARGO AS PART OF THE RED
RIVER WATERSHED

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Erika Leigh Olson

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By

Erika Leigh Olson

The Supervisory Committee certifies that this *disquisition* complies with North Dakota
State University's regulations and meets the accepted standards for the degree of

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SUPERVISORY COMMITTEE:

Dr. Christina Hargiss

Chair

Dr. Jack Norland

Dr. Peter Bergholz

Approved:

November 18, 2020

Date

Edward Shawn DeKeyser

Department Chair

ABSTRACT

Little is known about the spatial and temporal changes in water quality and *E. coli* throughout stormwater systems. The objectives of this study are to: 1) assess surface water within detention basins and retention ponds and adjacent groundwater to determine *E. coli* movement within the system; 2) determine how precipitation events impact water quality and *E. coli*; and 3) genetically source track *E. coli* and pathogens to better understand the impact on humans. Methods of this study include sampling surface water during major storm events, followed by sampling of groundwater and surface water one week later. Additional samples were taken to assess *E. coli*. We concluded that *E. coli* quantities are high during storm events, often higher in detention basins compared to retention ponds, and *E. coli* is present but not consistent in groundwater.

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LIST OF ABBREVIATIONS

Cu.....	Copper
Cd.....	Cadmium
CFU.....	Colony Forming Units
Cr.....	Chromium
CWA.....	Clean Water Act
DIN.....	Dissolved Inorganic Nitrogen
DON.....	Dissolved Organic Nitrogen
DNA.....	Deoxyribonucleic Acid
FC.....	Fecal Coliforms
Fe.....	Iron
FIB.....	Fecal Indicator Bacteria
FS.....	Fecal Streptococci
ICP-MS.....	Inductively Coupled Plasma-mass Spectrometry
MDS.....	Multiparameter Display System
MST.....	Microbial Source Tracking
MPCA.....	Minnesota Pollution Control Agency
NDAWN.....	North Dakota Agricultural Weather Network
NDCC.....	North Dakota Century Code
NDDEQ.....	North Dakota Department of Environmental Quality
NH ₃	Ammonia
NH ₄ ⁺	Ammonium
NO ₂ ⁻	Nitrite
NO ₃ ⁻	Nitrate

NMDS	Nonmetric Multi-dimensional Scaling
NRCS	Natural Resources Conservation Service
P	Phosphorus
Pb	Lead
PCBs	Polychlorinated Biphenyls
PERMANOVA	Permutation Multivariate Analysis of Variance
PHDI	Palmer Hydrological Drought Index
PON.....	Particulate Organic Nitrogen
PVC.....	Polyvinyl Chloride
SAS	Statistical Analysis System
SPSS.....	Statistical Package for the Social Sciences
TDS	Total Dissolved Solids
TFP	The Fargo Project
TKN	Total Kjeldahl Nitrogen
TSS.....	Total Suspended Solids
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
YSI	Yellow Springs Instruments
Zn	Zinc

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CHAPTER 1: LITERATURE REVIEW

Urbanization and Water

Urbanization and urban sprawl are increasing across the world (McKinney 2008; Gaston 2010). Urbanization, as a concept, was introduced in 1937 by one of the first city planners, Earle Draper (Nechyba and Walsh 2004). Urban ecology is a relatively new field of study and has only been around since the late 1990s (Gaston 2010). Urban ecology incorporates an understanding of humans as part of an ecosystem and links environmental pollution and hazards, which are important aspects to consider for urban planners. According to the United Nations, the urban population is expected to increase from the 2008 numbers of 50 percent living in urban areas to 66 percent by 2050 (United Nations 2014). Due to the continuous increase in population, the footprint of urban areas has grown, causing issues with urban sprawl (United Nations 2014). Rapid urban growth can threaten sustainable development when the necessary infrastructure or policies are not in place to ensure the city can function properly (United Nations 2014). This can lead to issues with health, the economy, and the environment. Due to urbanization, there has been an introduction of physical, chemical, and biological pollutants from various anthropogenic activities, especially in water systems (Goonetilleke et al. 2005). This has caused an increase in the quantity of urban runoff, as well as a decrease in the water quality of stormwater runoff in large urban areas.

Increasing size in urban areas has greatly influenced the amount of urban runoff from highly developed areas (USGS 1996). Issues with flooding in urban areas have been shown to cause water quality issues due to the deposition and washout of various urban pollutants (Burant et al. 2018). Left untreated these excess contaminants can reach other bodies of water, and if concentrations become high enough, eutrophication or toxicity is likely to occur (Seelig and

Dekeyser 2006; MPCA 2007; Dahl 2011). Current research suggests that over 650 identified compounds are present in trace concentrations in stormwater (Gasperi et al. 2014). Organic pollutants found in urban areas can include pesticides, flame retardants, polycyclic aromatic hydrocarbons, and corrosion inhibitors (Burant et al. 2018). There are also higher levels of toxicity due to heavy metals, total suspended solids, and legacy organic contaminants such as petroleum hydrocarbons, legacy pesticides, and polychlorinated biphenyls (PCBs) (Burant et al. 2018). Due to disturbances, even naturally occurring elements are found in higher concentrations across many urban areas (Neary et al. 1988; NRCS 2006; MPCA 2007). The USGS conducted a national survey looking at water quality in rural and urban areas across the United States (USGS 1999). The USGS findings show that rivers and streams surrounded by agricultural land and urban development contained medium-to-medium high concentrations of nitrogen, phosphorous, herbicides, and insecticides. Rivers near urban areas showed higher concentrations of insecticides and phosphorus. While agricultural land near rivers nearby contained higher concentrations of nitrogen and phosphorus (USGS 1999). Land-use changes resulting from increased urbanization have a strong correlation with the increases in a variety of water chemistry parameters (Tran et al. 2010; Hettiararchchi et al. 2011; Khatri and Tyagi 2015).

Khatri and Tyagi (2015) discussed three land use categories that influence water quality including: 1) natural (wind deposition, geology, climate, weathering, etc.); 2) rural (agriculture, runoff from croplands, feedlots, mining operations, pasture land); and 3) urban (industrial discharge, municipal discharge, landfills, domestic effluent, impervious surfaces). They found nitrates, phosphates, total dissolved solids, and heavy metals were the main factors different between rural and urban water quality. These differences can be traced back to the various rural and urban sources of contamination (Khatri and Tyagi 2015).

Hettiarchchi et al. (2011) studied water quality surrounding the city of Colombo, Sri Lanka during a five year period. Their findings show that water quality declined over time as the city's population grew. Water quality parameters such as phosphates, fecal coliforms, and heavy metals exceeded water quality standards for the area. The area surrounding the city was impacted by the town's domestic wastewater which degraded water quality drastically over a short period (Hettiarchchi et al. 2011).

Vitro et al. (2017) studied the relationship between population growth and the increase in fecal coliforms (FC) in rivers and streams in North Carolina, USA. For North Carolina, the two largest contributors of FC are biota of unknown sources (i.e. undetermined biological sources) and mercury. In this area, FC is the third largest cause of impairment to their rivers and streams. The increase in FC found in the study can be contributed to their rapid population growth from ~6.6 million in 1993 to ~9.6 million in 2010. Additionally, the study found that with an increase in open water, the in-stream FC measurements can decrease, and it may help to utilize open water as a buffer in urban areas (Vitro et al. 2017).

Taylor et al. (2005) found that phosphorus is often the limiting nutrient contributing to the eutrophication of receiving waters around the world. Dissolved Inorganic Nitrogen (DIN) has the greatest impact on water bodies because it is readily available for uptake by simple organisms. Specific DIN species include ammonia (NH_3), nitrite (NO_2^-), and nitrate (NO_3^-). An increase in DIN leads to eutrophication, hypoxia, and loss of biodiversity and habitat in many urban areas. Taylor et al. (2005) found that there is more variability during storm events caused by variation in the aerial deposition, rainfall quality, catchment soils, and past and present catchment activities. There was not a clear relationship between runoff and nitrogen species concentrations during major storm events. Researchers expected to see the higher concentrations

of Particulate Organic Nitrogen (PON) during the storms due to washing off by high flows, but their research failed to prove this (Taylor et al. 2005).

Prior stormwater research in North Dakota showed there is an increase in contaminants in runoff from highly urbanized areas (McCarthy 2009). Worldwide, studies have also shown as urbanization increases, water quality decreases, and rivers and streams that pass through the urban system are greatly impacted (Goonetilleke et al. 2005; European Environmental Agency 2006; Fletcher et al. 2013). With urbanization comes increased land-use modifications such as removal of vegetation and an increase in impervious surfaces which lead to surface runoff changes and increased stormwater runoff volume and peak flows (Goonetilleke et al. 2005). Stormwater found in industrial areas has been found to contain higher metal concentrations, which can be traced back to denser roads and human populations (Burant et al. 2018). Human activity generates wastes and pollutants that have the potential to be washed into bodies of water during storm events (Barbosa et al. 2012). Surface runoff has now become an area of focus for many urban planners.

Runoff

Working to protect water quality, urban developers look at ways to limit urban runoff and reduce pollutant holdings (USEPA 1996). The amount of runoff is increased in urban areas due to increased impermeable surfaces, which also contain large quantities of pollutants (Liu et al. 2013). Pollutant build-up, and subsequent wash-off processes during precipitation events, are influenced by a range of catchment characteristics including size, land use, and area of impervious surfaces (Liu et al. 2013).

Biofiltration systems, also commonly known as biofilters or bioretention systems, are frequently used to reduce pollution by improving the quality of stormwater runoff by

intercepting the first flush (Davis et al. 2001; Shammaa et al. 2002; Kim et al. 2003; Hatt et al. 2007; Henderson et al. 2007). When implementing a biofiltration system it is important to select the right type of vegetation for the geographic region by considering the durability and ability to survive harsh conditions in a detention basin (Bratieres et al. 2008). In most cities stormwater runoff is released into a larger body of water (river, lake, reservoir) untreated, which can cause issues in that water body. This has been a large problem in urban areas around the world and has been dubbed “urban stream syndrome” (Wallace et al. 2013).

First Flush Phenomenon

The first flush phenomenon is well established in the literature as the initial runoff after rainfall that is washed off impervious surfaces and contains a large number of pollutants (Deletic 1998; Stanley 1996; Lee et al. 2002; McCarthy 2009). During the first flush the concentration of pollutants is considerably higher than later points in the storm (Lee et al. 2002). This first flush can be influenced by many factors such as the watershed size, rainfall intensity, the amount of impervious area, and previous dry weather periods (Wanielista and Yousef 1993; Gupta and Saul 1996). In urban areas the first flush runoff can include rainwater from rooftops, roads, and other impermeable surfaces, as well as discharge from separate and combined sewage systems (Deletic 1998). The receiving waters of this first flush are getting larger quantities of oil, grease, and toxic chemicals from vehicles, nutrients and pesticides from turf management, viruses and bacteria from failing septic systems, road salts, and heavy metals (USEPA 1996). The contaminants found in urban stormwater carry large quantities of heavy metals such as Pb, Zn, Cu, and Cd as well as suspended solids (Davis et al. 2001; Fritioff and Greger 2003; Hatt et al. 2007; Henderson et al. 2007).

Retention Ponds and Detention Basins

Treatment of stormwater is a popular practice and in many areas is required to protect the receiving water's quality. Utilizing retention ponds and detention basins can help minimize the number of pollutants flowing into the receiving waters (Stanley 1996; Wu et al. 1996; Starzec et al. 2005; Zhu et al. 2020). The main difference between detention basins and retention ponds is the amount of time the water is held within the site.

Detention basins hold water temporarily and limit the outflow of water in the process, minimizing flooding and hydrological disturbances downstream from large flushes of water (Roy et al. 2008). Detention basins can reduce the number of pollutants that are picked up and transported during rainfall events (Davis et al. 2001; Zhu et al. 2020). These basins are often designed to mitigate the negative effects of a set of water quality indicators including: total suspended solids, biochemical and chemical oxygen demand, nitrates, phosphates, and fecal coliforms (Wu et al. 1996; Carleton et al. 2000; Scholes et al. 2008; Burns and Meiburg 2012). Stormwater held in detention basins often carries sediment and pollution and moves them into other larger bodies of water (Colford et al. 2012). The temporary storage of this water allows at least some of the larger solids and pollutants to settle out of the runoff (Middleton and Barrett 2008). A study by Starzec et al. (2005) showed that more than 80% of metals, 70% of phosphorus, and 30% of nitrogen during certain periods can be removed with detention basins.

Many detention basins serve multiple functions and are tasked with both reducing the number of pollutants, as well as reducing the pressure on downstream systems by releasing the held stormwater slowly (Starzec et al. 2005). Schueler (1994) studied a variety of detention basins and found generally good removal of suspended solids, an inconsistent removal of phosphorus, and poor removal efficiency for nitrogen. Similarly, Zhu et al. (2020) found that Fe

and Pb have a faster settling velocity followed by Cu and Cr. They also discovered that particles carrying Fe can be used as indicators to identify trace metals contamination areas (Zhu et al. 2020).

Retention ponds are areas that hold water year-round and remain wet even during dry seasons, essentially serving as urban ponds. Retention ponds are usually found in areas where the water table is relatively high, making it easier for that area to remain wet even during the drier seasons (Fischer et al. 2003). Retention ponds help improve water quality by suspended materials. In a study by Davis et al. (2001) they found retention ponds can remove 80% zinc, 87% iron, and 93% total suspended solids (TSS) from stormwater runoff. The long retention times and larger surface area of urban retention ponds have proven to be effective in the removal of particulate matter (Davis et al. 2001). Previous researchers found that during a retention time of 18 hours, 60% of TSS, lead, and hydrocarbons; and 45% of total biochemical oxygen demand, copper, and phosphates had been removed by the retention pond (Davis et al. 2001).

E. coli

Stormwater runoff often contains bacteria from the surrounding urban landscape, most commonly found in fecal coliform bacteria, particularly *Escherichia coli* (*E. coli*) (Chen and Chang 2014). Waterborne virus outbreaks can be associated with recreational water, treated drinking water, and groundwater posing a threat to human health and quality of life due to water-related gastrointestinal illnesses (Gibson 2014). Urban bodies of water are expected to have higher levels of most pollutants due to their proximity to human activity and the increase in impervious surfaces (Chen and Chang 2014). Understanding the influence and concentrations of *E. coli* can assist in the management of microbial contaminations in urban waters. Waters containing elevated levels of *E. coli* can negatively affect the water supply, recreation, and

aquatic habitat (Chen and Chang 2014). Identifying the factors that influence *E. coli* within stormwater helps to control/manage the sources of *E. coli*, as well as provide information to better predict levels of *E. coli* during different conditions. The health threat from human fecal contamination is well documented in the literature. Animals, both domestic and agricultural, can also spread pathogens through fecal matter including *Salmonella*, *E. coli* 0157:H7, *Campylobacter jejuni*, *Giardia spp.*, *Cryptosporidium spp.*, and hepatitis E virus (Field and Samadpour 2007). Fecal coliform bacteria, particularly the species *Escherichia coli* (*E. coli*), is most often used as an indicator of feces in bodies of water (Chen and Chang 2014). *E. coli* can be transported in water in many ways including by particulate matter, adsorbed onto suspended particles, or have an attraction to sediments in the water (Chen and Chang 2014). An increase in *E. coli* means a greater possibility the body of water has been polluted by feces and associated pathogens (Chen and Chang 2014). During the warmer seasons of the year, with warmer temperatures and less streamflow, tend to have higher growth rates and survival of *E. coli* (Chen and Chang 2014). In urban areas, higher *E. coli* concentrations can be associated with increases in animal and human outdoor activity due to warmer temperatures (Chen and Chang 2014).

Microbial Source Tracking

Utilizing technology such as genetic source tracking, researchers are better able to control and mitigate bacteria. Genetic source tracking of *E. coli* is a useful method of assessing fecal contamination in water since the standard method using fecal indicator bacteria (FIB) counts does not identify the source of contamination (Field and Samadpour 2007; Harwood et al. 2014; Henry et al. 2016). Microbial source tracking (MST) relies on two aspects to identify bacteria sources; having a source with distinct microbial community composition, or fingerprint, as well as the ability to back-calculate source samples by comparing its fingerprint to a range of source

fingerprints (McCarthy et al. 2017). Micro-organisms of fecal origin can be present in stormwater due to septic tank seepage, sewer leakage and overflow, and domestic and agricultural animal feces. Chemical and microbial markers allow researchers to distinguish fecal pollution sources, i.e. human, dog, bird, bovine, etc. (Jardè et al. 2018). Significant findings of human fecal contamination in the waters would be more concerning due to the threat to human health as compared to animal sources (Soller et al. 2010; Harwood et al. 2014).

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CHAPTER 2: UNDERSTANDING *E. COLI* AND WATER QUALITY IN STORMWATER RETENTION PONDS AND DETENTION BASINS

Abstract

Little is known about the spatial and temporal changes that occur with water quality and *E. coli* in urban stormwater systems. The goal of this project was to assess urban stormwater detention basins and retention ponds to: determine water quality differences and similarities between the two; assess *E. coli* levels during storms and normal water flows to see how it moves through the system; and determine genetic sources of the *E. coli* and pathogens to better understand potential impacts on humans. Surface water quality was sampled at three detention basins and five retention ponds during major storm events in the summers of 2018 and 2019. One week after each storm groundwater and surface water were sampled. Additionally, molecular source tracking samples were taken from storm events and normal flows, for both surface and groundwater, to determine the genetic source(s) of the *E. coli*. Results indicate that *E. coli* quantities are often higher in detention basins than retention ponds, but other water quality parameters are not significantly different between the two. *E. coli* across all sites was found to be extremely high during storm events, especially if a significant amount of time has passed since the last precipitation event. This research is important to researchers, scientists, and water managers seeking to understand water quality in urban systems. Special attention should be paid to water quality in urban areas where stormwater ponds are being utilized or retrofitted to meet recreational needs.

Introduction

Urban development is expanding rapidly as the human population grows. Urbanization is considered one of the leading contributors to water quality related issues in the United States

(USEPA 1996). Urbanization transforms the natural environment by removing vegetation and increasing impervious surfaces, surface runoff, and stormwater volumes and peak flows (Stoner et al 1998; Paul and Meyer 2001; Goonetilleke et al. 2005; Walsh et al. 2005). Untreated stormwater, and the pollutants it contains, are a threat to rivers, streams, and lakes (Brabec et al. 2002; Owens and Walling 2002), as the concept of urban stream syndrome and the decline of water quality in urban areas has been well documented across the globe (Goonetilleke et al. 2005; European Environmental Agency 2006; Fletcher et al. 2013; Wallace et al. 2013). While there is a wealth of knowledge on declines in urban water quality systems overall, less is known about water quality in stormwater retention ponds and detention basins.

Stormwater that falls in urban areas often has little chance to infiltrate into the ground due to impervious surfaces (Lee et al. 2002; Goonetilleke et al. 2005; Liu et al. 2013). Stormwater retention ponds and detention basins are utilized in many urban areas to reduce the impact stormwater has on the urban population and the natural waters it flows into. Detention basins are designed to hold water temporarily and limit the outflow of water, thus minimizing flooding and hydrologic disturbances downstream from large flushes of water (Roy et al. 2008). Additionally, the longer the basin retains water before releasing it to a larger system, the more pollutants will settle out (Roy et al. 2008). Retention ponds, on the other hand, are ponds that hold water year-round and remain wet during the dry seasons, all while helping mitigate stormwater runoff (Fischer et al. 2003). Retention ponds and detention basins also help reduce pollutants such as suspended solids, phosphates, hydrocarbons, and metals, in addition to reducing runoff and effects on downstream systems (Stanley 1996; Wu et al. 1996; Davis et al. 2001; Starzec et al. 2005; Zhu et al. 2020). As the urban population grows and cities become denser, there is a need to utilize open areas within larger cities for recreational use (Konrad and

Booth 2005). When designed correctly, detention basins and retention ponds can be utilized for recreation and green space, while still serving the typical functions of a stormwater basin (Konrad and Booth 2005). However, little is known about water quality in these areas and potential impacts on humans utilizing the area.

Urbanization has been shown to affect land use (McDonnell and Pickett 1990; Grimm et al. 2000), increase the nutrient load (Brabec et al. 2002), and deliver fecal indicator bacteria to waterways (Parker et al. 2010). The first flush phenomenon is well established in the literature and is known to be the initial period of stormwater runoff in which the concentration of pollutants is considerably higher than later points in the storm (Stanley 1996; Deletic 1998; Lee et al. 2002; McCarthy 2009). Studies in other parts of the world have shown that detention basins and retention ponds have high levels of phosphates, fecal coliforms (FC), and heavy metals that often exceed water quality standards (Taylor et al. 2005; Hettiarchchi et al. 2011; Vitro et al. 2017). Stormwater runoff often contains bacteria from the surrounding urban landscape, most commonly found in fecal coliform bacteria, particularly *Escherichia coli* (*E. coli*) (Chen and Chang 2014). *E. coli* can be transported in water in many ways including by particulate matter, adsorbed onto suspended particles, or have an attraction to sediments in the water (Chen and Chang 2014). *E. coli* are also thought to be capable of establishing growing populations in the sediments of water sources, including lakes and rivers, during the warm season (Byappanahalli et al. 2003). However, to the authors' knowledge, no study to date has compared retention ponds and detention basins water quality to understand *E. coli* presence, abundance, and movement in urban stormwater catchments.

The source of bacteria can be as or more important than presence and abundance. Most strains of *E. coli* are harmless and live in humans and animals already, however strains such as

O157:H7 have been found to cause severe illnesses in humans (CDC 2020). Utilizing technology such as genetic source tracking, researchers are better able to control and mitigate bacteria. Through microbial source tracking (MST), chemical and microbial markers allow researchers to distinguish fecal pollution sources, i.e. human, dog, bird, bovine, etc. (Jardè et al. 2018). When able to identify the main sources of fecal pollution it is easier to predict health risks and mitigate impacts, however this is not always possible. In general, human fecal contamination in an area, including water, would be more concerning due to the threat to human health as compared to animal sources (Soller et al. 2010; Harwood et al. 2014). While MST has been used in tracking bacterial and chemical markers in headwater and coastal areas (Vitro et al. 2017; Jardè et al. 2018), and springs and wells in karst regions of the Midwest (Zhang et al. 2014), to researchers knowledge it has yet to be used on stormwater in urban areas.

The goal of the current study is to determine water quality differences in detention basins and retention ponds and to determine how runoff impacts these parameters. The specific objectives of the project include:

- 1) Determine water quality analyte similarities and differences between retention ponds and detention basins during storm events and times of normal water.
- 2) Evaluate *E. coli* changes temporally and spatially in retention ponds and detention basins.
- 3) Assess sources and temporal changes of *E. coli* and the presence of pathogens utilizing genetic source tracking.

Materials and Methods

Study Area

Sampling for the project took place during 2018 and 2019. Three detention basins were randomly selected that had similar characteristics in size and catchment basin, as well as related to residential and commercial surroundings. Also, five retention ponds with similar characteristics were randomly selected to compare differences in stormwater retention ponds vs. detention basins (Figure 1).

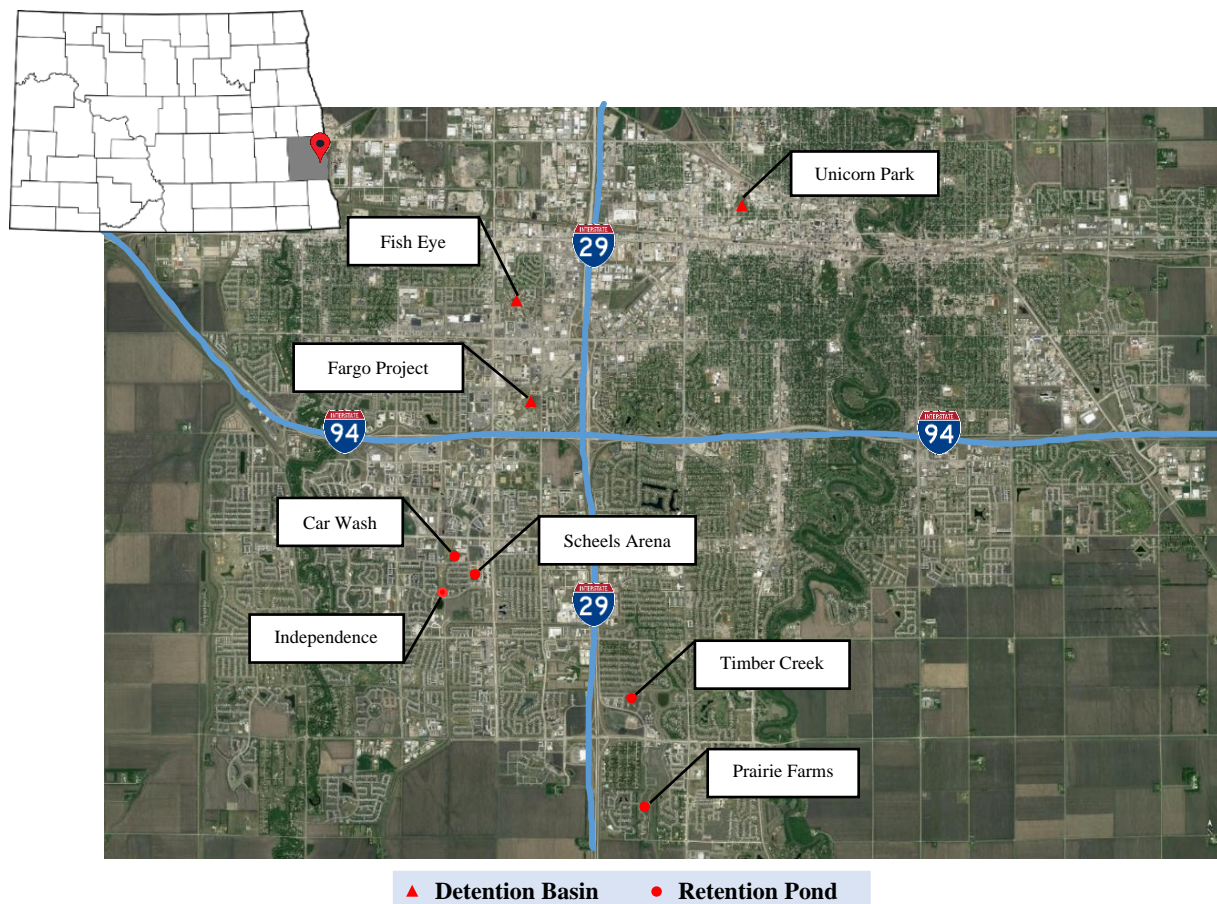


Figure 1. Map of North Dakota showing Cass County in gray and Fargo with a red pin with a black dot. The Fargo-Moorhead metro area is shown in the aerial photo. Detention basins are indicated by red triangles and retention ponds are indicated by red dots.

In detention basins, stormwater is held anywhere from 12-48 hours depending on the size of the rain event. Once the water drains from the site, all nutrients, metals and other materials in

the runoff flow into the Red River which forms the border between North Dakota and Minnesota, USA. The river then flows north into Lake Winnipeg, Canada. The numerous jurisdictions surrounding the Red River cause difficulties determining ways to manage water entering the river and impacts on the ecosystem.

The Fargo-Moorhead metropolitan area, where the study took place, has a population of 171,000 and includes the communities of Fargo and West Fargo, North Dakota, USA; and Moorhead, Minnesota, USA (United States Census Bureau 2020). Table 1 shows the percent of land use surrounding the different basins in Fargo, ND. The proportion of the land use surrounding each basin was calculated from information provided by the City of Fargo. A 500 meter buffer was identified around each basin, and the land use was calculated within the buffer. Two land use types were calculated. The proportion of impervious cover within the buffer. With the impervious cover being where there is no infiltration of water due to built structures like roofs, sidewalks, and roads. The impervious cover was calculated separately from other land uses. The second land use type was the proportion of different land uses within the buffer for each basin. The land use was categorized as commercial, high density residential, low density residential, vacant, public lands and parks. The proportion for each category was calculated from the total amount of land use within the buffer. The commercial was defined as businesses, warehouses, shops, and other infrastructure related to commerce including industrial sites. Vacant land included land that had no built structures and included land still used for agriculture. This information is important to consider since the Fargo Moorhead area continues to grow quickly.

Table 1. Percent of land use surrounding the different basins within a 500m buffer. Impervious cover is calculated separately from the other land uses. GIS layer information obtained from City of Fargo website.

Site	Commercial	High Density Residential	Low Density Residential	Public Lands and Parks	Vacant	Impervious
Carwash	16.1	27.0	16.4	15.6	24.9	15.7
Fisheye	26.7	65.3	0.1	7.5	0.4	55.1
Independence	0.3	41.4	31.1	14.1	13.2	10.8
Prairie Farms	2.1	0.9	49.4	26.3	21.2	9.8
Scheels	25.6	11.1	30.1	15.3	17.9	13.0
TFP	49.0	32.0	4.1	10.8	4.0	55.4
Timber Creek	4.5	0.0	39.5	42.8	13.2	19.2

According to the 2010 census Fargo, North Dakota has a population of approximately 105,549 people, with the projected July 1, 2019 population of 124,662, and a population percent change from April 1, 2010 to July 1, 2019 of 18.0% with approximately 2,162 people living per square mile according to the 2010 census (United States Census Bureau 2020). According to the 2010 census Moorhead, Minnesota has a population of 38,065 people, with a projected July 1, 2019 population estimate of 43,652, and a population percent change from April 1, 2010 to July 1, 2019 of 10.7%, and approximately 1,922.3 people living per square mile according to the 2010 census (United States Census Bureau 2020). The area has an average warm season from May to September with monthly highs around 24°C and lows around 11°C (NDAWN 2020). The average rainfall from May to September is approximately 7.62 cm (NDAWN 2020).

Climate and Precipitation

The Palmer Hydrological Drought Index (PHDI) measures the hydrologic impacts of drought on reservoir and groundwater levels, which tend to take longer to recover from a drought. Positive values in PHDI represent above-normal moisture conditions for that location and negative values suggest below-normal soil moisture conditions. Figure 2 shows the PHDI for

the study area for 2018 and 2019. The 2018 sampling season for the project had close to average but below normal soil moisture conditions. In 2019, the study area had above normal soil moisture conditions, with higher moisture conditions happening towards the end of the sampling season. Figure 3 shows the monthly average rainfall for the study area from January 2018 until December 2019. In 2018, the sampling season received the most rain in June, and in 2019 received the most rainfall in July.

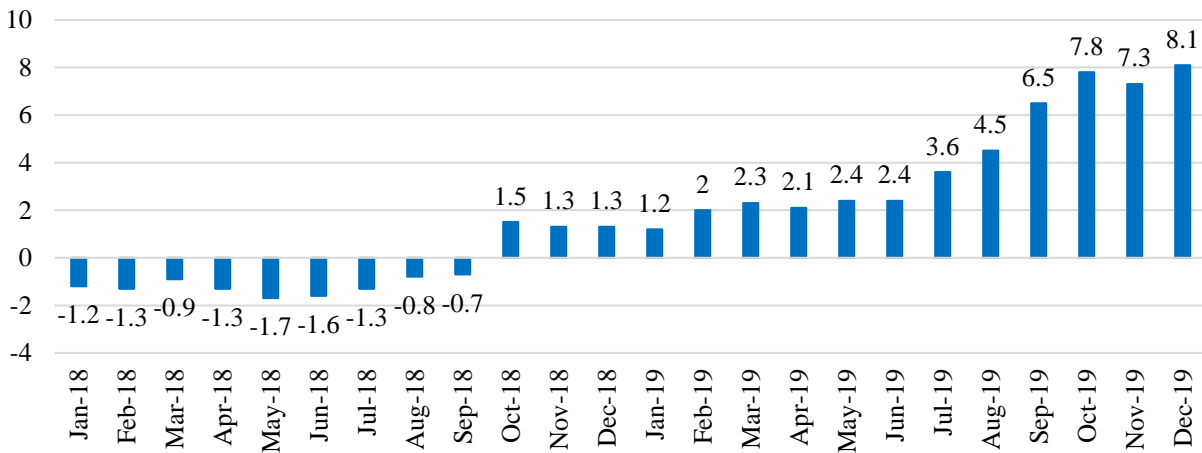


Figure 2. Divisional Palmer Hydrological Drought Index for the study area. Positive values represent above normal moisture levels, whereas negative numbers are below normal soil moisture conditions.

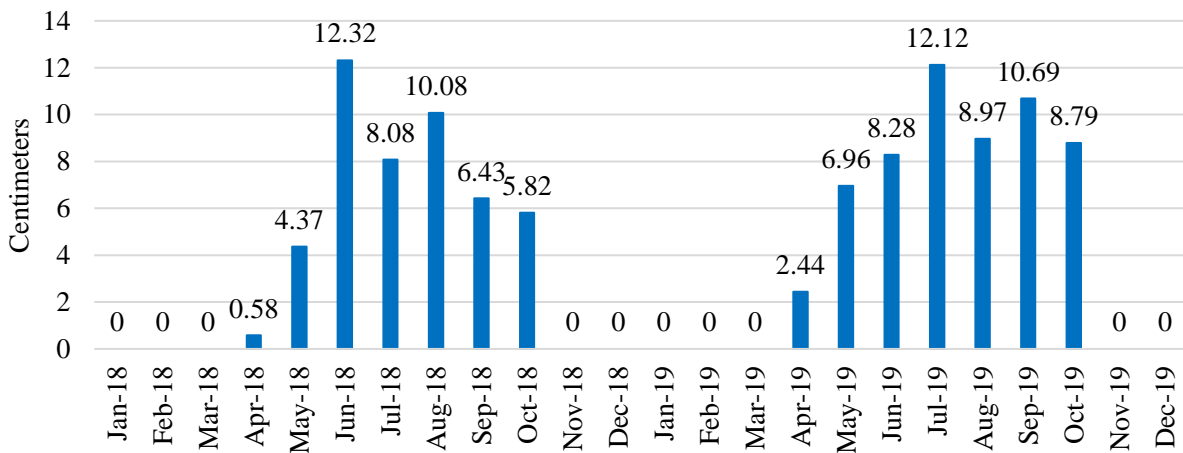


Figure 3. Monthly rainfall averages in centimeters from January 2018 to December 2019 for the study area.

Water Sampling

Water sampling for the project took place from May until September 2018 and 2019. Piezometers, that would be used later for groundwater collection, were installed in April 2018. Samples were collected during major storm events at the five retention ponds and three detention basins. Major storm events, for the purposes of this study, were defined as events receiving over 1.905 centimeters of rain in one hour. The North Dakota Agricultural Weather Network (NDAWN) measurements from Fargo, North Dakota were utilized to obtain an accurate estimate of precipitation. During each of the summers of 2018 and 2019 three separate events were sampled. Once dangerous weather had cleared from the area, researchers immediately took surface samples from all eight sites, no matter the time of day (Event, Table 2). In addition to the storm surface samples, one week following the storm event surface samples were collected at all eight sites, as well as groundwater samples from all piezometers (Post, Table 2). If there were no storm events in a given month surface and groundwater samples were obtained at least once during the month from all eight sites (Dry, Table 2). Exact dates of sampling can be found in Appendix A.

Table 2. Water sampling totals showing the number of occurrences for events, post event samples and dry month samples from summers 2018 and 2019.

Type of Sample	Summer 2018			Summer 2019		
	Event	Post	Dry	Event	Post	Dry
Number of Occurrences	3	3	1	3	3	2

All water quality samples were gathered in compliance with the North Dakota Department of Health protocol (NDDEQ 2011). On-site measurements were recorded in the field during the 2018 field season using a Yellow Spring Instrument Co. YSI model 650 MDS data logger combined with model 600 QS Sonde to measure temperature, electrical conductivity, pH,

and dissolved oxygen (YSI Incorporated, Yellow Springs, Ohio, USA). The same parameters were measured in 2019 using a newer model YSI ProDSS handheld multiparameter meter (YSI Incorporated, Yellow Springs, Ohio, USA). All surface samples were collected using a Bel-Art long-handled dipper cup (Bel-Art SP Scienceware; Wayne, New Jersey, USA). Samples were preserved according to NDDEQ protocol, cooled on ice, and transported to the NDDEQ's lab in Bismarck, North Dakota for analysis. Parameters analyzed include total suspended solids (TSS), nutrients complete (TKN, NO₂, NO₃, NH₃, NH₄, and P), major cations and anions, trace metals, and *E. coli*. Piezometers were installed using a 7.62-centimeter bucket auger, similar to those utilized in soil sampling, to dig the holes for the piezometer installation. Piezometers consisted of a 7.62 cm by 1.52 meter long white 40 PVC pipe with 0.245 mm slot well screen (Atlantic Screen and Mfg., Inc.; Milton, DE, USA) (Figure 4).

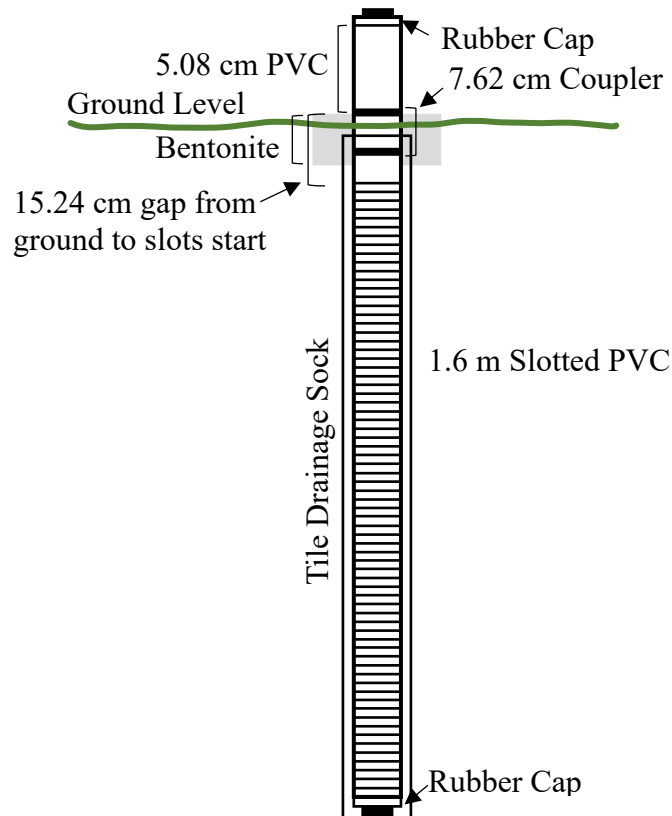


Figure 4. Piezometer design diagram.

The piezometers were encased to ground level in a 10.16 cm drain sleeve to prevent large particles from flowing into the system. The slotted PVC started 15.24 cm from the surface to ensure surface water was not entering the tube unless it infiltrated the 15.24 cm of soil. Finely ground bentonite powder was placed at ground level around the installed PVC to prevent surface water from infiltrating the piezometer, as well as to secure the PVC in place. Construction of the piezometers included a 5.08 cm coupler that attached the slotted PVC to a 5.08 cm piece of PVC. To ensure water did not infiltrate through the top of the piezometer, male cleanout mechanical test plugs were installed. This allowed the top to be easily removed for sampling and to be sealed once finished. Piezometers at the Fargo Project shown in image C (largest detention basin) were installed near the inlets, the outlet, and where the two inlet flows meet (confluence) (Figure 5). Piezometers were also installed at Fisheye shown in image B (detention basin outlet) and Independence shown in image A (retention pond inlet) to assess the movement of *E. coli* in the groundwater (Figure 5). At each piezometer location, a set of piezometers were installed at 0 m, 2.5 m, and 5 m from the surface water or channel.

One week after each storm event groundwater samples from all piezometers were sampled. Water was extracted using a plastic hand-operated water and chemical siphon/drum pump with 6.35 mm clear plastic tubing (Cole-Parmer North America; Vernon Hills, Illinois, USA). Samples were preserved, cooled, and transported to the NDDEQ's lab for analysis. Parameters analyzed for groundwater include nutrients complete (TKN, NO₂, NO₃, NH₃, NH₄, and P), major cations and anions, trace metals, and *E. coli*.



Figure 5. Locations where piezometers are installed: a) Independence; b) Fisheye; and c) the Fargo Project. Each red triangle represents the general piezometers location, at each triangle there are piezometers installed at 0 m, 2.5 m, and 5 m.

Source Tracking

Water samples for genetic source tracking were taken from the Fargo Project storm event on August 26th, 2018 and south inlet on September 4th, 2018. In the summer of 2019 additional funding was available for analysis; therefore, six different collection times and dates were collected. All 2019 samples were taken from the Fargo Project, the six samples included a storm event on July 9th; storm event on July 17th; west outlet post event on July 23rd; storm event on August 27th; west outlet post event on September 17th; and south piezometer at 0 m on September 17th.

Samples were submitted to Source Molecular Corporation (Miami Lakes, Florida, USA) and analyzed for the presence and concentration of fecal host-associated biomarkers from bird, dog, human, and *E. coli* O157:H7 in 2018. These biomarkers were chosen as they were the most likely sources to contribute *E. coli* for this system. Goose, gull, and sewage markers were added in 2019 based on 2018 findings of bird and human biomarkers. All samples were cooled on ice and transported to the NDDEQ's lab where they were filtered using a 0.4-micron Pall (Pall Corporation, Washington, NY) pre-sterilized filter and cooled to -20°C as requested by Source Molecular. The NDDEQ lab stored the samples until the end of the sampling season and then shipped them to Source Molecular. Once received by Source Molecular, each sample is filtered through a 0.45-micron membrane filter, and each filter is placed in a separate sterile 2mL disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for one minute and the DNA is extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, New Jersey, USA). Samples were then analyzed for the presence and concentration of fecal host-associated biomarkers.

Statistical Analysis

The number of samples of *E. coli* above the recreational standard (126 CFU) North Dakota Century Code (61-28-04. 33-16-02.1-09) sets standards for surface water classifications, mixing zones, and numeric standards. The recreational standard in North Dakota for Class I, IA, II, and III streams and lakes and reservoirs follow the EPA recreational standard for *E. coli* (EPA 2020) and is followed from May 1st until September 30th. The samples of *E. coli* were analyzed using a chi-square test in SPSS (IBM SPSS Statistics for Windows, version 26, IBM Corp., Armonk, New York, USA). Individual sites and events were compared together using a z-test adjusting the p values using the Bonferroni method.

The *E. coli* data collected in surface samples were analyzed as a two-factor random design with site and event as the two fixed factors using Proc GLM in the SAS software system ([SAS/STAT] software, Version 9.4 for Windows 10, Copyright © [2014] SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA). The data was log transformed prior to analysis. Multiple comparisons were made using LSMEANS procedure and the Tukey adjustment. The *E. coli* data collected in the piezometers were analyzed with piezometer location nested under site with site and event as main fixed factors using Proc Mixed in the SAS software system. Multiple comparisons were made using LSMEANS procedure and the Tukey adjustment.

The ten water quality factors found in Appendix B were analyzed using multivariate methods. The piezometer and surface water samples were analyzed using Permutation Multivariate Analysis of Variance (PERMANOVA) in PRIMER-e™ software (Quest Research Limited) (see Anderson et al. 2008 for an explanation of the procedure). The piezometer data

were analyzed with the location as a nested factor under site with site and event as main fixed factors. The surface data were analyzed with site and event as the main fixed factors. Both analyses used the relative Euclidian distance measure. Paired comparisons for all the factors reported the unadjusted P-values as suggested by Anderson et al. (2008).

Nonmetric Multi-Dimensional Scaling (NMDS) was used to ordinate and visually present both the surface and piezometer data grouping the main factors of site and events. The NMDS analysis used PC-ORD version 7 (MjM Software Design, Gleneden Beach, OR). A relative Euclidian distance measure was used similar to the PERMANOVA analysis. The NMDS used these circumstances: 1) 500 iterations in PC-ORD to reduce from six axes to two or one, 2) a significant Monte Carlo test ($p \leq 0.05$) to select axes from random, 3) stress < 25 , 4) instability < 0.0001 , 5) selection was halted when the next axis did not reduce stress by at least five.

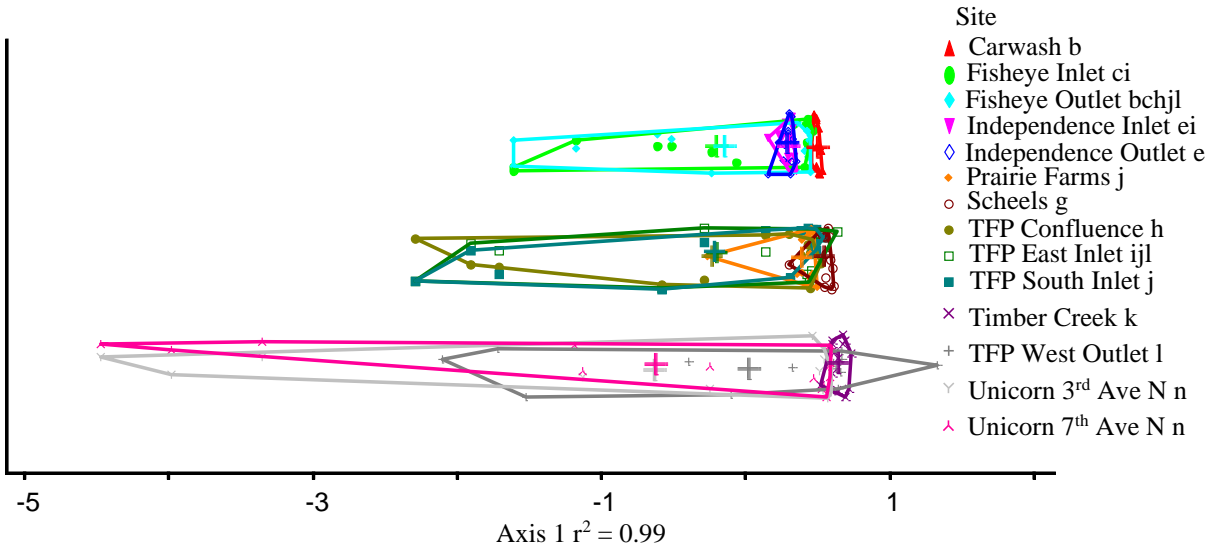
Results and Discussion

Surface

Water Quality

The NMDS analysis of the ten water quality parameters for surface water (Appendix B) produced a final solution with one dimension (Final Stress = 5.3; Final Instability < 0.0001 ; Number of Iteration = 45). The PERMANOVA analysis determined that both site and event were significantly different ($p < 0.001$) (Figures 6 and 7). This resulted in most of the sites being grouped far to the right, but the Unicorn sites were further to the left and had a wide range of values. Suspended solids were positively correlated with Axis 1, whereas anion and cation sum, conductivity, dissolved solids, sodium and sodium adsorption ratio were negatively correlated. Surface samples were highly variable; some sites had high levels of salts during one event, then low levels of salts during another. Similar results, in regards to salt variability in samples, have

been found in many urban water quality studies (Barbosa et al. 2012; Khatri and Tyagi 2015; Burant et al. 2018). When areas with large impervious surfaces are dry for an extended period, then receive a large amount of rain, there tend to be higher concentrations of pollutants (Stanley 1996; Deletic 1998; Lee et al. 2002; McCarthy 2009). The current study found differences in water quality parameters to be driven by the site rather than the event. Sites in industrial landscapes, as opposed to a suburban neighborhood, tended to have an increase in certain pollutants. For example, Unicorn Park is located in a primarily industrial area, leading to higher levels of pollutants in the surface water samples. Whereas Independence is located in a large neighborhood with lots of grass yards and parks, which lead to lower pollutant levels. The dry events, and the majority of the post-storm events, tended to be concentrated together due to the first flush of pollutants affecting the storm event samples.



Negatively correlated:

Anion Sum=0.54, Cation Sum=0.57, Conductivity= 0.57,

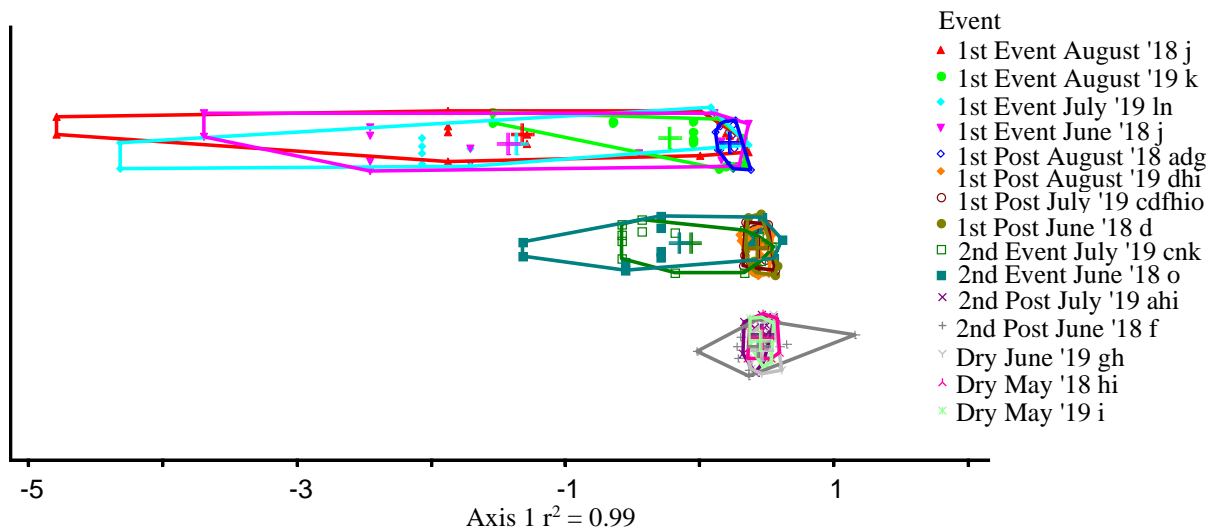
Dissolved Solids=0.52, Sodium=0.59,

Sodium Adsorption Ratio=0.59

Positively Correlated:

Suspended Solids=-0.77

Figure 6. Nonmetric Multi-Dimensional Scaling (NMDS) graph of the multivariate analysis of surface water quality parameters showing sites with convex hull polygons. The sites were split into three different displays to increase legibility. Normally the sites would be clustered together, but given that only one axes were chosen jittering the display does not decrease interpretation. Legend items followed by different letters are significantly different at $p < 0.05$. The coefficient of determination between ordination distances and distances in the original n -dimensional space is shown in the axis label. Correlations between parameter abundance and the axis scores are shown in the boxes.



Negatively correlated:	Positively Correlated:
Anion Sum=0.54, Cation Sum=0.57, Conductivity= 0.57,	Suspended Solids=-0.77
Dissolved Solids=0.52, Sodium=0.59,	
Sodium Adsorption Ratio=0.59	

Figure 7. Nonmetric Multi-Dimensional Scaling (NMDS) graph of the multivariate analysis of surface water quality parameters showing events with convex hull polygons. The events were split into three different displays to increase legibility. Normally the events would be clustered together but given that only one axes were chosen jittering the display does not decrease interpretation. Legend items followed by different letters are significantly different at the $p < 0.05$. The coefficient of determination between ordination distances and distances in the original n-dimensional space is shown in the axis label. Correlations between parameter abundance and the axis scores are shown in the box.

The USEPA (1996) reported that the first flush receives a larger quantity of oil, grease, and toxic chemicals from vehicles; nutrients and pesticides from turf management; viruses and bacteria from failing septic systems; road salts; and heavy metals. Large quantities of heavy metals have also been reported in previous literature including Pb, Zn, Cu, and Cd, as well as suspended solids (Davis et al. 2001; Fritioff and Greger 2003; Hatt et al. 2007; Henderson et al. 2007). Additionally, studies have found naturally occurring elements, such as phosphorous, fluoride, sulfate, total dissolved solids, manganese, and iron are found in higher concentrations in urban areas due to disturbance (Neary et al. 1988; NRCS 2006; MPCA 2007). The current study assessed 52 different trace elements, including metals, but at no time did any of the levels cross the threshold for water quality parameters as determined by the state of North Dakota for stormwater levels.

A goal of this study was to understand the differences in water quality between retention ponds and detention basins. Results did not produce any significant differences between retention ponds and detention basins when assessing nutrients and trace elements. Both detention basins and retention ponds are utilized for recreation, green space, and natural habitat, all while mitigating flood impacts and removing contaminants found in the stormwater runoff (Stanley 1996; Wu et al. 1996; Starzec et al. 2005; Zhu et al. 2020). Schueler (1994) found detention basins generally are able to remove suspended solids, but inconsistently remove phosphorus, and poorly remove nitrogen. The current study did not find elevated levels of phosphorus and nitrogen, therefore the basins are likely either effectively filtering the stormwater as it settles, or there is generally a small amount of nitrogen and phosphorus within the system. Davis et al. (2001) found that longer retention times and larger ponds helped with the removal of particulate matter, this could also be contributing to the low numbers.

E. coli

This study took place in North Dakota, as per North Dakota Century Code (NDCC), which is law in the state, the *E. coli* standard is related to surface water quality, and is meant to ensure that water is safe for boating, swimming, fishing, and other water recreational use. Samples from the detention basins and retention ponds had *E. coli* levels rarely falling below the recreational standard of 126 CFU/100 mL (Table 3). While researchers do not encourage people to ever swim in stormwater, this is the best and only standard in the area for comparison. Additionally, within and surrounding the study area for the project some sites allow for paddle boating, kayaking, and fishing, therefore those waters would need to meet the standard. Though we never fully assessed sites for the recreational standard, which would have required taking a minimum of five samples in one month, the comparison of research results to the standard is valid. The standard sets a maximum level of *E. coli* (409 CFU) that cannot be exceeded more than 10% of the time, and mean value (126 CFU), and the sites in this project often did not meet these criteria.

The Fargo Project (TFP) confluence never once during any sampling period in either year met the recreational standard for Class I, IA, II, and III streams, lakes, and reservoirs (NDCC 61-28-04. 33-16-02.1-09). Additionally, TFP east, south, and west sampling sites all had the majority of readings over 126 CFU. The Independence inlet and outlet have *E. coli*, but only 27% of samples exceeded 126 CFU. In general, it did not matter where the detention basin or retention pond was located within the study area, all of the sites during some sampling period had readings over the recreational limit. Of the eight retention ponds and detention basins, TFP had the highest incidence of samples over 126 CFU, while Independence and Prairie Farms had the lowest. The catchment of the Independence site is a mix of residential and commercial lots

with a fair amount of impermeable surfaces but had some of the lowest incidences of *E. coli*.

Likely, the green space within the catchment, including backyards, schools and public parks with permeable surfaces contributed to the low levels of *E. coli* due to the infiltration of water into permeable surfaces.

Table 3. The number of times samples from detention and retention sites were above the recreational limit, total samples taken, and percent over the recreational limit. Sites with different letters were significantly different at $p < 0.05$.

	Above 126 CFU	Total Samples	% over 126 CFU
TFP Confluence	14 a	14	100
Unicorn Outlet	14 ab	15	93
TFP West	13 ab	14	93
TFP South	13 ab	14	93
Unicorn Inlet	13 abc	15	87
Fisheye Inlet	12 abc	14	86
TFP East	12 abc	14	86
Fisheye Outlet	11 abc	15	73
Carwash	7 abc	15	47
Scheels	7 abc	15	47
Timber Creek	7 abc	15	47
Prairie Farms	5 bc	15	33
Independence Inlet	4 c	15	27
Independence Outlet	4 c	15	27
TOTAL	136	205	66

Additionally, Prairie Farms had the other lowest incidence of *E. coli*, this is likely due to being a newer development in the peri-urban area of the city that contains a large amount of green space. Steinman (2017) found that pond sites in peri-urban areas with a large amount of green space have more consistently low samples, and the green space is likely serving as a buffer. The amount of bacteria on impermeable surfaces can be high, yielding higher levels of bacterial and pathogens to surface drains and urban receiving waters (Ellis 2004). Other research has shown that fecal bacteria densities are directly related to the density of housing, population, development, percent impervious area, and apparent domestic animal density (Young and

Thackston 1999; Field and Samadpour 2007; Hettiatchchi et al. 2011; Vitro et al. 2017). Young and Thackston (1999) specifically found that fecal coliform counts were much higher during rain events in urban areas and showed a direct relationship between various land uses and the amount of bacterial found.

The amount of *E. coli* at sites was averaged over all study samples, and 100% of sites were over the 126 CFU recreational limit (Table 4), though standard deviations were large due to extreme variability in sampling periods. Some sites were significantly different from others based on the geometric mean of *E. coli*, and from the lowest site to the highest there was a 33 times increase in *E. coli*. The highest sites had catchments that were a mix of residential and commercial, while the lowest were mainly residential areas. However, no matter the land use type, *E. coli* was high during some sampling periods. The large variation of *E. coli* within sites between sampling dates is characteristic of all sites. Such variation means that any site studied is capable of events or samples that can have high, even beyond the detection limit of 24,000 CFU, or low values depending on the size of stormwater events or length of dry periods since rainwater contributions. In reference to recreational use, this could mean that the site may be useable (under 126 CFU standard) for several readings but then might skyrocket to 24,000 CFU or more after a rain event. Other research has shown similar results, in that during dry weather bacteria are not usually a problem, unlike pollutants such as metals and organics, but during wet weather, bacteria become more of a problem (Young and Thackston 1999; Hatt et al. 2007; Henderson et al. 2007; Hettiarchchi et al. 2011; Burant et al. 2018). Young and Thackston (1999) found a relation between higher bacteria counts during wet weather high flows compared to the dry weather low flows. Similar to McCarthy et al. (2012) findings, our peak *E. coli* concentrations

were found during storm events, likely due to a build-up of sediment and fecal matter on stormwater pipes and impervious surfaces within the catchment basin.

Table 4. The geometric means of *E. coli* (CFU) for each site. Sites followed by different letters are significantly different at $p < 0.05$.

	Geometric Mean of <i>E. coli</i>	Significant Differences $p < 0.05$	Geometric Std Dev Factor
TFP Confluence	2227.1	a	4.0
TFP West	1740.4	ab	5.7
TFP South	1470.2	abc	7.2
TFP East	1182.0	abc	7.9
Unicorn Outlet	926.8	abc	6.3
Unicorn Inlet	896.3	abc	6.6
Fisheye Outlet	769.4	abcd	4.8
Fisheye Inlet	714.4	abcd	5.6
Timber Creek	288.2	abcd	19.8
Scheels	158.3	bcd	12.4
Carwash	103.1	cd	12.8
Prairie Farms	97.1	cd	13.8
Independence Outlet	72.4	d	8.5
Independence Inlet	66.9	d	8.6

Regardless of location (Table 3) or stormwater rain events (Table 5) that result in the basins rising or filling almost always have samples above the recreational limit. Our results specifically indicate a trend of high *E. coli* levels in stormwater events that are preceded by long dry periods. Regardless of the type of basin, storm events almost always have values above the 126 CFU recreational limit. Research indicates that urban runoff contains higher amounts of bacteria (Young and Thackston 1999) and those amounts have been shown to be higher following rainfall (Goonetilleke et al. 2005; Barbosa et al. 2012; Burant et al. 2018). The literature also suggests that over 650 identified compounds are present in stormwater in a variety of concentrations (Gasperi et al. 2014). Though our study did not test for these, it has been shown that pesticides, flame retardants, polycyclic aromatic hydrocarbons, corrosion inhibitors,

among other organic materials can be found in the surface and groundwater in urban areas (Burant et al. 2018).

Table 5. The number of times samples from different events that were above recreational limit, total samples taken, and percent over the recreational limit. Sites with different letters were significantly different at $p < 0.05$.

	Above 126 CFU	Total Samples	% over 126 CFU
1st Event August '18	14 a	14	100
1st Event August '19	14 a	14	100
1st Event July '19	14 a	14	100
2nd Event June '18	14 a	14	100
1st Event June '18	11 ab	14	79
2nd Event July '19	11 ab	14	79
1st Post August '18	9 ab	14	64
2nd Post June '18	9 ab	14	64
1st Post June '18	8 ab	14	57
Dry June '19	8 ab	14	57
2nd Post July '19	7 ab	14	50
Dry May '18	7 ab	14	50
1st Post August '19	4 b	13	31
Dry May '19	4 b	14	29
1st Post July '19	2 b	10	20
TOTAL	136	205	

Results showing an increase in *E. coli* in stormwater after a dry period indicates the first flush phenomenon is at work within these systems (Table 6). The first flush phenomenon is well established in the literature as the initial runoff that is washed off impervious surfaces after a rainfall event that contains the most pollutants (Stanley 1996; Deletic 1998; McCarthy 2009; Lee et al. 2002). Similar to the current study, in the Jardè et al. (2018) study, rainfall was shown to wash the landscape and bring contaminants to a larger body of water, in our case stormwater basins. It is important to understand contaminants, their concentrations, and the timing of their movement to better manage and mitigate problems within the system.

Table 6. The geometric means of *E. coli* (CFU) for each event. Sites followed by different letters are significantly different at $p < 0.05$.

	Geometric Mean of <i>E. coli</i>	Significant Differences $p < 0.05$	Geometric Std Dev Factor
1st Event August '18	7456.2	a	2.9
1st Event August '19	6124.7	ab	2.2
1st Event July '19	5422.1	ab	1.6
2nd Event July '19	1799.4	bc	12.9
2nd Event June '18	1712.0	cd	3.1
1st Event June '18	556.2	de	9.2
1st Post August '18	318.7	de	7.0
2nd Post June '18	242.6	e	8.2
1st Post June '18	117.5	e	7.6
Dry June '19	114.7	e	5.6
Dry May '18	105.2	e	5.5
1st Post August '19	87.9	e	14.3
2nd Post July '19	75.0	e	6.3
1st Post July '19	63.3	e	2.8
Dry May '19	51.2	e	3.9

Groundwater

Water Quality

The NMDS analysis of the ten water quality parameters for groundwater (Appendix B) produced a final solution with two dimensions where Axis 1 represents 80% of the variation in the data and axis 2 represents 19% of the variation in the data (Final Stress = 2.2; Final Instability < 0.0001; Number of Iteration = 142). The PERMANOVA analysis determined significant differences exist among the different events and sites $p < 0.05$ (Figure 8 and 9). Of the sites, only the Fisheye and TFP east inlet were not significantly different, while all the others were significantly different (Figure 8). Two of the TFP sites, confluence and west, were located at the axis with the least concentrations of the water quality parameters. The sampling events did not have much spread, therefore differences between events were less common than between sites (Figure 9). However, the dry May events were significantly different from the 1st August

post-storm event in 2018. Regardless of the site or event, higher concentrations of water quality parameters were associated with the negative side of Axis 1. These lower concentrations at TFP confluence and west are likely attributed to both of the piezometer areas being in soil that is almost pure sand lenses, as it has been shown that sands tend to contribute fewer ions and conductivity to groundwater (Tutmez et al. 2006). Previous research on stormwater has found pollutants such as heavy metals, pesticides, total suspended solids, and some organic contaminants in urban areas (Goonetilleke et al. 2005; Barbosa et al. 2012; Burant et al. 2018). Our findings for metals and total suspended solids in groundwater did not indicate high levels of these pollutants.

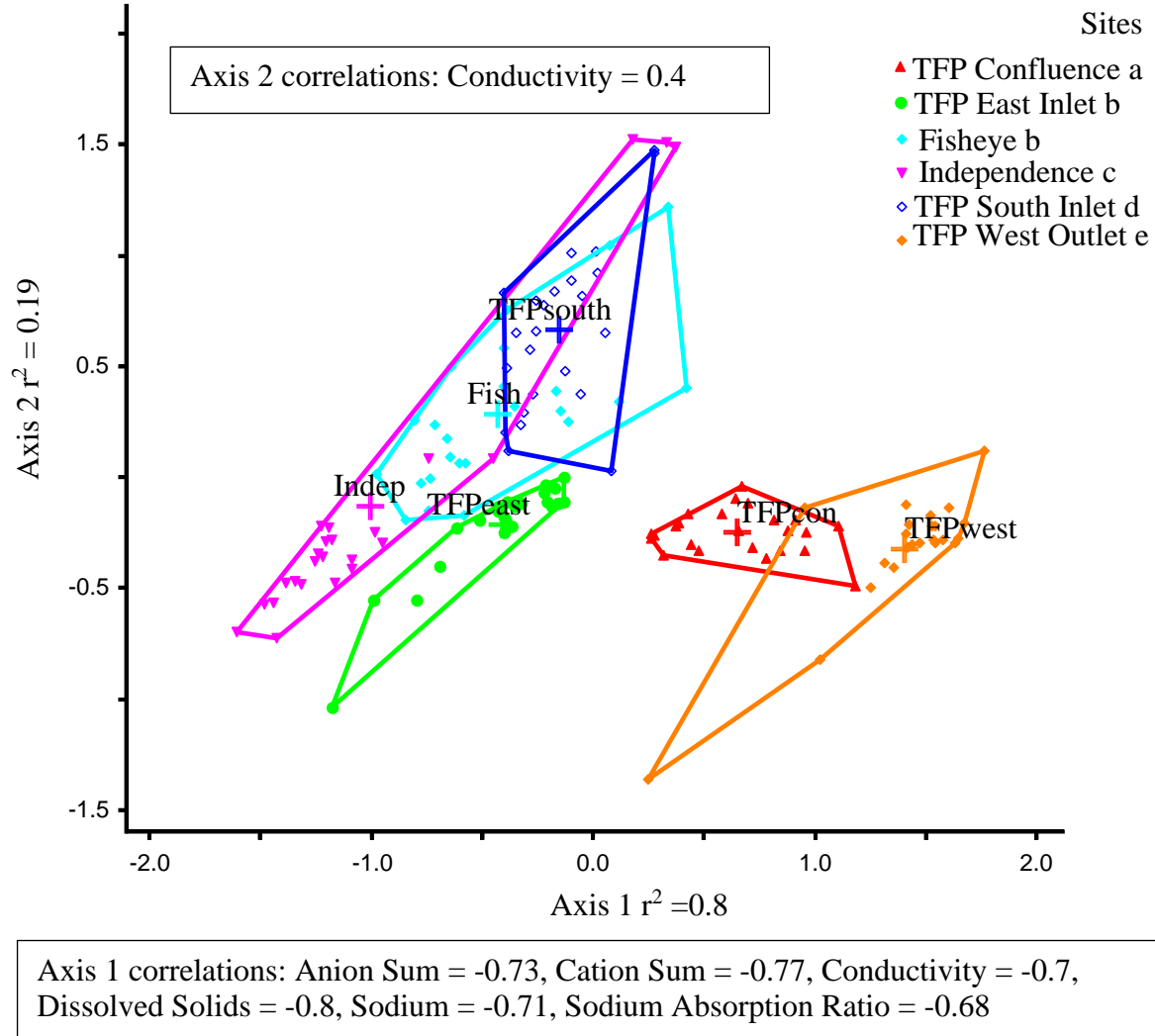


Figure 8. Nonmetric Multi-Dimensional Scaling graph of the multivariate analysis of piezometer water quality parameters showing sites with convex hull polygons. Legend items followed by different letters are significantly different at $p < 0.05$. The coefficient of determination between ordination distances and distances in the original n -dimensional space is shown in the axis label. Correlations between parameter abundance and the axis scores are shown in the boxes.

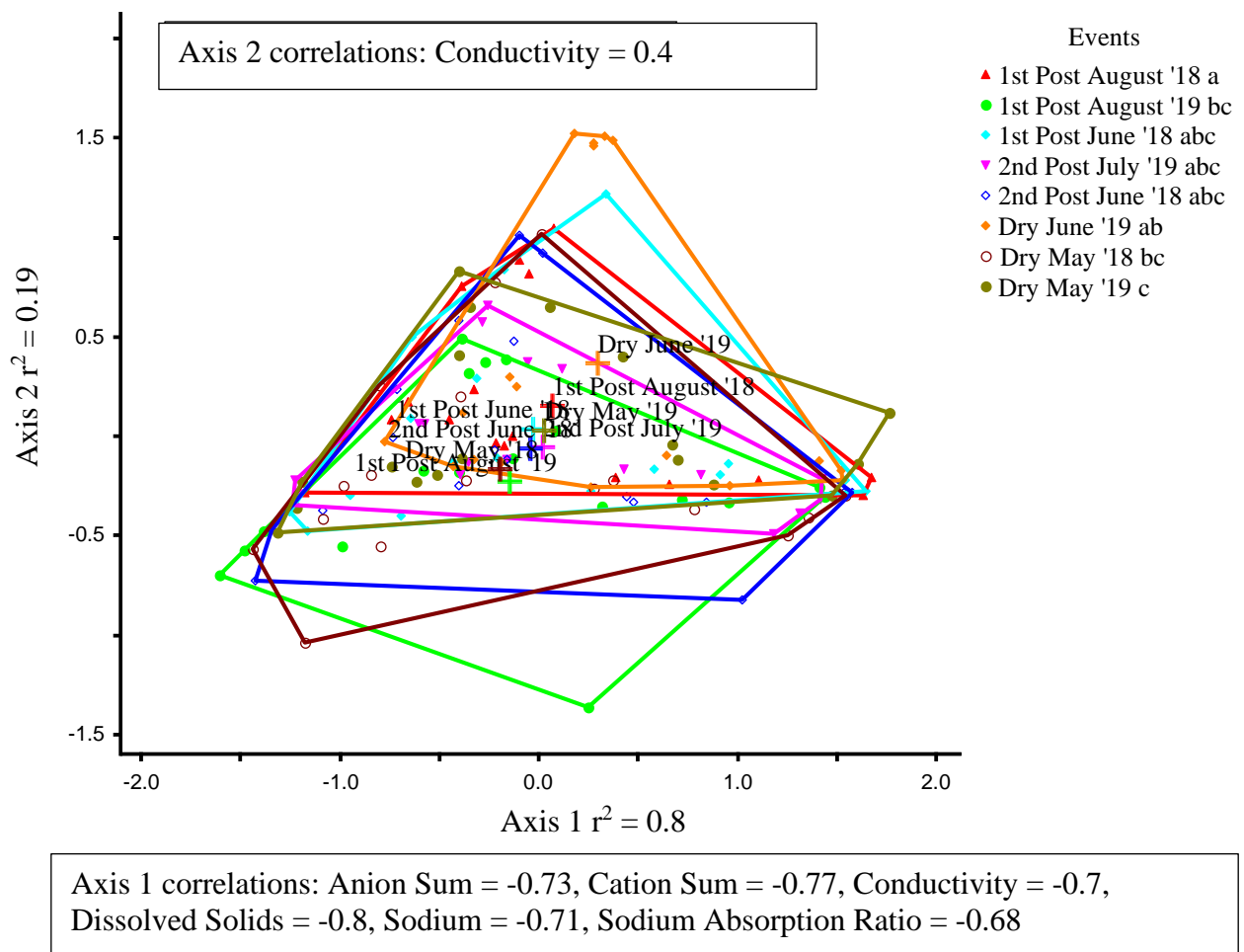


Figure 9. Nonmetric Multi-Dimensional Scaling graph of the multivariate analysis of piezometer water quality parameters showing events with convex hull polygons. Legend items followed by different letters are significantly different at $p < 0.05$. The coefficient of determination between ordination distances and distances in the original n -dimensional space is shown in the axis label. Correlations between parameter abundance and the axis scores are shown in the boxes.

E. coli

Many sites *E. coli* samples taken from groundwater piezometers were over the 126 CFU recreational limits, but compared to the surface samples, the number of incidences was reduced (Tables 7). Independence only once had one piezometer sample over 126 CFU, whereas Fisheye had 62.5% of the groundwater samples over the recreational limit. Regardless of where the piezometers are located in Fargo, there is at least one occurrence of samples being over 126 CFU. The amount of *E. coli* found at each piezometer sites was in the same order as the number of occurrences over 126 CFUs (Table 8).

Table 7. The number of *E. coli* piezometer site samples over 126 CFU. Sites followed by different letters were significantly different at $p < 0.05$.

	Above 126 CFU	Total Samples	% over 126 CFU
Fisheye	15 a	24	62.5
TPF West	11 ab	23	47.8
TFP East	9 abc	24	37.5
TPF South	6 abc	24	25
TFP Confluence	3 bc	24	12.5
Independence	1 c	24	4.2
Total	45	143	

Table 8. The geometric means of *E. coli* (CFU) for each piezometer site. Sites followed by different letters are significantly different at $p < 0.05$.

	Geometric Mean of <i>E. coli</i>	Significant Differences $p < 0.05$	Geometric Std Dev Factor
Fisheye	236.8	a	5.8
TPF West	119.8	ab	7.7
TFP East	95.8	ab	8.0
TPF South	43.1	b	5.4
TFP Confluence	23.4	b	4.0
Independence	20.1	b	2.9

The amount of *E. coli* found in the groundwater at Fisheye is 11 times greater than the amount found at Independence. Events compared to the sites were highly variable, there were no differences found in the frequency analysis (Table 9). This was similar to the analysis of the

amounts of *E. coli* though there were differences between the events with the lowest and the events with the highest means by 7 times (Table 10). There was no discernable trend in the presence and movement of *E. coli* through groundwater regardless of the 0m, 2.5m, and 5m distances from the surface water. Therefore, regardless of the distance from surface water, it appears there is still a risk of *E. coli* in groundwater in both detention basins and retention ponds. It has been shown that soil and plants can provide natural filtration for the removal of bacteria (Vacca et al. 2005) and soil properties have been shown to affect the movement of bacteria through saturated soils (Conboy and Goss 2000). Additionally, research has shown that in temperate climates *E. coli* is persistent in soils for more than nine years, essentially naturalizing in the environment (Brennan et al. 2010). In reference to the current study, *E. coli* was found to be prevalent regardless of distance from the surface water. Leading researchers to believe there is potentially a natural filtration process taking place from surface water, however *E. coli* that is present is likely naturalized and persistent in the soil.

Table 9. The number of times samples from different events that were above recreational limit, total samples taken, and percent over the recreational limit. Sites with different letters were significantly different at $p < 0.05$.

	Above 126 CFU	Total Samples	% over 126 CFU
1st Post August '18	9 a	17	52.9
2nd Post July '19	9 a	18	50
2nd Post June '18	8 a	18	44.4
Dry June '19	7 a	18	38.9
1st Post June '18	5 a	18	27.8
1st Post August '19	3 a	18	16.7
Dry May '18	3 a	18	16.7
Dry May '19	1 a	18	5.6
Total	45	143	

Table 10. The geometric means of *E. coli* (CFU) piezometers for each sampling event. Sites followed by different letters are significantly different at $p < 0.05$.

	Geometric Mean of <i>E. coli</i>	Significant Differences $p < 0.05$	Geometric Std Dev Factor
1st Post August '18	119.0	a	5.3
Dry June '19	114.8	a	10.8
2nd Post July '19	98.0	ab	7.1
2nd Post June '18	88.0	ab	7.8
1st Post June '18	64.4	ab	5.6
Dry May '18	29.6	ab	6.2
1st Post August '19	27.9	ab	4.9
Dry May '19	16.7	b	3.0

Molecular Source Tracking

Micro-organisms of fecal origin in stormwater can be from septic tank seepage, sewer leakage and overflow, and domestic and agricultural animal feces (USEPA 1996; Field and Samadpour 2007; Khatri and Tyagi 2015; Jardè et al. 2018). Previous research has shown that developed stormwater basins can have high levels of dog waste due to an abundance of dogs living in urban areas and owners not effectively picking up waste (Young and Thackston 1999). Additionally, dependent on the amount of rain during the season there can be higher levels of *E. coli* (Chen and Chang 2014). The current study found that *E. coli* was present in both the groundwater and surface water, and in both detention basins and retention ponds regardless of the surrounding landscape. Therefore, our study wanted to investigate the source of the *E. coli*.

Samples from field seasons 2018 and 2019 were used to determine the genetic source(s) of *E. coli*. Due to the prohibitive costs, samples were only taken at TFP. In 2018, two samples were taken, one from a storm and one week after a storm at TFP, to sample for genetic contributions of *E. coli* from human, dog, bird, and the pathogen O157:H7 (Table 9). In 2018,

the bird marker was detected in both the storm and regular flow samples, but human and dog were only detected during storm events, and O157:H7 was never detected.

Table 11. Genetic source tracking results for 2018.

Event	Human	Dog	Bird	O157:H7
Storm Event TFP	Detected	Detected	Detected	Not detected
Post TFP	Not detected	Not detected	Detected	Not detected

In the summer of 2019, with an increased sampling budget, seven markers were tested including human, dog, bird, gull, goose, sewage, and the pathogen O157:H7. Samples were taken at TFP at six different times, three storms, two regular flows (one week after a storm), and one from a piezometer (groundwater) (Table 10). Bird and sewage markers were detected in all six samples, but goose was never detected. The piezometer sample showed that birds and sewage markers were present in the groundwater, but no other sources. Human, dog, bird, and sewage were present during all storm events, but human and dog were not detected when the water returned to its normal level, except in one instance where dog markers were detected in a post-storm regular flow sample.

Table 12. Genetic source tracking results for 2019.

Event	Human	Dog	Bird	Sewage	Goose	Gull	O157:H7
Storm Event TFP	Detected	Detected	Detected	Detected	Not detected	Not detected	Not detected
Storm Event TFP	Detected	Detected	Detected	Detected	Not detected	Not detected	Not detected
Storm Event TFP	Detected	Detected	Detected	Detected	Not detected	Detected	Not detected
Post TFP	Not detected	Detected	Detected	Detected	Not detected	Not detected	Not detected
Post TFP	Not detected	Not detected	Detected	Detected	Not detected	Not detected	Not detected
Piezometer TFP	Not detected	Not detected	Detected	Detected	Not detected	Not detected	Not detected

Chemical and microbial markers allow researchers to distinguish fecal pollution sources, i.e. human, dog, bird, bovine, etc. (Jardè et al. 2018). Field and Samadpour (2007) and Chen and Chang (2014) assessed *E. coli* in urban water quality, but few studies have assessed the genetic source(s) of the *E. coli*, and when they have they only assess one potential source, such as human vs. non-human investigated in Field and Samadpour (2007). There is little research currently on genetic source tracking in stormwater. Molecular source tracking (MST) is a relatively new technology and literature will grow over time, however current research is lacking. The MST technology has been used on beaches in coastal areas to investigate the higher levels of enterococci, suggestive of possible fecal contamination (Henry et al. 2016). Additionally, Jardè et al. (2018) assessed bacterial and chemical markers to study the seasonal change in the intensity and sources of fecal contamination in three very different French headwater and coastal catchments within an agricultural ranching landscape and showed high levels of bovine *E. coli*. To the authors' knowledge, this is the first study of its kind to utilize molecular source tracking of multiple markers to assess *E. coli* in stormwater.

In general, human bacteria from fecal matter is considered more dangerous than fecal matter from other sources (Soller et al. 2010), though contamination from cattle and other ruminants is most common (Fairbrother and Nadeau 2006). The O157:H7 pathogen can have a dire impact on humans, especially children, and can lead to detrimental health effects including kidney problems and death (Tarr et al. 1997). This pathogen is spread by both domestic and agricultural animal's fecal matter (Field and Samadpour 2007). Previous research shows the detection of bacterial densities and pathogens in impermeable surface runoff is generally from domestic animals, rodents and birds (Ellis 2004). Young and Thackston (1999) determined that

fecal coliforms (FC) and fecal streptococci (FS) in runoff was likely more from animals rather than human contamination.

Conclusion

E. coli was found to be highest during storm events when compared to all other sampling times. This is likely due to the build-up of *E. coli* sources on the ground level and attachment to sediment or substrate which are then washed into the stormwater after the first flush of rain. Consequently, storm event samples were almost always over the recreational standard of 126 CFU, regardless of the location or type of basin. In general, *E. coli* was higher in detention basins compared to retention ponds after storm events and during dry times, this is likely due to a dilution effect provided by the retention ponds. Water quality analysis comparing detention basins and retention ponds showed more differences amongst events than sites but did not bring to light other major concerns.

Molecular source tracking of *E. coli* showed genetic markers for humans and dogs were always present in stormwater runoff, whereas bird and sewage genetic markers were found in all samples including stormwater runoff, standing water and groundwater. This indicates that there is a potential danger to humans who may interact with the water for recreation, such as swimming, kayaking, or fishing. Luckily, when samples were tested for the *E. coli* O157:H7 pathogen, it was never found. Due to the fact that the O157:H7 strain lives in the intestines of infected humans and cattle, and there are no livestock farms near these stormwater catchments, it is not surprising that O157:H7 was not found.

In the future, it will be important to assess all sites planned for recreational use to understand the potential impacts of *E. coli* and water quality on humans. A sampling of one or two sites, if stormwater is sampled at all, and assuming results encompass all basins in a region

is a cheap and standard practice. However, this study exemplifies the need for the sampling of all sites, if they are to have human interaction with the water, as significant differences exist across basins and events. Additionally, as urban areas expand and residents and city leaders look to stormwater retention ponds and detention basins for recreation, it is important to understand the risks that may be involved. Authors recommend in areas where this is being considered or currently happening, that monitoring is done during both storm events and dry times regularly to make sure water is safe. Additionally, more research should be done to assess how the design of stormwater detention basins and retention ponds impacts *E. coli* and how this changes spatially across the urban landscape.

Results from this project make an important contribution to understanding water quality and *E. coli* in stormwater in urban areas. As human populations and urban areas around the world grow, the importance of utilizing green and water spaces within our cities grows. However, it is important for city planners, water managers, researchers, and scientists to understand the dangers that may exist and to make informed decisions when improving urban green infrastructure.

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APPENDIX A. DATES SAMPLING TOOK PLACE IN 2018 AND 2019

Table A1. 2018 Sampling Season

Events	Post	Dry
June 5 th	June 12 th and 13 th	May 29 th and 30 th
June 29 th	July 10 th and 11 th	
August 26 th	September 4 th and 5 th	

Table A2. 2019 Sampling Season

Events	Post	Dry
July 9 th	July 16 th	May 21 st
July 17 th	July 23 rd and 24 th	May 26 th and 27 th
August 26 th	September 16 th and 17 th	

APPENDIX B. WATER QUALITY PARAMETERS STUDIED

Table B1. Bold items are the water quality parameters we analyzed.

Field Measurements	General Chemistry	Detection Limits	Nutrients	Detection Limits	Biological	Detection Limits
pH	Alkalinity	3.30 mg/L	Ammonia	0.030 mg/L	<i>E. coli</i>	10 #/100 mL
Temperature	Anion Sum	NL ²	Nitrate-nitrite	0.030 mg/L		
Dissolved Oxygen	Bicarbonate	1 mg/L	Total Kjeldahl Nitrogen	0.061 mg/L		
Specific Conductance	Calcium	2.00 mg/L	Total Nitrogen	0.015 mg/L		
	Carbonate	1 mg/L	Total Phosphorus	0.004 mg/L		
	Cation Sum	NL ²	Sodium Adsorption Ratio	NL ²		
	Chloride	0.300 mg/L				
	Fluoride	4.00 mg/L				
	Hardness	NL ²				
	Hydroxide	1 mg/L				
	Iron	0.050 mg/L				
	Magnesium	1.00 mg/L				
	Manganese	0.010 mg/L				
	Potassium	1.00 mg/L				
	Silica	2.00 mg/L				
	Sodium	3.00 mg/L				
	Sulfate	0.300 mg/L				
	Total Dissolved Solids	NL ²				
	Total Suspended Solids	5 mg/L				

APPENDIX C. SOURCE MOLECULAR FINDINGS FROM 2018 AND 2019

2018 Source Molecular Results

Table C1. Bird Fecal Quantification ID

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-9A04075	18-R1829 F5	Bird Fecal ID	DNQ	Detected
SM-9A04077	18-R1901 G8	Bird Fecal ID	DNQ	Detected

Table C2. Dog Fecal Quantification ID

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-9A04073	18-R1829 F5	Dog Bacteroidetes ID: EPA 1	7.57E+03	Detected
SM-9A04074	18-R1901 G8	Dog Bacteroidetes ID: EPA 1	ND	Not Detected

Table C3. E. coli O157:H7 ID

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-9A04079	18-R1829 F5	E.coli O157:H7 ID Target 1	ND	Not Detected
SM-9A04080	18-R1901 G8	E.coli O157:H7 ID Target 1	ND	Not Detected
SM-9A04079	18-R1829 F5	E.coli O157:H7 ID Target 2	ND	Not Detected
SM-9A04080	18-R1901 G8	E.coli O157:H7 ID Target 2	ND	Not Detected

Table C4. Human Fecal Quantification ID

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-9A04081	18-R1829 F5	Human Bacteroidetes ID: Dorei	DNQ	Detected
SM-9A04082	18-R1901 G8	Human Bacteroidetes ID: Dorei	ND	Not Detected

2019 Source Molecular Results

Table C5. Bird Fecal Quantification ID Test Results Report

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM20B13015	FP Storm 7/9/19	Bird_GFD	DNQ	Filter
SM20B13016	FP Storm 7/17/19	Bird_GFD	5.75E+03	Filter
SM20B13017	FP Storm 7/23/19	Bird_GFD	4.67E+04	Filter
SM20B13019	FP Storm 8/27/19	Bird_GFD	4.34E+04	Filter
SM20B13020	FP West 9/17/19	Bird_GFD	5.05E+04	Filter
SM20B13021	FP S-Piez 0m 9/17/19	Bird_GFD	3.02E+03	Filter

Table C6. Dog Fecal Quantification ID Test Results Report

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM20B13007	FP Storm 7/9/19	Dog_BacCan-UCD	1.84E+04	Filter
SM20B13008	FP Storm 7/17/19	Dog_BacCan-UCD	4.09E+03	Filter
SM20B13009	FP Storm 7/23/19	Dog_BacCan-UCD	DNQ	Filter
SM20B13012	FP Storm 8/27/19	Dog_BacCan-UCD	4.52E+03	Filter
SM20B13013	FP West 9/17/19	Dog_BacCan-UCD	ND	Filter
SM20B13014	FP S-Piez 0m 9/17/19	Dog_BacCan-UCD	ND	Filter

Table C7. E. coli O157:H7 ID Test Results Report

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM20B13028	FP Storm 7/9/19	E.coli O157:H7 (D/ND)	ND	Filter
SM20B13029	FP Storm 7/17/19	E.coli O157:H7 (D/ND)	ND	Filter
SM20B13030	FP Storm 7/23/19	E.coli O157:H7 (D/ND)	ND	Filter
SM20B13031	FP Storm 8/27/19	E.coli O157:H7 (D/ND)	ND	Filter
SM20B13032	FP West 9/17/19	E.coli O157:H7 (D/ND)	ND	Filter
SM20B13033	FP S-Piez 0m 9/17/19	E.coli O157:H7 (D/ND)	ND	Filter

Table C8. Goose Fecal Quantification ID Test Results Report

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM20B13022	FP Storm 7/9/19	Goose_CGOF1	ND	Filter
SM20B13023	FP Storm 7/17/19	Goose_CGOF1	ND	Filter
SM20B13024	FP Storm 7/23/19	Goose_CGOF1	ND	Filter
SM20B13025	FP Storm 8/27/19	Goose_CGOF1	ND	Filter
SM20B13026	FP West 9/17/19	Goose_CGOF1	ND	Filter
SM20B13027	FP S-Piez 0m 9/17/19	Goose_CGOF1	ND	Filter

Table C9. Gull Fecal Quantification ID Test Results Report

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM20B14008	FP Storm 7/9/19	Gull_Gull-4	ND	Filter
SM20B14009	FP Storm 7/17/19	Gull_Gull-4	ND	Filter
SM20B14010	FP Storm 7/23/19	Gull_Gull-4	ND	Filter
SM20B14011	FP Storm 8/27/19	Gull_Gull-4	4.19E+03	Filter
SM20B14012	FP West 9/17/19	Gull_Gull-4	ND	Filter
SM20B14013	FP S-Piez 0m 9/17/19	Gull_Gull-4	ND	Filter

Table C10. Human Fecal Quantification ID Test Results Report

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM20B13001	FP Storm 7/9/19	Human_HF183	1.78E+04	Filter
SM20B13002	FP Storm 7/17/19	Human_HF183	DNQ	Filter
SM20B13003	FP Storm 7/23/19	Human_HF183	ND	Filter
SM20B13004	FP Storm 8/27/19	Human_HF183	DNQ	Filter
SM20B13005	FP West 9/17/19	Human_HF183	ND	Filter
SM20B13006	FP S-Piez 0m 9/17/19	Human_HF183	ND	Filter

Table C11. Sewage/Pipe Quantification ID Test Results Report

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	Sample Type
SM20B19009	FP Storm 7/9/19	Sewage Marker BacV4V5-1	2.17E+04	Filter
SM20B19010	FP Storm 7/17/19	Sewage Marker BacV4V5-1	2.58E+04	Filter
SM20B19011	FP Storm 7/23/19	Sewage Marker BacV4V5-1	2.47E+06	Filter
SM20B19012	FP Storm 8/27/19	Sewage Marker BacV4V5-1	6.93E+04	Filter
SM20B19013	FP West 9/17/19	Sewage Marker BacV4V5-1	1.66E+05	Filter
SM20B19015	FP S-Piez 0m 9/17/19	Sewage Marker BacV4V5-1	4.12E+04	Filter
SM20B13034	FP Storm 7/9/19	Sewage Marker BacV6- 21	2.67E+03	Filter
SM20B13035	FP Storm 7/17/19	Sewage Marker BacV6- 21	4.89E+03	Filter
SM20B13036	FP Storm 7/23/19	Sewage Marker BacV6- 21	7.57E+05	Filter
SM20B13037	FP Storm 8/27/19	Sewage Marker BacV6- 21	7.59E+03	Filter
SM20B13038	FP West 9/17/19	Sewage Marker BacV6- 21	3.79E+04	Filter
SM20B13039	FP S-Piez 0m 9/17/19	Sewage Marker BacV6- 21	8.39E+03	Filter