Duluth Streams Bacterial Source Identification Study Final Report

City of Duluth, Public Works and Utilities

Duluth Streams Bacterial Source Identification Study
Project No. 118320

8/19/2020

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Final Report

prepared for

City of Duluth, Public Works
Duluth Streams Bacterial Source Identification Study
Duluth, MN

Project No. 118320

8/19/2020

prepared by

Burns & McDonnell Engineering Company, Inc.
San Diego, CA

TABLE OF CONTENTS

Page No.

LIST OF TABLES

Page No.

LIST OF FIGURES

Page No.

LIST OF ABBREVIATIONS

Abbreviation	Term/Phrase/Name
°C	degrees Celsius
BMP	best management practice
City	City of Duluth
cfs	cubic feet per second
CFU	colony forming unit
cm	centimeter
COC	chain of custody
Ct	cycle threshold
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
EPA	U.S. Environmental Protection Agency
g	gram
gpm	gallons per minute
m	meter
MCA	microbial community analysis
mL	milliliter
mm	millimeter
MPN	most probable number
MS4	municipal separate storm sewer system
NPDES	National Pollutant Discharge Elimination System
Pace Laboratory	Pace Analytical Services, Inc.

Abbreviation	Term/Phrase/Name
PBS	phosphate buffer solution
PCR	polymerase chain reaction
SE	standard error
SWMP	Stormwater Management Plan
TMDL	total maximum daily load
TSS	total suspended solids
µL	microliter
µm	micrometer
Weston Laboratory	Weston Solutions
UMN	University of Minnesota, Saint Paul, Minnesota
UMD	University of Minnesota, Duluth, Minnesota

1.0 INTRODUCTION

The Duluth Urban Area Streams Total Maximum Daily Load (TMDL) (MPCA, 2018) addresses stream impairments in the Duluth Urban Area in northeastern Minnesota including a portion of the St. Louis River major watershed (Hydrologic Unit Code [HUC] 04010201) and a portion of the Lake Superior South Watershed (HUC 04010102). The TMDL includes all of the developed areas in the Duluth area and surrounding communities. There are eleven streams assessed in the Duluth Urban Area Streams TMDL, including Keene Creek and Tischer Creek (Figure 1-1). Water quality monitoring data indicate that water quality standards for recreational uses are not being attained in Keene Creek and Tischer Creek, based on exceedances of numeric criteria for *E. coli*, which is a common fecal indictor bacteria.

The applicable water quality standards for *E. coli* are described in amendments to Minnesota's Rule 7050 and are summarized in Table 1-1. There are two standards established by the rule for *E. coli*: the single sample water quality standard of 1,260 most probable number (MPN)/100 milliliters (mL) and the geometric mean water quality standard of 126 MPN/100 mL.

Table 1-1: Applicable Water Quality Standards for *E. coli* in Keene Creek and Tischer Creek

Parameter	Units	Water Quality Standard[(a)]	
E. coli[b]	#/100 mL	Single Sample	1,260 in < 10% of samples[c]
		Geometric Mean	< 126[d]

Source: Amendments to Minnesota Rule 7050
(a) The standard applies only between April 1 and October 31.
(b) *E. coli* standards apply only between April 1 and October 31
(c) Standard shall not be exceeded by more than 10% of the samples taken within any calendar month
(d) Geometric mean based on minimum of five samples taken within any calendar month

The City of Duluth has a National Pollutant Discharge Elimination System (NPDES) permit for the municipal separate storm sewer system (MS4) within its jurisdictional boundaries (MS400086) and is responsible for identifying the sources of *E. coli* in the watersheds and meeting the regulatory goals of the TMDL. In an effort to address the impairment and better understand the sources of *E. coli* causing exceedances, the City and its partner, the Minnesota Pollution Control Agency (MPCA) (through a Clean Water Fund grant), has initiated this Duluth Streams Bacterial Source Identification Study for Keene Creek and Tischer Creek (Study). The Study is focused on identifying the sources of *E. coli* within those portions of the Keene Creek and Tischer Creek watersheds within the jurisdictional boundary of the City. The study areas within each of the two watersheds are identified in Figure 1-2 and Figure 1-3 for Keene and Tischer Creek, respectively.

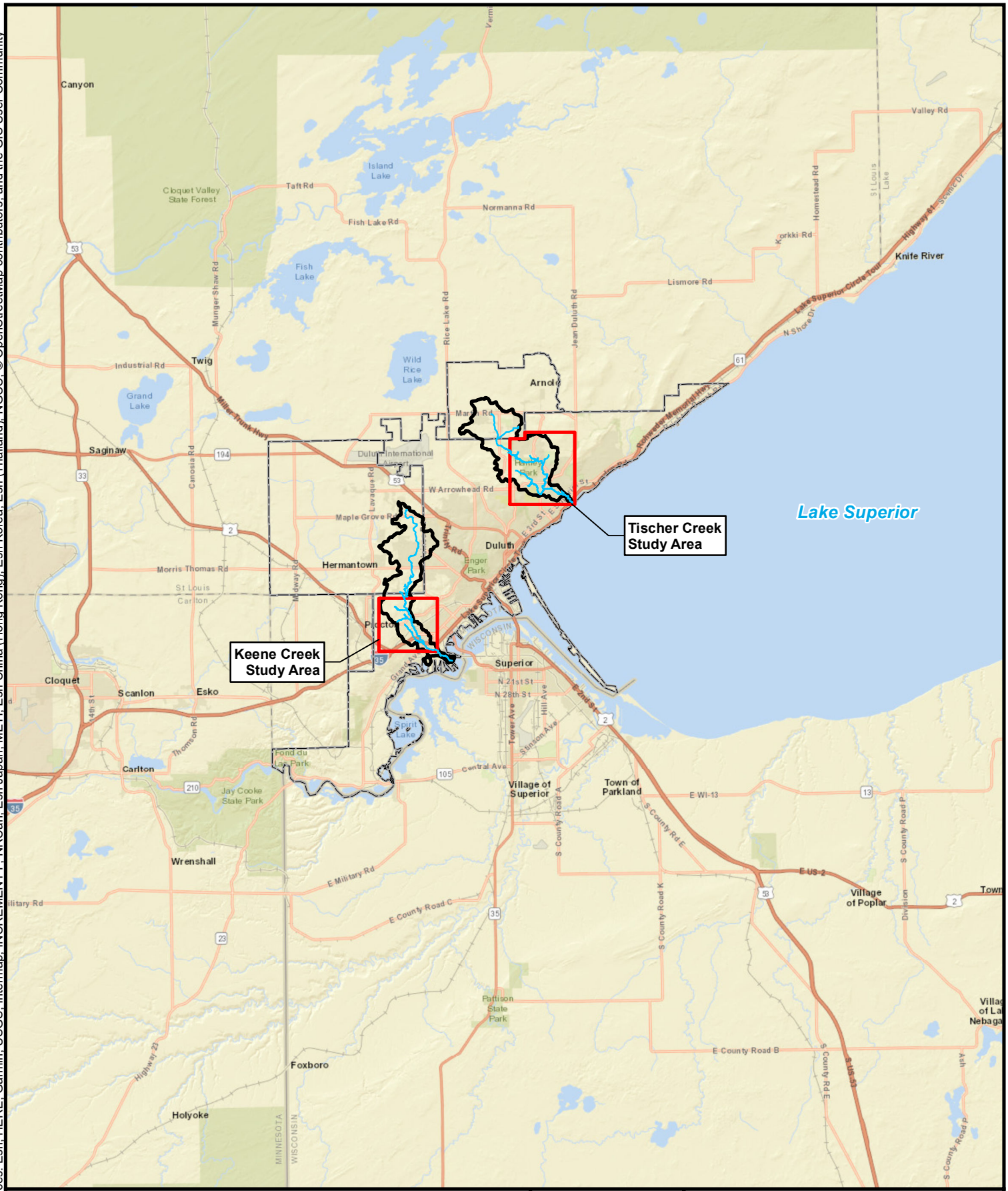

Figure 1-1: Locations of the Keene Creek and Tischer Creek Watersheds and Study Areas

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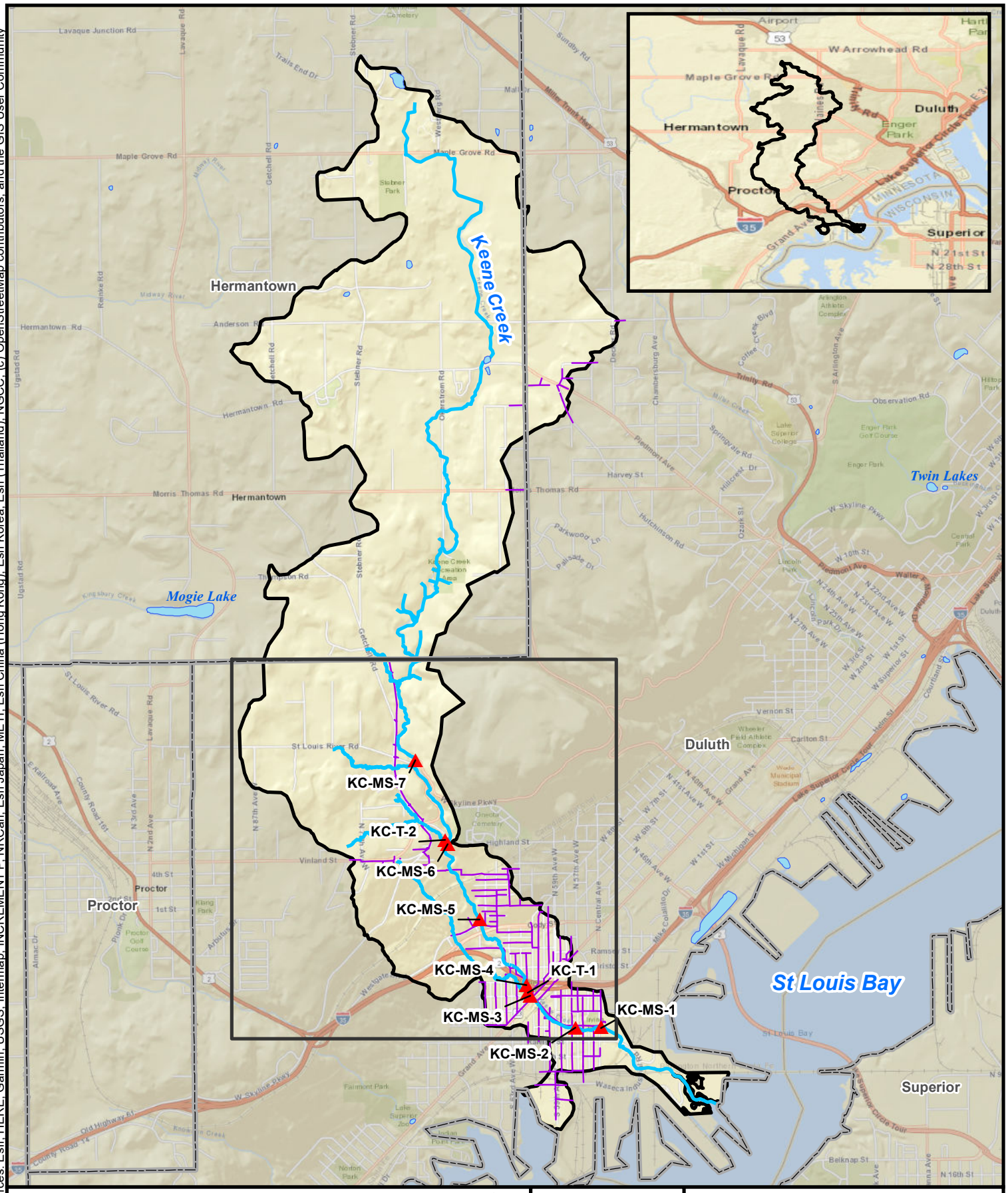

Figure 1-2:
Keene Creek Watershed
and Study Area

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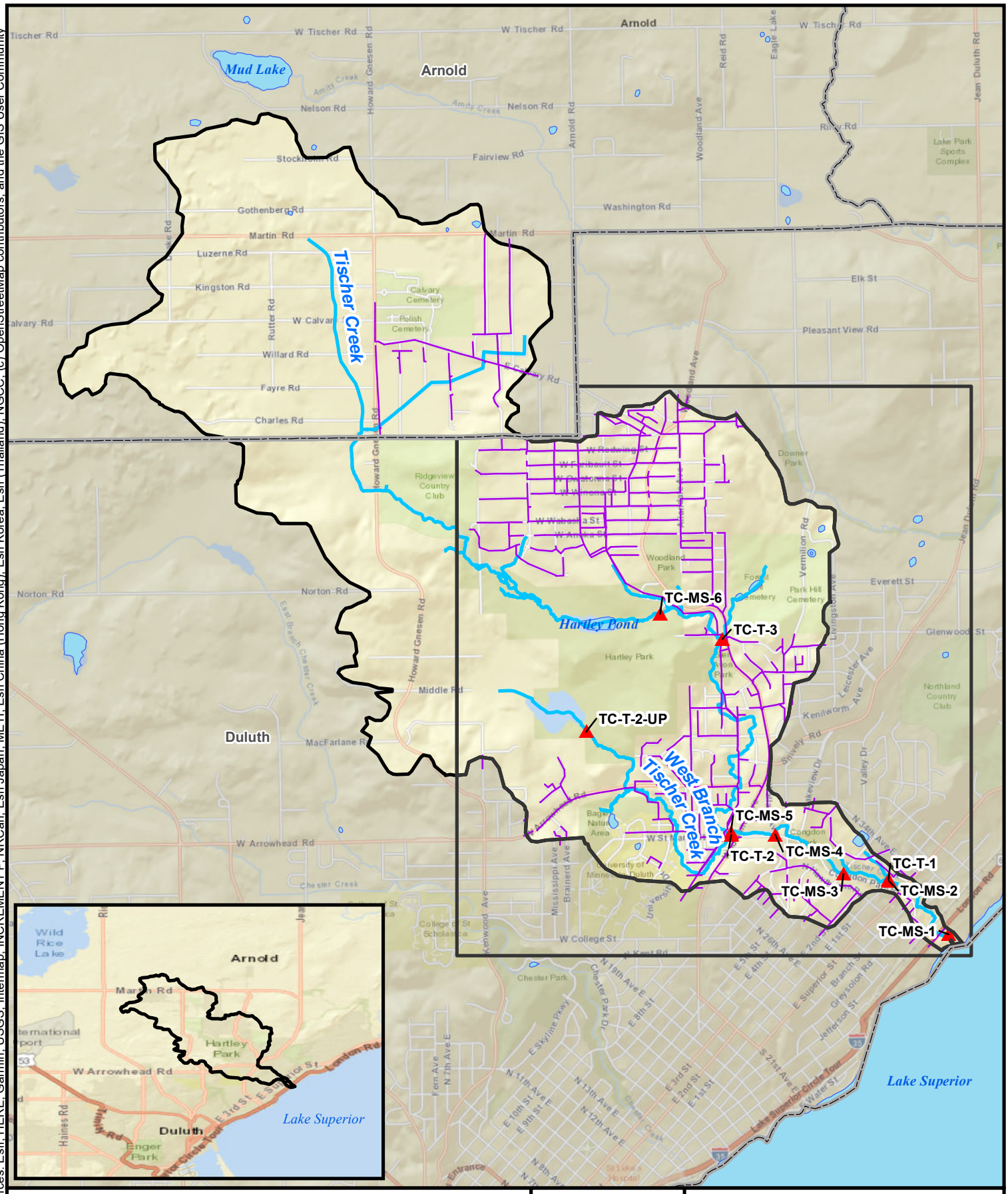

Figure 1-3:
Tischer Creek Watershed
and Study Area

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1.1 Study Objectives

The overall objective of the Study is to provide the City with information on the sources of *E. coli* bacteria that may be causing exceedances of state water quality standards in Keene Creek and Tischer Creek receiving waters and to use the information gathered from the Study to provide recommendations on best management practices (BMPs) that can be used to achieve the TMDL reduction targets. All monitoring, sample collection, and assessments for the Study were conducted during periods of dry weather only, at least 48 hours after a storm event, from August through October 2019.

1.1.1 Dry Weather Study Questions

Based on a review of existing data, the study design for this dry weather assessment was developed to answer the following study questions:

1. What are the potential sources of *E. coli* in Keene Creek and Tischer Creek (e.g., local wildlife, domestic animals, leaking sewer or septic lines, other human sources, natural, etc.)?
2. How does bacteria survival, propagation, or re-growth contribute to *E. coli* levels in the storm drain system (e.g., leaf litter and grass clippings along curb lines or ditches) and discharge to surface waters of the creek?
3. Does the *E. coli* in the Study Areas originate from human sources?
4. How can the City adapt current management practices to reduce levels of *E. coli?*

1.2 Description of Study Areas

E. coli concentrations in creeks are often heavily influenced by land use practices. Land use in the Keene Watershed (4,029 acres) and Tischer Watershed (4,767 acres) (depicted in Figure 1-4 and Figure 1-5, respectively) consists primarily of forest and other natural land covers (71 and 63 percent, respectively) with smaller urbanized portions of the watersheds consisting of developed/disturbed land cover (29 and 37 percent, respectively) (MPCA, 2018). Areas of land use transition in a watershed (e.g., rural to urban, pervious to impervious) are often key drivers for establishing monitoring locations for microbial source tracking studies.

In Tischer Creek, the reach impaired by *E. coli* is relatively short (approximately Woodland Avenue to Lake Superior) and consists primarily of an urbanized land use. In contrast, Keene Creek is impaired from the headwaters to the St. Louis River; however, only a small portion of the creek (primarily downstream of the railroad crossing just upstream of Cody Street) is urbanized. These differences in land use characteristics and degree of impairment were important factors in the design of the Study to understand the sources of *E. coli* in the Keene Creek and Tischer Creek watersheds.

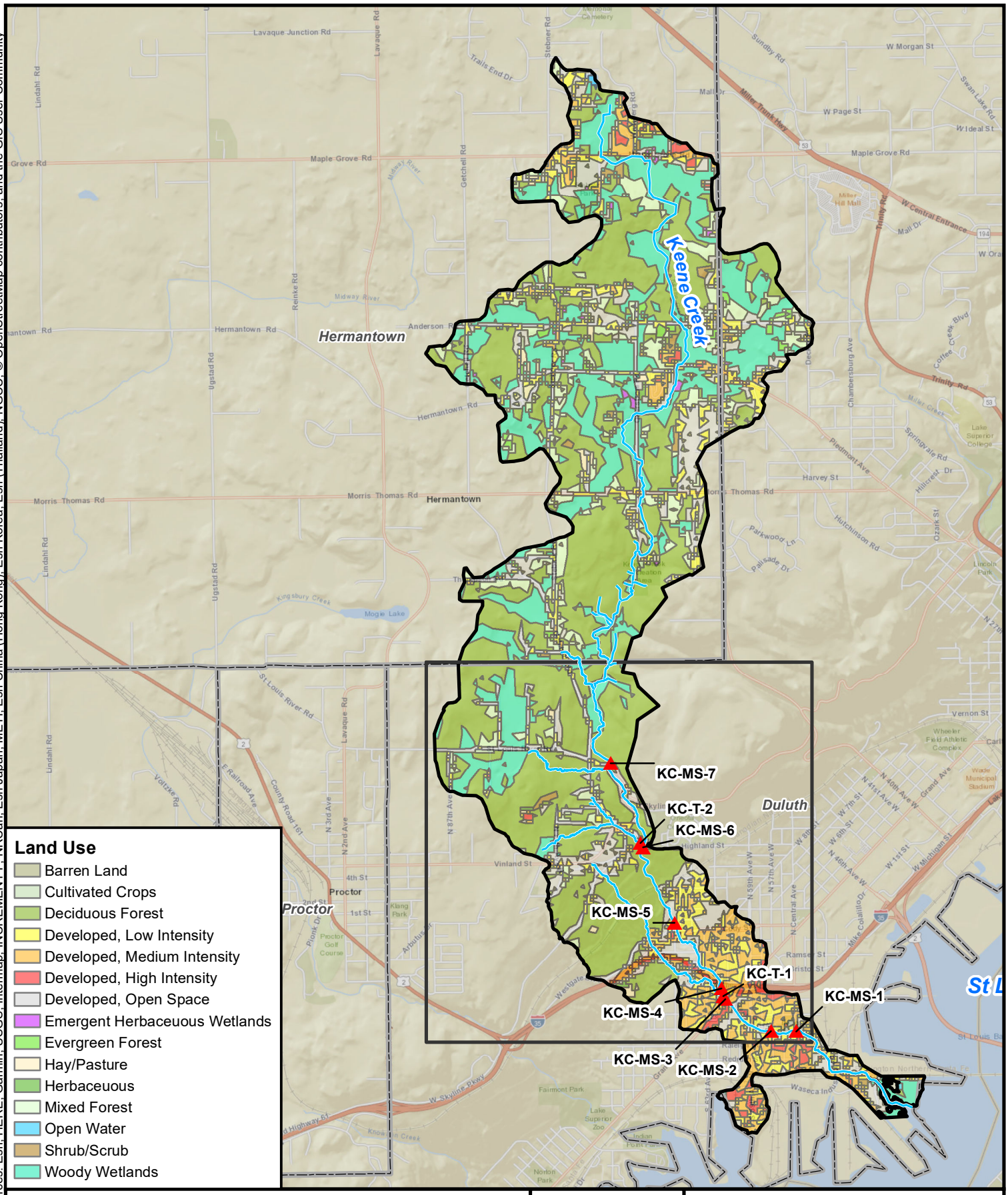

Figure 1-4:
Land Use in the Keene Creek Watershed and Study Area

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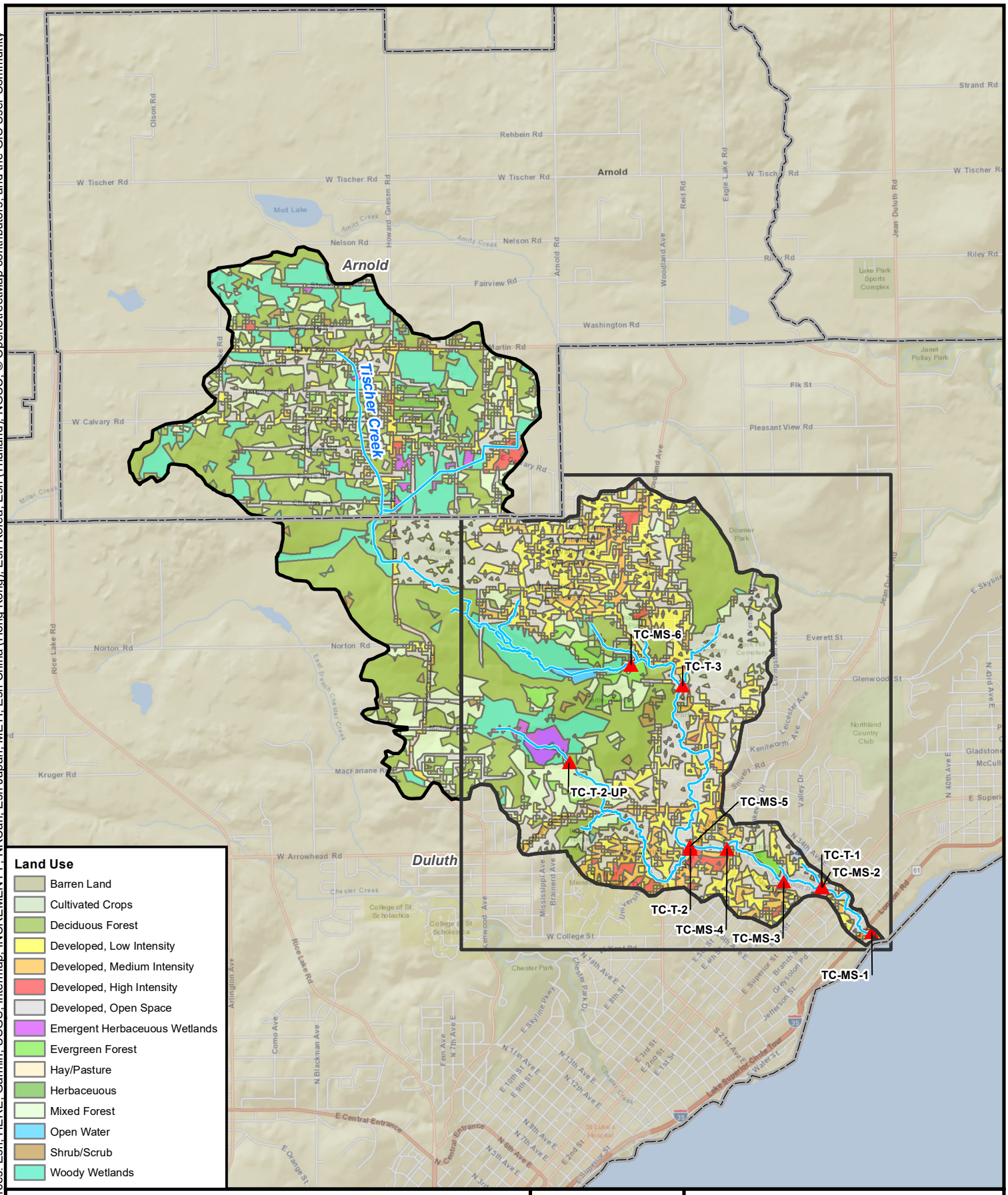

Figure 1-5:
Land Use in the Tischer Creek Watershed and Study Area

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1.3 Study Design

The design used to conduct the Study was based on similar studies conducted in other regions of the country for identifying sources of indicator bacteria (e.g., *E. coli*) in urban watersheds. The design uses three approaches that have been shown to be effective in identifying sources of bacteria in urban watersheds throughout the country (Griffith et al., 2013). The study design is (1) phased, (2) tiered, and (3) adaptive. Each of these design approaches is described briefly below.

1.3.1 Phased Approach

In order to identify the sources of bacteria in the two watersheds, the study was phased to focus first on dry weather conditions (at least 48 hours following precipitation). Identifying and remediating sources of bacteria is much simpler under dry weather conditions than wet weather conditions, particularly when the Study Area has not been thoroughly characterized or monitored (Urban Water Resources Research Institute, 2014). Thus, using a phased approach, this Study focused initially on dry weather conditions only.

The information gained from the dry weather phase, may be used to inform the study design and study questions for a potential future wet weather phase, providing a focused assessment of suspected sources and a more efficient use of limited resources. Moreover, separating the study into dry and wet weather phases provides a more meaningful approach to identifying pollutant-reduction BMPs because effective solutions during dry weather are often very different than wet weather BMPs. In addition, dry weather BMPs can be compromised during wet weather when the receiving waters can be overwhelmed with numerous sources.

1.3.2 Tiered Approach

The tiered approach uses a stepwise procedure of assessing the Study Area and identifying sources of bacteria in a prioritized, progressive process. For both Keene Creek and Tischer Creek, a series of sequential steps were implemented to focus the assessment on high priority sources of bacteria first, followed by additional steps as the study progressed. This tiered approach has been developed from similar monitoring programs (Griffith et al., 2013) with elements specific to the Keene and Tischer Creek watersheds.

The following tiered steps were implemented in the Study:

1. Characterize the watershed by obtaining infrastructure maps, examining historical monitoring data for spatial and temporal trends, and conducting visual inspections during a site

reconnaissance to develop a list of potential fecal contamination sources and transport mechanisms.

2. Based on the watershed characterization, develop a list of study questions to be addressed by the assessment that are specific to the conditions within that drainage.
3. Conduct initial monitoring to produce a more detailed picture of spatial and temporal patterns in the drainage.
4. Test ambient waters for human source specific genetic markers (even if traditional tools have not identified a leaking sanitary system). Place high priority on either detecting or confirming a human fecal source, as this source may pose the greatest relative health risk.
5. Where there is indication of leakage from a sanitary system, investigate it using traditional tools such as closed-circuit television inspections or dye testing.
6. Where human sources have been accounted for and the relative human loadings are better understood, and/or a likely animal fecal pollution source (e.g., runoff from a dog park) has been identified, test ambient waters using non-human (animal) source-specific genetic markers.
7. Where source-specific genetic markers have yet to be developed for the suspected source(s), test ambient waters and potential sources using microbial community analysis (MCA) methods.

The basic steps listed above were used in the dry weather assessment for this Study and were modified to meet the specific characteristics of the two Study Areas.

1.3.3 Adaptive Approach

Bacterial source identification studies can be difficult to conduct due to the ubiquitous nature of bacteria in the environment, the multiple sources within a given watershed, and the potential for regrowth of bacteria outside the host animal. For these reasons, source identification studies often do not lend themselves to prescriptive monitoring plans where the details of each monitoring element are determined prior to the initiation of the study. Instead, the most effective source identification studies often rely on a basic monitoring framework with elements developed from the tiered approach discussed above. The details of each monitoring element are adaptive, whereby the results of the first element are used to focus the design for subsequent elements in the study. The adaptive approach allows the design of each element of the study to build upon the results of the previous element, resulting in an increasingly focused approach to identifying the sources of bacteria in a defined study area. The end result is a comprehensive and efficient assessment of potential bacterial sources in the drainage, leading to multiple lines of evidence for identifying those sources that have the greatest impact on water quality. These results also allow for focused recommendations on the most effective and efficient BMPs to remediate the bacterial source.

In this Study, primary study elements were developed specifically for the two Study Areas and monitoring protocols were established to answer the drainage-specific study questions for dry weather conditions. When the results from the primary study elements were analyzed, special studies were designed and implemented to further address unanswered components of the study questions. This adaptive approach maximizes the efficiency of limited resources to conduct the Study and produces a focused assessment of the sources of *E. coli* in both Keene and Tischer creeks during dry weather conditions.

1.4 Report Organization

This Study used a weight of evidence approach to identify the sources of *E. coli* bacteria in the Keene Creek and Tischer Creek receiving waters. Because two watersheds were assessed in the Study, portions of some chapters were combined for both watersheds and some were separated to allow for a focused discussion of each watershed. The report contains separate sections for each watershed within the Materials and Methods Chapter (Chapter 2.0), but combined sections for field methods and laboratory methods. This chapter discusses the means to achieve the Study objectives. The Results Chapter (Chapter 3.0), which summarizes the Study's findings, has separate sections for each watershed. As does the Conclusions Chapter (Chapter 5.0), which identifies the salient points of the Study. In the Discussion and Recommendations chapters (Chapter 4.0 and Chapter 6.0, respectively), the results from the two watersheds have been integrated to facilitate ease of discussion on how the results of the Study enhance our understanding of the sources of *E. coli* in the watersheds and how potential BMPs might be implemented to reduce *E. coli* levels in the creeks and meet the goals of the TMDL.

1.4.1 Dry Weather Study Elements

The dry weather study design was organized to focus on several primary Study elements first, followed by special studies based on the initial results. The primary dry weather Study elements were the same for both watersheds and included the following:

- Baseline Monitoring
- Sanitary Survey Investigation
- Special Study – Water and Sediment Characterization

2.0 MATERIALS AND METHODS

2.1 Baseline Monitoring

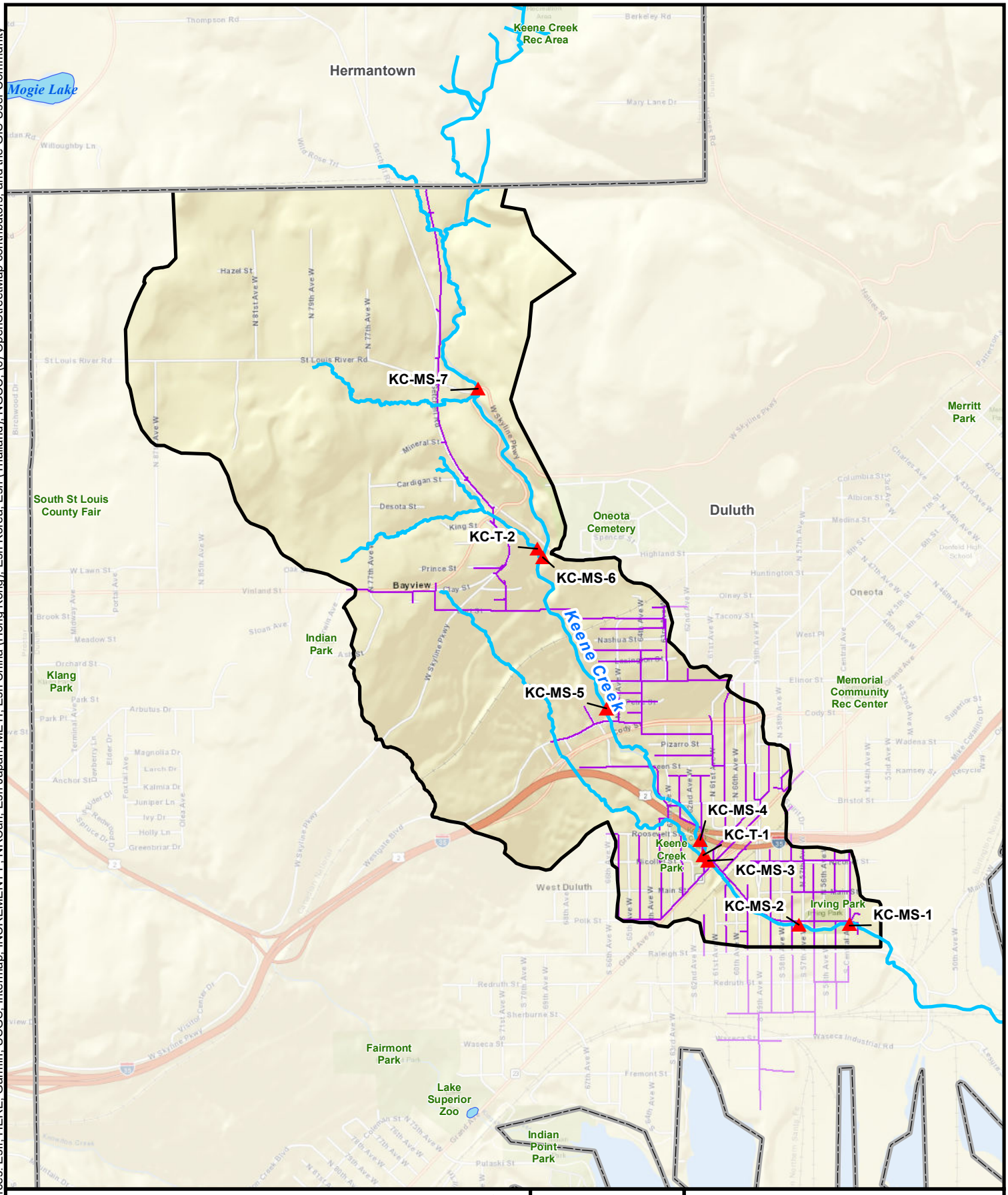
The site locations and procedures for the Baseline Monitoring are presented below for Keene Creek and Tischer Creek.

2.1.1 Keene Creek Monitoring Sites

The Study Area within the Keene Creek Watershed lies within the municipal boundary of the City. The baseline monitoring sites within the Keene Creek Study Area consist of seven mainstem sites (designated as MS-#) and two tributary sites (designated as T-#). The locations are summarized in Table 2-1 and presented graphically on Figure 2-1. The locations were selected to provide spatial coverage along the mainstem of Keene Creek and to account for bacteria sources contributed to the mainstem from the main tributaries within the Study Area.

Table 2-1: Descriptions of Keene Creek Baseline Monitoring Sites in the Study Area

Site Name	Latitude	Longitude	Elevation (feet)	Description
MS-1	46.732431	-92.166296	602	On mainstem, just downstream of South Cedar Avenue
MS-2	46.732391	-92. 169355	605	On mainstem, just upstream of South 57th Avenue West
MS-3	46.735199	-92. 175073	618	On mainstem, upstream of Grand Avenue and just downstream of Keene Creek Dog Park
MS-4	46.736099	-92. 175525	651	On mainstem, in Keene Creek Park across from picnic tables
MS-5	46.741783	-92. 181303	729	On mainstem, upstream of Westgate Boulevard at large boulders on left bank
MS-6	46.748289	-92. 185141	954	On mainstem, approximately 300 feet downstream of Highway 89 Bridge, upstream of confluence with Site T-2
MS-7	46.755519	-92. 189055	1,139	On mainstem, just downstream of West Skyline Parkway off Saint Louis River Road
T-1	46.735442	-92. 175353	623	Tributary to mainstem from right bank at Keene Creek Dog Park, just upstream of confluence with mainstem (borders the northwest border of Keene Creek Dog Park)
T-2	46.748653	-92. 185468	969	Tributary to mainstem from right bank, approximately 150 feet upstream of confluence with mainstem at the walking trail bridge



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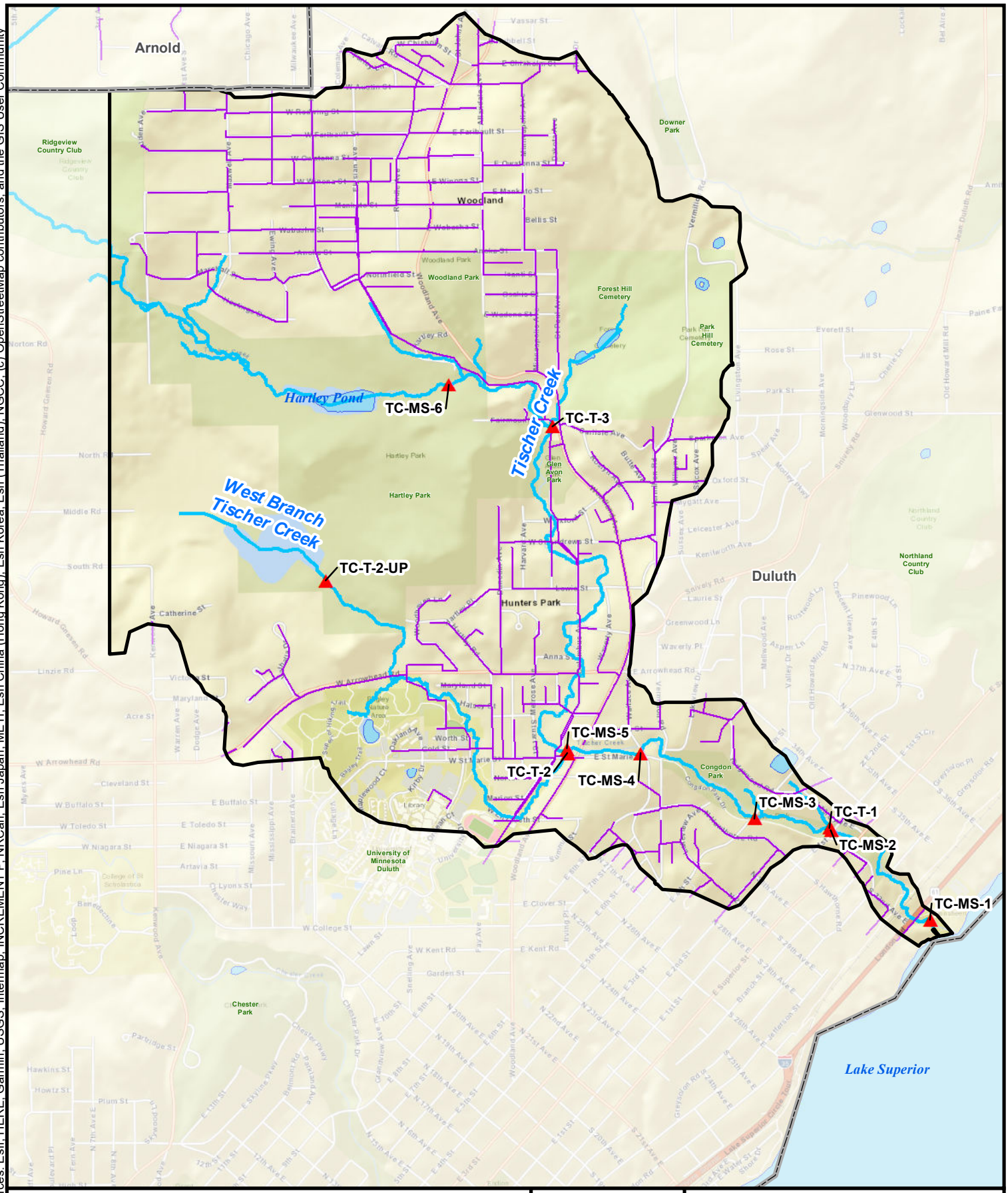
2.1.2 Tischer Creek Monitoring Sites

The Study Area within the Tischer Creek Watershed also lies within the municipal boundary of the City. The baseline monitoring sites within the Tischer Creek Study Area consist of six mainstem sites (designated as MS-#) and two tributary sites (designated as T-#). The locations are summarized in Table 2-2 and presented graphically on Figure 2-2. As with Keene Creek, the Tischer Creek monitoring locations were selected to provide spatial coverage along the mainstem of Tischer Creek and to account for bacteria sources contributed to the mainstem from the main tributaries within the Study Area.

Table 2-2: Descriptions of Tischer Creek Baseline Monitoring Sites within the Study Area

Site Name	Latitude	Longitude	Elevation (feet)	Description
MS-1	46.814288	-92.052083	596	On mainstem, 200 feet downstream of London Road, 100 feet upstream of walking trail bridge
MS-2	46.818833	-92.058182	709	On mainstem, 400 feet upstream of East Superior Street, just upstream of T-1 waterfall
MS-3	46.819393	-92.063008	840	On mainstem, just downstream of East 4th Street
MS-4	46.822268	-92.070059	1,038	On mainstem, just upstream of Wallace Avenue culvert, adjacent to East Saint Marie Street
MS-5	46.822512	-92.074481	1,050	On mainstem, 125 feet upstream of Woodland Avenue, adjacent to East Saint Marie Street, upstream of confluence with T-2
MS-6	46.838154	-92.081735	1,179	On mainstem in Hartley Nature Center, just downstream of walking bridge of mainstem leading from parking lot
T-1	46.818956	-92.058417	710	Tributary to mainstem, at waterfall just downstream of MS-2
T-2	46.822332	-92.074632	1,051	Tributary to mainstem at MS-5 (also known as the West Branch of Tischer Creek), just downstream of West Saint Marie Street, behind Domino's Pizza on Woodland Avenue, upstream of confluence with mainstem
T-3	46.836199	-92.075367	1,125	Tributary to mainstem, just downstream of Fairmont Street, adjacent to Woodland Avenue
T-2-Up	46.829028	-92.088653	1,169	Tributary T-2 to mainstem (West Branch of Tischer Creek) in Hartley Park below beaver dam, approximately 250 feet upstream of wooden walking bridge that crosses creek



Monitoring Locations
Storm Drain
Tischer Creek
Tischer Creek Study Area
City Boundary

NORTH

0 1,000 2,000
Scale in Feet

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Figure 2-2:
Tischer Creek Study Area with Baseline Monitoring Sites

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The sites in both watersheds were monitored during dry weather (at least 48 hours after a rain event of 0.1 inch or greater) from August through October 2019. The public data provided by the National Weather Service (2019) was used to determine dates of sampling events, based on precipitation forecasts.

2.1.3 Field Methods

Water samples were collected at the locations identified above for analysis by both culture and molecular techniques. The methods used to collect water samples differ by technique and are discussed below.

2.1.3.1 Sample Collection for Analysis of Bacteria by Culture Techniques

Water samples from the mainstem and tributary baseline monitoring sites (Figure 2-1 and Figure 2-2) were collected by field technicians wearing sterile latex gloves and hip waders. Samples were collected from the thalweg of the stream in sterile, EPA-approved 100-mL plastic bottles containing sodium thiosulfate (to counteract any chlorine that might be present in the water). Sample containers were kept in clear re-sealable food-grade plastic bags until use. Just prior to sampling, the bag and sample container were opened. Both container and lid were held facedown to prevent airborne contamination. Facing upstream, the field technician submerged the bottle approximately 6 inches below the surface of the water. The bottle was then filled and capped. No sediment or debris from the streambed was allowed to enter the sample bottle. All observations during site visits were recorded on field observation forms.

Each bottle was labeled in the field with the project title, appropriate site identification number, date, time, and initials of collector using black, waterproof ink. The sample container was then sealed in the re-sealable plastic bag. The samples were stored on ice in the dark in a closed cooler from the time of sample collection until delivery to the analytical laboratory. All samples were delivered to Pace Analytical Services, Inc. in Duluth (Pace Laboratory) within the required 6-hour holding time. The samples were transferred to the laboratory using standard chain of custody (COC) procedures discussed in Section 2.4. The cooler and sampling equipment were cleaned with biodegradable soap prior to use.

2.1.3.2 Sample Collection for Analysis of Bacteria by Molecular Techniques

Water samples for molecular analyses were collected from the same baseline monitoring sites discussed above, using 250-milliliter (mL) sterile (irradiated), nuclease-free, plastic bottles. Extreme care was taken to avoid sample contamination. Samples were collected exclusively by technicians specifically trained in the “clean hands” aseptic technique.

In the laboratory, each bottle was sealed inside two sterile, plastic bags and placed in a sterilized cooler that had been dedicated for molecular samples only. In the field, field technicians wearing sterile, latex gloves removed the bottle from the plastic bags and labeled it with a unique sample name, location, date,

time, and name of collector using black, waterproof ink. Gloves and outside plastic surfaces were sprayed with DNA *AWAY*™, a deoxyribonucleic acid (DNA) destabilizing reagent, and wiped dry prior to opening sample bottles to remove any potential contamination from human contact. The bags were placed back in the cooler and the capped bottle was carried to the monitoring site. While the sample bottle was open, the cap was held facedown to prevent aerial contamination. After sampling, excessive water was removed from the outside of the sample container, and using clean gloves, the outside of the sample bottle was sprayed with DNA *AWAY*™ and wiped dry prior to placing it in the inner re-sealable plastic bag. The sample bottle sealed in re-sealable plastic bags were placed in a clean, dedicated cooler with-ice and transported to the Pace Laboratory within 2 - 3 hours of collection. Samples for MCA analysis were delivered the University of Minnesota, Saint Paul (UMN) within 48 hours of collection.

To verify proper sampling technique, field blanks were collected during each sampling event (a rate of approximately 10 percent of the overall samples per field event). Field blanks were collected using the sampling technique described above except that reagent-grade, nuclease-free water was substituted for the water sample. Samples were delivered to the laboratory at the same time as the samples for culture analyses (described above) following standard COC procedures discussed in Section 2.4.

2.1.4 Laboratory Methods for Analysis of Bacteria by Culture Techniques

Samples delivered to Pace Laboratory were analyzed by standard methods for total coliforms and *E. coli* following the analytical parameters described in Table 2-3.

Table 2-3: Bacterial Analyte and Corresponding Analytical Parameters for Culture Techniques

Analyte	Method	Units[(a)]	Reporting Limit	Sample Volume	Container (#, Size, Type)	Preservation	Holding Time
E. coli	IDEXX Colilert-18	MPN/ 100 mL	1.0 MPN	100 mL	1, sterile,100-mL plastic	$Na_2S_2O_3$ < 0 to 10 °C[b]	6 hours
Total Coliform	IDEXX Colilert-18	MPN/ 100 mL	1.0 MPN	100 mL	1, sterile, 100-mL plastic	$Na_2S_2O_3$ < 0 to 10 °C[b]	6 hours

(a) MPN – Most Probable Number
(b) °C = degrees Celsius

2.1.5 Laboratory Methods for Analysis of Bacteria by Molecular Techniques

The laboratory analysis procedures for molecular analyses included sample filtration, DNA extraction, and DNA amplification by real-time polymerase chain reaction (PCR). Sample filtration was completed at the Pace Laboratory. DNA extraction and amplification were completed at Weston Solutions in Carlsbad, California (Weston Laboratory).

The Pace Laboratory was responsible for initial sample filtration, as summarized below. Prior to filtration, all surface and equipment were sterilized using DNA *AWAY*™. A 2-mL extraction tube (GeneRite DNA EZ kit) for each sample was labelled with the sample information and placed on a drying rack. Pre-packaged filter funnels (Pall Microfunnels) were removed from the packaging and placed in a sterilized vacuum filter manifold. The polycarbonate filter was 47-millimeter (mm) in diameter with a 0.22-micrometer (µm) mesh size. The water sample was shaken, and 100 mL was pipetted into the funnel using a sterile pipettor. The vacuum was turned on, and the sample was extracted through the filter. The sides of the funnel were rinsed with sterile phosphate buffer solution (PBS), and filtration continued until all fluid had been pulled through. The funnel was then removed from the filter base, exposing the filter. The filter was removed with sterilized forceps, rolled into a cylinder, and inserted into the labelled extraction tubes. The extraction tube cap was secured and frozen at -20 °C. The filters were then placed in a cooler on dry ice and shipped by overnight courier to the Weston Laboratory in Carlsbad, California, following standard COC procedures discussed in Section 2.4.

Once the filters had been received by the Weston Laboratory, they were prepared for DNA extraction and amplification as follows. DNA was extracted and purified using the GeneRite DNA-EZ Kit according to the manufacturer's protocol. Purified DNA was stored at -80 °C until PCR analysis. A blank filter was processed as an extraction blank during every set of extractions (about 1 blank per 12 sample extractions). Extracted DNA was analyzed by real-time PCR for three molecular markers: human marker (HumanBacteroidales-HF183TqamanCAMan), dog marker (DogBacteroidales-DogBact), and bird marker (AvianHelicobacter-GFDSYBRAvian), as described in Boehm et al. (2013). Positive controls for the human marker used genomic *Bacteroides. dorei* DNA (DSMZ 17855), and positive controls for the dog and bird markers used plasmid DNA. DNA was quantified on a Nanodrop 2000 UV-Vis spectrophotometer (Thermo-Scientific, Wilmington, Delaware). Each DNA sample was tested for PCR inhibition with the HumMST assay *B. dorei* DNA added to HF183 Taqman PCR reactions that contained extracted sample DNA at (a) full strength and (b) extract diluted 1:10 by molecular-grade water. Sample DNA was considered inhibited if the cycle threshold (Ct) between the undiluted and diluted extracts differed by more than 1.5 cycles.

Samples were processed on a BioRad CFX96 Real-time PCR Detection System and used default quality control data analysis settings (efficiency 90 to 110 percent, standard curve $r^2 \geq 0.980$), baseline subtracted curve fit with fluorescence drift correction, and baseline threshold set to 100.

2.2 Sanitary Survey Investigation

The purpose of the sanitary survey investigations was to identify any potential sources of *E. coli* within the Study Area of each of the two watersheds (Keene and Tischer Creek). Numerous potential sources were considered at the onset of the investigations, following a review of the existing data, land use, and documentation in the TMDL regarding potential sources. Based on the information available and the characteristics of the watershed, a list of the sources in the Study Area that had the potential to impact receiving waters in either of the creeks was developed. The list of potential sources considered in the Sanitary Surveys is presented in Table 2-4.

Table 2-4: Potential Bacterial Sources Considered within the Study Area for the Sanitary Survey Investigation

General Category	Potential Source/Activity
Municipal Sanitary Infrastructure (piped)	Sanitary sewer overflows
	Combined sewer overflows; regulated under NPDES
	Leaky sewer pipes (exfiltration) (see Sercu et al., 2011)
	Illicit sanitary connections to MS4
Other Human Sanitary Sources (some also attract urban wildlife)	Leaky or failing septic systems
	Homeless encampments
	Temporary toilets (e.g., Porta-Potties)
	Dumpsters (e.g., diapers, pet waste, urban wildlife)
	Trash cans
	Garbage trucks
Domestic Pets	Dogs, cats, other domestic or feral wildlife
Urban Wildlife (naturally occurring and human attracted)	Rodents/vectors (rats, raccoons, squirrels, opossums)
	Birds (gulls, pigeons, swallows, etc.)
	Open space (coyotes, foxes, beavers, feral cats, etc.)
Other Urban Sources (including areas that attract vectors)	Food processing facilities
	Outdoor dining
	Restaurant grease bins
	Bars/stairwells (wash-down areas)
Urban Non-stormwater Discharges (potentially mobilizing surface-deposited bacteria)	Power washing
	Excessive irrigation/overspray
	Car washing
	Pools/hot tubs
	Reclaimed water/graywater (if not properly managed)
MS4 Infrastructure	Illegal dumping

General Category	Potential Source/Activity
	Illicit sanitary connections to MS4 (*also listed above*)
	Leaky sewer pipes (exfiltration) (*also listed above*)
	Biofilms/regrowth
	Decaying plant matter, litter, and sediment in the storm drain system
Recreational Sources	Bathers and/or boaters
	RVs (mobile)
Natural Open Space/Forested Areas	Wildlife populations
	Grazing
Other Naturalized Sources	Plants/algae, sand, soil (naturalized *E. coli*)

Source: Modified from Armand Ruby Consulting (2011)

For each of the two watersheds, the surveys were conducted by dividing the Study Area into drainages that influenced each of the designated monitoring sites (e.g., within the reach or reaches upstream of the monitoring site). The drainages were established by reviewing the storm drain infrastructure within the Study Area and defining areas upstream of a baseline monitoring site.

Using the list of potential bacterial sources identified in Table 2-4, each drainage area was thoroughly surveyed by field technicians in cars and on foot. Field personnel were provided with maps of the drainage area, sanitary survey field observations forms, sample collection gear, and digital cameras to document any potential sources of bacteria within the Study Area that could introduce *E. coli* to the receiving waters. Each street of the drainage area was observed for potential bacterial sources and the results were documented on sanitary survey field observation forms. In addition to visual observations, spot samples were collected from any suspected source of bacteria in the drainage that had the potential to be transported to the creek (e.g., water in gutters from irrigation, car washing, etc.). The location, date/time, and a description of the sample was recorded on the field observation forms.

Samples were collected following protocols described in Subsection 2.1.1.1 for analysis by culture techniques and Subsection 2.1.1.2 for analysis by molecular techniques.

2.3 Special Study – Water and Sediment Characterization

As part of the adaptive study design described in Chapter 1.0, special studies were conducted to address the extent to which streambed sediment, soil from the streambank and riparian area of the creek, and water sources from outside of the creek receiving waters influenced *E. coli* levels in Keene Creek and Tischer Creek receiving waters. Within each of the two study areas, the two most impacted stream reaches (identified by monitoring results and urban landuse) were characterized and compared to a site

with the least urban influence (referred to as a reference site for comparative purposes). The characterization consisted of physical, chemical, and biological parameters for both water and sediment in the three reaches of each Study Area.

2.3.1 Field Methods

The sampling locations for the sediment special study in the Keene Creek and Tischer Creek Study Areas along with the water and sediment collection procedure are discussed in this Subsection.

2.3.1.1 Keene Creek Monitoring Sites

For Keene Creek, two sites (KC-MS-1 and KC-MS-2) were determined to have the greatest *E. coli* concentrations and greatest number of potential *E. coli* sources (due primarily to urbanization) (see Figure 2-1). Samples were collected from three locations within each of the two reaches (MS-1 reach and MS-2 reach): at the bottom (designated as sample A), middle (sample B), and top (sample C) of each reach. In addition, three similar samples were collected from the reach above Site KC-MS-7, which has very little urban influence and is referred to here as a relative "reference" site to compare to the urbanized reaches of KC-MS-1 and KC-MS-2.

At each location within a reach (A, B, and C of each of the three reaches), a single composite sample (consisting of three randomly selected areas for a given location) was collected for sediment analysis. Thus, each reach was characterized by three samples, represented as A, B, and C. An analogous sampling regimen was used to collect water samples from the creek. These samples were considered to be "sinks", for which sources in the watershed and creek were identified and assessed. The site names given to the sinks for Keene Creek are identified in Table 2-5 for the three reaches assessed.

Table 2-5: Keene Creek Special Study Monitoring Sites

Site Name	
Sediment	**Water**
KC-MS-1-Sed-A	KC-MS-1-Wat-A
KC-MS-1-Sed-B	KC-MS-1-Wat-B
KC-MS-1-Sed-C	KC-MS-1-Wat-C
KC-MS-2-Sed-A	KC-MS-2-Wat-A
KC-MS-2-Sed-B	KC-MS-2-Wat-B
KC-MS-2-Sed-C	KC-MS-2-Wat-C
KC-MS-7-Sed-A	KC-MS-7-Wat-A
KC-MS-7-Sed-B	KC-MS-7-Wat-B
KC-MS-7-Sed-C	KC-MS-7-Wat-C

In addition to the samples collected above identified as sinks, several potential sources throughout the Study Area were identified. These sources included sediment at storm drain outfalls or from organically rich wetlands or bogs either in the creek or adjacent to it, soil in the streambank and riparian areas adjacent to the creek, and water from numerous potential sources identified in the Sanitary Survey, such as wetlands, bioswales, ponded water (e.g., in catch basins with accumulate organic debris and other sources), and storm drain effluent. Composited samples consisting of three randomly-selected areas within each potential source were collected and analyzed for physical, chemical, and biological parameters, similar to those conducted for sinks. Fecal samples were also collected as potential sources from goose waste, dog waste, and human sewage.

2.3.1.2 Tischer Creek Monitoring Sites

In Tischer Creek, samples were collected in the same way as described above for Keene Creek above, but were collected from reaches associated with mainstem Site TC-MS-5 and tributary Site TC-T-2, which represented the impacted sites (based on monitoring results and landuse), as well as Site TC-T-2-Up in Hartley Park, which represented the reference site (see map on Figure 2-2). The site names given to the sinks for Tischer Creek are identified in Table 2-5 for the three reaches assessed.

Table 2-6: Tischer Creek Special Study Monitoring Sites

Site Name	
Sediment	**Water**
TC-MS-5-Sed-A	TC-MS-5-Wat-A
TC-MS-5-Sed-B	TC-MS-5-Wat-B
TC-MS-5-Sed-C	TC-MS-5-Wat-C
TC-T-2-Sed-A	TC-T-2-Wat-A
TC-T-2-Sed-B	TC-T-2-Wat-B
TC-T-2-Sed-C	TC-T-2-Wat-C
TC-T-2-Up-Sed-A	TC-T-2-Up-Wat-A
TC-T-2-Up-Sed-B	TC-T-2-Up-Wat-B
TC-T-2-Up-Sed-C	TC-T-2-Up-Wat-C

Potential source samples were also collected within the Tischer Creek Study Area, as discussed above for Keene Creek.

2.3.1.3 Sample Collection

In order to characterize the chemical, physical, and biological conditions within each reach that may contribute to elevated *E. coli* concentrations, samples were collected for analyses of water quality,

sediment quality, and biological community parameters (both water and sediment). Water samples were collected from potential source and sink sites using the methods discussed in Subsection 2.1.3. Water samples for *E. coli* (culture) analysis and chemical analyses were delivered to the Pace Laboratory in Duluth. Water samples for microbial community analyses (MCA; see below) were delivered to the UMN.

A series of sediment and soil samples were collected at each site identified in Table 2-5 and Table 2-6 (as well as potential sediment sinks, such as wetlands). At each site, a series of streambed sediment and soil samples were collected from three discrete zones, defined as follows:

1. Streambed sediment – the bottom of the streambed as close to the thalweg as possible
2. Streambank soil – the unvegetated soil bank above the high-water mark of the creek
3. Riparian soil – the vegetated riparian area above the streambank

Three discrete samples were collected and composited from each zone for analysis of a suite of chemical constituents, grain size, *E. coli* (culture), and MCA. Samples were collected with a sterile, plastic scoop. Sediment and soil samples for chemical analyses were placed in pre-labelled glass jars with Teflon lids (supplied by Pace Laboratory), samples for grain size and MCA were placed in pre-labelled sterile plastic bags, and samples for *E. coli* (culture) were placed in pre-labelled sterile 100-ml plastic bottles (same bottles used for water sampling). The top one to two centimeters of sediment and soil was collected at each site with the sterile plastic scoop and placed in the appropriate containers for each analysis.

All samples were placed on ice in coolers and transported to the laboratory following COC procedures discussed in Section 2.4. Sediment and soil samples for *E. coli* (culture) analysis, chemical analyses, and grain size analysis were delivered to the Pace Laboratory in Duluth. Sediment and soil samples for MCA were delivered to the UMN.

2.3.2 Laboratory Methods

The laboratory methods used to analyze the samples collected as part of the Special Study are discussed in this Subsection.

2.3.2.1 Water and Sediment Chemistry

Samples were collected and analyzed for a suite of water quality constituents: Total Kjeldahl Nitrogen (TKN), nitrate plus nitrite (listed as NO_3), total phosphorus (TP), total organic carbon (TOC), total suspended solids (TSS), and *E. coli*. Sediment and soil samples were analyzed for the same constituents, except TSS. The analytical parameters for water and sediment and soil samples are described in Table 2-7.

Table 2-7: Chemical Analyte and Corresponding Analytical Parameters for Water and Sediment and Soil Samples

Analyte	Method	Units[a]	Reporting Limit	Method	Units[a]	Reporting Limit
	Water			Sediment and Soil		
TKN	EPA 351.2 rev2	mg/L	0.50	EPA 351.2	mg/kg	78
NO_3	EPA 353.2 rev2	mg/L	0.02	EPA 353.2	mg/kg	0.34
TP	EPA 365.3	mg/L	0.05	EPA 365.1	mg/kg	4.0
TOC	SM 5310C	mg/L	1.0	EPA 9060	mg/kg	2220
TSS	USGS I-3765-85	mg/L	10.0	NA[b]	NA	NA
E. coli	IDEXX Colilert-18	MPN/ 100 mL	1.0	SM 9221B	MPN/ 100 g	NA
Percent Moisture	NA	NA	NA	ASTM D 2974-87	%	0.1

(a) MPN – Most Probable Number, mg/L – milligrams per Liter, mg/kg – milligrams per kilogram
(b) NA – Not Applicable

Grain size analyses of sediment and soil samples were conducted using Method ASTM D 6913. Data were reported as percent gravel (coarse and fine), percent sand (coarse, medium, and fine), and percent fines (silt and clay).

2.3.2.2 Microbial Community Analysis

Water, sediment and fecal samples were processed for MCA at the UMN using the following methods. Water samples were filtered through 0.22-µm-pore size mixed cellulose esters filters, whereas fecal slurries and effluent samples were pelleted. Filters, sediment, and fecal/effluent pellets were stored at -20º C prior to DNA extraction. The DNeasy PowerSoil Pro Kit (Qiagen; Hilden, Germany) was used to extract DNA from water filters, added directly to PowerBead tubes, or 0.25 grams of sediment/ fecal pellets according to the manufacturer's instructions. The V4 hypervariable region of the 16S rRNA gene was amplified using the 515F/806R primer set (Caporaso et al., 2012). Illumina (San Diego, CA) sequencing adapters and indices were then added using the dual index method (Gohl et al., 2016). Sterile water negative controls were carried through amplification and sequencing. Samples were paired-end sequenced at a read length of 300 nucleotides on the Illumina MiSeq platform.

Sequence processing was performed using QIIME v. 1.8.0 (Caporaso et al. 2010b). Raw data, as fastq files, were trimmed to 250 nucleotides to remove lower-quality regions (< Q30) using Trimmomatic v. 3.2 (Bolger et al. 2014) and paired-end joined using the fastq-join script (Aronesty, 2013). Chimeras were identified and removed using UCHIME v. 6.1 (Edgar et al. 2011). Taxonomy was assigned version 14

release from the Ribosomal Database Project at a bootstrap confidence cutoff of 80% (Cole et al., 2009). Operational taxonomic units (OTUs) were clustered at 97 percent similarity using UCLUST, and taxonomic assignments were made against the SILVA v.132 16S rRNA gene database using PyNast (Caporaso et al. 2010a; Edgar 2010; Quast et al. 2013). For comparisons among samples (Gihring et al., 2012), the numbers of sequence reads per sample were rarefied by random subsample to 20,000 reads per sample.

Alpha diversity (species richness with a sample) measures were calculated using observed species, and Shannon H indices. Bray-Curtis dissimilarity matrices were used for principal coordinates analysis and to assess differences in beta diversity (number of species that are not the same between samples) by analysis of similarity. Canonical correspondence analysis (CCA) was performed to determine which parameter best explained the variation in microbial community structure within water and sediment samples. All statistics were evaluated at $\alpha = 0.05$, unless corrected for multiple comparisons as noted.

The amount of source contribution was determined using default parameters of SourceTracker software version 0.9.8 (Knights et al., 2011). This software employs an iterative Bayesian approach to determine which OTUs in sink communities are attributable to those in source communities. The fraction of reads that cannot be assigned to a source at a significance threshold of $\alpha = 0.001$ is assigned to an "unknown" category.

2.4 Chain of Custody Procedures

COC procedures were used for all samples throughout the collection, transport, and analytical process. Samples were considered to be in custody if they were: (1) in the custodian's possession or view, (2) retained in a secured place (under lock) with restricted access, or (3) placed in a container and secured with an official seal such that the sample could not be reached without breaking the seal.

COC procedures were initiated during sample collection. A COC record was provided with each sample or group of samples. Each person who had custody of the samples signed the form and confirmed the samples were not left unattended unless properly secured. Documentation of sample handling and custody includes the following:

- Sample identifier
- Sample collection date and time
- Any special notations on sample characteristics or analysis
- Initials of the person collecting the sample
- Date the sample was sent to the analytical laboratory

Completed COC forms were placed in a plastic envelope and kept inside the container containing the samples. Once delivered to the analytical laboratory, the COC form was signed by the laboratory personnel receiving the samples. The condition of the samples was noted and recorded by the receiver.

2.5 Quality Assurance / Quality Control

For culture analyses, field blanks were collected at a rate of one sample per sampling event. Field blanks were used to verify that no contamination originating from the collection, transport, or storage of environmental samples occurred. For molecular analyses, at least one sterile field blank was collected by each sampling field technician during each sampling event. Once in the laboratory, care was taken to avoid contamination during sample processing. Surfaces and instruments were first cleaned with ethanol and DNA *AWAY*™. The outsides of the sample bottles were wiped down with DNA *AWAY*™ and dried with Kimwipes® prior to being brought to the filtration area.

Laboratory controls included the following: (1) laboratory blanks, (2) no-template controls, (3) positive controls, and (4) inhibition controls. In addition to field blanks, a laboratory blank was processed for every set of molecular samples. Laboratory blanks were filtered similarly to samples, except that molecular-grade water was substituted for the water sample. No-template controls (two to three per plate) consisted of PCR reactions set up with molecular-grade water replacing sample DNA. Positive controls consisted of plasmid or genomic DNA.

Samples were tested for inhibition using a matrix spike consisting of *B. dorei* DNA added to HF183 Taqman PCR reactions that contained extracted sample DNA (not crude lysate) at full strength (1:1) and extract diluted 1:10 by molecular-grade water. Sample DNA was considered inhibited if the Ct between the undiluted and diluted extracts differed by more than 1.5 cycles. For samples analyzed by only the HF183 Taqman assay, each sample was accompanied by a matrix spike. If results had indicated inhibition, the sample DNA would have been diluted 1:5 and re-analyzed. No inhibition was observed for the samples analyzed during this study.

A field or laboratory blank or no-template control found positive by PCR analysis would have invalidated the samples for that PCR set. No field or laboratory blanks tested positive by PCR during the entire course of this study. Lack of amplification of a positive control would have invalidated the PCR run, and the samples would have been analyzed again. No positive controls failed to amplify for the entire study.

3.0 RESULTS

3.1 Keene Creek Dry Weather Assessment

The results of the Keene Creek dry weather assessment are described in this Section.

3.1.1 Baseline Monitoring

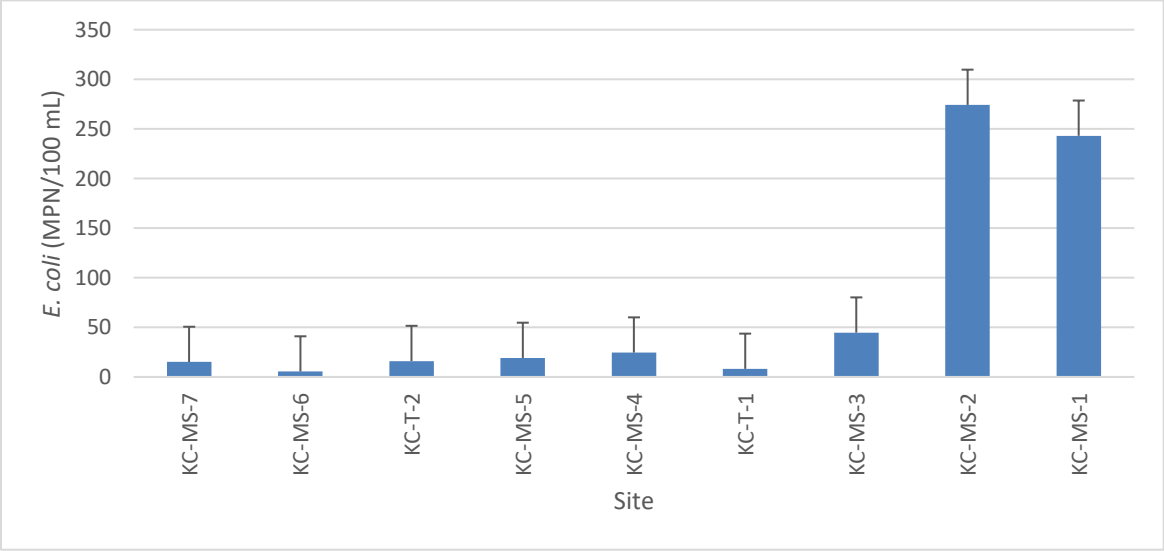
The baseline monitoring results for *E. coli* and molecular markers are presented below.

3.1.1.1 *E. coli* Concentrations

A total of 56 dry weather samples (at least 48 hours after the last rain event) were collected and analyzed for *E. coli* from Keene Creek during the baseline monitoring. Samples were collected and analyzed from seven mainstem monitoring sites (KC-MS-1 through KC-MS-7) and two tributary sites (sites KC-T1 and KC-T2) over seven monitoring events from August 22 to September 26, 2019 (not all sites were monitored during all events). Spatial patterns of *E. coli* concentrations among the Keene Creek baseline monitoring sites are depicted as geometric mean concentrations plus one standard error (SE) on Figure 3-1.

Mean concentrations from samples collected over the course of the dry weather monitoring varied little and were low between Sites KC-MS-7 (upper-most portion of the Study Area) and KC-MS-3 (Keene Creek Dog Park). All individual samples except one were less than 100 MPN/100 mL at these sites and the geometric means were less than 50 MPN/100 mL. In contrast, geometric mean concentrations were much greater at the two sites at the bottom of the Keene Creek Study Area at Sites KC-MS-2 and KC-MS-1, which had geometric mean concentrations of 274.2 and 243.1 MPN/100 mL, respectively.

Figure 3-1: *E. coli* Geometric Mean Concentrations (+1 SE) at Keene Creek Monitoring Sites



3.1.1.2 Molecular Markers

The results of the samples collected for molecular analyses during the Keene Creek baseline monitoring are summarized in Table 3-1. A total of 27 samples were collected over the course of three sampling events (September 24, September 26, and October 8) for molecular analyses from the three baseline monitoring sites with the greatest *E. coli* concentrations (MS-1, MS-2, and MS-3). All samples were analyzed for the three molecular markers (bird, dog, and human). Among the samples collected, two were positive for the bird marker (22.2 percent), none were positive for the dog marker (including samples collected from the Keene Creek Dog Park), and three were positive for the human marker (33.3 percent). All positive samples were collected during the October 8 monitoring event. All blank samples collected during the monitoring were negative for all three markers.

Table 3-1: Keene Creek Baseline Monitoring Results for Molecular Markers

Marker	Sample ID	Date Sampled	Sample Result	Percent Positive
Bird	MS-1	09/24/19	No	22.2%
	MS-2		No	
	MS-3		No	
	MS-1	09/26/19	No	
	MS-2		No	
	MS-3		No	
	MS-1	10/08/19	No	
	MS-2		**Yes**	
	MS-3		**Yes**	
Dog	MS-1	09/24/19	No	0.0%
	MS-2		No	
	MS-3		No	
	MS-1	09/26/19	No	
	MS-2		No	
	MS-3		No	
	MS-1	10/08/19	No	
	MS-2		No	
	MS-3		No	
Human	MS-1	09/24/19	No	33.3%
	MS-2		No	
	MS-3		No	
	MS-1	09/26/19	No	
	MS-2		No	
	MS-3		No	
	MS-1	10/08/19	**Yes**	
	MS-2		**Yes**	
	MS-3		**Yes**	

3.1.2 Sanitary Surveys

Sanitary surveys were conducted over the entire Keene Creek Study Area from September 16 through 19, 2019. Each drainage area corresponding to the seven mainstem sites and two tributary sites were assessed through visual observations and photo-documentation as well as "spot samples" collected from suspected sources of *E. coli* in the drainage. Survey methods are described in Subsection 2.2.1 and the results are presented below.

3.1.2.1 Observations

The results of the sanitary surveys are summarized in Table 3-2 with select photos provided in Figure 3-2. There was no evidence of bacterial sources originating from the municipal sanitary system. The field team did not observe evidence of leaky sewer pipes, illicit connections to the MS4, septic systems/leach fields, or any other evidence of leaking sanitary systems that could convey *E. coli* from human origin to the creek receiving waters. There was some evidence of a potential homeless encampment in the trees adjacent to the left bank of Keene Creek (middle of the MS-2 reach) at South 58th Avenue West. However, there were no people observed in the area and no evidence of human feces were observed at this site or anywhere else throughout the Study Area.

One car washing episode was observed in the alley off Raleigh Street, west of South 59th Avenue West (adjacent to the local school). The field team observed the discharge from the back of a garage as the person washing the car had just finished. Wash water flowed down the dirt alley carrying sediment with it to Raleigh Street in front of the school and then flowed to the creek via gutters on South 59th Avenue West. The discharge path was well-worn, suggesting that the cleaning may be a frequent occurrence (a similar flow can also be seen on Google Earth).

Decaying plant material in the catch basin inlets was a frequent observation in reaches MS-1 and MS-2 of Keene Creek. Leaf litter and sediment clogged catch basin inlets were observed where Keene crosses South Central Avenue, South 57th Avenue West, and South 59th Avenue West. Flow from these catch basins discharges directly to the creek. Similar observations of excessive street debris were noted at Waseca Industrial Road (near South Central Avenue, reach MS-1), the end of South 56th Avenue (right bank, reach MS-2), and North 61st Avenue West and Roosevelt Street (reach MS-4).

Relatively few sightings of dogs (on leash or otherwise) were observed in the Study Area. Dogs on leash were observed at Irving Park soccer field at South 57th Avenue West and in the neighborhood north of Site MS-4 along Green Street. No dog waste was observed anywhere in the Study Area except at the Keene Creek Dog Park. Numerous dogs were observed at the park and dog waste was observed in the

grassy area of the park on separate locations, but there did not appear to be an obvious flow path to the creek. A smaller buffer strip of unmown grass and vegetation was observed along the right bank of the creek adjacent to the dog park, which may prevent flow from the park from reaching the receiving waters. The vast majority of the waste was properly disposed of in trash cans and doggie bags located inside the fenced-in off-leash area. A small tributary with ephemeral flow (Tributary T-1) runs adjacent to the northwest side of the dog park. It was not flowing the last two weeks of August 2019 and the first week of September, with minor flow after that. A vegetated buffer strip also lines the right bank of the stream (similar to the mainstem), which should help prevent sheet flow from transporting *E. coli* from dog waste to the Keene Creek receiving waters.

A variety of songbirds and crows were observed in the Study Area, but sightings were relatively minimal. A population of Canadian geese was observed consistently at Irving Park (MS-2 reach) and large amounts of goose waste were observed in the soccer field and adjacent park area. There is a small detention basin to the west of the soccer field with a catch basin inlet for overflow water and goose waste was observed in and around the basin. There is a also a small catch basin inlet on the south side of the soccer field. It is unclear how these catch basins drain to Keene Creek, but there is a small six-inch PVC line directly on the other side of the riparian buffer from the southern catch basin that discharges directly to the creek. Minimal flow was emanating from the pipe during the sanitary survey (and subsequent observations) and the storm drain appeared to be flooded. The drain may be partially clogged, but when flowing, would represent a pathway for *E. coli* associated with the goose waste from the soccer field, park, and detention basin to enter Keene Creek. Approximately 300 feet downstream from this discharge is another six-inch blue PVC pipe that appears to also originate from the soccer field. The pipe was not flowing during any of the observation days and no catch basin inlet could be found in the soccer field or adjacent area. No other wildlife (including other birds) were observed in large numbers anywhere in the Study Area.

There were several other potential sources of *E. coli* identified in the Keene Creek Study Area, as discussed below:

- **Paper mill tributary (MS-1 reach) –** A tributary originating from the paper mill property that lies to the north of the mouth of Keene Creek discharges to the creek (left bank) approximately 100 feet above the historical MS-1 monitoring site (S004-968). Water quality appeared to be very poor in this discharge water, which had a thick, oily sheen on the surface and very loose, anoxic streambed sediments.

- **Wetland discharge (MS-1 reach) –** Approximately 350 feet upstream of Site S004-968, is an outfall on the left bank of Keene Creek that drains water from a wetland on the opposite side of the bike path that parallels the creek. The wetland contained large amounts of ponded water with decaying organic debris and degraded habitat that can serve as a source of *E. coli* via bacterial regrowth. The outfall had a minor but persistent flow from the wetland to the creek during the sanitary survey.

- **Non-MS4 storm drain outfalls (MS-1 reach) –** Approximately 550 feet upstream of Site S004-968 are two PVC outfalls (approximately 12-inch diameter) that discharge to the right bank of Keene Creek. The pipes originate from the back side of a warehouse at 117 South Central Avenue, but were not flowing during the sanitary survey or during other dry weather observations.

- **Erosion at wooden stairs (MS-2 reach) –** At the end of South 56th Avenue West on the right bank of Keene Creek are a set of wooden stairs that lead from the end of the street to the streambank. A stormdrain outfall that drains the street and discharges adjacent to the stairs has produced severe erosion in the streambank. This can act as a source of *E. coli* to the creek (due to naturalized *E. coli* in the soil and attachment of bacteria to sediment particles), particularly during storm events.

- **Wetland discharge (MS-2 reach) –** On the left bank of the creek where it crosses South 57th Avenue West, lies a wetland that contains large amounts of ponded water with decaying organic debris and degraded habitat that can serve as a source of *E. coli* via bacterial regrowth. Ponded water with large amounts of organic debris were also observed in the gutter of South 57th Avenue West that was flowing into the wetland. It is unclear how this wetland drains to the creek (no outfall could be found), but the wetland is a potential source of *E. coli* to the creek that may be considered for further investigation.

- **Degraded habitat (MS-2 reach) –** The lower portion of reach MS-2 (from South 57th Avenue West to South 59th Avenue West) is severely degraded. This portion of the reach flows directly under two transmission line towers whose foundation impede flow and trap sediment. The streambanks are severely eroded in several places and decaying vegetation has filled the stream. Several storm drain outfalls discharge directly to this part of the MS-2 reach and it is characterized by fine-grained sediment in the streambed, sluggish flow, and very turbid water.

- **Severe erosion (MS-2 reach) –** At the top of reach MS-2, just downstream of Grand Avenue is an abandoned railroad line. The area where the railroad crosses Keene Creek is characterized by severe erosion on the upstream side of the crossing (Particularly the left bank) and decaying vegetation has filled the stream in several areas.

Keene Creek above Site MS-4 (upstream of U.S. Route 2), including tributary T-2, has a steeper gradient, stable, vegetated banks, and much less urban land use than reaches below Site MS-4. There was only one obvious source of *E. coli* observed in the Study Area upstream of Site MS-4. Just upstream of Site MS-6, Keene Creek crosses under Highland Street (State Route 89), which is supported by a large bridge. The area under the bridge is large (due to the depth of the ravine formed by the creek) and the bridge girders are exposed. Very large amounts of bird droppings were found on the rocks and bike path under the bridge presumably from birds roosting on the bridge girders. The bird waste covered the bank of the creek, which was composed of rip rap and concrete. Concentrations of *E. coli* during dry weather monitoring at Site MS-6 (just 100 feet downstream from the bridge) were very low throughout the baseline monitoring period, suggesting that the fecal matter under the bridge is an unlikely source of *E. coli* to Keene Creek during dry weather conditions.

Table 3-2: Potential Bacterial Sources Identified in the Sanitary Survey Investigation of the Keene Creek Study Area

General Category	Potential Source/Activity	Observation
Municipal Sanitary Infrastructure (piped)	Sanitary sewer overflows	Not observed
	Combined sewer overflows (CSOs); regulated under NPDES/LTCP	Not observed
	Leaky sewer pipes (Exfiltration)	Not observed
	Illicit sanitary connections to MS4	Not observed
	Wastewater Treatment Plans regulated under NPDES	Not observed
	Leaky or failing septic systems	Not observed
MS4 Infrastructure	Illegal dumping	Not observed
	Biofilms/regrowth	Observed at mainstem sites and at tributary sites T-2 and T-4
	Decaying plant matter, litter and sediment in the storm drain system	Observed throughout the Study Area in street gutters at multiple locations
Other Human Sanitary Sources (some also attract urban wildlife)	Homeless encampments	Signs of potential homeless at MS-2 and South 58th Avenue West (left bank)
	Temporary toilets (e.g., Porta-Potties)	Observed at Irving Park soccer field east of South 57th Avenue West (good condition)
	Dumpsters (e.g., diapers, pet waste, urban wildlife)	Dumpsters were observed in mixed use areas, but all were well-maintained
	Trash cans	Trash cans were observed throughout the Study area, particularly at parks, but all were well maintained including those at the Keene Creek Dog Park
	Garbage trucks	Not observed on days when sanitary surveys were conducted
	Other wildlife attracted to human sources (deer, coyotes, feral cats, etc.)	No other wildlife was observed in the Study Area attracted to human sanitary sources
Other Urban Sources (including areas that attract vectors)	Food processing facilities	Not observed, but a tributary from the paper plant was observed to be flowing to the mainstem just upstream of Site MS-1 (left bank). The surface water was discolored and appeared to have a thick sheen on the surface of the water.
	Outdoor dining	Not observed
	Restaurant grease bins	Not observed

General Category	Potential Source/Activity	Observation
	Bars/stairwells (wash-down areas)	Not observed
Urban Non-stormwater Discharges (potentially mobilizing surface-deposited bacteria)	Power washing	Not observed
	Excessive irrigation/overspray	Not observed
	Car washing	One car washing episode was observed in the alley off Raleigh Street, west of South 59th Avenue West. The discharge led directly to the right bank of the creek via street runoff. Appears to be a frequent occurrence.
	Pools/hot tubs	Not observed
	Reclaimed water/graywater (if not properly managed)	Not observed
Domestic Pets	Dogs, cats, etc.	Dog waste was observed at the Keene Creek Dog Park, but there was no evidence of runoff to the creek. No dog waste was observed elsewhere in the Study Area and dog walking was minimal.
Urban Wildlife (naturally occurring and human attracted)	Rodents/vectors (rats, raccoons, squirrels, rabbits, opossums)	Minimal evidence of urban wildlife and no feces from these animals were observed. No evidence of rats, raccoons, or opossums was observed.
	Birds (geese, ducks, gulls, crows, pigeons, songbirds, etc.)	A variety of songbirds and crows were observed in the Study Area, but relatively minimal. A population of Canadian geese and goose waste were observed at Irving Park soccer field. Two small drains in the field discharge directly to the creek.
Recreational Sources	Bathers and/or boaters	Not observed
	RVs (mobile)	Not observed
Open Space/ Forested Areas	Wildlife populations	Other than birds, no other wildlife observed
	Grazing	Not observed
Other Sources	Plants/algae, soil (naturalized *E. coli*)	Severe erosion at stairs at the end of South 58th Avenue West (right bank), throughout reach MS-2, and downstream of Grand Avenue at the railroad crossing. Very degraded habitat throughout reach MS-2.

Source: Modified from Armand Ruby Consulting (2011)

Figure 3-2: Photographs of Potential Bacterial Sources Observed During Sanitary Surveys of the Keene Creek Study Area

Inlet from paper plant at MS-1

Inlet from wetland at MS-1

Clogged catch basin at MS-1

Geese in Irving Park at top of MS-1

Catch basin at soccer field

Clogged inlet at MS-2

Degraded habitat at MS-2

Bank erosion at top of MS-2

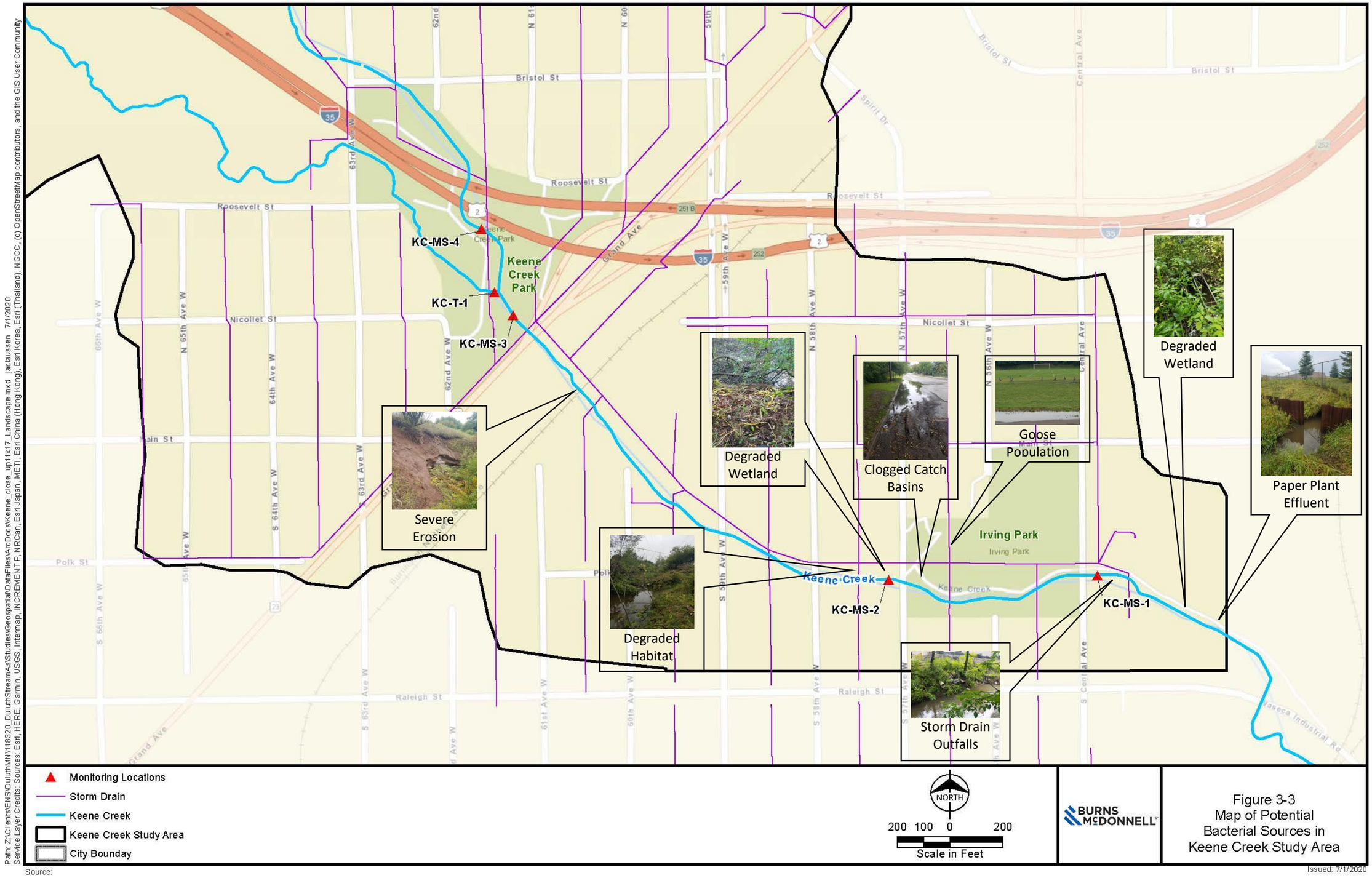
3.1.2.2 *E. coli* Concentrations

In addition to the visual observations conducted during the sanitary survey investigation, a limited number of spot samples were collected from various sources within the Study Area. All samples were collected on September 18, 2019. The results are summarized in Table 3-3. The greatest concentrations among the spot samples were collected from catch basins in the streets either adjacent to or directly on top of Keene Creek and ponded water in Irving Park. These sites were often clogged with sediment, leaf litter, and other debris and contained ponded or slowly draining water that flowed directly to the surface waters of Keene Creek. Lower concentrations were observed from the wetland on the left bank of the MS-1 reach and the outfall from the warehouse off Central at the outfall from the warehouse on South Central Avenue.

Table 3-3: *E. coli* results from the Keene Creek Sanitary Survey Investigation

Sample ID	*E. coli* (MPN/100 mL)	Reach	Site Description
KC-MS-1-A	687	MS-1, Left Bank	Paper mill tributary upstream of Waseca Industrial Road
KC-MS-1-B	190	MS-1, Left Bank	Ponded water from wetland just upstream of paper mill tributary
KC-MS-1-C	7	MS-1, Left Bank	Outfall to Keene Creek from wetland upstream of paper mill tributary
KC-MS-1-D	> 2,420	MS-1, Midstream	South Central Avenue – catch basin above creek
KC-MS-1-E	35	MS-1, Right Bank	PVC outfall, off South Central Avenue behind Moline warehouse
KC-MS-2-B	> 2,420	MS-1, Left Bank	Ponded water in Irving Park soccer field
KC-MS-2-C	> 2,420	MS-1, Left Bank	Detention basin in park to west of soccer field
KC-MS-2-D	2,420	MS-1, Right Bank	South 56th Avenue West – storm drain outfall near bank erosion at stairs
KC-MS-2-A	> 2,420	MS-2, Left Bank	57th Avenue West – catch basin adjacent to creek

A map of the potential sources of *E. coli* identified in the Keene Creek Study Area is shown on Figure 3-3.


KC-MS-4
KC-T-1
KC-MS-3
KC-MS-2
KC-MS-1
Keene Creek Park
Irving Park
Keene Creek
Bristol St
Roosevelt St
Nicollet St
Raleigh St
Polk St
Main St
Grand Ave
Spirit Dr
Central Ave
Degraded Wetland
Clogged Catch Basins
Goose Population
Degraded Wetland
Paper Plant Effluent
Severe Erosion
Degraded Habitat
Storm Drain Outfalls
Monitoring Locations
Storm Drain
Keene Creek
Keene Creek Study Area
City Bounday
NORTH
200 100 0 200
Scale in Feet
BURNS McDONNELL
Figure 3-3
Map of Potential Bacterial Sources in Keene Creek Study Area
Issued: 7/1/2020
Source:
Path: Z:\Clients\ENS\Duluth\MN\118320_Duluth\StreamAssStudies\GeospatialDataFiles\ArcDocs\Keene_close_up11x17_Landscape.mxd jaclaussen 7/1/2020
Service Layer Credits: Sources: Esri, HERE, Garmin, USGS, Intermap, INCREMENT P, NRCan, Esri Japan, METI, Esri China (Hong Kong), Esri Korea, Esri (Thailand), NGCC, (c) OpenStreetMap contributors, and the GIS User Community

3.1.3 Special Study – Water and Sediment Characterization

Using the adaptive approach discussed in Chapter 1.0, a special study was designed that was based on the results of the baseline monitoring and sanitary survey. For Keene Creek, two sites (KC-MS-1 and KC-MS-2) were determined to have the greatest *E. coli* concentrations and greatest number of potential sources (due primarily to urbanization). Samples were collected from three locations within each of the two reaches (MS-1 reach and MS-2 reach): at the bottom (designated as sample A), middle (sample B), and top (sample C) of each reach. In addition, three similar samples were collected from the reach above Site 7, which has very little urban influence and is referred to here as a relative "reference" site to compare to the urbanized reaches of MS-1 and MS-2. In order to characterize the chemical, physical, and biological conditions within each reach that may contribute to elevated *E. coli* concentrations, samples were collected for analyses of water quality, sediment quality, and biological community parameters (both water and sediment). The results of the Water and Sediment Characterization Special Study are presented below.

3.1.3.1 Water Chemistry

The results of the Keene Creek Water Characterization Special Study are presented in Table 3-4. Samples were collected and analyzed for a suite of water quality constituents: Total Kjeldahl Nitrogen (TKN), nitrate plus nitrite (listed as NO_3), total phosphorus (TP), total organic carbon (TOC), total suspended solids (TSS) and *E. coli*. Mean values are arithmetic means for chemical constituents and geometric means for *E. coli.*

Table 3-4: Keene Creek Water Characterization Results

Site	TKN (mg/L)	NO_3 (mg/L)	TP (mg/L)	TOC (mg/L)	TSS (mg/L)	*E. coli* (MPN/100 mL)
KC-MS-1-Wat-A	1.60	0.00	0.17	12.0	70.6	1,160
KC-MS-1-Wat-B	0.81	0.03	0.16	11.2	69.8	52
KC-MS-1-Wat-C	0.82	0.02	0.08	11.9	58.0	285
Mean:	**1.08**	**0.02**	**0.14**	**11.7**	**66.1**	**499.0**
KC-MS-2-Wat-A	1.50	0.10	0.56	11.4	44.6	119
KC-MS-2-Wat-B	0.64	0.06	0.17	14.3	28.4	41
KC-MS-2-Wat-C	0.50	0.06	0.08	11.4	214.0	185
Mean:	**0.88**	**0.07**	**0.27**	**12.4**	**95.7**	**115.0**
KC-MS-7-Wat-A	0.51	0.06	0.02	12.3	26.6	16
KC-MS-7-Wat-B	0.54	0.04	0.04	12.4	5.0	18
KC-MS-7-Wat-C	0.59	0.06	0.02	13.1	13.5	17
Mean:	**0.55**	**0.05**	**0.03**	**12.6**	**15.0**	**16.9**

Mean TKN, TP, and TSS in Keene Creek surface waters were lower at the reference site (MS-7) than the urbanized sites (MS-1 and MS-2) while NO_3 and TOC concentrations were similar among all sites. Mean concentrations of *E. coli* were seven to thirty times lower at the reference site (MS-7) than mean concentrations at sites MS-2 and MS-1, respectively.

3.1.3.2 Sediment Chemistry

The results of the Keene Creek Sediment Characterization Special Study are presented in Table 3-5. The chemistry patterns in sediment did not reflect those observed in the water samples. Mean concentrations of TKN, TP, and TOC were lowest in sediment at Site MS-1. Concentrations of NO_3 were below detection limit in all samples except one sample at KC-MS-1-Sed-C, which had a concentration of 0.41 mg/kg. Sediment concentrations of *E. coli* were lowest at the T-2-Up reference site with geometric mean concentrations two to seven times lower than those at the urbanized sites.

Table 3-5: Keene Creek Sediment Characterization Results

Site	TKN (mg/kg)	NO_3 (mg/kg)	TP (mg/kg)	TOC (mg/kg)	*E. coli* (MPN/100 g)
KC-MS-1-Sed-A	84.1	ND	156.0	3,140	2,800
KC-MS-1-Sed-B	88.9	ND	164.0	2,600	7,100
KC-MS-1-Sed-C	134.0	0.41	172.0	6,400	13,000
Mean:	**102.3**	**0.41**	**164.0**	**4,047**	**7,633**
KC-MS-2-Sed-A	837.0	ND	219.0	15,000	3,300
KC-MS-2-Sed-B	139.0	ND	181.0	4,230	12,000
KC-MS-2-Sed-C	226.0	ND	169.0	8,330	26,000
Mean:	**400.7**	**ND**	**189.7**	**9,187**	**13,766**
KC-MS-7-Sed-A	315.0	ND	227.0	5,880	6,400
KC-MS-7-Sed-B	235.0	ND	148.0	5,250	2,100
KC-MS-7-Sed-C	328.0	ND	187.0	7,110	3,100
Mean:	**292.7**	**ND**	**187.3**	**6,080**	**3,866**

Results are reported on a dry weight basis, adjusted for percent moisture, sample size, and any dilutions

3.1.3.3 Sediment Grain Size

The results of the Keene Creek streambed sediment grain size analyses are presented in Table 3-6. The differences between grain size at the refence site (MS-7) compared to the urbanized sites were substantial. Streambed sediments collected from the reference site tended to have a larger grain size, with greater percentages of coarse gravel, fine gravel, coarse sand, and medium sand than either of the two urban sites. Streambed sediment at the urbanized sites tended to consist of finer-grained sediment, with greater percentages of fine sand and silt/clay than the reference site.

Table 3-6: Keene Creek Sediment Grain Size Results (values represent the percent abundance of each fraction per site)

Site	Coarse Gravel	Fine Gravel	Coarse Sand	Medium Sand	Fine Sand	Silt/ Clay
KC-MS-1-Sed-A	0.0	0.0	0.0	27.4	72.0	0.6
KC-MS-1-Sed-B	0.0	0.0	0.0	0.3	94.0	5.7
KC-MS-1-Sed-C	0.0	0.0	0.1	17.9	79.2	2.8
Mean:	**0.0**	**0.0**	**0.0**	**15.2**	**81.7**	**3.0**
KC-MS-2-Sed-A	0.0	0.0	0.1	0.8	80.6	18.5
KC-MS-2-Sed-B	0.0	0.1	0.5	19.3	70.7	9.4
KC-MS-2-Sed-C	0.0	0.0	0.1	3.0	77.4	19.5
Mean:	**0.0**	**0.0**	**0.2**	**7.7**	**76.2**	**15.8**
KC-MS-7-Sed-A	0.0	19.7	6.1	26.0	39.5	8.7
KC-MS-7-Sed-B	6.5	20.4	15.6	27.5	28.4	1.6
KC-MS-7-Sed-C	0.0	0.1	4.0	65.7	29.0	1.2
Mean:	**2.2**	**13.4**	**8.6**	**39.7**	**32.3**	**3.8**

3.1.3.4 Canonical Correspondence Analysis (CCA)

The results of the CCA analysis of samples collected from Keene Creek are presented on Figure 3-4 for water samples and Figure 3-5 for sediment samples. Three water samples were collected from each of the three reaches and analyzed with the water chemistry and *E. coli* results. Similarly, sediment samples from the three sites were compared to sediment chemistry, *E. coli*, and grain size results. Figure 3-4 shows that the receiving water samples tended to group together by site (MS-1 sites grouped together, MS-2 sites grouped together, and MS-7 sites grouped together). In addition, MS-1 samples were associated with elevated concentrations of *E. coli*, TKN, TSS, and TP.

Sediment samples also tended to cluster by site. In streambed sediment, MS-1 and MS-2 samples tended to be associated with elevated concentrations of *E. coli*, and NO_3, as well as higher percentages of fine-grained sediment (fine sand and silt).

Figure 3-4: Keene Creek Canonical Correspondence Analysis Results for Water Samples

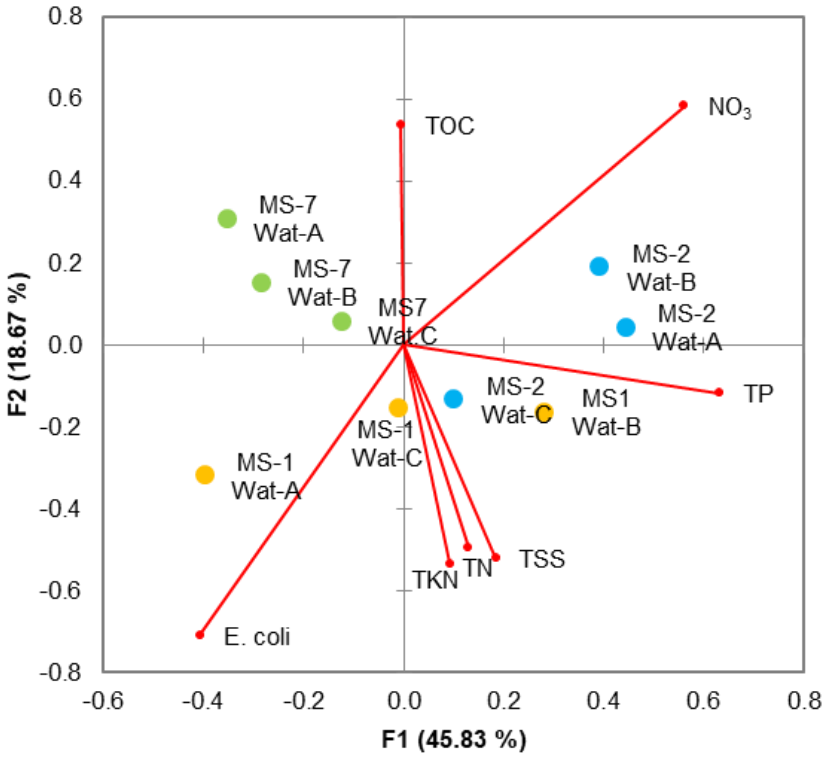
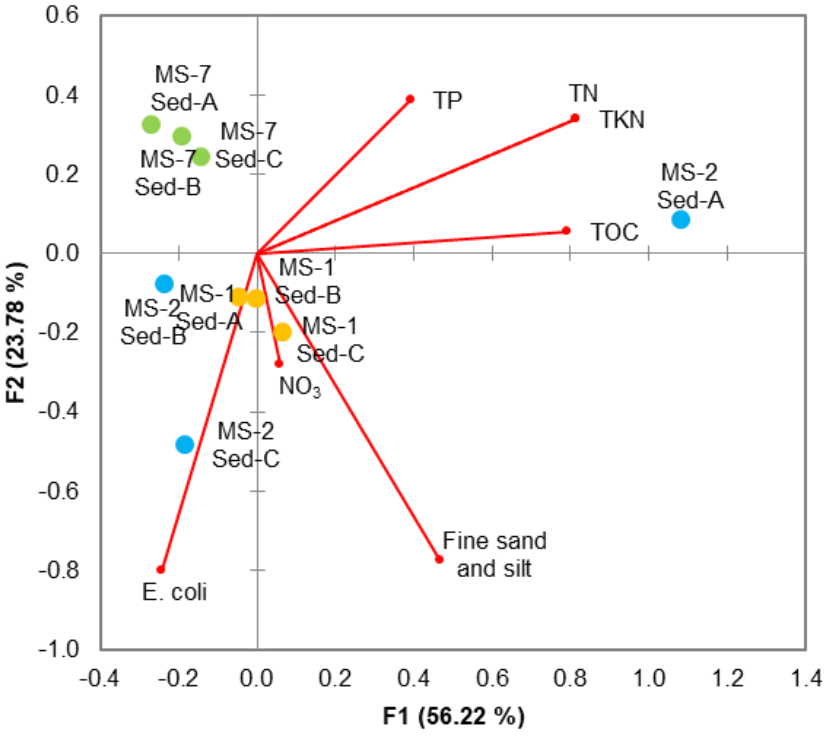


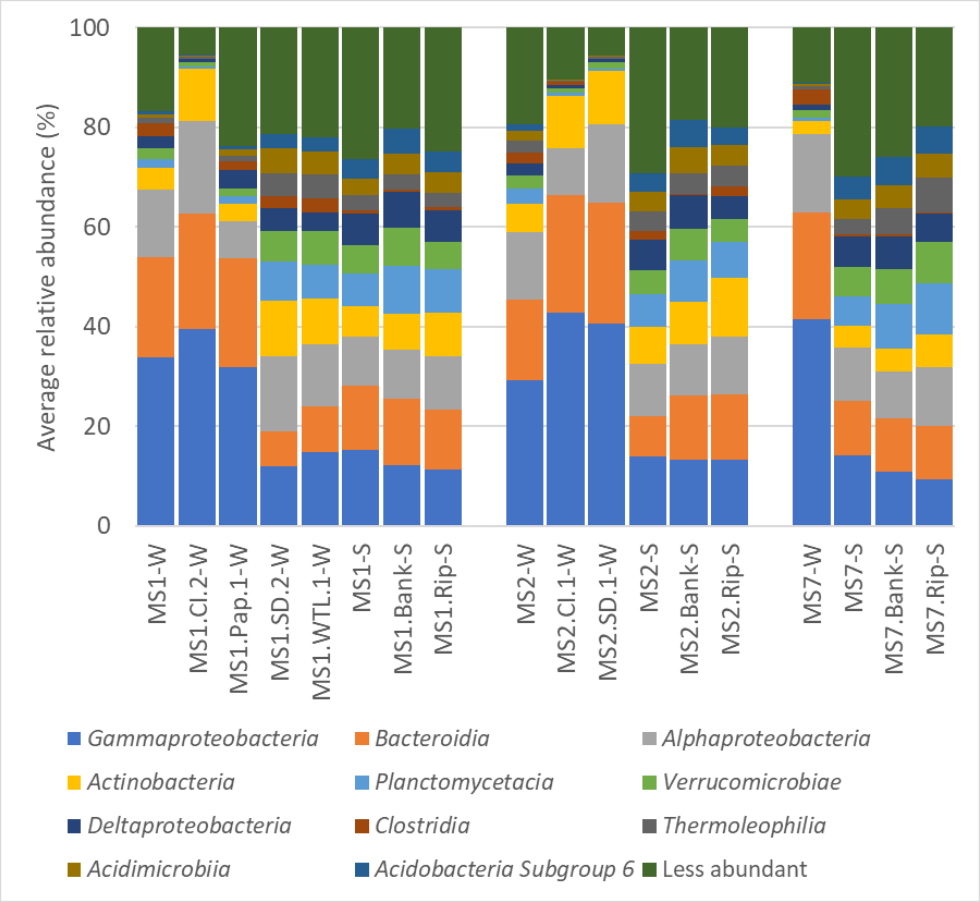
Figure 3-5: Keene Creek Canonical Correspondence Analysis Results for Sediment Samples



3.1.3.5 Bacterial Community Composition

The results of the bacterial community composition analysis are presented on Figure 3-6. Bacterial communities in water and sediment samples mostly consisted of members of the classes *Gammaproteobacteria*, *Bacteroidia*, *Alphaproteobacteria* and *Actinobacteria*. In general, water samples harbored a greater relative abundance of *Gammaproteobacteria*, whereas sediment samples were enriched with *Planctomycetacia*, *Verrucomicrobiae*, *Deltaproteobacteria*, *Thermoleophila*, *Acidimicrobiia*, and *Acidobacteria* Subgroup 6. The genus *Escherichia-Shigella* was detected in water samples from catch basin inlets and storm drains in both MS-1 and MS-2 reaches (data not shown). Microbial community patterns were generally similar for receiving water samples collected from MS-1 (MS-1-W), MS-2 (MS-2-W), and MS-7 (MS-7-W), although the MS-7 water samples tended to be slightly less diverse than the urbanized sites. Similarly, sediment samples tended to have similar microbial communities regardless of the reach from which it was collected. The exception to this was water samples collected from MS-1-SD-2 (storm drain) and MS-1-WTL-1 (wetland), both of which had microbial communities similar to those observed in sediment samples.

Figure 3-6: Keene Creek Bacterial Community Composition (Class Level)



* Samples were grouped by sampling location; W: Water, S: Sediment

3.1.3.6 Source Tracker Analysis

The results of the SourceTracker analysis of water and samples collected from Keene Creek are presented graphically on Figure 3-7 and numerically in Table 3-7. SourceTracker software was used to determine which sources of bacteria (from samples collected from a variety of suspected sources in MS-1, MS-2, and MS-7 reaches) were the major source contributors for a given "sink", where sink is defined as either Keene Creek surface water at sites MS-1, MS-2, or MS-7 or as sediment at sites MS-1, MS-2, or MS-7. Colors in the stacked bar chart on Figure 3-7 and values in Table 3-7 represent the mean percent contribution of each suspected source for a given sink. The means were derived from three samples collected from each suspected source. For each sink, the two identified sources with the highest percent contribution are highlighted in red text.

SourceTracker analysis revealed that the major sources of bacteria to Keene Creek surface waters in the MS-1 reach were water from the paper mill effluent (18.7 percent) and effluent from the MS-1 storm drain outfall at South Central Avenue (16.3 percent). The major sources to receiving water collected in the MS-2 reach were storm drain effluent from the outfall at South 59th Avenue West (26.1 percent) and, streambed sediment from reach MS-7 (14.4 percent). The major identified sources to receiving water collected in MS-7 was MS-7 sediment (36.5 percent), but the largest proportion at this site was from unknown sources.

The major sources of all sediment sinks originated from sediment sources. For example, the major sources of bacteria to Keene Creek streambed sediment in the MS-1 reach were streambed sediments collected from MS-2 (23.1 percent) and MS-7 (19.3 percent). For MS-2 streambed sediment, the major sources were identified as streambed sediment form MS-7 (23.1 percent) and bank sediment from MS-2 (16.6 percent). For MS-7 streambed sediment, the major identified source was MS-7 bank sediment (28 percent), with a large proportion of unknown sources. Contributions from suspected water sources to streambed sediment were small at all three sites.

Figure 3-7: Graphic of Mean Percentage of Source Contributions to Keene Creek

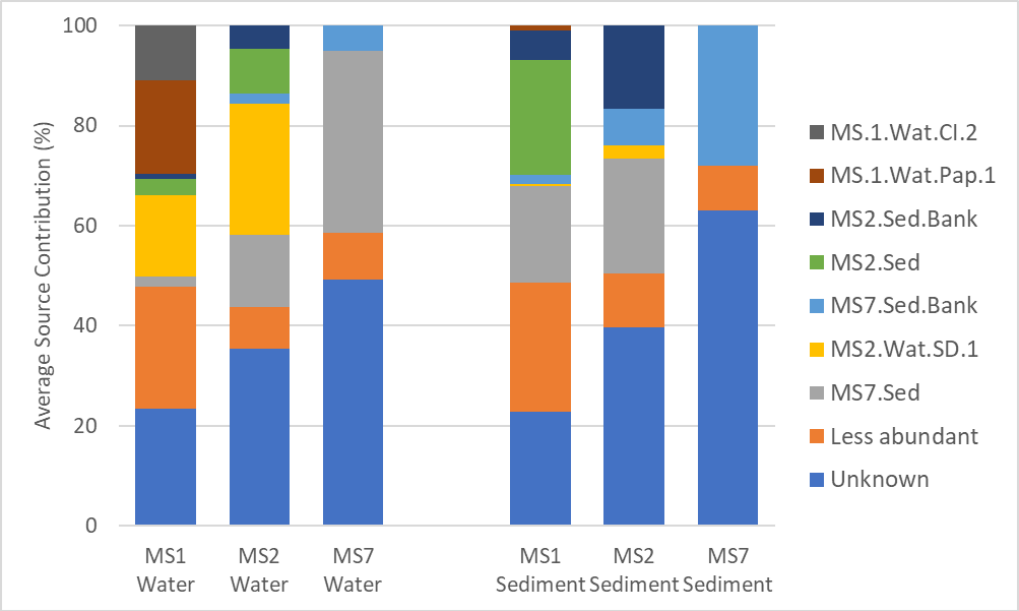


Table 3-7: Table of Mean Percentage of Source Contributions to Keene Creek

Source Label	Description and Reach	Sink					
		Water			Sediment		
		MS1	MS2	MS7	MS1	MS2	MS7
MS1.Wat.CI.2	Catch basin inlet, MS-1	10.9	NA	NA	0.0	NA	NA
MS1.Wat.Pap.1	Paper plant effluent, MS-1	18.7	NA	NA	1.0	NA	NA
MS1.Wat.SD.2	Storm drain outfall, MS-1	5.1	NA	NA	5.8	NA	NA
MS1.Wat.WTL.1	Wetland effluent, MS-1	8.7	NA	NA	5.4	NA	NA
MS1.Sed	Streambed sediment, MS-1	6.3	NA	NA	NA	NA	NA
MS1.Sed.Bank	Bank sediment, MS-1	0.1	NA	NA	6.7	NA	NA
MS1.Sed.RIP	Riparian sediment, MS-1	0.0	NA	NA	3.4	NA	NA
MS2.Sed	Streambed sediment, MS-2	3.3	8.8	NA	23.1	NA	NA
MS2.Sed.Bank	Bank sediment, MS-2	1.0	4.7	NA	5.8	16.6	NA
MS2.Sed.RIP	Riparian sediment, MS-2	2.2	7.5	NA	2.0	8.8	NA
MS2.Wat.CI.1	Catch basin inlet, MS-2	2.1	0.0	NA	0.4	0.1	NA
MS2.Wat.SD.1	Storm drain inlet, MS-2	16.3	26.1	NA	0.4	2.6	NA
MS7.Sed	Streambed sediment, MS-7	1.9	14.4	36.5	19.3	23.1	NA
MS7.Sed.Bank	Streambank sediment, MS-7	0.0	2.2	5.0	1.8	7.3	28.0
MS7.Sed.Rip	Riparian sediment, MS-7	0.0	1.0	4.6	2.0	1.7	8.9
Sewage	Raw human sewage, MS-1	0.0	0.0	0.0	0.0	0.0	0.0
Dog	Dog waste, MS-3	0.0	0.0	0.0	0.0	0.1	0.0
Goose	Goose waste, MS-1	0.0	0.0	4.7	0.0	0.1	0.0
Unknown		23.4	35.3	49.2	22.9	39.6	63.1

NA- Indicates that the source was not included in library configuration

3.2 Tischer Creek Dry Weather Assessment

The results of the Tischer Creek dry weather assessment are described in this Section.

3.2.1 Baseline Monitoring

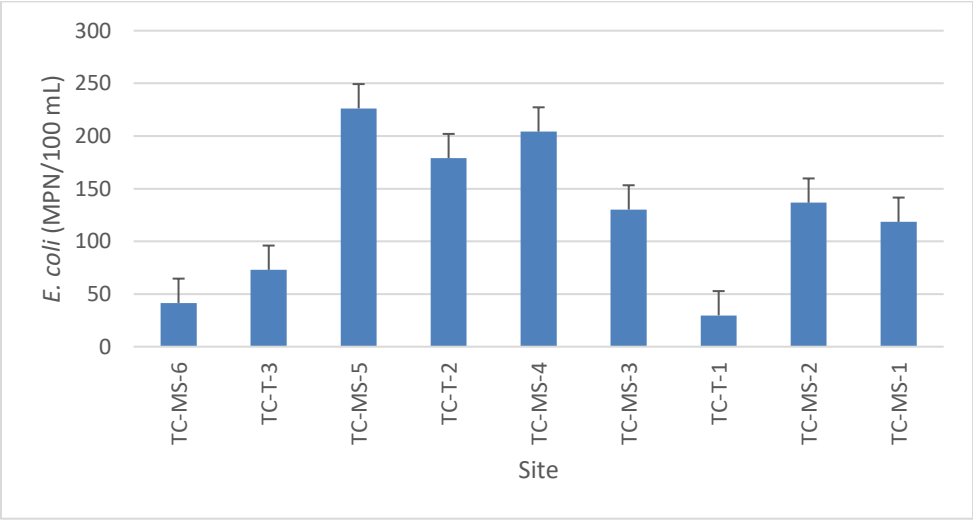
The baseline monitoring results for *E. coli* and molecular markers are presented below.

3.2.1.1 *E. coli* Concentrations

A total of 49 dry weather samples (at least 48 hours after the last rain event) were collected and analyzed for *E. coli* from Tischer Creek during the baseline monitoring. Samples were collected and analyzed from six mainstem monitoring sites (TC-MS-1 through TC-MS-6) and three tributary sites (sites TC-T1, TC-T2, and TC-T3) over six monitoring events from August 23 to September 24, 2019 (not all sites were monitored during all events). Spatial patterns of *E. coli* concentrations among the Tischer Creek baseline monitoring sites are depicted as geometric mean concentrations plus one standard error (SE) on Figure 3-8.

Mean concentrations from samples collected over the course of the dry weather monitoring at Tischer Creek were lowest at the upper-most mainstem site in the Study Area (Site TC-MS-6) and tributary sites TC-T-1 and TC-T-3. Over the course of the baseline monitoring at these three sites, concentrations from individual samples were less than 100 MPN/100 mL except for one sample collected at TC-T-3 (which had a value of 102 MPN/100 mL). The greatest mean concentrations were observed at mainstem sites TC-MS-5, TC-MS-4 (just downstream of TC-MS-5) and tributary site TC-T-2. Geometric mean values for these sites were 222.6 MPN/100 mL, 204 MPN/100 mL, and 178.9 MPN/100 mL, respectively. The remaining sites had generally lower concentrations.

Figure 3-8: *E. coli* Geometric Mean Concentrations (+1 SE) at Tischer Creek Monitoring Sites



3.2.1.2 Molecular Markers

The results of the samples collected for molecular analyses during the Tischer Creek baseline monitoring are summarized in Table 3-8. A total of 27 samples were collected over the course of three sampling events (September 24, September 26, and October 8) for molecular analyses from mainstem Site TC-MS-5 and tributary site TC-T-2 (which had the greatest *E. coli* concentrations during the baseline monitoring) and from the base of the Study Area at mainstem Site TC-MS-1. All samples were analyzed for the three molecular markers (bird, dog, and human). Among the samples collected, five were positive for the bird marker (55.6 percent), none were positive for the dog marker, and four were positive for the human marker (44.4 percent). The human marker was positive in all three samples collected from tributary Site TC-T-2 and from one sample collected from mainstem Site TC-MS-1 at the base of the watershed. All blank samples collected during the monitoring were negative for all three markers.

Table 3-8: Tischer Creek Baseline Monitoring Results for Molecular Markers

Marker	Sample ID	Date Sampled	Sample Result	Percent Positive
Bird	MS-1	09/24/19	No	55.6%
	T-2		**Yes**	
	MS-5		No	
	MS-1	09/26/19	No	
	T-2		**Yes**	
	MS-5		No	
	MS-1	10/08/19	**Yes**	
	T-2		**Yes**	
	MS-5		**Yes**	
Dog	MS-1	09/24/19	No	0.0%
	T-2		No	
	MS-5		No	
	MS-1	09/26/19	No	
	T-2		No	
	MS-5		No	
	MS-1	10/08/19	No	
	T-2		No	
	MS-5		No	
Human	MS-1	09/24/19	No	44.4%
	T-2		**Yes**	
	MS-5		No	
	MS-1	09/26/19	No	
	T-2		**Yes**	
	MS-5		No	
	MS-1	10/08/19	**Yes**	
	T-2		**Yes**	
	MS-5		No	

3.2.2 Sanitary Survey

A Sanitary Survey was conducted over the entire Tischer Creek Study Area from September 16 through 19, 2019. Each drainage area corresponding to the six mainstem sites and three tributary sites were assessed through visual observations and photo-documentation and "spot samples" were collected from suspected sources of *E. coli* in the drainage. Survey methods are described in Subsection 2.2.1 and the results are presented below.

3.2.2.1 Observations

The results of the Sanitary Survey are summarized in Table 3-9 with select photos provided on Figure 3-9. There was no evidence of bacterial sources originating from the municipal sanitary system. The field team did not observe evidence of leaky sewer pipes, illicit connections to the MS4, septic systems/leach fields, or any other evidence of leaking sanitary systems that could convey *E. coli* from human origin to the creek receiving waters. There was also no evidence of homeless encampments observed in the watershed.

Relatively few sightings of dogs (on leash or otherwise) were observed in the Tischer Creek Study Area and we are not aware of any dog parks in the Study Area. Dogs on leash were observed on the University of Minnesota, Duluth (UMD) campus near the stadium, along Ewing Avenue and West Owatonna Street, and West Louis Street and Dunedin Avenue. All dogs observed in the residential neighborhoods were on leash and there was no evidence of dog waste anywhere in the Study Area. Several dogs were observed on the walking trail in Hartley Park (accessed at the trailhead at the end of Hartley Road, just west of Woodhaven Lane. Dogs were observed both on and off leash, but owners were present whenever a dog was observed. Signage and doggie bags were observed at the trailhead and there were no observations of dog waste anywhere in Hartley Park. We are not aware of a dog park within the Tischer Creek Study Area. A variety of songbirds and crows were observed in the Study Area, but sightings were relatively minimal. No other wildlife (including other birds) were observed in large numbers anywhere in the Study Area.

Decaying plant material and sediment in the catch basin inlets, degraded habitat, stagnant water, and wetland bogs were frequent observation in Reach T-2 (also known as the West Branch of Tischer Creek, see Figure 2-2). The T-2 tributary reach makes a large meander from the confluence of the tributary with the mainstem. From the mouth, the tributary crosses under West Saint Marie Street at Woodland Avenue, runs southwest towards Elizabeth Street, then north along the eastern side of the UMD campus where it crosses under West Saint Marie Street again near Midway Avenue and the entrance to the UMD campus. This reach of tributary T-2 had several locations where potential *E. coli* sources were identified, primarily

associated with the potential for regrowth of *E. coli* in the environment that has been associated with fine-grained sediments and stagnant water (see Chapter 4.0). The potential *E. coli* sources identified in this reach of the T-2 tributary are discussed below as one moves upstream from the confluence of the tributary with the mainstem just upstream of Woodland Avenue.

- **Storm drain outfall –** There are two storm drain outfalls on the downstream side of West Saint Marie Street where find-grained sediment and organically-rich ponded water has accumulated. The outfalls discharge on either side of the main flow of the creek. The grade is very flat in this region (as it is throughout the reach) and it appears to be an area of sediment deposition.

- **Eroding banks and organic debris –** Between West Saint Marie Street and North Street, there are several areas along the right bank of the T-2 tributary where eroded banks were observed. The largest area is behind the Republic Bank, where the asphalt in the alley has been severely eroded, forming a small sink hole.

- **Degraded Pond –** Just downstream of Norton Street, the T-2 tributary passes through a stagnant pond where organic debris and fine-grained sediment has accumulated (see photos on Figure 3-9). Sampling of the pond revealed very fine-grained sediment with a gelatinous consistency and a foul (hydrogen sulfide) odor. The pond is adjacent to the foundation of a house and appeared to be formed by a debris dam (appeared to be organic) and emergent vegetation just downstream. Flow through this area of the creek was extremely slow and the water had stagnated. Concentrations of *E. coli* collected from the pond were very high (see Subsection 3.2.2.2).

- **Fouled storm drain infrastructure** – Organic debris and sediment that has accumulated in the streets in this area were also identified as potential sources of *E. coli*. Storm drain catch basins along Norton Street, Waverly Avenue, and Marion Street (which parallel the left bank of the tributary) were nearly completely clogged with debris, primarily leaf litter and organics, but also sediment from front lawns and sidewalks. In some areas along Waverly Avenue south of Norton Street, the curb had been destroyed, and large amounts of sediment clogged the gutter and catch basin inlet.

- **Mulch stockpiles** – South of Marion Street along Waverly Avenue, there is a large stockpile of organic mulch directly on the bank of the creek. BMPs had been installed between the stockpile and the creek, but close proximity of the organic stockpile and the creek receiving water suggest that the stockpile may be a source of E. coli to the creek, particularly during storm events.

- **Storm drain outfall from campus –** South of Marion Street at Waverly Avenue, the tributary turns north and runs along the east side of the UMD campus. On the right bank of the creek, approximately 250 feet upstream of the intersection between Waverly Avenue and Elizabeth Street, there is a PVC pipe (approximately 12-inch diameter) that sticks out from the bank, apparently originating from the campus parking lot. The pipe was not flowing during the Sanitary Survey, but may be a source of *E. coli* during storm events.

- **Ponded water –** On the left bank of the tributary at Norton Street and Carver Avenue, there is a large area of ponded water adjacent to the park on the west side of Carver Avenue (see photos on Figure 3-9). This ponded area is full of organic material and sediment and water was present throughout the duration of the study. It is unclear if there is a catch basin inlet beneath the water surface that may be plugged, but the water had very high *E. coli* concentrations.

- **Grassy swale –** The ponded water at Norton Street and Carver Avenue drains to tributary T-2 via a grassy swale directly west of Carver Avenue and adjacent to the creek. The grassy swale drains directly to the T-2 tributary through a wetland bog and also had high *E. coli* concentrations (see Subsection 3.2.2.2).

- **Wetland bog –** directly north of the grassy swale described above is a wetland bog that the T-2 tributary flows through (see photos on Figure 3-9). This area is characterized by a large amount of organic material, fine-grained sediments, and debris jam (organic material) that has created stagnant water to build up in the area. There was some evidence of beaver activity in this area as well, although it did not appear to be recent and there was no sign of beavers in the area. High concentrations of *E. coli* were documented from both sediment and water samples collected from the wetland bog (see Subsection 3.2.2.2).

The gradient above the portion of the T-2 reach described above increases substantially upstream of East Saint Marie Street and Midway Avenue. The riparian habit is well-developed upstream of East Saint Marie Street and there were no indications of eroded banks or fouled storm drain infrastructure. Samples were collected in this area (including the tributary from Rock Pond on the UMD campus and sites north of West Arrowhead Road) and *E. coli* concentrations were low (see Subsection 3.2.2.2).

In addition to tributary Site T-2, potential *E. coli* sources were observed in reach MS-5 at several locations (Table 3-10), as described below.

- **Mulch stockpile –** Just upstream from the confluence of the mainstem site MS-5 with the T-2 tributary (West Branch of Tischer Creek), on the left bank is a cul de sac at the southern end of

Columbus Avenue. A large stockpile of what appeared to be fertilizer and/or mulch was piled up at the end of the cul de sac, which sits at the top of the bank of Tischer Creek. Standing water had pooled behind it on the creek-side of the stockpile and it was apparent that water from the stockpile had flowed down the bank toward the creek. There were no BMPs in place to prevent runoff to the creek. Water samples collected from the pooled water had very high *E. coli* concentrations (see Subsection 3.2.2.2).

- **Storm drain outfall –** There are relatively few storm drain outfalls in the lower portion of the MS-5 reach that discharge directly to Tischer Creek. One of the larger outfalls is just downstream of West Arrowhead Road on the right bank, which drains a fairly large area in this part of the Study Area (see Figure 2-2). Fined-grained sediment and organic debris has accumulated at the base of the outfall, creating a pool of stagnant water and accumulated debris. Other outfalls in the reach did not appear to have the same conditions. In addition to the storm drain outfall at this site, there are several homes with lawns directly adjacent to the stream bank with no buffer strip or BMPs to prevent sheet runoff from the lawns to the creek during storm events or periods of irrigation. High *E. coli* concentrations are often associated with residential lawns, thus these areas may be a source of *E. coli* to the creek.

- **Wetland bog –** There is a large bog that discharges to mainstem of Tischer Creek at West Louis Street and Harvard Avenue. The bog originates at two small ponds located on West Saint Louis Street and Harvard Avenue. Water from the ponds flows downgradient to the southeast through an organically rich series of wetland pools and marshes. The bog discharges to the mainstem just upstream of a stone walking bridge at West Hardie Street and Columbus Avenue. Water samples collected from the bog at the point of discharge to the creek had vey high E. coli concentrations (see Subsection 3.2.2.2).

- **Construction debris** – During the Sanitary Survey, road construction (apparently associated with cable laying operations) was taking place in the upper part Woodland Avenue between West Oxford Street and Saint Paul Avenue (see Figure 2-2). Sediment from the construction activities had filled the gutters along Woodland Avenue (both side of the road) with soil, which also covered the road in this area (Figure 3-9). Catch basin BMPs (filter socks) had been installed at some locations, but had not been maintained and were no longer preventing sediment from entering the storm drain. Major road construction was also taking place during the Sanitary Survey at Woodland Avenue and Calvary Road in the upper part of the Study Area; however, it was unclear if sediment from the construction site was entering the storm drain infrastructure.

Table 3-9: Potential Bacterial Sources Identified in the Sanitary Survey Investigation of the Tischer Creek Study Area

General Category	Potential Source/Activity	Observation
Municipal Sanitary Infrastructure (piped)	Sanitary sewer overflows	Not directly observed during the sanitary survey, but the team was informed of a sewage leak that occurred on September 9, 2019 upstream of Site TC-MS-5
	Combined sewer overflows (CSOs); regulated under NPDES/LTCP	Not observed
	Leaky sewer pipes (Exfiltration)	Not observed
	Illicit sanitary connections to MS4	Not observed
	Wastewater Treatment Plans regulated under NPDES	Not observed
	Leaky or failing septic systems	Not observed
MS4 Infrastructure	Illegal dumping	Not observed
	Biofilms/regrowth	Observed at mainstem sites and at tributary site T-2
	Decaying plant matter, litter and sediment in the storm drain system	Observed throughout the Study Area in street gutters at multiple locations
Other Human Sanitary Sources (some also attract urban wildlife)	Homeless encampments	Not observed
	Temporary toilets (e.g., Porta-Potties)	Not observed
	Dumpsters (e.g., diapers, pet waste, urban wildlife)	Dumpsters were observed in mixed use areas, but all were well-maintained
	Trash cans	Trash cans were observed throughout the Study Area, particularly at parks, but all were well maintained
	Garbage trucks	Not observed on days when sanitary surveys were conducted
	Other wildlife attracted to human sources (deer, coyotes, feral cats, etc.)	No other wildlife was observed in the Study Area attracted to human sanitary sources
Other Urban Sources (including areas that attract vectors)	Food processing facilities	Not observed
	Outdoor dining	Not observed
	Restaurant grease bins	Not observed
	Bars/stairwells (wash-down areas)	Not observed

General Category	Potential Source/Activity	Observation
Urban Non-stormwater Discharges (potentially mobilizing surface-deposited bacteria)	Power washing	Not observed
	Excessive irrigation/overspray	Not observed
	Car washing	Not observed
	Pools/hot tubs	Not observed
	Reclaimed water/graywater (if not properly managed)	Not observed
Domestic Pets	Dogs, cats, etc.	Dog waste was not observed in the Study Area and dog walking was infrequent, except on Hartley Road Trail where numerous dog walkers were observed. Dog waste signage and dispensers were available at the trail head off Hartley Road near Woodhaven Lane.
Urban Wildlife (naturally occurring and human attracted)	Rodents/vectors (rats, raccoons, squirrels, rabbits, opossums)	Minimal evidence of urban wildlife and no feces from these animals were observed. No evidence of rats, raccoons, or opossums was observed.
	Birds (geese, ducks, gulls, crows, pigeons, songbirds, etc.)	A variety of songbirds and crows were observed in the Study Area, particularly in less urban areas, such as Hartley Nature Center and trail.
Recreational Sources	Bathers and/or boaters	Not observed
	RVs (mobile)	Not observed
Open Space/ Forested Areas	Wildlife populations	Other than birds, no other wildlife observed
	Grazing	Not observed
Other Sources	Plants/algae, soil (naturalized *E. coli*)	Extensive road construction along Woodland Avenue between West Oxford Street and Saint Paul Avenue and at Woodland Avenue and Calvary Road. Soil erosion prevent BMPs appeared to be inadequate along Woodland Avenue. Very turbid water was apparent in the mainstem below Site TC-T-3 during one of the reconnaissance visits, possibly due to construction-related soil. A large wetland bog is located off West Louis Street and Harvard Avenue that discharges to the mainstem near Columbus Avenue. Severely degraded habitat and poorly maintained catch basins at TC-T-2 at Norton Street and Waverly Avenue. Wetland bogs, large ponded areas, and swales that drain to mainstem at Carver Avenue and Norton Street.

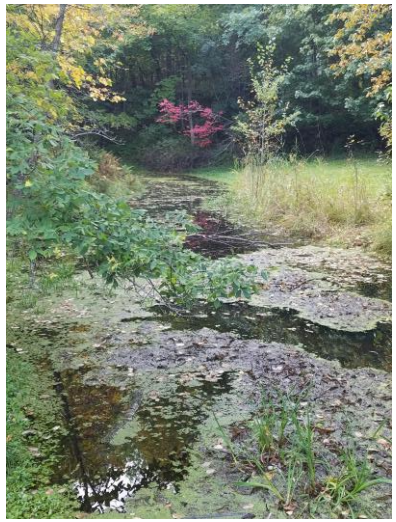
Source: Modified from Armand Ruby Consulting (2011)

Figure 3-9: Photographs of Potential Bacterial Sources Observed During Sanitary Surveys of the Tischer Creek Study Area

Clogged catch basin at MS-5

Construction sediment at MS-5

Storm drain outfall at MS-5

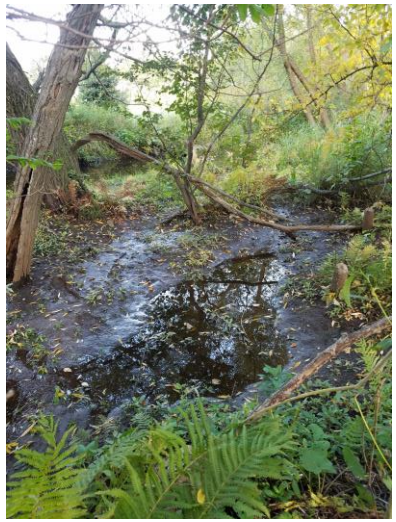


Wetland bog at upper MS-5

Degraded pond at T-2

Ponded water that drains to T-2

Grassy swale at T-2



Wetland bog at T-2

3.2.2.2 *E. coli* Concentrations

In addition to the visual observations conducted during the sanitary survey investigation, a limited number of spot samples were collected from various sources within the Study Area. All samples were collected on September 18 and 19, 2019. The results are summarized in Table 3-10. The samples were separated into three groups based on location in the Study Area and the potential source:

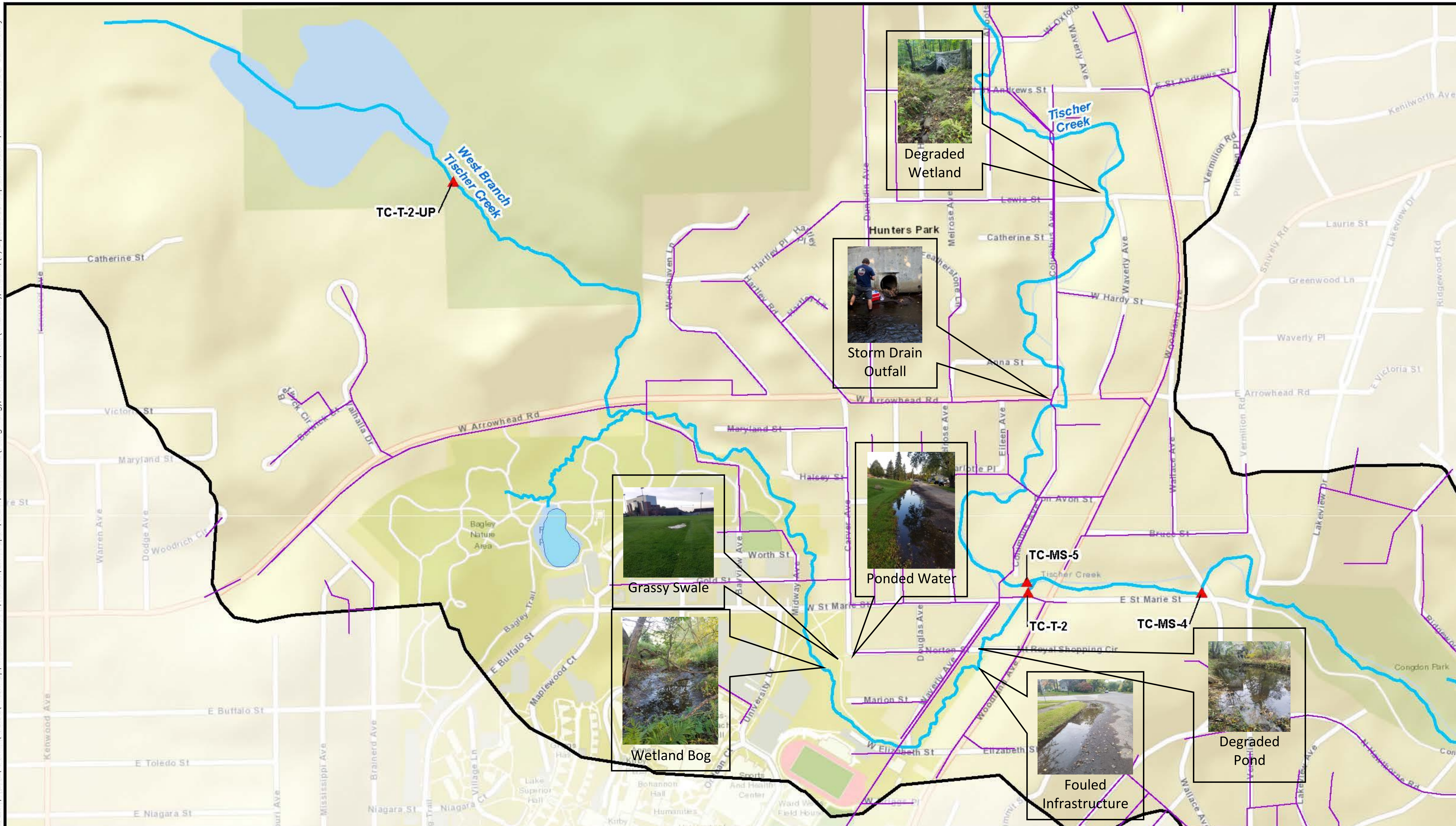
1. Sites in reach TC-T-2 near UMD campus (off stream potential sources)
2. Sites in upper reach TC-T-2 upstream of UMD campus (in stream)
3. Sites in reach TC-MS-5 (off stream potential sources)

The first group consisted of puddles, swales, wetland bogs, and catch basins that were located adjacent to Tischer Creek near the UMD campus and had the potential to influence creek surface waters (Table 3-10). The results were extremely variable, and ranged from 28 MPN/100 mL to > 2,420 MPN/100 mL. the greatest concentrations were associated with roadside puddles that drained to the creek, a swale at Carver avenue and Norton Street, water form a catch basin inlet at Waverly Avenue and Marion Street, and a small wetland in a creek meander off Carver Avenue. All of these sites drain directly to the T-2 tributary that winds through the campus and are potential sources of *E. coli* to the creek receiving waters.

Table 3-10: *E. coli* results from the Tischer Creek Sanitary Survey Investigation

Sample ID	*E. coli* (MPN/100 mL)	Reach	Site Description
Sites in reach TC-T-2 near University of Minnesota Duluth campus (off stream potential sources)			
TC-T2-A	> 2,420	TC-T-2	Pond at Norton St. & Waverly Ave.
TC-T2-D-1	> 2,420	TC-T-2	Ponded water across from stadium, left bank
TC-T2-E	422	TC-T-2	Ponded water at swale at Carver Ave. & Norton St
TC-T2-C	308	TC-T-2	Grassy swale across from stadium, left bank
TC-T2-F	28	TC-T-2	Grassy swale across from stadium, right bank
TC-T2-D-2	> 2,420	TC-T-2	Grassy swale, Carver Ave. and Norton St.
TC-T2-B	1,986	TC-T-2	Catch basin inlet at Waverly Ave. & Marion St.
TC-T2-G	1,565	TC-T-2	Wetland, left bank, off Carver Ave.
Sites in upper reach TC-T-2 upstream of University of Duluth campus (in stream)			
TC-T2-I	54	TC-T-2-Up	Beaver Pond just upstream of T-2-UP
TC-T2-H	93	TC-T-2 Trib	Rock Pond tributary at confluence with T-2
TC-T2-J	62	TC-T-2	T-2 mainstem, just upstream of W. Arrowhead Rd.
TC-T2-K	53	TC-T-2	T-2 mainstem just upstream of W. St. Marie St.
TC-T2-L	91	TC-T-2	T-2 mainstem just downstream of W. St. Marie St.
Sites in reach TC-MS-5 (off stream potential sources)			
TC-MS5-A	6,867	MS-5	Ponded water at end of Columbus Street
TC-MS5-B	6,867	MS-5	Major bog at Lewis and Harvard
TC-MS5-C	301	MS-5	Detention basin at Hartley Nature Center

A map of the potential sources of *E. coli* in the Tischer Creek Study Area is shown on Figure 3-10.



▲ Monitoring Locations
Storm Drain
Tischer Creek
Tischer Creek Study Area
City Boundary

500 250 0 500
Scale in Feet
NORTH

BURNS McDONNELL

Figure 3-10: Map of Potential Bacterial Sources in Tischer Cfreek Study Area

Path: Z:\Clients\ENS\DuluthMN\118320_DuluthStreamAs\Studies\Geospatial\DataFiles\ArcDocs\Tischer_close_up11x17_Landscape.mxd jaclaussen 7/1/2020
Service Layer Credits: Sources: Esri, HERE, Garmin, USGS, Intermap, INCREMENT P, NRCan, Esri Japan, METI, Esri China (Hong Kong), Esri Korea, Esri (Thailand), NGCC, (c) OpenStreetMap contributors, and the GIS User Community

Source:

Issued: 7/1/2020

3.2.3 Special Study – Water and Sediment Characterization

Using the adaptive approach discussed in Chapter 1.0, a special study was designed that was based on the results of the baseline monitoring and sanitary survey. For Tischer Creek, two sites (TC-MS-5 and TC-T-2) were determined to have the greatest *E. coli* concentrations and greatest number of potential sources (due primarily to urbanization). Samples were collected from three locations within each of the two reaches (MS-5 reach and T-2 reach): at the bottom (designated as sample A), middle (sample B), and top (sample C) of each reach. In addition, three similar samples were collected from the reach near the top of the T-2 tributary in Hartley Park, which has very little urban influence and is referred to here as a relative "reference" site (T-2-Up) to compare to the urbanized reaches of MS-5 and T-2. In order to characterize the chemical, physical, and biological conditions within each reach that may contribute to elevated *E. coli* concentrations, samples were collected for analyses of water quality, sediment quality, and biological community parameters (both water and sediment). The results of the Water and Sediment Characterization Special Study for Tischer Creek are presented below.

3.2.3.1 Water Chemistry

The results of the Tischer Creek Water Characterization Special Study are presented in Table 3-11. Samples were collected and analyzed for a suite of water quality constituents: TKN, nitrate plus nitrite (listed as NO_3), TP, TOC, TSS and *E. coli*. Mean values are arithmetic means for chemical constituents and geometric means for *E. coli.*

Nearly all the mean concentrations of the chemical constituents assessed and *E. coli* were lowest at the T-2-Up reference site compared to the urbanized sites at MS-5 and T-2 (mean TOC at Site T-2, 10.9 mg/L, was slightly lower than that at T-2-Up, 10.7 mg/L) (Table 3-11). In general, TKN concentrations were two to four times lower at the reference site than the urbanized sites, NO_3 concentrations were below detection limit in all three reference site samples, TOC concentrations were half that observed at Site MS-5, and TSS concentrations at the reference site were below detection limit in two of the three samples, with a mean concentration two to three times lower than mean concentrations observed at the urbanized sites. The biggest differences between the three sites was for *E. coli*. *E. co*li concentrations in the three samples collected at the T-2-Up reference site were 20 MPN/100 mL or lower with a geometric mean concentration of 7 MPN/100 mL. in the two urbanized sites (MS-5 and T-2), *E. coli* concentrations ranged from 185 MPN/100 mL to over 11,000 MPN/100 mL. Geometric mean concentrations at the urbanized sites were 60 to 360 times greater than the mean concentration at the reference site.

Table 3-11: Tischer Creek Water Characterization Results

Site	TKN (mg/L)	NO_3 (mg/L)	TP (mg/L)	TOC (mg/L)	TSS (mg/L)	*E. coli* (MPN/100 mL)
TC-MS-5-Wat-A	0.57	0.19	0.04	21.20	0.76	185
TC-MS-5-Wat-B	1.20	0.22	0.20	23.90	1.40	1,722
TC-MS-5-Wat-C	1.60	0.15	1.10	15.40	1.70	231
Mean:	**1.12**	**0.19**	**0.45**	**20.17**	**1.29**	**419**
TC-T-2-Wat-A	0.63	0.18	0.07	9.90	0.80	2,613
TC-T-2-Wat-B	3.70	0.09	9.50	11.50	3.80	11,199
TC-T-2-Wat-C	2.30	0.05	0.71	10.70	2.40	583
Mean:	**2.21**	**0.11**	**3.43**	**10.70**	**2.33**	**2,574**
TC-T-2-Up-Wat-A	0.53	ND	0.05	10.90	0.54	16
TC-T-2-Up-Wat-B	ND	ND	0.04	10.90	ND	20
TC-T-2-Up-Wat-C	ND	ND	0.07	10.90	ND	1
Mean:	**0.53**	**ND**	**0.05**	**10.90**	**0.54**	**7**

3.2.3.2 Sediment Chemistry

The results of the Tischer Creek Sediment Characterization Special Study are presented in Table 3-12. Similar to the Keene Creek sediment characterization results, the chemistry patterns in Tischer Creek sediment did not reflect those observed in the water samples. Mean concentrations of TKN, TP, and TOC were lowest in sediment at Site MS-5. Concentrations of NO_3 were below detection limit in all samples except one sample at TC-MS-5-Sed-B, which had a concentration of 0.28 mg/kg. Urbanized Site T-2 had the greatest concentrations of TP and TOC, and had a TKN value only slightly less than the reference site. Sediment concentrations of *E. coli* were two to three times lower at the reference site (T-2-Up) than at the urbanized sites.

Table 3-12: Tischer Creek Sediment Characterization Results

Site	TKN (mg/kg)	NO_3 (mg/kg)	TP (mg/kg)	TOC (mg/kg)	*E. coli* (MPN/100 g)
TC-MS-5-Sed-A	361.0	ND	246.0	16,300	700
TC-MS-5-Sed-B	142.0	0.28	236.0	3,500	600
TC-MS-5-Sed-C	629.0	ND	307.0	14,400	25,000
Mean:	**377.3**	**0.28**	**263.0**	**11,400**	**2,190**
TC-T-2-Sed-A	753.0	ND	276.0	50,800	2,300
TC-T-2-Sed-B	3,010.0	ND	568.0	57,400	20,000
TC-T-2-Sed-C	1,540.0	ND	313.0	20,800	16,000
Mean:	**1,767.7**	**ND**	**385.7**	**43,000**	**9,029**
TC-T-2-Up-Sed-A	2,150.0	ND	248.0	13,200	2,200
TC-T-2-Up-Sed-B	2,030.0	ND	331.0	34,200	1,000
TC-T-2-Up-Sed-C	1,330.0	ND	243.0	35,900	900
Mean:	**1,836.7**	**ND**	**274.0**	**27,767**	**1,256**

Results are reported on a dry weight basis, adjusted for percent moisture, sample size, and any dilutions

3.2.3.3 Sediment Grain Size

The results of the Tischer Creek streambed sediment grain size analyses are presented in Table 3-13. Similar to the results of the Keene Creek grain size analysis, streambed sediment at the reference site in Tischer Creek (Site TC-T-2-Up) tended to have a larger grain size, with greater relative percentages of coarse gravel, fine gravel, and coarse sand than the two urbanized sites (MS-5 and T-2). Streambed sediment at the urbanized sites tended to consist of finer-grained material than the reference site, with greater proportions of fine sand and silt/clay.

Table 3-13: Tischer Creek Sediment Grain Size Results (values represent the percent abundance of each fraction per site)

Site	Coarse Gravel	Fine Gravel	Coarse Sand	Medium Sand	Fine Sand	Silt/ Clay
TC-MS-5-Sed-A	0.0	5.7	6.8	57.2	27.4	2.9
TC-MS-5-Sed-B	0.0	0.1	1.0	66.1	32.0	0.8
TC-MS-5-Sed-C	0.0	0.2	1.3	9.7	79.1	9.7
Mean:	**0.0**	**2.0**	**3.0**	**44.3**	**46.2**	**4.5**
TC-T-2-Sed-A	0.0	1.5	5.5	25.7	50.3	17.0
TC-T-2-Sed-B	0.0	0.1	1.4	9.6	34.4	54.5
TC-T-2-Sed-C	0.0	0.7	0.8	17.5	57.4	23.6
Mean:	**0.0**	**0.8**	**2.6**	**17.6**	**47.4**	**31.7**
TC-T-2-Up-Sed-A	7.7	18.6	21.7	35.2	9.8	7.0
TC-T-2-Up-Sed-B	14.2	34.5	11.7	19.7	13.7	6.2
TC-T-2-Up-Sed-C	0.0	0.4	5.1	33.8	49.5	11.2
Mean:	**7.3**	**17.8**	**12.8**	**29.6**	**24.3**	**8.1**

3.2.3.4 Canonical Correspondence Analysis (CCA)

The results of the CCA analysis of samples collected from Tischer Creek are presented on Figure 3-11 for water samples and Figure 3-12 for sediment samples. Three water samples were collected from each of the three reaches (MS-5, T-2, and T-2-Up) and analyzed with the water chemistry and *E. coli* results. Similarly, sediment samples from the three sites were compared to sediment chemistry, *E. coli*, and grain size results. Figure 3-11 shows that the receiving water samples in general tended to group together by site (MS-5 sites grouped together, T-2 sites grouped together, and T-2-Up sites grouped together). In addition, T-2 samples (particularly sample T-2-B) were associated with elevated concentrations of *E. coli*, TKN, TSS, and TP. These results are vey similar to those observed for the urbanized site MS-1 in Keene Creek (see Figure 3-4).

Tischer Creek sediment samples also tended to cluster by site, although more loosely than the clusters seen for water. In streambed sediment, Sample T-2-Sed-B tended to be associated with elevated concentrations of *E. coli*, TP, TOC, as well as higher percentages of fine-grained sediment (fine sand and silt). Sample T-2-Sed-B was collected from the badly degraded pond (see Figure 3-9) upstream of the mouth of the T-2 tributary with the Tischer Creek at Norton Street. Sediments in the pond appeared to have large amounts of decaying organic material, a very fine grain size, and had a hydrogen sulfide odor.

Figure 3-11: Tischer Creek Canonical Correspondence Analysis Results for Water Samples

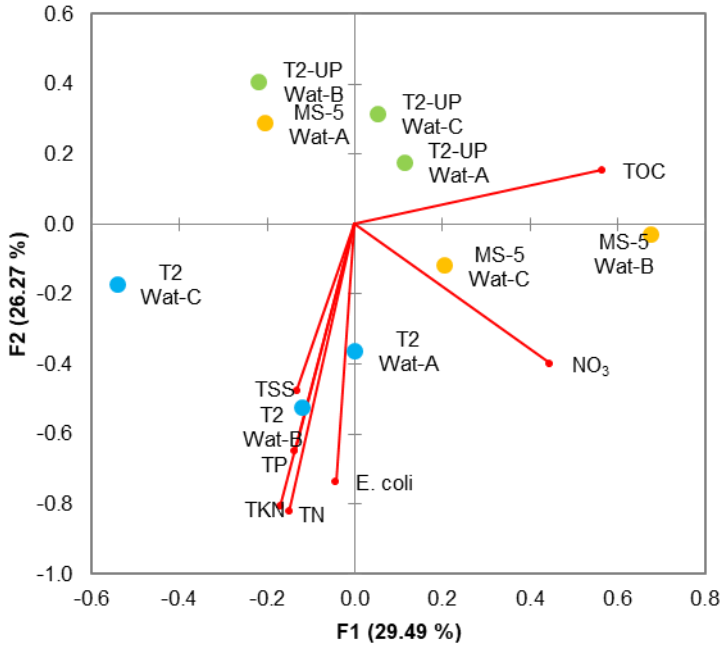
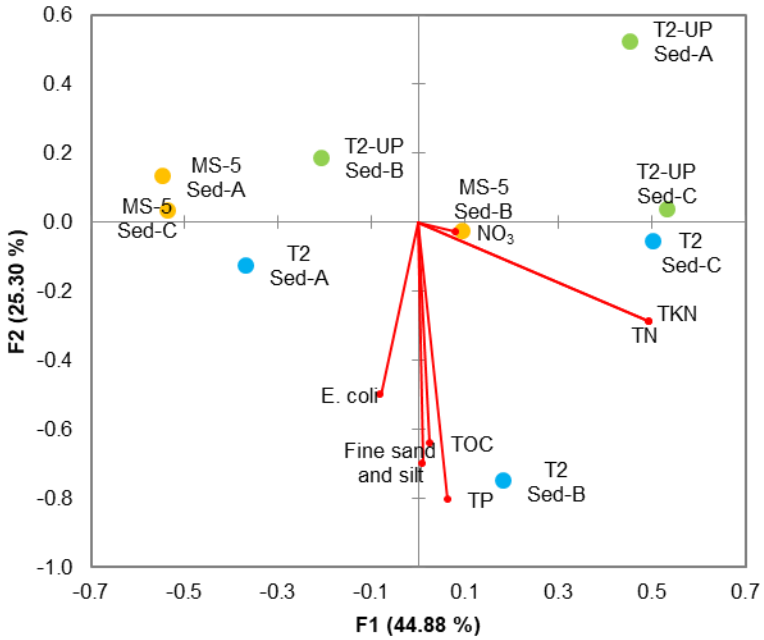


Figure 3-12: Tischer Creek Canonical Correspondence Analysis Results for Sediment Samples

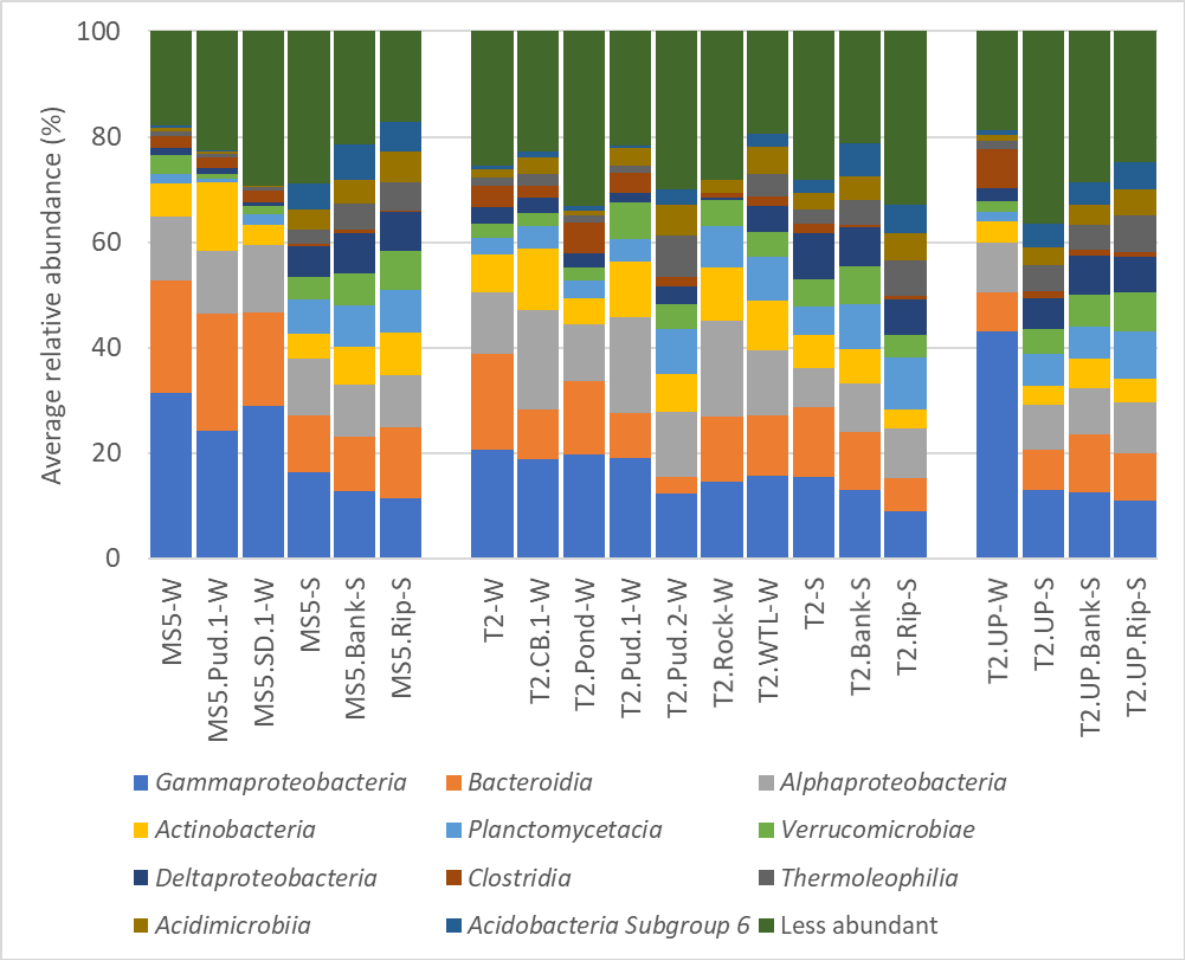


3.2.3.5 Bacterial Community Composition

The results of the bacterial community composition analysis are presented on Figure 3-13. Bacterial communities in water and sediment samples mostly consisted of members of the classes *Gammaproteobacteria*, *Bacteroidia*, *Alphaproteobacteria* and *Actinobacteria* (as well as the less abundant class-level taxa, represented as a mix of other class-level taxa on Figure 3-13). These results are very similar to those observed in Keene Creek.

Microbial community patterns in sediment were generally similar across all sites (MS-5, T-2, and T-2-UP) (Figure 3-13). Microbial community patterns in water also were similar among sites with a generally lower proportion of *Gammaproteobacteria* and *Bacteroidia* than was found in sediment samples and a larger proportion of more diverse taxa. The exception to that was reach T-2, where little difference was observed in microbial community structure between water and sediment samples.

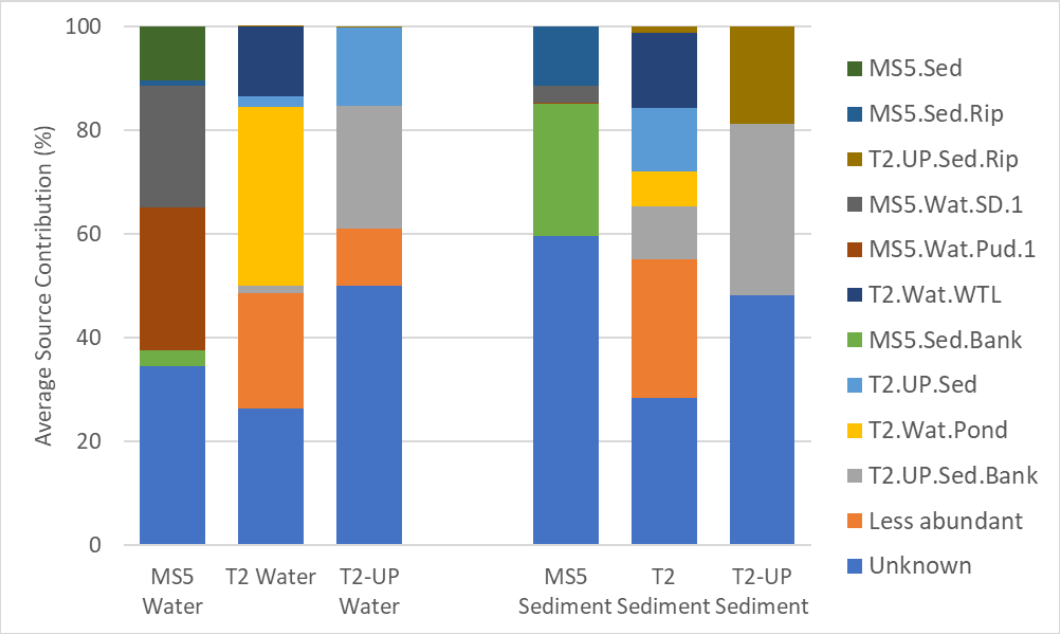
Figure 3-13: Tischer Creek Bacterial Community Composition (Class Level)



3.2.3.6 Source Tracker Analysis

The results of the SourceTracker analysis of water and samples collected from Tischer Creek are presented graphically on Figure 3-14 and numerically in Table 3-14. SourceTracker software was used to determine which sources of bacteria (from samples collected from a variety of suspected sources in MS-5, T-2, and T-2-Up reaches) were the major source contributors for a given "sink", where sink is defined as either Tischer Creek surface water at sites MS-5, T-2, or T-2-Up or as sediment at sites MS-5, T-2, or T-2-Up. Colors in the stacked bar chart on Figure 3-14 and values in Table 3-14 represent the mean percent contribution of each suspected source for a given sink. The means were derived from three samples collected from each suspected source. For each sink, the two identified sources with the highest percent contribution are highlighted in red text.

Figure 3-14: Graphic of Mean Percentage of Source Contributions to Tischer Creek



SourceTracker analysis revealed that the major sources of bacteria to Tischer Creek surface waters in the MS-5 reach were water from samples collected within the reach from ponded water at the southern end of Columbus Avenue (27.7 percent) and storm drain effluent from the storm drain outfall at West Arrowhead Road (23.3 percent) (Table 3-14). The major sources to receiving water collected in the T-2 reach were ponded water at Norton Street and Waverly Avenue (34.4 percent) and the wetland adjacent to the creek off Carver Avenue (13.5 percent). The major identified sources to receiving water collected at T-2-Up was T-2-Up bank sediment (23.7 percent) and T-2-Up streambed sediment (15.1 percent), but the largest proportion at this site was from unknown sources (50.1 percent).

Similar to the Keene Creek sediment results, the major sources of most sediment sinks in Tischer Creek originated from sediment sources. For example, the major sources of bacteria to Tischer Creek streambed sediment at the bottom of the MS-5 reach was streambank and riparian sediment at MS-5 (25.5 and 11.5 percent, respectively) (Table 3-14). For T-2 streambed sediment in Tischer Creek, the major sources were identified as the water from the wetland adjacent to the T-2 tributary off North Street (14.5 percent) and streambed sediment from T-2-Up (12.3 percent). For streambed sediment at the T-2-Up reference site, the major sources were the bank and riparian sediments at T-2-Up (33.2 and 18.7 percent, respectively).

Table 3-14: Table of Mean Percentage of Source Contributions to Tischer Creek

Source	Description and Reach	Sink					
		Water			Sediment		
		MS5	T2	T2-UP	MS5	T2	T2-UP
MS5.Wat.Pud.1	Puddle at end of Columbus St., MS-5	27.7	NA	NA	0.2	NA	NA
MS5.Wat.SD.1	Storm drain inlet, MS-5	23.3	NA	NA	3.3	NA	NA
MS5.Sed	Streambed sediment, MS-5	10.3	NA	NA	NA	NA	NA
MS5.Sed.Bank	Streambank sediment, MS-5	3.0	NA	NA	25.5	NA	NA
MS5.Sed.Rip	Riparian sediment, MS-5	1.1	NA	NA	11.5	NA	NA
T2.Wat	Receiving water, T-2	NA	NA	NA	NA	4.7	NA
T2.Wat.CB.1	Catch basin inlet, T-2	NA	3.6	NA	NA	3.1	NA
T2.Wat.Pond	Degraded pond, T-2	NA	34.4	NA	NA	6.7	NA
T2.Wat.Pud.1	Puddle 1, T-2	NA	3.3	NA	NA	4.7	NA
T2.Wat.Pud.2	Puddle 2, T-2	NA	1.5	NA	NA	3.0	NA
T2.Wat.Rock	Rock Pond, T-2	NA	8.5	NA	NA	0.0	NA
T2.Wat.WTL	Wetland, T-2	NA	13.5	NA	NA	14.5	NA
T2.Sed	Streambed sediment, T2	NA	3.5	NA	NA	NA	NA
T2.Sed.Bank	Streambank sediment, T2	NA	1.7	NA	NA	7.9	NA
T2.Sed.Rip	Riparian sediment, T2	NA	0.0	NA	NA	3.2	NA
T2.UP.Sed	Streambed sediment, T-2-Up	NA	2.0	15.1	NA	12.3	NA
T2.UP.Sed.Bank	Streambank sediment, T-2-Up	NA	1.5	23.7	NA	10.2	33.2
T2.UP.Sed.Rip	Riparian sediment, T-2-Up	NA	0.0	0.2	NA	1.2	18.7
Sewage	Raw human sewage, MS-1	0.0	0.0	1.1	0.0	0.0	0.0
Dog	Dog waste, MS-3	0.0	0.0	0.0	0.0	0.0	0.0
Goose	Goose waste, MS-1	0.0	0.0	9.8	0.0	0.0	0.0
Unknown		34.5	26.5	50.1	59.6	28.5	48.1

NA- Indicates that the source was not included in library configuration

4.0 DISCUSSION

The purpose of this Study was to identify the sources of *E. coli* that may be causing exceedances of water quality standards in Keene Creek and Tischer Creek and to use the information gathered from the Study to provide recommendations on bacterial-reduction BMPs that can be implemented to meet TMDL reduction targets. The project team used a weight of evidence approach to gather information on numerous potential sources of *E. coli* in each of the two Study Areas and applied a phased, tiered, and adaptive approach that has been shown to be successful in identifying bacterial sources in urban streams (City of Minneapolis, 2019; Goodwin et al., 2016; Griffith et al., 2013; Gruber et al., 2005). The design for this Study combined primary studies shown to be effective in other investigations with site-specific special studies based on the initial findings. The results indicate the sources of *E. coli* in Keene Creek and Tischer Creek are influenced by a dynamic process involving several factors, including insufficient maintenance of storm drain infrastructure, environmental reservoirs of *E. coli* with varying transport mechanisms that deliver bacteria to the creek, sources of *E. coli* originating from wildlife, soil from construction activities, degraded habitat, and likely contributions from naturalized *E. coli* in the environment.

4.1 *E. coli* Sources in the Keene Creek Watershed

The baseline monitoring conducted in Keene Creek revealed a strong spatial pattern of *E. coli* concentrations among the monitoring sites within the Study Area (Figure 3-1). Sites located in the lower portion of the watershed along the mainstem of Keene Creek (KC-MS-1 and KC-MS-2) consistently had the highest *E. coli* concentrations in all of the synoptic baseline monitoring events, suggesting that this portion of the watershed was contributing the majority of the *E. coli* to the Keene Creek receiving waters. This lower portion of the watershed is characterized by a flatter gradient compared to upstream reaches, urbanized land use that results in a large number of storm drain outfalls draining the urban infrastructure of the City to the receiving waters, and degraded habitat that was identified below Grand Avenue (i.e., below KC-MS-3 at Keene Creek Dog Park). Geometric mean concentrations of *E. coli* were approximately five times greater in the lower part of the watershed (sites KC-MS-1 and KC-MS-2) than all the other sties in the Study Area. These results suggest that future BMPs designed to reduce *E. coli* concentrations in Keene Creek should be focused on this area of the watershed.

The results of the molecular marker analyses conducted in Keene Creek indicate that the bird markers was positive in 22% of the samples collected (Table 3-1), suggesting that birds were a likely contributor to the *E. coli* in the receiving waters, but not necessarily a dominant source. The goose population (and associated goose waste) was prevalent at the Irving Park soccer field and the detention basin and catch

basin on the south side of the park provide a means of transporting elevated levels of *E. coli* to the Keene Creek mainstem. This source and associated transport mechanism represent an area where focused BMPs should be considered. In contrast, all or the samples analyzed for the dog marker were negative, indicating that dog waste was an unlikely contributor to *E. coli* in the receiving waters. These results are consistent with the baseline monitoring, which indicate that *E coli* concentrations were low at tributary Site KC-T-1 (adjacent to Keene Creek Dog Park) and at Site KC-MS-3 (just downstream of Keene Creek Dog Park). It is also consistent with the results of the Sanitary Survey conducted in the Keene Creek Watershed, where very minimal evidence of dog waste that may contribute to *E. coli* levels in Keene Creek were found. Together, the results indicate that dogs were an unlikely source of *E.coli* to the receiving waters of Keene Creek.

There was little evidence that human sewage was contributing to elevated *E coli* levels in Keene Creek. There was no evidence of failing septic systems or sewage infrastructure anywhere in the watershed and there were no signs that active homeless encampments were present. However, the percentage of positive results for the human marker in Keene Creek (33.3%) was higher than is typically seen in urban watersheds where failing sewage infrastructure is not present (Goodwin et al., 2016, Gruber et al., 2005, City of Minneapolis, 2019, Griffith et al., 2013). The sample size for the molecular monitoring was low for this element of Study (nine samples collected over three separate monitoring events) and all the positive samples were collected on the same day (see Table 3-1), so the results may not be reflective of true conditions in the watershed. Additional monitoring and investigation of the sewage infrastructure in the lower port of Keene Creek may be needed to fully address the extent to which *E. coli* originating from human sources is present.

The Sanitary Survey in Keene Creek did identify several areas in the lower portion of the watershed where degraded habitat and poorly-maintained stormwater infrastructure (e.g., clogged catch basin inlets) were present. Although degraded habitat may not be thought of as a source of *E. coli* in the traditional development and interpretation of fecal indicator bacteria (especially when compared to sources identified by molecular markers, which signal bacterial host origin), the presence of naturalized *E. coli* in the environment associated with both sediment and water sources is well-documented (see discussion below) and is considered in this assessment as potential source of *E. coli* to the receiving waters of both Keene and Tischer creeks.

Degraded habitat, severe erosion, and discharges from wetland bogs and the paper mill were found in several areas in the lower reaches of the watershed (and in many cases elevated levels of E. *coli*), particularly in reach MS-2, where severe erosion downstream of Grand Avenue and degraded habitat

upstream of South 57th Avenue West were particularly evident. Exposed streambank soil and degraded habitat characterized by stagnant, organically rich conditions can act as sources of *E. coli* by sequestering bacteria delivered from upstream sources and creating an environment that can amplify bacterial regrowth. In Keene Creek, degraded habitat, storm drain outfalls, and eroded banks in reaches MS-1 and MS-2 were identified as the dominant sources of *E. coli* in the Study Area.

The Water and Sediment Characterization Special Study demonstrated how the more urbanized reaches (e.g., MS-1 and MS-2, see Figure 1-4) provide an environment conducive to regrowth of *E. coli*. Keene Creek water in the urban areas had greater concentrations of nutrients and TSS and much greater concentrations of *E. coli* than the upstream reference site (this was also demonstrated in the CCA for water, see Figure 3-4). The main effect of urbanization on streambed sediments was observed in the differences in grain size between the urbanized and upstream reference site. Urban streambed sediments had a much smaller grain size than the sediments at the reference site, with much higher relative percentages of fine sand and silt/clay. A smaller grain size creates a larger surface area to volume ratio, which increases the potential for bacterial-binding. Thus, smaller gain size was the likely driver for the higher concentrations of *E. coli* observed in the Keene Creek sediments. Smaller grain size particles in the streambed are also more likely to be entrained in the water column than larger particles, which is consistent with the elevated TSS concentrations (and *E. coli* concentrations) observed in the Keene Creek water samples from the urbanized sites (MS1 and MS-2).

4.2 *E. coli* Sources in the Tischer Creek Watershed

In Tischer Creek, the baseline *E. coli* monitoring also revealed an important spatial pattern, although it was not as strong as that observed in Keene Creek. In Tischer Creek, mean *E. coli* concentrations were greatest at mainstem Site MS-5 and at the tributary site T-2 (Figure 3-8), both of which are upstream of mainstem Site MS-4 (which also had elevated *E. coli* concentrations compared to other sites). These results indicate that in Tischer Creek, these two areas of the watershed should be prioritized for bacterial-reduction BMPs. Several potential sources of E. coli were identified in these two reaches. The results of the molecular marker analyses conducted in Tischer Creek indicated that over 55 percent of the samples were positive for the bird marker. This is twice the percentage observed in Keene Creek and suggests that birds are likely an important source of E. coli to the receiving waters. Similar to Keene Creek, none of the samples in Tischer Creek analyzed for the dog marker were positive. These results are consistent with the Sanitary Survey in which no dog waste was observed in the Study Area and suggests that dogs are an unlikely source of *E. coli* to the receiving waters.

Similar to the results of the Keene Creek assessment, there was little evidence that human sewage was contributing to elevated *E coli* levels in Tischer Creek. There was no evidence of failing septic systems or sewage infrastructure anywhere in the watershed and there were no signs that active homeless encampments were present. However, the percentage of positive results for the human molecular marker was high. Four out of the nine samples analyzed from the Tischer Creek Study Area were positive for the human marker (Table 3-8), including all three of the samples collected from tributary Site T-2. Although the sample size was small for this element of the Study, future monitoring should be considered to determine the extent to which sewage infrastructure may be contributing to elevated *E. coli* levels in this reach of Tischer Creek.

The Sanitary Survey conducted in Tischer Creek also revealed several areas of degraded habitat, ponded water associated with insufficient storm drain infrastructure, and wetland bogs, all of which are likely contributors to elevated *E. coli* concentrations in the MS-5 and T-2 reaches. The largest potential source of this kind identified in the MS-5 reach was in the upper portion of the drainage at West Louis Street and Harvard Avenue. This large wetland area produced very high concentrations of *E. coli* that produced dry weather flows directly to the Tischer Creek receiving waters. The MS-5 reach was also characterized by storm drain outfalls with accumulated organic debris and stockpiles of mulch on the streambank without pollution prevention BMPs.

However, the largest potential source of *E. coli* in the reach was found in the upper portion of the drainage along Woodland Avenue between West Oxford Street and Saint Paul Avenue (see Figure 2-2). Cable-laying construction activities in this area generated a large amount of soil that had severely impacted the gutters, storm drain inlets, and adjacent street in this area. *E. coli* in the environment has been shown to adsorb rapidly to soil particles of all types, particularly soils with high clay content (Nola et al. 2005, Ling et al., 2003, Abu-Ashour and Lee, 1999) and can be released to receiving waters during rain events or other transport mechanisms (City of Minneapolis, 2019; Ling et al., 2009, Muirhead et al., 2006, Schillinger and Gannon, 1985). Thus, the soil generated form construction activities can act as a reservoir for *E. coli* that can be transported to the receiving waters when pollution prevention BMPs are not in place. The City of Minneapolis (2019) quantified the potential impact of construction-related soil on *E. coli* levels in downstream receiving waters as part of a larger scale bacterial source identification study. The study was designed to determine the extent to which construction-related soil and organic debris in the street gutters of the study area contained *E. coli*. The results suggested that *E. coli* levels in street gutter runoff containing soil associated with a cable installation project were thirty times greater than gutters without soil debris and the *E. coli* could be easily transported directly to the MS4 via runoff to the storm drain inlets. Similar results have been found in other studies (Skinner et al, 2010). Thus,

constructed-related soil (and organic debris) in the street gutters, when not properly managed, can act as a reservoir of *E. coli* (albeit temporary during the time of construction activities) that can be transported to local creeks through over-irrigation or storm events.

The tributary reach T-2 had the most degraded habitat observed in the Tischer Creek Study Area. The reach between the mouth of the tributary at the confluence with the mainstem just downstream of Site MS-5 and West Saint Marie Street near Midway Avenue and the entrance to the UMD campus had several areas of degraded habitat and other conditions that are the likely source of *E. coli* to the water of the creek. This reach was characterized by an accumulation of organic debris at storm drain outfalls, eroding banks, debris dams causing an accumulation of organically-rich sediment and stagnant water, ponded water due to insufficient drainage, bioswales, and wetland bogs. All of these areas had high *E. coli* concentrations in the water (and sediment in some cases) and act as potentially large sources of *E. coli* that can cause exceedances of water quality standards in the receiving waters of the tributary as well as downstream reaches of the Tischer Creek mainstem. Based on our assessment, the Tischer Creek T-2 reach should be considered as a high priority for potential restoration activities.

4.3 *E. coli* Sources in Stream Sediment and Soil

The concept that degraded habitat can be a source of *E. coli* to receiving waters is well-documented. The City of Minneapolis (2019) quantified *E. coli* in streambed sediment, streambanks, and riparian soil of an urban creek and found high concentration in all three of these zones, which act as environmental reservoirs that can introduce *E. coli* to the creek receiving waters. These results are similar to those of other studies in both tropical and temperate areas, where *E. coli* has been found in high concentrations in stream sediment, streambank soil, and riparian soil (Byappanahalli et al., 2012; Silyn-Roberts, 2012; Byappanahalli et al., 2006; Ishii and Sadowsky, 2008; Ishii et al., 2006; Gruber et al., 2005; Byappanahalli et al., 2003; Roll and Fujioka, 1997; Hardina and Fujikoa, 1991). For example, Byappanahalli et al. (2003) studied an urban stream in Michigan and found high concentrations of *E. coli* in these environmental reservoirs correlated significantly with those in the creek receiving waters and accounted for continuous loading of bacteria to the creek.

Byappanahalli et al. (2006) found frequent occurrence of *E. coli* in temperate forest soils contained within exclosures designed to prevent direct fecal deposition from wildlife. Using genetic techniques, they determined that *E. coli* can exist for extended periods of time in forest soil, independent of input from wildlife sources, and that the soil *E. coli* populations formed a cohesive phylogenetic group compared to *E. coli* from fecal sources. The authors concluded that soil-borne *E. coli* should be treated as a background concentration in source identification investigations. Thus, even in the absence of a known

contamination source, *E. coli* levels in streams may remain high as a result of input from adjacent soil reservoirs. Direct fecal input inadequately explained the widespread and consistent occurrence of *E. coli* in the watershed and suggested that long-term survival of *E. coli* in the sediment and soil habitats or multiplication in the environment was likely. Byappanahalli et al. (2012) found high densities of *E. coli* in a variety of soil types in Hawaii. In mesocosm studies, they demonstrated that *E. coli* inoculated on sterilized soil samples from the region increased two orders of magnitude (100-fold) in 4 days. They concluded that the *E. coli* identified in the stream sediment and streambank soil was part of a natural soil microfauna that had the potential to influence the quality of the stream receiving waters.

Ishii and Sadowsky (2008) described a conceptualized life cycle of *E. coli* in secondary habitats, such as water, sediment, and soil. *E. coli* is released from the primary host (warm-blooded animals) to the environment through direct deposition of fecal matter. The majority of the bacteria die due to environmental stresses outside the host, but some of them are able to survive longer as they become attached to physical structures in the environment, such as soil, sediment, or the surfaces of vegetation. In some cases, these strains can grow and maintain their populations long enough to survive and replicate and thus become adapted or "naturalized" to the environment.

High concentrations of *E. coli* found in sediment and soils in the sreambeds, streambanks, and riparian areas of both Keene Creek and Tischer Creek suggest that these areas act as large reservoirs for potential input of bacteria to the creek receiving waters. The extent to which the *E. coli* in these environmental reservoirs may be naturalized to the environment remains to be determined; however, the results from this Study and others suggest that these reservoirs can have a dramatic influence on *E. coli* levels in the creek receiving waters.

4.4 *E. coli* Sources in Biofilms

The storm drain infrastructure itself can also serve as a reservoir of *E. coli* to the receiving waters of urban creeks. Biofilms are matrices of bacteria and other microbes that form on various solid surfaces in the environment exposed to a liquid (Characklis and Marshall, 1990). Storm drain infrastructure with periodic urban flows, a steady supply of nutrients, and dark environments protected from ultraviolet radiation and desiccation are ideal environments for biofilm growth (Sylin-Roberts, 2012; Tiefenthaler et al., 2008). Storm drain systems therefore have the potential to act as reservoirs for *E. coli* and other fecal indicator bacteria within the biofilm matrix, and several studies have identified regrowth of fecal indicator bacteria within the urban MS4 infrastructure (City of Minneapolis, 2019; Goodwin et al., 2013; Balzer et al., 2010; Langmark et al., 2007; Silyn-Roberts, 2012; Griffith and Ferguson, 2012; Schultz-Fademrecht et al., 2008; Gruber et al., 2005). When environmental conditions are favorable for growth, bacteria in the

biofilm can replicate to high levels and eventually slough off, to be released into the water column where it can be transported downstream and become an intermittent or persistent source of bacteria to the receiving waters (Tiefenthaler et al., 2008). The extensive storm drain infrastructure and large number of storm drain outfalls in both the Keene Creek and Tischer Creek Study Areas where E. coli concentrations were greatest (Figure 1-4 and Figure 1-5, respectively) demonstrate the large potential for inputs of *E. coli* to the creeks in urbanized areas of the watersheds.

4.5 Urban Stream Syndrome

According to the US Environmental Protection Agency (EPA), the term "urban stream syndrome" describes the consistently observed ecological degradation of streams draining urban land (Walsh et al., 2005). Streams in urbanized areas are characterized by flashier hydrograph, elevated concentrations of nutrients and contaminants, altered channel morphology and particle size in the streambed, and increased suspended solids (TSS) in the water column. The mechanisms driving the syndrome are complex, but are primarily a result of impervious services in the urban landscape and an efficient drainage system that directs runoff rapidly to streams. Although the impacts of the urban stream syndrome have been well-studied, the effects of urbanization on levels of fecal indicator bacteria in the water column (e.g., *E. coli*) have not.

There are several characteristics of urban streams that may result in elevated *E. coli* concentrations in the receiving waters.

- Storm drain infrastructure in urbanized areas short circuit the natural attenuation of bacteria that occurs in un-urbanized watersheds that occurs through infiltration.

- Storm drain infrastructure in urbanized creeks promotes the growth of biofilms that act as a continuous reservoir of *E. coli* and other microbes that can be delivered to the creek receiving waters during high flow events.

- An increase in impervious surfaces and a storm drain infrastructure designed to efficiently move water away from structures and roads often leads to hydromodification of urban creeks, which erodes streambanks and exposes soil that contains *E. coli* to the receiving waters.

- Runoff from developed areas can alter the chemical makeup of the streambed sediment resulting in higher nutrient concentrations that may promote the growth of *E. coli* within the urbanized stream ecosystem.

- Runoff from urbanized areas can also change the physical characteristics of the streambed sediment by delivering fine-grained sediments to the creek, which increases the surface area to volume ratio of streambed sediment, essentially creating habitat for *E. coli* (and other microbes) within the urban stream.

The results of this Study suggest that these characteristics associated with the urbanized streams are the major factors that have increased the concentrations of *E. coli* in the receiving waters of both Keene Creek and Tischer Creek. One large review of the urban stream syndrome (Kominkova, 2012) emphasized that restoration is the only way to achieve good ecological status (health) of waterways affected by urbanization.

5.0 CONCLUSIONS

Several conclusions can be drawn from the Study. Conclusions presented below are organized by the study questions posed for the Keene Creek and Tischer Creek assessments.

Keene Creek:

1. **What are the potential sources of *E. coli* in Keene Creek (e.g., local wildlife, domestic animals, leaking sewer or septic lines, other human sources, natural, etc.)?**
   - Synoptic monitoring of seven mainstem and two tributary sites within the Study Area revealed that the greatest *E. coli* concentrations in Keene Creek during dry weather were found near the bottom of the watershed in reaches MS-1 and MS-2.
   - In general, *E. coli* concentrations were low at mainstem and tributary sites over the course of the Study, with no exceedances of the single sample water quality standard during dry weather.
   - Several potential sources of *E. coli* that were considered unlikely sources to Keene Creek include the homeless population, septic systems and sewer lines, illegal dumping, trash operations, outdoor dining and wash-down, and wildlife populations other than birds.
   - Car washing (possibly a persistent occurrence) was observed at one location in the watershed (in the alley off Raleigh Street, west of South 59th Avenue West) and transport of sediment-laden water to the creek was documented.
   - The Sanitary Survey, molecular markers, and spatial monitoring of Keene Creek Dog Park indicate that dogs are an unlikely source of E. *coli* to Keene Creek.
   - Birds were present throughout the Study Area, but only identified in large numbers at the Irving Park soccer field. Goose waste in this area is a likely source of *E. coli* to the creek.
   - Several areas of degraded habitat and eroded streambanks were observed in reaches MS-1 and MS-2 and likely act as a source of *E. coli* to the receiving waters.
   - The small tributary that apparently originates from the pulp mill had degraded water quality, high *E. coli* concentrations, and was shown to be a source of bacteria to the downstream receiving waters.

2. **How does bacteria survival, propagation, or re-growth contribute to *E. coli* levels in the storm drain system (e.g., leaf litter and grass clippings along curb lines or ditches) and discharge to surface waters of the creek?**

- Several locations were identified in the lower portion of the Study Area where leaf litter, organic debris, and soil had accumulated in the catch basin inlets.
- Ponded water associated with the clogged infrastructure was identified as a source of *E. coli* to the creek receiving waters and is a likely location for regrowth of *E. coli* to occur.
- Stagnant water created by debris dams (mostly organic) in reach MS-2 is a likely source of *E. coli* to the receiving waters.
- Streambed sediment in urbanized areas contained high concentrations of *E. coli* (potentially naturalized *E. coli*) and are likely source of bacteria to the receiving waters.

3. Does the *E. coli* in the Study Area originate from human sources?

- There was no evidence of active homeless encampments, leaking sewage infrastructure, septic systems, or other sources of *E. coli* from human waste, except temporary toilets in some locations observed anywhere in the Study Area, suggesting that *E. coli* from human source origin is unlikely.
- However, the percentage of positive results for the human molecular marker was higher than would be expected in an urban stream. The sample size for this element of the Study was small and additional assessment may be necessary to fully address this question.

4. How can the City adapt current management practices to reduce levels of *E. coli*?

- Several management practices that may contribute to elevated levels of *E. coli* in the creek were identified (see below) and include better maintenance of street infrastructure to prevent clogged storm drain inlets, management of goose waste to prevent introduction to the creek, stabilization of eroded streambanks, and restoration of degraded habitat.

Tischer Creek:

1. What are the potential sources of *E. coli* in Tischer Creek (e.g., local wildlife, domestic animals, leaking sewer or septic lines, other human sources, natural, etc.)?

- Synoptic monitoring of six mainstem and three tributary sites within the Study Area revealed that the greatest *E. coli* concentrations in Tischer Creek during dry weather were found at mainstem Site MS-5 and tributary Site T-2.
- In general, *E. coli* concentrations were low at mainstem and tributary sites over the course of the Study, with no exceedances of the single sample water quality standard during dry weather.

- Several potential sources of *E. coli* that were considered unlikely sources to Tischer Creek include the homeless population, septic systems and sewer lines, illegal dumping, trash operations, outdoor dining and wash-down, car washing, and wildlife populations other than birds.
- The Sanitary Survey and molecular marker results indicate that dogs are an unlikely source of E. *coli* to Tischer Creek.
- Birds were present throughout the Study Area, but were not identified in large numbers at any particular location. Over 55 percent of the bird molecular marker samples were positive, suggesting that birds are a likely source of *E. coli* to Tischer Creek.
- Several areas of degraded habitat, ponded water, eroded streambanks, and discharges from wetland bogs were observed in reaches MS-5 and T-2 and likely act as source of *E. coli* to the receiving waters.
- Soil from construction activities and insufficient BMPs in the upper portion of the MS-5 reach are likely sources of *E. coli* to the receiving waters.

2. How does bacteria survival, propagation, or re-growth contribute to *E. coli* levels in the storm drain system (e.g., leaf litter and grass clippings along curb lines or ditches) and discharge to surface waters of the creek?

- Several locations were identified in reaches MS-5 and T-2 where leaf litter, organic debris, and soil had accumulated in the catch basin inlets.
- Ponded water associated with the clogged infrastructure is a likely source of *E. coli* to the creek receiving waters and is a likely location for regrowth of *E. coli* to occur.
- Wetland bogs in reach MS-5 and T-2 are likely sources of regrowth of *E. coli* and had high concentrations of *E. coli* that were sources to the receiving waters.
- Streambed sediment in urbanized areas contained high concentrations of *E. coli* (potentially naturalized *E. coli*) and are likely source of bacteria to the receiving waters.

3. Does the *E. coli* in the Study Area originate from human sources?

- There was no evidence of active homeless encampments, leaking sewage infrastructure, septic systems, temporary toilets, or other sources of *E. coli* from human waste observed anywhere in the Study Area, suggesting that *E. coli* from human source origin is unlikely.
- However, the percentage of positive results for the human molecular marker was higher than would be expected in an urban stream. The sample size for this element of the Study was small and additional assessment may be necessary to fully address this question.

4. How can the City adapt current management practices to reduce levels of *E. coli*?

- Several management practices that may contribute to elevated levels of *E. coli* in the creek were identified (see below) and include better maintenance of street infrastructure to prevent clogged storm drain inlets, increased enforcement of construction BMPs to minimize soil (and associated *E. coli*) from entering the MS4, stabilization of eroded streambanks, and restoration of degraded habitat.

6.0 RECOMMENDATIONS

Based on the Study conclusions, the following recommendations are offered for consideration by the City.

General Recommendations for both Keene Creek and Tischer Creek.

- Assess and consider enhancing the street sweeping program to remove leaf litter and soil in street gutters, which were shown to be sources of *E. coli*.
- Implement and/or enforce BMPs for construction crews (contractor and City) to prevent construction-related soil from entering the storm drain system.
- Implement inlet protection at City parks (e.g., the soccer field at Irving Park in the Keene Creek Study Area) and other public facilities to prevent flow from grassy areas from entering the storm drain system during irrigation activities and storm events.
- Assess the use of fertilizer on City-owned properties and replace manure-based fertilizers with synthetic fertilizers, as appropriate.
- Implement and/or continue education and outreach BMPs that focus on preventing *E. coli* from entering the MS4. Messaging may include dog waste control (e.g., dog waste dispensers and signage), water conservation (preventing irrigation overflow from entering the MS4), and minimizing the accumulation of organic debris (leaf litter and grass clippings) in street gutters.
- Enhance the City's illicit discharge program to identify sources of *E. coli* in dry weather flows within the Study Area and implement BMPs as appropriate.
- Consider additional studies to better understand the potential health risks associated with *E. coli* in Keene Creek and Tischer Creek (such as a quantitative microbial risk assessment) and an associated assessment of the applicability of the existing standards.

Specific Recommendations for Keene Creek.

- **Prioritization:** the first priority in improving water quality in creeks impaired by *E. coli* is to identify the extent to which *E. coli* concentrations represent a threat to human health. In Keene Creek, the percentage of molecular samples that were positive for the human marker were relatively high, suggesting the potential presence of *E. coli* from human sewage. The sample size for the molecular marker testing in this Study was small and additional assessments of the potential for human sewage in reaches MS-1, MS-2, and MS-3 should be conducted first. The assessments should include the use of the human molecular marker (along with standard culture methods to enumerate *E. coli*) collected from the same sites used in this study in the lower reaches, collected synoptically, during dry weather. After several rounds of testing, assess the

data to determine the frequency of positive results for the human marker and determine if spatial patterns exist. These data can be used to determine if specific areas within the lower three reaches are consistently positive for the human marker, which would indicate a potential sewage source. If an area can be isolated, then further assessments should be conducted, such as an evaluation of sewer line integrity in the area (or nearby upstream areas). If sewage infrastructure problems are identified, repairing them as quickly as possible should be the major priority.

- The second priority in Keene Creek would be to implement the general BMPs outlined above (again, focusing on the lower three reaches of the Study Area). These BMPs represent the "low hanging fruit" because they are the easiest and most cost-effective to implement and because some of them are already established and may need to be enhanced or modified. These general strategies can often be the most effective in reducing *E. coli* concentrations in urban streams because they focus on source control of non-point sources that are common throughout urbanized areas.
- The third priority in Keene Creek is to implement structural BMPs and restoration activities that focus on restoring the integrity and natural stream processes that help attenuate *E. coli* levels in un-urbanized streams.
    - Identify areas where streambank erosion has occurred and implement streambank stabilization BMPs. In Keene Creek, streambank erosion was identified in the lower two reaches, as discussed in Subsection 3.1.2 and identified on the map on Figure 3-3. The most obvious area of streambank erosion was just downstream of Grand Avenue at the railroad overpass in the upper area of Reach MS-2.
    - Identify areas of degraded habitat where restoration activities could be prioritized and implemented. In Keene Creek, several areas were identified where degraded habitat was a likely contributor to elevated *E. coli* levels (see Subsection 3.1.2 and Figure 3-3). The most obvious areas in need of restoration is the lower portion of Reach MS-2 (between North 57th Avenue West and North 59th Avenue West) and the degraded wetland area and paper plant effluent downstream of South Central Avenue.
    - Identify areas where riparian buffers are minimal or not present and enlarge buffers where possible to prevent sheet flow runoff from adjacent grassy areas to the creek. In Keene Creek, areas that may be considered for riparian buffer improvements are Reach MS-3 Keene Creek Park (both at the Keene Creek Dog Park and just upstream across from the picnic tables) and at Irving Park where sheet flow from the grass fields is a likely contributor to elevated *E. coli* levels in the creek.

- **BMP Effectiveness Monitoring:** As BMPs are implemented, it is important to monitor their effectiveness in reducing *E. coli* levels in the receiving waters. BMP effectiveness monitoring typically consists of measuring *E. coli* concentrations upstream and downstream of the BMP or before and after implementation. The study design should be sufficiently robust (e.g., number and frequency of samples) to provide a statistical comparison of changes in *E. coli* concentrations due to BMP implementation.
- **Monitoring Program.** The effectiveness of specific BMPs in reducing *E. coli* concentrations should be one part of an overall strategy to improve water quality in Keene Creek and meet the goals of the TMDL. Water quality improvement strategies are typically incorporated into a stormwater management plan (SWMP) that outlines the goals and specific steps needed to achieve them for the watershed. It is recommended that for Keene Creek, the monitoring program should build off of this Study, using the results as a baseline for future assessments. Because *E. coli* concentrations were low in the upper part of the Study Area, we recommend that the City focus future monitoring in reaches MS-1, MS-2, and MS-3. Synoptic, dry weather (at least 24 hours after a rain event) surveys at the sites used in this Study should be considered for future monitoring programs for a consistent evaluation of water quality conditions over time (we recommend that wet weather assessments be considered after dry weather assessments and BMP implementation). Typically, monthly evaluations are sufficient to assess changes in water quality, but more frequent monitoring may be needed, depending on specific goals. The monitoring program in the SWMP should be considered as a living document with three basic steps: 1. Plan development, 2. BMP implementation, and 3. Assessment. These three steps are repeated to reach the overall goals of the SWMP.

Specific Recommendations for Tischer Creek.

- **Prioritization:** As with Keene Creek, the first priority in improving water quality in Tischer Creek is to identify the extent to which *E. coli* concentrations represent a threat to human health. In Tischer Creek, all of the samples collected form tributary Site T-2 were positive for the human marker, which suggests the potential presence of *E. coli* from human sewage in the T-2 tributary (West Branch of Tischer Creek). The sample size for the molecular marker testing in Tischer Creek was small and additional assessments of the potential for human sewage in the T-2 reach should be conducted first. The assessment should include the use of the human molecular marker (along with standard culture methods to enumerate *E. coli*) collected from the mouth of the T-2 tributary and several other locations within the reach. As with Keene Creek, the samples should be collected synoptically during dry weather. After several rounds of testing, assess the data to

determine the frequency of positive results for the human marker and determine if spatial patterns exist. These data can be used to determine if specific areas within the T-2 Reach are consistently positive for the human marker, which would indicate a potential sewage source. If an area can be isolated, then further assessments should be conducted, such as an evaluation of sewer line integrity in the area (or nearby upstream areas). If sewage infrastructure problems are identified, repairing them as quickly as possible should be the major priority.

- The second priority in Tischer Creek would be to implement the general BMPs outlined above. These general BMPs should be considered for all of Reach T-2, as well as Reach MS-4 and MS-5. Good housekeeping BMPs are a particular priority in Reach T-2 where storm drain infrastructure was clogged with debris (primarily leaf litter and organics), but also sediment from front lawns and sidewalks. In some areas along Waverly Avenue south of Norton Street, the curb had been destroyed, and large amounts of sediment clogged the gutter and catch basin inlet. These areas should be considered a high priority for the general recommendations outlined above.
- The third priority in Tischer Creek is to implement structural BMPs and restoration activities that focus on restoring the integrity and natural stream processes that help attenuate *E. coli* levels in un-urbanized streams.
    - Identify areas where streambank erosion has occurred and implement streambank stabilization BMPs. In Tischer Creek, streambank erosion was identified just upstream of the mouth of the T-2 tributary between West Saint Marie Street and North Street (See Subsection 3.2.2). Failing asphalt was observed along the road that parallels the creek and streambank stabilization should be considered as a high priority along this entire area.
    - Identify areas of degraded habitat where restoration activities could be prioritized and implemented. In Tischer Creek, several areas were identified where degraded habitat was a likely contributor to elevated *E. coli* levels (see Subsection 3.2.2 and Figure 3-10). The most obvious areas were in Tributary T-2, particularly at degraded pond just downstream of Norton Street, which had very poor habitat and was shown to be a source of *E. coli* to downstream receiving waters. Other areas in Reach T-2 that are in need of habitat restoration include the ponded water at Norton Street and Carver Avenue and the wetland bog in the creek just south of this area, which was clogged with organic debris and degraded habit. In Reach MS-5, the most degraded habitat was observed at West Saint Louis Street and Harvard Avenue. This large area adjacent to the creek had very poor habitat with high *E. coli* concentrations that are likely contributing to elevated levels in the Tischer Creek mainstem.
- **BMP Effectiveness Monitoring:** As discussed above for Keene Creek, it is important to monitor BMP effectiveness in reducing *E. coli* levels in the receiving waters. BMP effectiveness

monitoring typically consists of measuring *E. coli* concentrations upstream and downstream of the BMP or before and after implementation. The study design should be sufficiently robust (e.g., number and frequency of samples) to provide a statistical comparison of changes in *E. coli* concentrations due to BMP implementation.

- **Monitoring Program.** The effectiveness of specific BMPs in reducing *E. coli* concentrations should be one part of an overall strategy to improve water quality in Tischer Creek and meet the goals of the TMDL. It is recommended that for Tischer Creek, the monitoring program should build off of this Study, using the results as a baseline for future assessments. Because *E. coli* concentrations were low in the upper part of the Study Area, we recommend that the City focus future monitoring in reaches T-2 and MS-5. Synoptic, dry weather (at least 24 hours after a rain event) surveys in these reaches, as well as Reach MS-4 should be considered for future monitoring programs for a consistent evaluation of water quality conditions over time (we recommend that wet weather assessments be considered after dry weather assessments and BMP implementation). Typically, monthly evaluations are sufficient to assess changes in water quality, but more frequent monitoring may be needed, depending on specific goals.

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