Final Report

Bacterial Source Tracking at Impaired Beaches in the St. Louis River Area of Concern

St. Louis River Area of Concern Remedial Action Plan Project 7.02

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AOC: Area of Concern
LAN: Local Area Network
LDES: Laboratory Data Entry System
LSRI: Lake Superior Research Institute
PI: Principal Investigator
PM: Project Manager
QA/QC: Quality Assurance and Quality Control
QAM: Quality Assurance Manager
QAPP: Quality Assurance Project Plan
RPD: Relative Percent Difference
SOP: Standard Operating Procedure
SS: Sanitary Survey
STDI: Sterile Deionized Water (Autoclaved, Milli-Q)
US EPA: United States Environmental Protection Agency
UWS: University of Wisconsin-Superior
WDNR: Wisconsin Department of Natural Resources
qPCR: Quantitative Polymerase Chain Reaction

1 Executive Summary

This final report details the results of the Wisconsin Department of Natural Resources (WDNR) Project *Bacterial Source Tracking at Impaired Beaches in the St. Louis River Area of Concern*, hereafter referred to as the *Bacterial Source Tracking Project*. This project is described in the St. Louis River Area of Concern (AOC) Remedial Action Plan (RAP) as Project 7.02, the goal of which was to conduct bacterial source identification via DNA analysis to determine if bacterial contaminants were of human origin, and if necessary, recommend additional restoration options. The field sampling portion of this project took place on six impaired beaches in the St. Louis River AOC, on the State of Wisconsin or State of Minnesota 2014 and 2016 303(d) lists of non-attaining waters due to contamination by pathogens. Primary environmental data generated during this project includes Sanitary Survey (SS) data, concentration of *Escherichia coli* in surface water samples, and origin of measured *E. coli* via quantitative polymerase chain reaction (qPCR) analysis. The SS and *E. coli* concentration data were generated by the Lake Superior Research Institute (LSRI,University of Wisconsin-Superior), while the DNA marker data were generated by the by Dr. Sandra McLellan's laboratory at the University of Wisconsin-Milwaukee's School of Freshwater Sciences. The data generated from this project will be used by the St. Louis River AOC Coordinators and Beneficial Use Impairment (BUI) 7 Technical Team to determine if BUI 7 removal targets have been met. Table 1 presents an overall summary of the two-year project's results, including a prioritization of beaches for further investigation by the St. Louis River AOC.

Table 1. Ranking of Bacterial Source Tracking Project Beaches for Further Investigation by the St. Louis River Area of Concern.

State	Beach	Frequency of *E. coli* Exceedances (n)	Frequency Positive Result for Human Fecal Contamination(n)	Priority Ranking for Further AOC Investigation
Wisconsin	Barker's Island Inner	45% (370)	18% (78)	High
	Wisconsin Point	28% (304)	0% (34)	No Further Investigation Needed
Minnesota	Clyde Avenue Boat Launch	31% (199)	5% (39)	Medium
	Leif Erikson Park	45% (276)	28% (46)	High
	Minnesota Point 15th Street (Harbor Side)	18% (394)	0% (21)	No Further Investigation Needed

2 Introduction and Objectives

The St. Louis River AOC RAP includes a “Beach Closings and Body Contact Restrictions” BUI. Several beaches within the AOC are included on the 2014 and 2016 303(d) lists of non-attaining waters in Wisconsin and Minnesota due to contamination by pathogens. Routine bacteria (*E. coli*) monitoring for human health advisories does not provide enough information for the removal target. In order for impairments to be removed, water bodies within the AOC must not be on the 303(d) list due to “controllable sources”, defined as human sources, of pathogens (St. Louis River AOC RAP Update; September 30, 2016). Therefore, further analysis was necessary to determine the genetic origin of pathogens measured (i.e., genetic origin of *E. coli*) at each of the impaired beaches. An initial site assessment was conducted at each of six impaired beaches in the St. Louis River AOC according to the procedures outlined in the *Great Lakes Beach Sanitary Survey User Manual* (US EPA Office of Water, 2008). Based on findings from initial site assessments, sampling plans were developed for each of the six beaches. Sample collection locations were selected in an effort to investigate the contribution of the suspected *E. coli* sources. Staff from the Lake Superior Research Institute (LSRI) collected SS data and an array of *E. coli* samples from the impaired beaches during routine and rain sampling events. The project team selected a subset of samples exceeding water quality standards,(i.e., *E. coli* >235 MPN/100 mL), with the highest potential of informing decisions about likely host organisms for qPCR analysis of specific DNA makers.

The primary objectives of this investigation were to determine:

1. The frequency of *E. coli* exceedances (i.e., >235 MPN/100 mL) and the potential sources contributing to those exceedances at each of the six impaired beaches included in the project.
2. Whether the measured *E. coli* in samples that exceeded 235 MPN/100 mL were of human origin, or controllable, according to the St. Louis River AOC RAP.

Note that exceedances were defined in this project using established federal regulatory criteria for the safe use of recreational waters. The action value used to trigger an advisory at recreational beaches is *E. coli* concentration >235 MPN/100 mL. Sampling locations within this project included recreational beaches, tributaries, and storm water outfalls. Regardless of sample origin, the federal regulatory criterion for beaches was used to calculate exceedance rates at each sampling site and to determine if samples would be filtered and processed for future DNA marker analysis.

3 Methods

3.1 Project Schedule and Overall Design

The sampling effort for this project was focused at six impaired beaches in the St. Louis River AOC: Clyde Avenue Boat Launch, Leif Erikson Park, Minnesota Point/15th Street Harbor Side, and Park Point/20th Street Hearding Island Canal (2015 only) in Duluth, Minnesota and Barker’s Island Inner and Wisconsin Point (from Dutchman’s Creek to Lot 8)in Superior, WI. The map in Figure 1 indicates the official routine monitoring locations as identified by WDNR and the Minnesota Pollution Control Agency (MPCA) that are also monitored for beach advisories and closures. During the 2015 and 2016 beach seasons (i.e., approximately the last weekend in May to the first weekend in September), LSRI staff collected surface water samples twice per week from the official routine monitoring locations identified in Figure 1. In order to evaluate potential sources of contamination contributing to elevated *E. coli* numbers at each routine monitoring location, samples were also collected from various locations within the immediate vicinity of each beach, including tributaries and/or storm drains with outflows located at or near each beach. The source tracking sampling locations at each beach were selected based upon data gathered during initial site assessments conducted May 14-20, 2015 following the method outlined in "Section 4 – Steps for Conducting a Beach Sanitary Survey" of the *Great Lakes Beach Sanitary Survey User Manual* (US EPA Office of Water, 2008). A site-specific sampling plan was developed for each of the six beaches that detailed the frequency and location of the monitoring, exploratory, and rain event samples collected

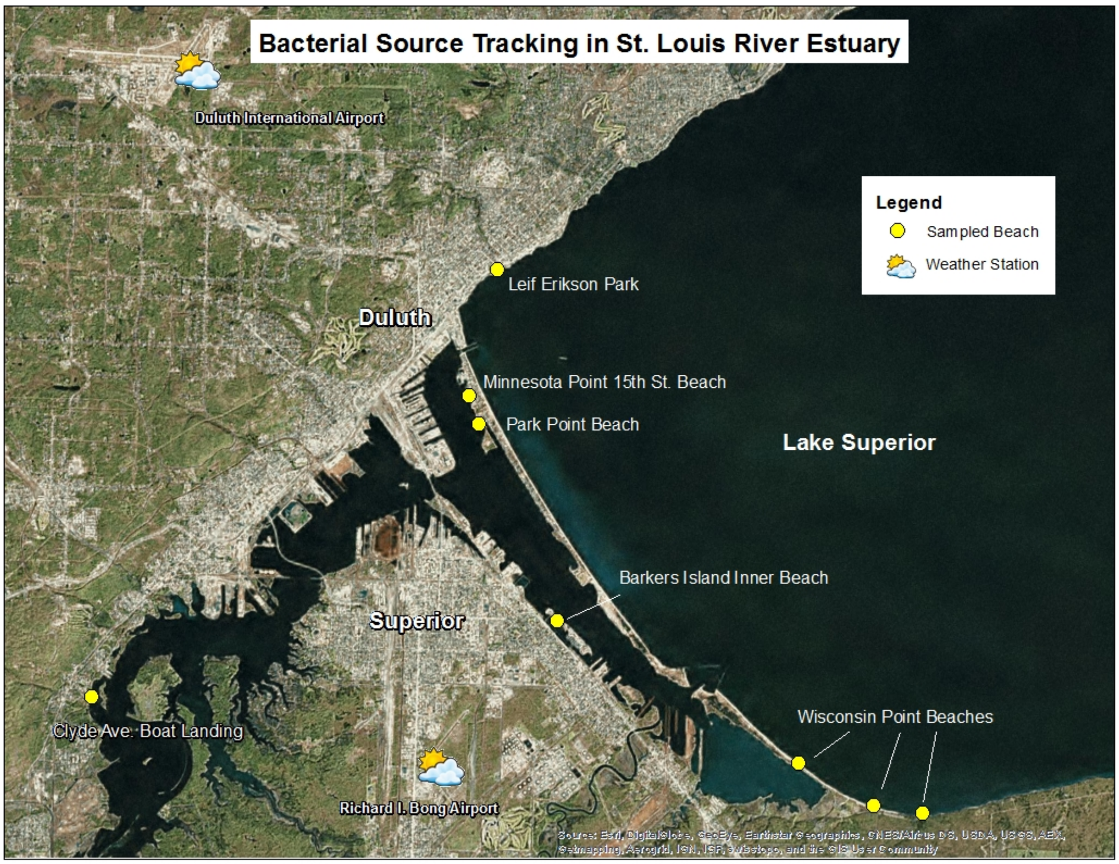

Figure 1. WDNR Bacterial Source Tracking Project Location. The official sampling locations of all six beaches are indicated by the yellow dots. Note that Park Point 20th Street/Hearding Island Canal was removed from the study in 2016. The weather stations that were used for rain event and other data are indicated by the sun/cloud icon.

LSRI staff collected and analyzed rainfall event samples within 48 hours of the conclusion of any rainfall event having over ½-inch of precipitation in the previous 24 hours. Hereafter, any mention of rain event meets this definition. The target was to collect samples within 48 hours of ten qualifying rainfall events at each beach over the two beach seasons. Each beach may be impacted by storm water runoff at different times after a relatively large rain event, therefore, LSRI staff made every effort to vary the timing of post-rain event sample collection to determine the effect of time on storm water runoff at each beach.

Precipitation data were obtained from using two methods, depending upon final use of the data:

1. To determine whether a qualifying rain event had occurred, precipitation totals (in inches) were monitored daily (i.e., obtained in real time) from weather stations located within a five-mile radius of each beach as noted in the Bacterial Source Tracking QAPP (Figure 1). To document relative precipitation amounts prior to each sampling event, the amount of rainfall that had occurred in the previous 72 hours was recorded on Sanitary Survey Datasheet.
2. To summarize precipitation data for reporting purposes and to obtain historical precipitation data for multiple linear regression model development, radar-estimated rainfall totals were obtained from the United States Geological Survey's Environmental Data Discovery and Transformation service. For model development, 24-hour, 48-hour, and 144-hour rainfall totals for each sample date were independent variables. In addition, previous 24-hour and previous 48-hour rainfall totals were independent variables used to develop each multiple linear regression model. Monitoring stations from which precipitation data were obtained are listed in Table 2.

Table 2. Precipitation Data Sources for each Beach's Reported Summary Data and Model Development.

Site Name	Site GPS Coordinates	Data Source	Station ID	Distance and Direction from Station to Site (Miles)
Clyde Avenue Boat Launch	46.70068, -92.20756	Applied Climate Information System	US1WIDG001	4.5 WSW
Leif Erikson Park	46.79662, -92.08185	Applied Climate Information System	US1MNSL0005	1.9 NE
Minnesota Point/15th Street Harbor Side	46.76888, -92.08964	Applied Climate Information System	US1MNSL0018	0.9 NNW
Barker Island Inner	46.76202, -92.05992	Applied Climate Information System	478349	1.9 SW
Wisconsin Point	46.67984, -91.94240 (WP3)	Applied Climate Information System	478349	7.1 NNW (from WP3)

Rain event samples were collected at the Minnesota beaches eleven times during the 2015 beach season and ten times in the 2016 season (Table 3). For Wisconsin beaches, rain event samples were collected 11 times in 2016 and nine times in 2016 (Table 4). Precipitation data reported in Tables 3 and 4 were obtained online via Weather Underground. (http://www.wunderground.com) using historical data for the two weather stations indicated in Figure 1, or the real-time data that was recorded on the Sanitary Survey Datasheets (2015)

Table 3. 2015 and 2016 Minnesota Rain Event Sampling Days and Amount of Rainfall (inches) in the Previous 24 Hours.

Collection Date (2015)	Rainfall (in)	Collection Date (2016)	Rainfall (in)
6/23/2015	0.86	6/27/2016	0.52

6/30/2015	0.60	7/12/2016	2.97
7/7/2015	1.87	7/13/2016	3.13
7/9/2015	0.88	7/22/2016	0.74
7/14/2015	0.94	8/3/2016	1.14
8/7/2015	0.83	8/16/2016	1.18
8/19/2015	1.33	8/29/2016	1.72
8/20/2015	1.77	9/6/2016	1.70
9/2/2015	0.74	9/7/2016	0.84
9/18/2015	0.84	9/8/2016	0.57
9/24/2015	1.86		

Table 4. 2015 and 2016 Wisconsin Rain Event Sampling Days and amount of rainfall (inches) in previous 24 hours.

Collection Date (2015)	Rainfall (in)	Collection Date (2016)	Rainfall (in)
6/23/2015	0.86	6/16/2016	0.61
6/30/2015	0.6	6/27/2016	0.52
7/7/2015	1.87	7/12/2016	2.97
7/9/2015	0.88	7/21/2016	0.53
7/14/2015	0.94	8/2/2016	0.53
8/7/2015	0.83	8/16/2016	1.18
8/19/2015	1.33	8/30/2016	0.59
8/20/2015	1.77	9/6/2016	1.7
9/2/2015	0.74	9/8/2016	0.57
9/18/2015	0.84		
9/24/2015	1.86		

The location and frequency of sample collection is described in more detail in the sections below and within each beach-specific sampling plans in Bacterial Source Tracking QAPP which available upon request. Note that the Park Point 20th Street/Hearding Island Canal location is no longer considered a beach and was removed from the study in 2016, therefore results are not included in this report.

3.2 Clyde Avenue Boat Launch Sampling Plan

3.2.1 Site Description

Clyde Avenue Boat Launch is located in the Smithville neighborhood of Duluth, MN, just past 92nd Avenue West. It is a widely-used public access site located on the St. Louis River, less than 10 miles from where the river meets Lake Superior (Figure 2). The public access provides a fishing pier, portable bathroom facilities, and a boat launch (Figure 3). Although there is no official swimming area at this location, people have been observed swimming at the boat launch.

Figure 2. Surface water sampling locations at Clyde Ave. Boat Launch.

Figure 3. Clyde Avenue Boat Launch and Fishing Pier.

3.2.2 Sampling Design and Justification

The potential sources of *E. coli* identified at Clyde Avenue Boat Launch based upon the initial site assessment and preliminary data collected during the first year of the *Bacterial Source Tracking Project* were:

1. Tributaries
   Stewart Creek has been listed by the MPCA as impaired for E. coli. Given that the Clyde Ave. Boat Launch is just downstream of where Stewart Creek enters the St. Louis River, it was hypothesized that E. coli and other contaminants would be carried from this tributary to the boat launch. In order to determine if Stewart Creek, the closest tributary was a contributing a human source of the E. coli detected at Clyde Ave. Boat Launch, baseline routine monitoring samples were collected twice weekly upstream from the mouth of the creek in Smithville park (2015 and 2016). In addition, rain event samples were collected both upstream and from the mouth of Stewart Creek. In 2016, samples were collected from the mouth of Stewart Creek weekly.
2. Waterfowl
   Geese and other waterfowl were identified as a potential source of E. coli at the boat launch because waterfowl frequently land and feed in the mowed grass adjacent to the boat launch. A careful count of waterfowl was conducted during each sanitary survey. On one occasion, a park maintenance worker reported to the sampling crew that he sweeps goose droppings off the dock into the water regularly.
3. Marina

Spirit Lake Marina and RV Park is located less than a half of mile downstream of the site. Improper disposal of human wastes from boats could contaminate surrounding waters. Although the marina is downstream of Clyde Avenue Boat Launch, Lake Superior seiche has the potential to transport contaminants upstream. In order to determine if the marina was contributing to the E. coli exceedances occurring at the boat landing, samples were collected in 2015, monthly or during a rain event, from the fishing pier (CLYD-FP) between the Marina and the boat landing.

4. Sewage Run-off/Leaky Pipes
   Buried sewage pipes are located in the area, and were identified as a potential source particularly in relation to Stewart Creek.
5. Storm Grate/Drain Pond /Portable Restroom
   The parking lot drains into a metal storm grate during large rain events. There is a portable restroom near the grate which runs across the boat launch just before the water (Figure 3,Right). The grate drains to the south of the boat launch into a small wooded area. During heavy rain, water accumulates in this small drain pond and it becomes a holding spot for bacteria. In order to determine if the portable restroom, parking lot, or drain pond was a contributing source of E. coli at the boat launch, water samples were collected from the drain pond when volume of water was sufficient to collect a sample using a 120 mL sterile sample bottle.

The sampling plan was designed to collect sufficient data to make a determination whether these five potential sources of *E. coli* were in fact contributing to *E. coli* concentrations measured at the boat launch. A sanitary survey was conducted and *E. coli* samples were collected at the boat launch location twice weekly. To correlate *E. coli* results from the boat launch with the each of the potential sources identified, routine monitoring (M), rain event (R), and exploratory (E) samples were collected with varying frequency during the 2015 and 2016 beach seasons as outlined in Table 5.

Table 5. 2015-2016 Surface Water Sampling Locations for Clyde Avenue Boat Launch, Including Routine Monitoring (M), Exploratory (E), and Rain Event (R) Sample Types.

LSRI Sample Site ID	GPS Coordinates	Description of Sample Collection Point	Sample Type: Justification for Site Selection
CLYD-BL	46.70068, -92.20756	On ramp at boat launch	M, R: Official site location; used for routine monitoring
CLYD-SC	46.69931, -92.21168	Stewart Creek Upstream: Access through Smithville Park off Clyde Ave.	M, R: Stewart Creek may be a potential source of *E. coli* contamination; it is on the MN 303(d) List
CLYD-FP	46.70115, -92.20724	Clyde Ave. Fishing Pier	R (2015 only); Between boat launch and marina
CLYD-ST	46.69931, -92.21159	Mouth of Stewart Creek	M[1], R: Stewart Creek may be a potential source of *E. coli* contamination; it is on the MN 303(d) List
CLYD-DP	46.700737, -92.207407	Parking lot storm drain outfall pool; only sampled when standing water is present	E,R: During heavy rain events standing water may be present in this pool; sample will indicate the contribution of storm water runoff from the parking lot

[1]Monitoring at CLYD-ST was conducted monthly in 2015 and once-per-week in 2016.

3.3 Leif Erikson Park Sampling Plan

3.3.1 Site Description

Leif Erikson Park is a popular park within the city limits of Duluth, MN near 11th Avenue East and London Road (Figure 4). The park borders Lake Superior, is a high-traffic location due to its close proximity to the Duluth Lakewalk, and is very popular with tourists and local residents. The beach is also a popular retreat for dog owners, and is also susceptible to runoff from the hillside city, which includes both residential and business districts in close proximity to the water. The shoreline is rocky but relatively gradual near the routine sampling locations (Figure 5). Away from the routine locations, the shoreline is much steeper and with very little beach shore. Elevation rises quickly with distance from the water (Figure 6).

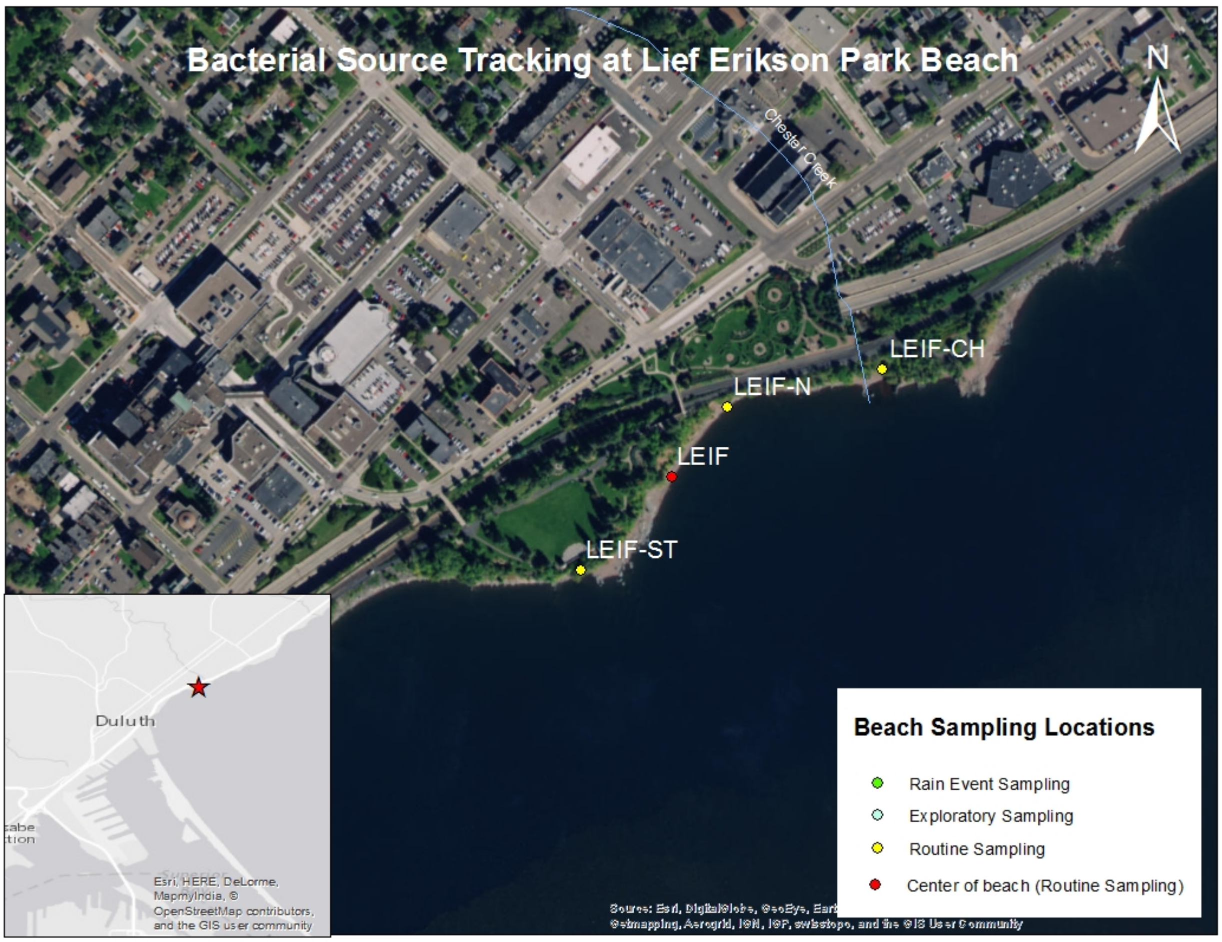

Figure 4. Surface water sampling locations at Leif Erikson Park. The Red Dot Indicates the Official Routine Monitoring Location for the Site.

Figure 5. South End (left) and Middle (right) of Leif Erikson Park Beach Swashzones.

Figure 6. North End of Leif Erikson Park Beach.

3.3.2 Sampling Design and Justification

The potential sources of *E. coli* identified at Leif Erikson Park beach based upon the initial site assessment and preliminary data collected during the first year of the *Bacterial Source Tracking Project* were:

1. Storm Water Runoff

In order to determine whether storm water runoff was contributing a human source to *E. coli* concentrations measured at the Leif Erikson Park center-of-beach location (LEIF), in addition to the baseline routine monitoring samples, samples were collected within 48 hours of a rain event. .There are two visible culverts near the beach. One culvert, drains directly to the beach from the city uphill (Figure 7). This culvert may potentially be a source of contaminants from nearby residences, businesses, or urban storm water. The other culvert, at the south end of the beach was actively flowing most of time, draining partially to the beach where the culvert is broken, and to the lake directly (Figure 8). Samples were collected here routinely and during rain events.

2. Parking Lot Runoff
   Due to the relatively impervious surface (i.e., boulders and gravel) that makes up the majority of the beach at Leif Erikson Park, the impact of runoff from the parking lot or the grassy areas of Leif Erikson Park may be a contributor at this site.
3. St. Louis River Estuary and Port Activity
   The beach's routine monitoring site (LEIF, Table 6) is located less than 1.5 miles from the St. Louis River Estuary and the Duluth Entry to the Duluth-Superior Harbor, which is home to the most active commercial docks within the Port of Duluth-Superior. Contamination transported from within the Estuary during weather events may contribute to exceedances. The northern-most point of the beach (LEIF-N, Table 6) was selected as a routine monitoring and rain event sample location in an effort to determine the directionality of *E. coli* contamination; *E. coli* originating from the north-northeast (potentially from LEIF-CH, Table 6) would in theory result in higher *E. coli* concentrations at LEIF-N than LEIF whereas *E. coli* originating from the south-southwest (potentially from sources within the Estuary or from LEIF-ST, Table 6) would result in higher *E. coli* concentrations at LEIF than LEIF-N.
4. Recreational Activities
   Visitors to the park may contribute sources of *E. coli* at the on this beach as it is often visited by tourists and residents alike with their pets. Improper disposal of pet waste may potentially source *E. coli* at the beach. The area near Chester Creek is frequented by overnight guests whose trash and materials are often left behind (Figure 9). The sanitary survey data were used to determine correlations between the numbers of people (in and out of the water), number of animals, and relative amount of trash/debris observed at the beach and *E. coli* concentration measured in samples collected at the center-of-beach location.

The sampling plan was designed to collect sufficient data to make a determination whether these four potential sources of *E. coli* were in fact contributing to *E. coli* concentrations measured at the beach. A sanitary survey was conducted and *E. coli* samples were collected at the center-of-beach location twice weekly to procure baseline data. In order to correlate *E. coli* results from the center-of-beach location with the each of the potential sources identified, routine monitoring (M), and rain event (R) samples were collected during the 2015 and 2016 beach seasons as outlined in Table 6.

Table 6. Surface Water Sampling Locations at Leif Erikson Park for the 2015 and 2016 Beach Seasons, Including Routine Monitoring (M), and Rain Event (R) Samples

Sample Site	GPS Coordinates	Description of Sample	Sample Type: Justification for Site Selection

ID		**Collection Point**	
LEIF	46.79662,-92.08185	Center of Beach between two rock formations	M, R: Official site location; used for routine monitoring
LEIF-CH	46.79753,-92.07941	Where Chester creek enters the lake; North of rose garden	M, R: Chester Creek on 303d List for *E. coli*, Storm water runoff may be a possible source of *E. coli* contamination
LEIF-N	46.79719,-92.08122	Northern most spot that you can walk to on Leif beach	M, R: Location to help determine directionality of *E. coli* contamination
LEIF-ST	46.79585,-92.08289	Small broken culvert/Storm drain on south side of beach.	M, R: Storm water runoff may be a possible source of *E. coli* contamination

Figure 7. Storm drain located near the routine (LEIF) Sample Location.

Figure 8. Storm Drain at south end of Leif Erikson Park Beach Where samples (LEIF-ST) are Routinely Collected.

Figure 9. Trash and Material Left Behind by Leif Erikson Park Visitors.

3.4 Minnesota Point/15th Street Harbor Side Sampling Plan

3.4.1 Site Description

During the beach season, Minnesota Point is among the most heavily used areas by both tourists and residents, and is a valuable asset the local Duluth community. The harbor side beach location near 15th Street (Figure 10) is designated as an Impaired Beach in the St. Louis Area of Concern (AOC). The 170-meter beach is a half mile across the harbor from Rice's Point, one of the most active ports in the Twin

Ports. The shoreline on the northwest end of the beach includes a large gravel parking area for the Duluth Boat Club (Figure 11), and the United States Coast Guard Station. The United States Army Reserve Center is on the south-east end of the beach (Figure 12). A large parking lot lies near the south-east end of the beach and drains to the bay.

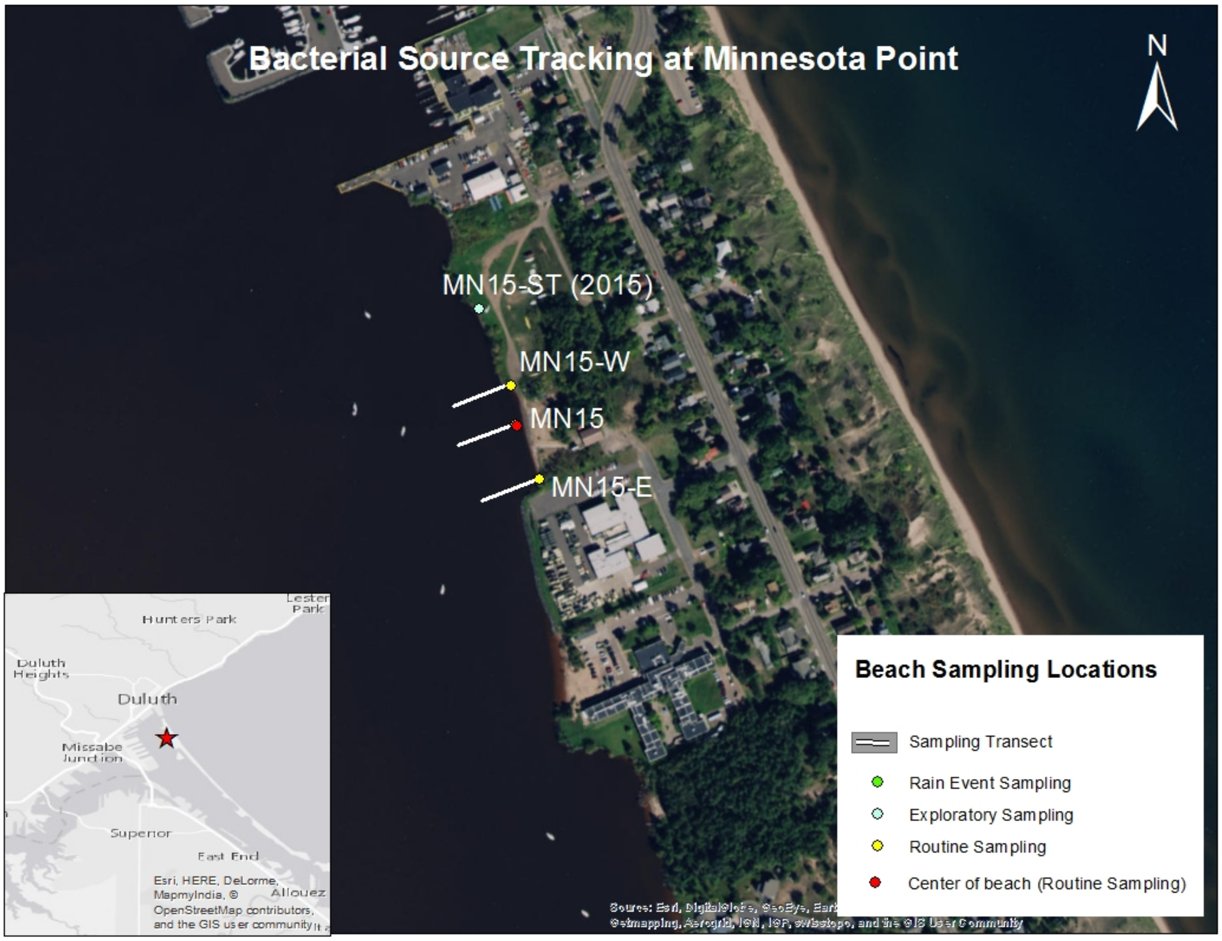

Figure 10. Map Indicating Minnesota Point's 15th Street Beach. Red Dot Indicates the Official Routine Monitoring Location. White Lines Depict Sampling Transects (2016).

Figure 12. View of East Side of Beach (MN15-E) and US Army reserve on East side of Beach; taken from beach center (MN15).

Figure 11. View of West Side of Beach (MN15-W); picture taken from beach center (MN15).

3.4.2 Sampling Design and Justification

The potential sources of *E. coli* investigated at Minnesota Point/15th Street based upon the initial site assessment and preliminary data collected during the first year of the project were:

1. Storm Water/Parking Lot Runoff
   The beach has one large paved area at the Army Reserve that drains to the lake and there is a large gravel parking lot on the east end of the beach. Gulls and geese that frequent the lots may contaminate the area with fecal matter, which may drain into the lake near the beachfront.
2. Recreational Activities
   This appear to be a location where many people launch watercraft such as kayaks and canoes. Visitors to the beach may potentially source *E. coli* at the sample site. A large park surrounds the beach area and improper disposal of trash and pet waste from dogs visiting with their owners may potentially source *E. coli* at the beach. There is also a high concentration of geese and ducks in the park area.
3. Ports
   The sample site is a half mile across the harbor from Duluth's most active ports. Contamination due to waste management or shipping practices may contribute to exceedances.

The sampling plan was designed to collect sufficient data to determine whether these three potential sources of *E. coli* were in fact contributing to *E. coli* concentrations measured at the beach. A sanitary survey was conducted and *E. coli* samples were collected at the center-of-beach location twice weekly. In order to correlate *E. coli* results from the center-of-beach location with the each of the potential sources identified, routine monitoring (M), rain event (R) samples were collected with varying frequency during the 2015 and 2016 beach seasons as outlined in Table 7. In order to determine whether storm water runoff was contributing a human source to *E. coli* concentrations measured at the center-of-beach location (MN15), in addition to sanitary survey data and the baseline routine monitoring samples, samples were collected within 48 hours of a rain event.

Table 7. 2015 and 2016 Surface Water Sampling Locations for Minnesota Point/15th Street Harbor Side, Including Routine Monitoring (M), and Rain Event (R) Sample Types.

LSRI Sample ID	GPS Coordinates	Description of Sample Collection Point	Sample Type: Justification for Site Selection
MN15	46.76888, -92.08964	In harbor just off walkway at boat club near US Army Reserve Transect sampling will be utilized at this site: shallow wading depth = 0.3 m, standard wading depth = 0.6 m, deep wading depth = 1.2 m	M, R: Official site location; used for routine monitoring M = Transect sampling (2016) R = Samples collected at standard wading depth of 0.6 cm
MN15-E	46.76859, -92.08953	End of beach near US Army Reserve Transect sampling will be utilized at this site: shallow wading depth = 0.3 m, standard wading depth = 0.6 m, deep wading depth = 1.2 m	M, R: Beach is long enough to necessitate sample collection at either end of beach M = Transect sampling (2016) R = Samples collected at standard wading depth of 0.6 m
MN15-W	46.76934, -92.08990	End of beach near United States Coast Guard port, and Harbor Cove Marina	M, R: Beach is long enough to necessitate sample collection at either end of beach

LSRI Sample ID	GPS Coordinates	Description of Sample Collection Point	Sample Type: Justification for Site Selection
		Transect sampling will be utilized at this site: shallow wading depth = 0.3 m, standard wading depth = 0.6 m, deep wading depth = 1.2 m	M = Transect sampling (2016) R = Samples collected at standard wading depth of 0.6 m
MN15-ST	46.76995, -92.09030	Culvert past northwest end of beach between beach and Coast Guard Station	R: Samples collected in 2015 to determine contribution of *E. coli* from the parking lot or storm water

3.5 Barker's Island Inner Sampling Plan

3.5.1 Site Description

Barker's Island is a small island in the Superior Bay of Lake Superior, off Highway 53 and Belknap Avenue in Superior, WI (Figure 13). The island is a predominant tourist location for the City of Superior; offering a city park, swimming beach, walking trails, and other recreational attractions. There is public access to the harbor, which includes a boat landing, fish cleaning station, and bathroom facilities. A large marina, a hotel, and several homes occupy the island as well.

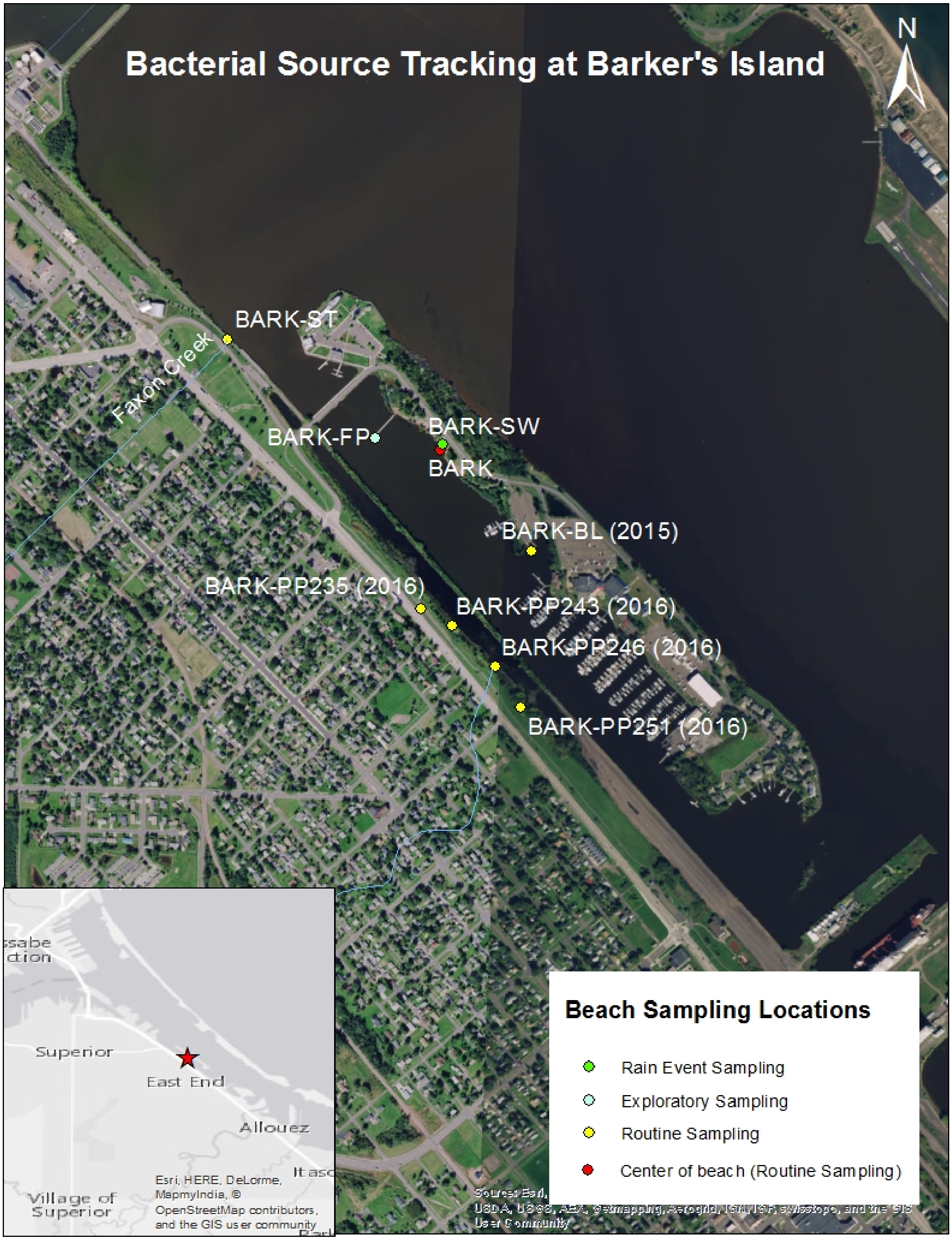

Figure 13. Map Showing the Location of Barker's Island within the St. Louis River Estuary (Superior, WI). Red dot (BARK) indicates the center-of-beach monitoring location.

3.5.2 Sampling Design and Justification

The potential sources of *E. coli* identified at Barker's Island Inner beach based upon the initial site assessment and preliminary data collected during the first year of the *Bacterial Source Tracking Project* were:

1. Storm Water Runoff
   Storm water from the City of Superior mainland drains to the inner bay along the beach near the fishing area via the large Faxon Creek culvert (Figure 14). Pickle Pond, a nine-acre man-made pond adjacent to Barker's Island and the Marina, also receives storm water discharges from the City of Superior's storm water system via five separate outfalls (Figure 15). Surrounding impervious surfaces, such as Highways 2 and 53, the Osaugie Trail, , as well as, large areas of mowed grass where geese often congregate do not permit sufficient infiltration of storm water runoff and allow for a direct path of storm water to Pickle Pond. Exchange of water between Pickle Pond and Barker's Bay occurs through two openings in the unused rail line that separates the two water bodies (Figure 16). Storm water also runs off the road and walking paths adjacent to the beach and accumulates in depressions near the beach. In order to determine whether storm water runoff was contributing to *E. coli* concentrations measured at Barker's Island Inner beach, baseline routine monitoring samples were collected twice weekly from the mouth of the Faxon Creek in both 2015 and 2016. In 2016, samples were also collected from Pickle Pond twice weekly. In addition, rain event samples were collected, from the mouth of Faxon Creek, the end of the fishing pier, Pickle Pond, and four of the five storm water outfalls draining into Pickle Pond (2016 only; samples collected only if water was actively flowing from outfall).

Figure 14. Bridge at Faxon Creek Culvert (BARK-ST) where Routine Samples were Collected Twice Weekly and During Rain Events.

Figure 15. Water Actively Flowing from the Pickle Pond Outfall Off of 14th Street and the Osaugie Trail.

Figure 16. Inactive Rail Road that Separate Pickle Pond and Barker's Bay.

2. Waterfowl
   There is a prominent presence of Canada Geese, gulls, and ducks on Barker's Island (Figure 17), and goose and/or duck feces litter the park and beach area. Visitors were often observed feeding the birds and on several occasions, more than 70 gulls, geese and ducks were present on site at a time. In order to determine the contribution of waterfowl to *E. coli* concentrations

measured at Barker's Island beach, the number and type of waterfowl observed were recorded during each sanitary survey conducted at the center-of-beach location (twice weekly). This observational data, along with the measured *E. coli* concentration from samples collected during each sanitary survey, were correlated to determine relative source contribution.

Figure 17. Photos of Waterfowl Present on the West Side of Barker's Island Swimming Beach and the Main Beach.

3. Recreational Activities

The park at Barker's Island sees a high influx of visitors during the summer months. Visitors have been seen using the park for a number of recreational activities, including swimming, running, fishing, and dog walking. The high number of visitors to the area may potentially source pathogens through improper disposal of pet or human waste. Additionally, improper disposal of other trash may attract animals to the beach area, where fecal bacteria may easily be transported into the water or sediment. The Barker's Island Marina is another potential source of *E. coli,* possibly through improper disposal of black water within recreational vessels docked at the facility. In order to determine the contribution of recreational activities to *E. coli* concentrations measured at Barker's Island beach, several parameters were carefully observed and recorded during the twice weekly sanitary survey done at the center-of-beach location, including: number of people out of the water (and type of activity), number of people in the water, number of dogs, and relative amount of trash present in and around the beach. This observational data, along with the measured *E. coli* concentration from samples collected during each sanitary survey, were correlated to determine relative contribution. In 2015, rain event samples were collected from the public boat launch, which is in close proximity to Barker's Island Marina in order to determine the relative concentration of *E. coli* at this location.

4. Lack of Infiltration/Standing Water on Beach
   The shoreline of the Barker's Island Inner beach is sandy and relatively flat. On either side of the swimming beach the incline is low with minimal vegetation in areas indicating significant erosion. There are some truncated areas in the foreshore that trap water during rain events causing stagnant water to catch droppings from geese and ducks along the shoreline (Figure 17). The pool of standing water can become quite large during the beach season with the potential for wave action and storm water runoff to create mixing between the standing water and the water at the swimming beach. In order to determine whether standing water due to lack of infiltration was a contributor of *E. coli* at Barker's Island beach, samples were collected each time a sanitary survey was conducted (twice weekly) at the center-of-beach location if the pool of water was sufficiently deep to allow for sample collection (as shown in Figure 18).

Figure 18. Standing Water Which Often Collected Along the Shoreline of Barker's Island Inner Beach.

The sampling plan was designed to collect sufficient data to make a determination whether these four potential sources of *E. coli* were in fact contributing to *E. coli* concentrations measured at the beach. A sanitary survey was conducted and *E. coli* samples were collected at the center-of-beach location twice weekly (Figure 1, red pin). In order to correlate *E. coli* results from the center-of-beach location with the each of the potential sources identified, routine monitoring (M), rain event (R), and exploratory (E) samples were collected with varying frequency during the 2015 and 2016 beach seasons as outlined in Table 8.

Table 8. Surface Water Sampling Locations for Barker's Island Inner during the 2015 and 2016 Beach Seasons, Including Routine Monitoring (M), Rain Event (R), and Exploratory Samples.

LSRI Sample Site ID	GPS Coordinates	Description of Sample Collection Point	Sample Type: Justification for Site Selection

LSRI Sample Site ID	GPS Coordinates	Description of Sample Collection Point	Sample Type: Justification for Site Selection
BARK	46.76202, -92.05992	Beach between open swimming beach and other small beach	M, R: Official site location; used for routine monitoring
BARK-BL	46.71829,-92.05721	Boat Landing near Barker's Island Marina	R(2015 Only): Possible source from of Marina
BARK-ST	46.72232, -92.06615	Faxon Creek storm water drain near Bong Heritage Center	M, R: Faxon Creek is a possible source of *E. coil* contamination
BARK-PP	46.76202, -91.95802	Pickle Pond entry from Barker's Island Marina or near railroad bridge	M, R: Pickle Pond receives storm water runoff and may be a possible source of *E. coli* contamination
BARK-PP235	46.71707, -92.06038	Pickle Pond Outfall off 11th St /Osaugie Trail	M, R: Collect samples during routine monitoring and rain event sampling only if water is actively flowing from outfall pipe.
BARK-PP243	46.71676, -92.05946	Pickle Pond Outfall off 12th St /Osaugie Trail	
BARK-PP246	46.71597, -92.05819	Pickle Pond Outfall off 13th St /Osaugie Trail	
BARK-PP251	46.71517, -92.05743	Pickle Pond Outfall off 14th St /Osaugie Trail	
BARK-FP	46.72045, -92.06183	Fishing pier	R: Fishing pier activities may contribute to *E. coli* contamination following heavy rain events
BARK-SW	Not Applicable	Standing water on beach; only sampled when standing water is present	E: Standing water is observed after rain events and may be a source of *E. coli* contamination

3.6 Wisconsin Point Sampling Plan

3.6.1 Site Description

Wisconsin Point is located at the east end of Superior, WI and is one of the largest freshwater sandbars in the world (229 acres). It provides nearly three miles of beach, a lighthouse, and a historical marker. It is a popular destination for bird watching, hiking, swimming, etc. Wisconsin Point's beaches are one of the more popular coastal beaches and the southeast portion of the point is also a historical nesting habitat for the endangered Piping Plover. There are six beaches on Wisconsin Point that are monitored for *E. coli*. This investigation focused on the beaches on the southeast end of the point due to a high historical frequency of *E. coli* exceedances that is focused primarily on Wisconsin Point 3 (WP3), Wisconsin Point 2 (WP2), and Wisconsin Point 1 (WP1) from southeast to northwest on the point (Figure 19); an area that stretches approximately 3,900 meters.

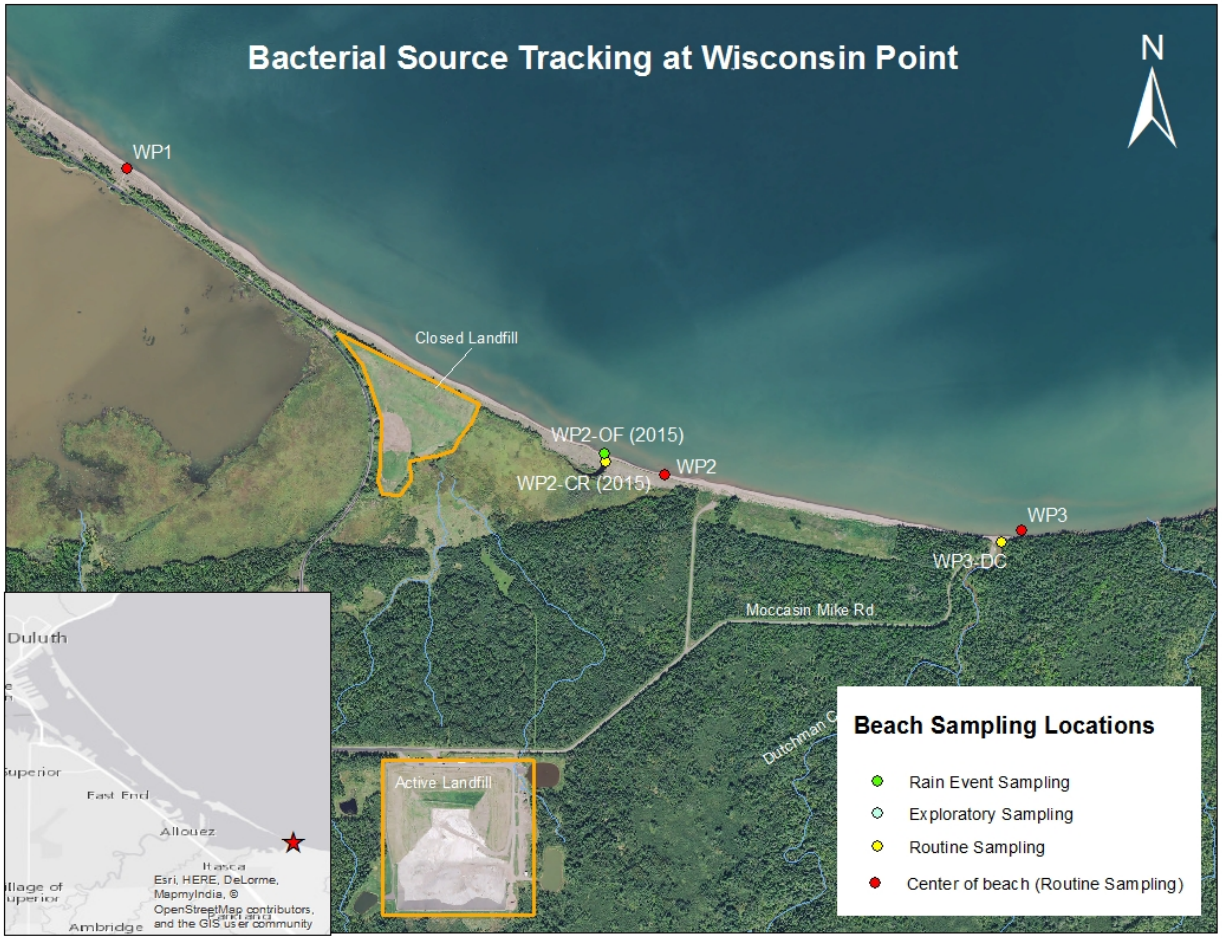

Figure 19. Map Indicating Wisconsin Point's Southeast Beaches and Additional Routine Monitoring Sites at Dutchman Creek (WP3-DC), and the WP2 locations sampled in 2015 only (WP2-CR and WP2-OF). Red pin indicates the official monitoring locations for WP3, WP2, and WP1.

3.6.1.1 Wisconsin Point 3 (WP3)

Wisconsin Point 3 beach is the furthest beach to the southeast on the point (Figure 19, WP3); accessible from Moccasin Mike Road. The City of Superior's landfill, which is an active open landfill, is located on Moccasin Mike Road less than 1.5 miles from WP3 beach. The mouth of Dutchman Creek intersects the beach (Figure 20). Dutchman Creek flows through rural areas located to the south of Wisconsin Point.

Figure 20. Photos of Wisconsin Point 3 Beach (LEFT) and Near the Mouth of Dutchman Creek (Right).

3.6.1.2 Wisconsin Point 2 (WP2)

Wisconsin Point 2 beach (Figure 21) is a historical nesting location for the endangered Piping Plover, and in recent years is not accessible to the public during this bird's nesting period from mid-May to mid-July (access to this beach was granted for purposes of this investigation). Accessible from Lake Shore Road, this beach is located less than one mile from a former municipal landfill that has been capped off. The capped off landfill is a gathering place for gulls and other seabirds.

Figure 21. Photos of Wisconsin Point 2 Beach (Left), Sample Collection at Wisconsin Point 2 Beach (Top Right), and a View of the Beach from the Bluff above.

3.6.1.3 Wisconsin Point 1 (WP1)

Wisconsin Point 1 beach (Figure 22) is located furthest to the northwest of all the Wisconsin Point beaches included in this investigation (Figure 19, WP1); of the three beaches it is the furthest from the mainland. This beach is accessible via Lot 8 off of Wisconsin Point Road. The beach bisects Allouez Bay to the south and Lake Superior to the north.

Figure 22. Photos from Wisconsin Point 1 Beach.

3.6.2 Sampling Design and Justification

The potential sources of *E. coli* investigated at the three Wisconsin Point beaches based upon the initial site assessment and preliminary data collected during the first year of the *Bacterial Source Tracking Project* were:

1. Landfill Drainage
   Runoff or leachate from the current, active municipal landfill or the former, capped-off landfill was identified as a potential source of pathogenic bacteria for both WP2 and WP1 (Figure 21). The active landfill is approximately 1,000 meters from Lake Superior located between WP2 and WP1. The closed landfill sits only a few tens of meters from Lake Superior also between WP2 and WP1. A few small creeks and wetlands are adjacent to both landfills, which is a possible source of transport to WP2 and WP1 beaches. In 2015, a wetland area or remnant creek near WP2 was sampled as it may accept runoff or leachate from the capped landfill.
2. Seabirds and Other Waterfowl
   Both landfills attract numerous gulls and other seabirds to the southeast end of Wisconsin Point. These seabirds transport solid waste from the active landfill to the beach, and they aggregate in extremely large groups on the beach itself (particularly at WP2). Feces are deposited onto the beach and are transported into the water via wave action or storm water runoff.
3. Recreation Beach Use
   Wisconsin Point beaches are a popular destination for recreational activities in the summer months. Litter from recreational beach use is a very common problem. The City of Superior uses a “pack out what you pack in” strategy for trash disposal on Wisconsin Point, therefore, there are no garbage cans present on/near any of the beaches. Improper disposal of trash, pet waste,

and possibly human waste on the beach and nearby foreshore can introduce pathogens into the sand and water via wave action and/or storm water runoff. Improper disposal of trash may also attract seabirds and other animals to the beach area, where fecal bacteria may be easily assimilated into the water or sediment.

4. Tributaries
   Dutchman Creek, which enters Lake Superior at WP3, may be a source of pathogenic bacteria as it may carry waste from agricultural activities and storm water runoff. Failing or nonfunctional septic systems may also contribute pathogens as the rural drainage area of the creek is prone to septic system issues due to the high clay content of the soils.

The sampling plan for Wisconsin Point's southeast beaches was designed to collect sufficient data to make a determination whether these four potential sources of *E. coli* were in fact contributing to *E. coli* concentrations measured at WP3, WP2, and WP1. A sanitary survey was conducted and *E. coli* samples were collected at the three beach monitoring locations twice weekly. In order to correlate *E. coli* results from the routine monitoring locations with the each of the potential sources identified, routine monitoring (M) and rain event (R) samples were collected with varying frequency during the 2015 and 2016 beach seasons as outlined in Table 9.

Table 9. 2015 - 2016 Surface Water Sampling Locations for Wisconsin Point, Including Routine Monitoring (M) and Rain Event (R) Sample Types.

LSRI Sample ID	GPS Coordinates	Description of Sample Collection Point	Sample Type: Justification for Site Selection
WP1	46.69027, -91.98167	Near parking lot 8; water is sampled in lake straight off trail to beach	M, R: Official site location; used for routine monitoring
WP2	46.68142, -91.95771	Walk beach to waypoint	M, R: Official site location; used for routine monitoring
WP2-CR	46.68177, -91.96030	Creek (Unknown name) northwest of WP2; close proximity of capped landfill	M, R(2015 Only): Creek in close proximity of capped landfill may contribute to *E. coli* on beach
WP2-OF	46.68203, -91.96038	Center of beach area where WP2-CR may enter the lake during a heavy rain event.	R (2015 Only): Collected during rain events to determine contribution of creek to high *E. coli* numbers at WP2 center of beach location.
WP3	46.67984, -91.94240	Cross the creek; area where Dutchman Creek meets the lake	M, R: Official site location; used for routine monitoring
WP3-DC	46.67982, -91.94353	Collect from Dutchman Creek on the same side of the creek as WP3	M, R: Dutchman Creek is a potential source of *E. coli* contamination; it has historically been on the WI 303(d) List.

3.7 Field Sampling Procedures and Requirements

During each sampling event, a suite of observational (qualitative) and quantitative data was collected in order to provide insight on the conditions during which elevated *E. coli* concentrations occur. Methods follow the U.S. Environmental Protection Agency's (EPA) Great Lakes Beach Sanitary Survey User Manual (May 2008) and can be found in more detail in the Bacterial Source Tracking QAPP. The primary data collection parameters included observational data such as weather conditions (e.g. rainfall, cloud cover, wave intensity) at the time of sample collection and an assessment of water quality (i.e., water color, water odor, and turbidity) and potential sources of pollution. Quantitative data collection included: Air temperature, wind speed and direction, longshore current speed and direction, wave height, water quality parameters (i.e., water temperature, turbidity, conductivity, dissolved oxygen and pH). The number of people at the beach as well as the number of waterfowl (i.e., gulls, ducks, and geese) present was also recorded. The sampling and analysis procedures and requirements for the *Bacterial Source Tracking Project* are outlined in Table 4 of the Bacterial Source Tracking QAPP. Collection of water samples are outlined in LSRI SOP *FS/38 – Collection of Water and Substrate Samples for Analysis of E. coli.* Briefly, samples were collected at knee depth, when possible, in sterile 1 L sample bottles and immediately placed on wet ice in a cooler. Samples were transferred to the LSRI Microbiology Laboratory on the day of collection and be placed into a refrigerator until the time of analysis (*E. coli;* typically within 6 hours of collection). Samples were held in the refrigerator until *E. coli* counts were determined and select samples were filtered for qPCR analysis.

3.8 Analytical Methods

E. coli analysis of routine monitoring, exploratory, and sand/substrate samples were conducted by trained LSRI staff; the LSRI Microbiology Laboratory received certification in May 2015 from the WI DATCP (and renewal in January 2016) to perform *E. coli* analysis in water samples. Samples were analyzed according to LSRI SOP *SA/56 – Analysis of Surface Water Samples and Extraction and Analysis of Sand/Substrate Samples for E. coli using IDEXX Colilert™*. Each 1-L water sample collected were analyzed for *E. coli*; the remaining sample volume was refrigerated for a maximum of 36 hours. In the event the *E. coli* analysis results are >235 MPN/100 mL after 24 hours of incubation at 35°C ± 0.5°C, a portion of the sample was filtered through a 47-mm, 0.22 µm nitrocellulose filter according to the procedure outlined in LSRI/SOP *SA/57 – Preparation of Surface Water Samples and Sand/Substrate Extract for qPCR Analysis.* The filters were placed into a 2-mL screw-cap microcentrifuge tube and frozen at -80°C until the end of the season when selection of samples for qPCR analysis took place. Preference was given to samples having an *E. coli* density of ≥1,000 MPN/100 mL, however, in order to investigate the sample sites thoroughly, any sample having an *E. coli* density of ≥235 MPN/100 mL was considered for qPCR analysis.

Select filters were shipped frozen to The University of Wisconsin-Milwaukee, School of Freshwater Sciences for qPCR analysis. The markers used for all qPCR assays were:

- *Human Bacteroides* (HF183)
- *Human Lachnospiraceae* (Lachno_2)
- *Enterococcus*
- *E. coli*
- Ruminant
- Gull

A portion (42 samples total) of the 2015 samples were analyzed only for the Human Lachno_3 marker, and data are not available for the above-named markers. The details of the qPCR analysis procedure can be found in Appendix 3 of the Bacterial Source Tracking QAPP. The data are reported in Excel format as copy numbers (CN) per 100 ml of original sample water, which was calculated by taking into account the original water volume sampled, the resulting volume following a DNA extraction, the volume of extracted DNA entering the qPCRs, and the relationship of the qPCR standard curve to the fluorescence product of the qPCR amplification in each sample.

3.9 Data Management and Data Analysis

3.9.1 Data Generation and Data Entry

Sample collection and analysis results were recorded by hand (using indelible ink) on pre-printed (on waterproof paper for field data) data collection forms or in a bound laboratory notebook as described in the QAPP. The original datasheets were scanned after completion and a .pdf file was created and stored on LSRI's secured Local Area Network (LAN).

Sanitary Survey and water quality data were entered into the *LSRI Bacterial Source Tracking Database* on a weekly basis following the procedure outlined in *LSRI/SOP/REC/16 – Entering Data in the LSRI Bacterial Source Tracking (BST) Database* (issued May 2016; Appendix 3). Count data for *E. coli* analysis were entered into the database after counts had been completed. *E. coli* density (reported as MPN/100 mL) was calculated directly in the database and the volume filtered for exceedances was tracked and entered into the database.

For the routine sampling points of the Wisconsin beaches monitored for beach closures (Figure 1), sanitary Survey and *E. coli* data were entered weekly into the Wisconsin Beach Health database, accessed via the public home page (http://www.wibeaches.us/apex/f?p=BEACH:HOME) following the procedure outlined in *LSRI/SOP/REC/17 – Entry of Sanitary Survey Data to the Wisconsin Beach Health Database* (issued May 2016;). Beach data from the Wisconsin Beach Health database was uploaded to SWIMS (Surface Water Integrated Monitoring System) at the end of each beach season.

3.9.2 Data Analysis

Water quality parameters measured at each sampling location were averaged over the two-year dataset and standard deviation of each parameter was determined. The two-year average *E. coli* concentration at each sampling location was also determined, with variability calculated as standard deviation. The two-year geometric mean *E. coli* concentration was calculated to provide a basis for comparison to regulatory standards, which are generally based upon geometric mean concentration.

Virtual Beach, version 3.0.6 (US EPA, USGS, Wisconsin Sea Grant) was used to develop multiple linear regression models for each beach. The *E. coli* concentration and sanitary survey data from each routine monitoring location were downloaded from Wisconsin Beach Health or from the LSRI-developed Microsoft Access Database for the project (i.e., for Minnesota beaches). Climate and tributary data, including data radar-estimated rainfall totals (24, 48, and 144-hour rainfall totals and previous 24-hour rainfall total) and nearby tributary discharge (where applicable; 7-day average flow rate and 24-hour total discharge) were obtained for each date sampled using the USGS Environmental Data Discovery and Transformation Service. The *E. coli* concentration, sanitary survey, climate, and nearby tributary discharge data were imported into Virtual Beach for analysis. If two years of *E. coli* concentration data were available from sampling locations adjacent to the routine monitoring location, those data were also imported into Virtual Beach. Data validation was performed using Virtual Beach to eliminate dates for which there were missing values. Outliers were investigated to ensure they were accurate values. Rare values, defined as values having an average of less than one during the two-year investigation were found to skew the model and were eliminated prior to model building. In all cases, the *E. coli* concentration each beach's routine monitoring location was the dependent variable. All *E. coli* data were transformed using Log_{10}. Independent variables were transformed only if the Pearson Coefficient associated with the transformed variable exceeded that of the untransformed variable by greater than 30%. Each multiple linear regression model was optimized using Akaike Information Criteria, Bayesian Information Criteria, and then R-Squared Criteria to weed out insignificant independent variables. Of the ten best-fit models provided by Virtual Beach, the multiple linear regression model having the lowest mean square of predicted errors was selected. The relative influence of each of the parameters making up the selected multiple linear regression model was calculated using the standardized coefficient of each parameter relative to the total. Two models were developed for each beach using this approach. The first model included the dependent variable, *E. coli* concentration measured at the routine monitoring location, and sanitary survey parameters, climate data, and tributary data (where applicable) as independent variables. The second model included *E. coli* concentration measured at sampling locations adjacent to the routine monitoring location (if two years of data were available) as additional independent variables. The first and second models could be compared relative to each other in order to gauge the overall effect of the potential *E. coli* sources present at the nearby sampling locations.

Quality control were calculated for *E. coli* analyses as described in the Bacterial Source Tracking at Impaired Beaches in the St. Louis River Area of Concern QAPP and are summarize in Appendix 1.

3.9.3 qPCR Data Interpretation

Guidance regarding interpretation of the qPCR results was provided by staff from Dr. Sandra McLellan's laboratory (University of Wisconsin-Milwaukee, School of Freshwater Sciences). Samples reported as zero were interpreted as non-detects. Samples reported as below the limit of quantification (BLQ) were considered to be detected but marker concentration was too low to quantify. The limit of quantification is dependent on the volume filtered. For a filtered volume of 200 mL BLQ equates to <225 CN/100 mL, and for a filtered sample volume of 400 mL BLQ equates to <112 CN/100 mL. Based on historical analysis of untreated sewage influent, benchmark ranges for the detection of untreated sewage have been established by Dr. Sandra McLellan's laboratory. Samples having a human *Bacteroides* (HF183) concentration ≥1,000 CN/100 mL or a human *Lachnospiraceae* (Lachno2) concentration ≥1,500 CN/100 mL were considered positive for the presence of human fecal contamination (Newton et al., 2011 and Sauer et al., 2011). Note that Lachno3 benchmarks equivalent to those established for HF183 and Lachno2 are not available, therefore, interpretation of the data from those 42 samples analyzed for Lachno3 marker cannot be put into context of human fecal presence versus diluted or undiluted sewage.

4 Results

4.1 Clyde Avenue Boat Launch

4.1.1 Water Quality

The water is dark, turbid and relatively slow moving at this location. Water quality parameters measured at the boat launch and the nearby sampling locations during the 2015 and 2016 beach seasons are summarized in Table 10. Over the two beach seasons, turbidity at the boat launch averaged 13.3 FNU, which is moderate relative to other beaches sampled as part of this investigation. At the routine monitoring location, air and water temperature averaged 22.4°C and 21.7°C, respectively, with wind speed averaging 2.9 mph during the 2015 – 2016 beach seasons. Wave action was minimal at the routine monitoring location, as evidenced by a two-year average of 0.16 ft.

Table 10. Average Values (± Standard Deviation) of Water Quality Parameters Measured at each Sampling Location during the 2015 and 2016 Beach Seasons. NM = Not Measured.

	CLYD-BL	CLYD-DP	CLYD-FP	CLYD-SC	CLYD-ST
Air Temp (°C)	22.4 (5.1)	NM	NM	NM	NM
Wind Speed (mph)	2.9 (2.3)	NM	NM	NM	NM
Wave Height (ft.)	0.16 (0.39)	NM	NM	NM	NM
Water Temp (°C)	21.7 (3.0)	20.1 (1.9)	19.6 (3.8)	16.6 (2.7)	20.1 (3.6)
Turbidity (FNU)	13.3 (8.7)	15.9 (12.2)	16.3 (7.9)	8.0 (14.5)	9.6 (15.4)
pH	7.6 (0.2)	7.8 (0.1)	7.5 (0.4)	7.6 (0.2)	7.5 (0.2)
Specific Conductivity (µS/cm)	185.2 (23.8)	50.6 (13.4)	200.7 (45.9)	334.8 (84.4)	254.2 (46.2)
Dissolved Oxygen (mg/L)	7.1 (1.1)	7.0 (1.3)	7.7 (0.8)	8.6 (1.3)	5.7 (2.4)

4.1.2 *E. coli* and qPCR Analysis Results and Discussion

4.1.2.1 Two-Year Summary and Monitoring Location Results

During the 2015 and 2016 beach seasons, there was a total of 199 surface water samples collected from the five Clyde Avenue sampling points (Table 11). Of the samples collected and analyzed for *E. coli*, 61 (31%) had *E. coli* values greater than 235 MPN/100 mL. Human *Bacteroides* (HF183) or Human *Lachnospiraceae* (LACHNO2) were detected in 24 of the 39 samples sent for qPCR analysis; three samples had HF183 and/or Lachno2 marker concentrations high enough to indicate the presence of human fecal contamination. This indicates that a human source of contamination, from one or more of the sampling points, could be contributing to the high *E. coli* numbers at the Clyde Ave. Boat Launch. All genetic markers assayed were detected at this location (Table 12).

Table 11. Summary of *E. coli* Results Clyde Ave. Boat Launch Location for the 2015 and 2016 Beach Seasons

	CLYD-BL	CLYD-DP	CLYD-FP	CLYD-SC	CLYD-ST	Combined All Sites
Total Number *E. coli* Analyses	66	19	16	64	34	199
Total Number of *E. coli* Exceedances (>235 MPN/100 mL)	12	17	2	17	13	61
Percent *E. coli* Exceedances >235 MPN/100 mL	18%	89%	13%	27%	38%	31%
Minimum *E. coli* MPN/100 mL	2	146	2	7.4	9.7	2
Maximum *E. coli* MPN/100 mL	>2,420	>24,196	1733	5,850	>2,420	>24,196
Average *E. coli* MPN/100 mL (±Std. Dev.)	242 (530)	6,418 (8,448)	169 (434)	456 (942)	503 (692)	939 (3,187)
Geometric Mean *E. coli* (MPN/100 mL)	76	2,480	38	123	202	192

Table 12. Summary of *E. coli* Concentration and Copy Number per Volume for Samples Selected for DNA Marker Analysis at Clyde Avenue Boat Launch. qPCR results for *E. coli* not included in this table.

Rainfall (in)	Collection Date	Sample Site ID	*E. coli* (MPN/100mL)	Human *Bacteroides* (CN/100mL)	Human *Lachnospiraceae* (CN/100mL)	Enterococcus (CN/100mL)	Gull2 (CN/100mL)	Ruminant (CN/100mL)
0.08	6/25/2015	CLYD-SC	248.9	BLQ	0	6125	0	0
0	7/2/2015	CLYD-BL	235.9	0	0	1278	0	0
0.83	8/7/2015	CLYD-SC	> 2419.6	BLQ	BLQ	104063	0	0
0.07	8/10/2015	CLYD-BL	151.5	0	0	1965	0	0
1.33	8/19/2015	CLYD-SC	1732.9	0	316	53084	0	479
1.33	8/19/2015	CLYD-ST	1413.6	2843	4539	44245	0	0
1.77	8/20/2015	CLYD-DP	2419.6	0	281	167883	0	0
1.77	8/20/2015	CLYD-ST	517.2	BLQ	310	9192	0	0
0.74	9/2/2015	CLYD-SC	5850	455	309	17630	0	0
0.84	9/18/2015	CLYD-ST	1119.9	0	0	12230	0	0
1.86	9/24/2015	CLYD-BL	248.9	BLQ	0	5389	0	0
1.86	9/24/2015	CLYD-DP	512	0	0	4632	0	0
1.86	9/24/2015	CLYD-FP	488.4	0	0	7460	0	0
1.86	9/24/2015	CLYD-SC	727	0	0	4937	0	0
1.86	9/24/2015	CLYD-ST	1299.7	0	BLQ	13235	0	0
0	6/6/2016	CLYD-BL	2419.6	BLQ	BLQ	1732	0	0
0	6/6/2016	CLYD-DP	8664	0	BLQ	5702	0	0
0.98	6/15/2016	CLYD-SC	686.7	BLQ	0	25389	0	0
0.98	6/15/2016	CLYD-ST	1119.9	BLQ	0	29201	0	0
0	7/6/2016	CLYD-BL	727	0	BLQ	1245	0	0

Rainfall (in)	Collection Date	Sample Site ID	*E. coli* (MPN/100mL)	Human *Bacteroides* (CN/100mL)	Human *Lachnospiraceae* (CN/100mL)	Enterococcus (CN/100mL)	Gull2 (CN/100mL)	Ruminant (CN/100mL)
0.1	7/11/2016	CLYD-SC	1203.3	BLQ	0	46639	0	0
2.97	7/12/2016	CLYD-BL	1553.1	BLQ	0	11858	0	0
2.97	7/12/2016	CLYD-DP	> 24196	0	0	216262	0	0
2.97	7/12/2016	CLYD-SC	547.5	BLQ	0	17136	0	0
2.97	7/12/2016	CLYD-ST	980.4	914	1070	10631	0	0
0	8/10/2016	CLYD-DP	8664	0	230	669085	0	0
0	8/10/2016	CLYD-SC	547.5	0	0	7552	0	0
0	8/15/2016	CLYD-BL	488.4	0	0	1762	0	0
1.18	8/16/2016	CLYD-SC	> 2419.6	0	BLQ	25071	0	0
1.18	8/16/2016	CLYD-ST	1986.3	BLQ	BLQ	28207	0	0
0	8/17/2016	CLYD-SC	1986.3	0	0	16735	0	0
1.72	8/29/2016	CLYD-BL	> 2419.6	0	0	7347	0	0
1.72	8/29/2016	CLYD-DP	2359	0	0	82572	0	0
1.72	8/29/2016	CLYD-SC	1732.9	0	BLQ	21439	0	0
1.72	8/29/2016	CLYD-ST	517.2	0	0	14018	0	0
1.7	9/6/2016	CLYD-SC	1732.9	0	0	39785	0	0
1.7	9/6/2016	CLYD-ST	816.4	0	BLQ	5948	0	0
0.57	9/8/2016	CLYD-SC	> 2419.6	0	0	28254	0	0
0.57	9/8/2016	CLYD-ST	2419.6	2431	7063	39078	0	0

To address the primary objectives of this investigation, based upon the data from the 2015 – 2016 beach seasons:

1. The frequency of *E. coli* exceedances (i.e., >235 MPN/100 mL) at Clyde Avenue Boat Launch was 18% (12 of the 66 samples collected), which ranks relatively low compared to the other beaches sampled for this project.
2. Human genetic markers (*Bacteroides* and/or *Lachnospiraceae*) were detected in four of the eight samples sent for qPCR analysis, however, in all cases the concentration of these genetic markers were below the method's limit of quantification.

The qPCR results may indicate a dilution effect of the human DNA from one of the potential sources by the time it reaches the boat launch or this result could be from recreational use of the water (i.e., people entering/exiting the water to launch their boats or people wading/swimming at the boat launch). For example, on July 12, 2016 after a significant rain event, Human *Bacteroides* and Human *Lachnospiraceae* were detected at the mouth of Stewart Creek with values at 914 CN/100 mL and 1,070 CN/100 mL, respectively. On this date, Human *Bacteroides* was also detected at levels below the limit of quantification at the boat launch, indicating a diluted human source (potentially from Stewart Creek near the mouth) may be contributing low levels of *E. coli* of human origin at the boat launch. Table 13 shows the multiple linear regression model for the boat launch, consisting of the combination of sanitary survey parameters and climate data that have the greatest influence on the *E. coli* concentration measured at that location. Rainfall (24-hour and 6-day total), wind speed, number of people at the boat launch (non-bathers), along-shore wind velocity, and water temperature comprise this model. Interestingly, all parameters have a positive correlation to *E. coli* concentration with the exception of 24-hour rainfall (i.e., the higher the rainfall total the lower the *E. coli* concentration), which supports the dilution effect theory when dilution is related to 24-hour rainfall total.

Table 13. Multiple Linear Regression Model Showing Relative Influence of Sanitary Survey and Climate Data on *E. coli* Concentration at Clyde Avenue Boat Launch.

Parameter	Relative Influence
24-Hour Total Rainfall	28.08%
6-Day Total Rainfall	27.29%
Wind Speed	13.56%
People at Beach (non-bathers)	11.40%
Along-Shore Wind Velocity	11.08%
Water Temperature	8.58%

4.1.2.2 Determination of E. coli Contribution from Stewart Creek – Upstream (CLYD-SC) and Mouth (CLYD-ST)

Of the 64 upstream Stewart Creek samples (Figure 23), 17 had *E. coli* concentrations above 235 MPN per 100 mL, and 14 of these exceedance samples were analyzed for several genetic markers via qPCR. Human *Bacteroides* was detected in seven of the samples, only one of which was above the limit of quantification (Table 14). Human *Lachnospiraceae* was detected in five of the 14 exceedance samples and was above the limit of quantification for two of the five detections (Table 14).

Figure 23. Sampling team collecting data at Stewart Creek in Smithville park (CLYD-SC).

Samples collected from the mouth of Stewart Creek (CLYDE-ST, Figure 24) had *E. coli* concentration >235 MPN/100 mL in 13 of the 34 samples; ten of these were analyzed for the presence of human and other genetic markers via qPCR. Human *Bacteroides* and/or Human *Lachnospiraceae* were detected in eight (80%) of the exceedance samples, two of which had human marker concentrations high enough to indicate the presence of human fecal contamination (bold values, Table 14). A third sample had human marker concentrations very near this benchmark (Table 14). The remaining samples that were positive for human marker had results at or near the method's limit of quantification (Table 14).

Figure 24. Railroad Bridge where Stewart Creek Enters the St. Louis River (CLYDE-ST).

Table 14. Summary *E. coli* and Genetic Marker copy numbers (CN) for samples sent for qPCR Analyses from the Stewart Creek upstream (CLYD-SC) and mouth (CLYD-ST) locations.

Rainfall (in)	**Collection Date**	**SAMPLE SITE ID**	**LSRI *E. coli* (MPN/100mL)**		**Human *Bacteroides* (CN/100mL)**	**Human *Lachnospiraceae* (CN/100mL)**	**Enterococcus (CN/100mL)**	**Gull2 (CN/100mL)**	**Ruminant (CN/100mL)**
0.08	6/25/2015	CLYD-SC		248.9	BLQ	0	6125	0	0
0.83	8/7/2015	CLYD-SC	>	2419.6	BLQ	BLQ	104063	0	0
1.33	8/19/2015	CLYD-ST		1413.6	**2843**	**4539**	44245	0	0
1.33	8/19/2015	CLYD-SC		1732.9	0	316	53084	0	479
1.77	8/20/2015	CLYD-ST		517.2	BLQ	310	9192	0	0
0.74	9/2/2015	CLYD-SC		5850	455	309	17630	0	0
0.84	9/18/2015	CLYD-ST		1119.9	0	0	12230	0	0
1.86	9/24/2015	CLYD-ST		1299.7	0	BLQ	13235	0	0

Rainfall (in)	Collection Date	SAMPLE SITE ID	LSRI *E. coli* (MPN/100mL)		Human *Bacteroides* (CN/100mL)	Human *Lachnospiraceae* (CN/100mL)	Enterococcus (CN/100mL)	Gull2 (CN/100mL)	Ruminant (CN/100mL)
1.86	9/24/2015	CLYD-SC		727	0	0	4937	0	0
0.98	6/15/2016	CLYD-ST		1119.9	BLQ	0	29201	0	0
0.98	6/15/2016	CLYD-SC		686.7	BLQ	0	25389	0	0
0.1	7/11/2016	CLYD-SC		1203.3	BLQ	0	46639	0	0
2.97	7/12/2016	CLYD-SC		547.5	BLQ	0	17136	0	0
2.97	7/12/2016	CLYD-ST		980.4	914	1070	10631	0	0
0	8/10/2016	CLYD-SC		547.5	0	0	7552	0	0
1.18	8/16/2016	CLYD-ST		1986.3	BLQ	BLQ	28207	0	0
1.18	8/16/2016	CLYD-SC	>	2419.6	0	BLQ	25071	0	0
0	8/17/2016	CLYD-SC		1986.3	0	0	16735	0	0
1.72	8/29/2016	CLYD-ST		517.2	0	0	14018	0	0
1.72	8/29/2016	CLYD-SC		1732.9	0	BLQ	21439	0	0
1.7	9/6/2016	CLYD-ST		816.4	0	BLQ	5948	0	0
1.7	9/6/2016	CLYD-SC		1732.9	0	0	39785	0	0
0.57	9/8/2016	CLYD-SC	>	2419.6	0	0	28254	0	0
0.57	9/8/2016	CLYD-ST		2419.6	**2431**	**7063**	39078	0	0

Following each beach season, samples were selected from dates during which the upstream sample point at Stewart Creek, the sampling point at the mouth of Stewart Creek, and the boat launch were all in exceedance of the *E. coli* regulatory standard in order to determine whether Stewart Creek is a contributing source of *E. coli* of human origin at the beach monitoring location. Dates selected for this purpose were September 24, 2015; July 12, 2016; and August 29, 2016. Results from qPCR analysis of samples collected from these three dates do not indicate a clear correlation between *E. coli* of human origin coming from Stewart Creek at the boat launch because concentrations of both human markers were below the method limit of quantification on two of the dates and neither of the human markers were detected on the third date in the boat launch sample (Table 13). A multiple linear regression model was developed to determine the sanitary survey, water quality, and climate parameters having the most influence on *E. coli* concentrations measured at the boat launch. Unlike the model presented in Table 12, the model in Table 14 includes the *E. coli* concentration measured at the upstream Stewart Creek sampling location. The other surrounding sampling locations were not included in the analysis, as there was only one year or less acquired data for those locations. The model (Table 14) indicates that previous 48-hour rainfall, wind speed, and along-shore wind velocity all have a high relative influence on *E. coli* concentration. There is no correlation between *E. coli* concentration measured at the upstream Stewart Creek location and *E. coli* concentration measured at the boat launch; this parameter did not influence the model. However, the combination of parameters that do influence the model suggest that rain and high wind velocity at the boat launch may cause *E. coli* coming from Stewart Creek to be diluted or carried away from the boat launch location.

Table 15. Multiple Linear Regression Model Showing Parameters (Including Stewart Creek Upstream Location) having the Greatest Relative Influence on *E. coli* Concentration Measured at the Clyde Avenue Boat Launch.

Parameter	Relative Influence
Previous 48-Hour Rainfall	41.90%
Wind Speed	13.03%
Along-Shore Wind Velocity	15.38%
Floatables	26.55%
6-Day Rainfall Total	3.14%

Following both beach seasons, samples were also selected for qPCR analysis in order to determine if *E. coli* of human origin is coming from an upstream location within Stewart Creek or near the mouth of the creek (August 19, 2015; June 15, 2016; August 16, 2016; and September 6 and 8, 2016). For six of the dates where both Stewart Creek locations were in exceedance, the samples collected from the mouth of Stewart Creek had a higher concentration of one or both human markers than samples collected from the upstream sampling location. There were two dates for which the presence of human fecal contamination was confirmed via the qPCR results at the creek mouth sampling location and either not detected or detected at concentrations at or near the quantification limit at the upstream location. These results suggest *E. coli* of human origin may be sourced from a location downstream from the upstream sampling location, perhaps near the mouth of the creek. The samples collected at the mouth of Stewart Creek were diluted with St. Louis River water. Higher values at the mouth could also indicate a possible source upstream in the St. Louis River, which was not investigated for this project.

Although high *E. coli* and incidence of human marker detection may be an AOC issue, in regards to Stewart Creek being on the 303d list. It may not be of utmost priority in regards to this project as the frequency of exceedances is low at the boat landing, and human signal was so low it was not quantifiable.

4.1.2.3 Determination of E. coli Contribution from Waterfowl

During the two beach seasons, a significant amount of goose droppings was observed on the docks and in the grass near the boat landing. While on average, the number of geese observed during the sanitary surveys was relatively low, there were a few days where as many as 20 geese were observed. During a sanitary survey, a private citizen informed staff conducting the sanitary survey that each morning this individual would sweep the goose feces off the boat launch dock into the water. High copy numbers of Enterococcus markers have been correlated to large goose populations (McLellan, personal communication). All measured qPCR values at the Clyde Ave. Site were greater than 1,000 CN/100 mL Enterococcus which could be an indication of geese contributing the contamination at this site.

4.1.2.4 Determination of E. coli Contribution from the Marina

Only two of the sixteen samples collected at the fishing pier in 2015 had high enough *E. coli* concentrations to be considered an exceedance and the one sent for qPCR analysis did not have any human markers detected (Table 13). The close proximity to the boat launch and the low number of exceedances led the sampling team to believe neither this location, nor the marina, were likely to be contributing to the high *E. coli* numbers at the boat launch, so sampling efforts were focused elsewhere in 2016.

4.1.2.5 Determination of E. coli Contribution from Sewage Run-off/Leaky Pipes

During the two beach seasons, active sewage run-off was never observed at or near Stewart Creek. However, a sewage odor was often noted during collection of the upstream samples. This odor could be from the Cloquet Pumping Station located on Knowlton Creek, which is less than two miles to the northeast of the upstream sampling location. The Cloquet Pumping Station receives all of the waste from the local paper mills, which creates a definite odor. Investigation further upstream of Stewart Creek or between CLYD-SC and CLYS-ST may be warranted due Stewart Creek mouth location expressing human signal more frequently than the upstream location.

4.1.2.6 Determination of E. coli Contribution via Storm Grate/Drain Pond (CLYD-DP)

Eighty-nine percent of the samples collected from the drainage pond near the boat launch were exceedances (i.e., >235 MPN/100mL *E. coli*). While no Human Bacteriodes were detected in any of the samples, Human *Lachnospiraceae* markers were detected in three of the six samples sent for qPCR analysis with the third sample having concentrations below the method's limit of quantification (Table 12). Given that there was a greater frequency of human marker detection at the boat launch than in samples collected from the drain pond, this location does not appear to contribute to *E. coli* of human origin at the monitoring location.

4.1.3 LSRI Recommendations

There is little evidence, based on this two-year investigation, that *E. coli* of human origin is an AOC issue at the Clyde Ave. Boat Launch monitoring point. However, *E. coli* concentrations at or above advisory-level were measured at the boat launch monitoring point in 18% of samples collected, and there are obvious sources of *E. coli* at the boat launch that are recommended for mitigation. First, it is recommended that the portable toilet be located further away from the water. Any vandalism or mishandling of these portable toilets would have a quick and direct pathway into the St. Louis River. Second, placing trash receptacles that are emptied on a regular schedule at the boat launch is recommended. Finally, it is recommended that a deterrent for waterfowl be installed, and that signage be placed in a highly-visible location to encourage the boat launch users to not sweep waterfowl feces into the water and discourage feeding of geese and ducks. Further investigation is recommended in order determine the source of *E. coli* in Stewart Creek and its "human" contribution to the Clyde Ave. Boat Launch. More data are needed in order to draw definitive conclusions with regard to source(s) and origin of *E. coli* in and around Stewart Creek.

4.2 Leif Erikson Park

4.2.1 Water Quality

Water is typically clear and wave energy is moderately high in this location, with an average wave height of 0.6 ft. (Table 16). Water quality parameters measured during the 2015 and 2016 beach seasons at Leif

Erikson Park sampling locations are summarized in Table 16. Over the two beach seasons, at the main beach monitoring location (LEIF), turbidity averaged 5.9 FNU, which is relatively low compared to other beaches located within the St. Louis River Area of Concern. Water quality can potentially change in extreme wind and weather as turnover occurs in the lake. Overall, air and water temperature averaged 21.6°C and 16.0°C, respectively, with wind speed averaging 3.5 mph during the 2015 – 2016 beach seasons.

Table 16 Average (± Standard deviation) Values of Water Quality Parameters Measured at each Leif Erikson Park Sampling Location during the 2015 and 2016 Beach Seasons of the Bacterial Source Tracking Project. NM = Not Measured.

	LEIF	LEIF-CH	LEIF-N	LEIF-ST
Air Temp (°C)	21.6 (5.8)	NM	NM	NM
Wind Speed (mph)	3.5 (2.2)	NM	NM	NM
Wave Height (ft.)	0.6 (0.7)	NM	NM	NM
Water Temp (°C)	16.0 (4.3)	16.4 (2.3)	16.1 (4.1)	15.7 (3.6)
Dissolved Oxygen (mg/L)	10.1 (1.1)	9.3 (0.8)	10.3 (1.1)	10.0 (1.2)
pH	7.9 (0.3)	7.8 (0.3)	8.0 (0.4)	7.8 (0.3)
Specific Conductivity (µS/cm)	126.7 (48.6)	532.3 (185.9)	135.1 (76.7)	516.3 (508.4)
Turbidity (FNU)	5.9 (7.7)	7.1 (14.8)	5.4 (6.9)	8.6 (10.6)

4.2.2 *E. coli* and qPCR Analysis Results and Discussion

4.2.2.1 Two-Year Summary and Monitoring Location Results

Overall, 45% of the 276 water samples, collected from the four Leif Erickson Park sampling locations during the 2015 and 2016 beach seasons, had greater than 235 MPN/100 mL *E. coli* (Table 17). The storm drain at the south end of the beach (LEIF-ST) and the Chester Creek outfall (LEIF-CH) had the most exceedances of all sites sampled for this project. The four Leif Erickson Park sampling locations had an average combined frequency of 89% for human genetic marker detection. All six of the genetic markers assayed were detected at this location (Table 18), however, ruminant marker was detected in only one sample (9/18/2015, LEIF-CH, Table 18) and ruminant contribution to measured *E. coli* concentration is considered negligible at this beach. Following the 2015 beach season, the preliminary results from this investigation did not point to parking lot runoff or activity from the Port of Duluth-Superior as contributing sources of *E. coli* to the beach. Rather, preliminary data suggested further investigation of the sampling points surrounding the beach center was necessary. The results presented below discuss these sources directly.

To address the primary objectives of this investigation, based upon the data from the 2015 – 2016 beach seasons:

1. The frequency of *E. coli* exceedances (i.e., >235 MPN/100 mL) at Leif Erikson Park, center-of-beach (LEIF) monitoring location was 10% (7 of the 69 samples collected), which is relatively low compared to the other beaches sampled for this project.

2. Human genetic markers (*Bacteroides* and/or *Lachnospiraceae*) were detected in 83% (5 of 6 samples) of samples sent for qPCR analysis; one of which was below the method's limit of quantification (BLQ).

Both *E. coli* and qPCR results indicate that there may be a dilution effect at the center of beach location, coming from two sources to the east that consistently had very high *E. coli* exceedances. For example, on August 7, 2015, samples collected from the north end of the beach and from Chester Creek had substantially higher *E. coli* concentrations than the sample collected from the beach monitoring location. The sample collected from Chester Creek had an *E. coli* concentration of >24,196 MPN/100 mL, moving west to the north end of the beach the *E. coli* concentration was reduced to 1,203 MPN/100 mL (a value that would trigger a beach closure), and moving further southwest the *E. coli* concentration at the beach monitoring location was reduced to about half at 613 MPN/100 mL (a value that would trigger a beach advisory). The DNA marker results from this date follow the same trend, while the *E. coli* measured from all three locations had quantifiable human markers, there was a higher concentration of human DNA marker in samples collected from Chester Creek and the north end of the beach than from the center-of-beach/beach monitoring location. This dilution effect at the beach monitoring location was also very evident on September 2 and 24, 2015 and August 10, 2016. This result indicates that the beach monitoring location may not be representative of the *E. coli* concentrations that bathers may be exposed to along the entirety of the beach, and that additional monitoring locations may be needed in order to generate a clear picture of exposure.

Table 17. Summary of *E. coli* Results for the 2015 and 2016 Beach Seasons at Leif Erikson Park.

	LEIF	LEIF-CH	LEIF-N	LEIF-ST	All Sites
Total Number *E. coli* Analyses	69	69	69	69	276
Total Number of *E. coli* Exceedances (>235 MPN/100 mL)	7	64	8	45	124
Percent *E. coli* Exceedances >235 MPN/100 mL	10%	93%	12%	65%	45%
Minimum *E. coli* MPN/100 mL	<1	98	1	23	<1
Maximum *E. coli* MPN/100 mL	1,414	36,540	1,733	>24,196	36,540
Average *E. coli* MPN/100 mL (±Std. Dev.)	114 (262)	2,375 (4,882)	141 (321)	2,616 (5,468)	1,325 (3837)
Geometric Mean *E. coli* MPN/100 mL	25	1283	33	6488	663

Table 18 Summary of *E. coli* values and Copy Numbers (CN) for all Leif Erikson park samples sent for qPCR analysis. qPCR results for *E. coli* not included in this table.

Rainfall (in)	Collection Date	Sample Site ID	*E. coli* (MPN/100 mL)	Human *Bacteroides* (CN/100 mL)	Human *Lachnospiraceae* (CN/100 mL)	Enterococcus (CN/100 mL)	Gull (CN/100 mL)	Ruminant (CN/100 mL)
0	6/2/2015	LEIF-ST	> 2419.6	1182	1205	28523	0	0
0.6	6/30/2015	LEIF-N	290.9	BLQ	110	5848	0	0
0.88	7/9/2015	LEIF-ST	> 2419.6	1009	24635	23641	0	0
0.94	7/14/2015	LEIF	248.1		255			0
0.94	7/14/2015	LEIF-N	307.6	178	916	9328	920	0
0	7/30/2015	LEIF-CH	1986.3	BLQ	131	516	0	0
0.02	8/4/2015	LEIF-CH	1986.3	354	BLQ	337	0	0
0.83	8/7/2015	LEIF	613.1	442	182	4585	0	0
0.83	8/7/2015	LEIF-CH	> 2419.6					
0.83	8/7/2015	LEIF-N	1203.3	479	232	3964	0	0
0.83	8/7/2015	LEIF-ST	816.4	285	1555	18649	0	0
0.07	8/10/2015	LEIF-CH	2419.6	647	571	19168	0	0
0	8/12/2015	LEIF-CH	> 2419.6	0	0	367	0	0
0	8/12/2015	LEIF-ST	> 2419.6					
0	8/18/2015	LEIF-CH	866.4	2324	910	11972	0	0
0	8/18/2015	LEIF-ST	344.8	0	2527	14895	0	0
1.33	8/19/2015	LEIF-CH	> 2419.6					
1.33	8/19/2015	LEIF-ST	> 2419.6					
1.77	8/20/2015	LEIF-ST	579.4	164	8747	3824	0	0
0.02	8/25/2015	LEIF-ST	> 2419.6	BLQ	307	3975	0	0
0	8/27/2015	LEIF-CH	866.4	906	337	4997	0	0
0.05	8/31/2015	LEIF-CH	3654	BLQ	0	585	0	0
0.74	9/2/2015	LEIF	1413.6	174	114	4022	0	0
0.74	9/2/2015	LEIF-CH	36540					
0.74	9/2/2015	LEIF-N	1732.9	270	240	8768	0	0
0.74	9/2/2015	LEIF-ST	19350					
0.84	9/18/2015	LEIF-CH	1624	13803	10161	30563	0	907
0.84	9/18/2015	LEIF-ST	1019	18689	3448	162927	0	0
1.86	9/24/2015	LEIF	980.4	0	0	2495	0	0
1.86	9/24/2015	LEIF-CH	1483					
1.86	9/24/2015	LEIF-ST	> 24196	4026	9287	346175	0	0
1.86	9/24/2015	LEIF-ST	19863	2360	4571	154236	0	0
0.98	6/15/2016	LEIF	307.6	BLQ	0	12261	639	0
0.98	6/15/2016	LEIF-CH	686.7	BLQ	BLQ	17282	498	0
0.98	6/15/2016	LEIF-N	727	BLQ	0	13889	659	0

Rainfall (in)	Collection Date	Sample Site ID	*E. coli* (MPN/100 mL)	Human *Bacteroides* (CN/100 mL)	Human *Lachnospiraceae* (CN/100 mL)	Enterococcus (CN/100 mL)	Gull (CN/100 mL)	Ruminant (CN/100 mL)
0.1	7/11/2016	LEIF-CH	3448	307	608	102313	5690	0
0.1	7/11/2016	LEIF-ST	3448	767	1430	262989	1207	0
2.97	7/12/2016	LEIF-CH	504	320	580	14398	0	0
2.97	7/12/2016	LEIF-ST	520	5258	5509	7150	0	0
0	7/20/2016	LEIF-ST	14136	0	0	31441	0	0
0	8/10/2016	LEIF	1046.2	BLQ	175	41821	0	0
0	8/10/2016	LEIF-CH	17329	1141	3837	43187	7481	0
0	8/10/2016	LEIF-N	727	0	BLQ	6623	0	0
0	8/10/2016	LEIF-ST	461.1	0	0	4833	0	0
0	8/24/2016	LEIF-ST	1986.3	0	0	6650	0	0

A multiple linear regression model was developed to determine the sanitary survey, water quality, and climate parameters having the most influence on *E. coli* concentrations measured at the beach center. The model (Table 19) indicates that previous 48-hour rainfall, wave height, and current speed all have a high relative influence on *E. coli* concentration at the beach center. This model also supports the theory that *E. coli* originating from Chester Creek to the north and/or the storm drain outfall to the south may be transported to the beach center through increased runoff (previous 48-hour precipitation), increased wave action, and increased current speed. In this scenario, concentrations of *E. coli* are increased at the beach center, but due to dilution, are lower than the *E. coli* concentration at the origin.

Table 19. Multiple Linear Regression Model Showing Relative Influence of Sanitary Survey Parameters on *E. coli* Concentration at Leif Erikson Park Beach Center.

Parameter	Relative Influence
Previous 48-Hour Rainfall	22.83%
Wave Height	16.21%
Water Temperature	14.98%
Cloud Cover	14.73%
Current Speed	12.90%
Water Clarity	9.47%
People at Beach	8.88%

4.2.2.2 Determination of E. coli Contribution from Storm Water and Parking Lot Runoff at Leif Erikson Park

All but one of the exceedances that occurred (August 10, 2016) at the center-of-beach location, occurred during a rain event (Table 18), indicating that rainfall is a contributing factor in the *E. coli* exceedances at this location. The multiple linear regression model (Table 19) shows previous 48-hour rainfall having the highest relative influence on the *E. coli* concentration measured at the beach center. A second multiple linear regression model, which included sanitary survey, water quality, and climate data, as well as, *E. coli* concentration at the north end of the beach, Chester Creek, and the storm drain outfall sampling locations, indicates that previous 48-hour rainfall still has a high influence on the model (Table 20) although the relative influence of this parameter on the model is less once the *E. coli* concentrations at the other sampling locations are factored in (Table 20). Overall *E. coli* counts were typically higher in all sampling locations after a rain event. However, runoff from the storm drain and Chester Creek is an issue even during non-rain events as exceedances were frequent at these locations and both are flowing continuously. People tend to utilize the length of the Leif Erikson Park beach, making the average of 45% exceedance rate for the four locations a concern.

4.2.2.3 Determination of E. coli Contribution from Leif Storm Drain (LEIF-ST)

Samples collected from the storm water outfall located at the south end of Leif Erikson Park beach were in exceeded 235 MPN/100 mL *E. coli* in 45 of the 69 total samples (Table 18). There were 25 samples having *E. coli* concentrations greater than 1,000 MPN/100 mL, the value typically used to issue a beach closure, which could be a human health concern. Interestingly, less than half (18) of the 45 exceedances occurred during rain events (Table 18); indicating this is not an issue only associated with rain. The storm drain varied in flow intensity and on one occasion (August 12, 2015) there was a flush of increased flow of cloudy water that occurred during sample collection. Human genetic marker was detected in 82% of the samples analyzed via qPCR; 53% of the samples analyzed via qPCR were positive for the presence of human fecal contamination. These results confirm that the discharge coming from the storm drain at the south end of Leif Erikson Park contains high *E. coli* concentrations that are of human origin. The storm drain at Leif Erikson Park was in fact the highest human marker point source found during the entire two-year investigation. The proximity of this point source to the beach is a human health concern.

4.2.2.4 Determination of E. coli Contribution from Chester Creek (LEIF-CH)

Ninety-three percent (64 of 69) of samples collected from the mouth of Chester Creek exhibited *E. coli* concentrations that exceeded 235 MPN/100 mL (Table 18). There were 44 samples with *E. coli* concentration greater than 1,000 MPN/100 mL, the value typically used to issue a beach closure. Of the 17 samples sent for qPCR analysis, Human *Bacteroides*, Human *Lachnospiraceae* (Lachno2), or Lachno3 were detected in 94% of the samples, and 25% percent of the samples had human DNA marker concentrations above the benchmark used to detect the presence of human fecal contamination. Throughout the two-year investigation, there was a trend of the highest *E. coli* concentration measured at Chester Creek and an apparent dilution effect as evidenced by decreasing *E. coli* concentrations moving west-southwest along the beach.

A second multiple linear regression model, which included sanitary survey, water quality, and climate data, as well as, *E. coli* concentration at the north end of the beach (Figure 25), Chester Creek, and the storm drain outfall sampling locations, indicates that when the other sampling locations are factored into the model it is the *E. coli* concentration measured at the north end of the beach that has the greatest influence at the *E. coli* concentration measured at the beach center. This confirms the directionality of the source; there is a strong positive correlation between *E. coli* concentration at the north end of the beach and *E. coli* concentration at the center of the beach.

Table 20. Multiple Linear Regression Model Showing Relative Influence of Sanitary Survey Parameters and *E. coli* Concentration at Surrounding Sampling Locations on *E. coli* Concentration Measured at Leif Erikson Park Beach Center.

Parameter	Relative Influence
[*E. coli*], North End of Beach	53.87%
Water Temperature	11.39%
Current Speed	10.21%
Previous 48-Hour Rainfall	10.18%
Along-Shore Wind Velocity	7.73%
Floatables	6.62%

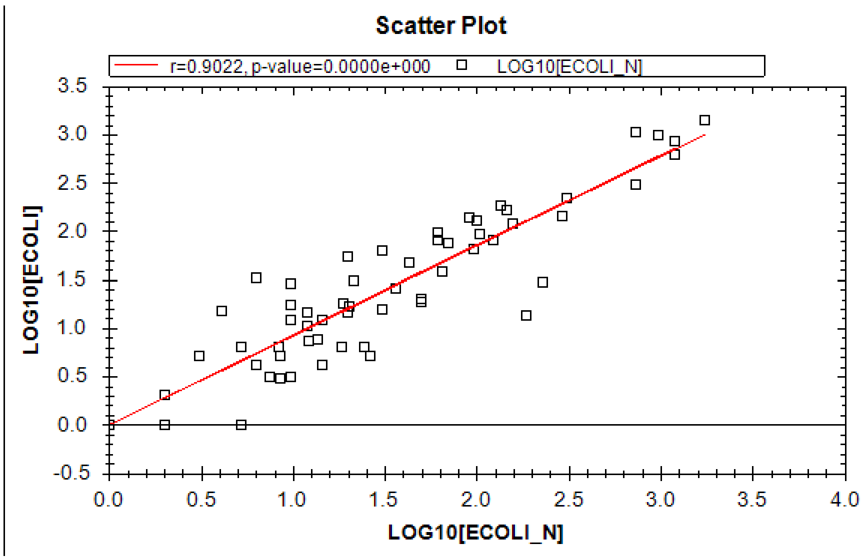

Figure 25. Scatter Plot Showing Positive Correlation Between *E. coli* Concentration at the North End of Leif Erikson Park Beach (ECOLI_N) and *E. coli* Concentration at the Beach Center (ECOLI).

The contribution from Chester Creek to *E. coli* exceedances at the main beach location is not only significant in regards to the frequency of exceedances, but more concerning is the level of contamination and the human health issues that could be associated with these high levels of bacteria. This outfall is contributing *E. coli* of human origin to the beach at Leif Erikson Park.

4.2.2.5 Contribution of Recreational Activity at Leif Erikson Park

It was commonly noted that people would camp on the beach at Leif Erikson Park or near Chester Creek. Trash was often left behind and although it was never observed, the possibility of human excrement being washed into the water is likely as it appeared there may have been homeless people staying here for extended periods of time. In fact, the number of people observed at the beach during the sanitary survey did influence the multiple linear regression model for the beach center (Table 19); when the other sampling locations are factored into the model the number of people observed is no longer a contributing factor but relative concentration of floatables (floating debris; an indicator of human activity) did influence this second model (Table 20).

4.2.3 LSRI Recommendations

Results from this investigation point to Chester Creek as a primary source of *E. coli* at Leif Erikson Park beach, given the strong correlation between *E. coli* measured at the north end of the beach (closest sampling point to Chester Creek) and *E. coli* measured at the center of the beach, as well as, the very high influence of *E. coli* concentration at the north end of the beach on the multiple linear regression model for the beach center. The storm water outfall that drains directly into Lake Superior at Leif Erikson Park beach also had frequent, high *E. coli* concentrations (often above levels that would be used for beach closure), which were of human origin. While the correlation between *E. coli* concentration at the storm water outfall and the beach center is less significant, it is nonetheless alarming that this source of human *E. coli* is draining directly adjacent to a swimming beach. Sampling the beach only at the routine monitoring location may be giving an inaccurate representation of the beach users' *E. coli* exposure, underestimating the actual exposure along the entire length of the beach and the risk to human health. It is recommended at this time that serious investigation be done, to determine the source of high *E. coli* concentrations coming from the storm drain/culvert at the south end of the beach and from Chester Creek. On one occasion at Chester Creek, *E. coli* values were as high as 36,540 MPN/100 mL. While *E. coli* is used as a pathogen indicator for routine monitoring purposes, this two-year investigation has confirmed that the origin of the *E. coli* is very often human. The fact that Human *Bacteroides* or Human *Lachnospiraceae* (Lachno2) values were above the benchmark used to detect the presence of human fecal contamination in 53% and 25% of samples collected at the storm drain and Chester Creek, respectively, indicate that the source of *E. coli* should be addressed as an AOC issue and sources should be controlled or mitigated before the beneficial use impairment is removed. These two locations should be considered a high priority for determining how to reduce human health risk.

4.3 Minnesota Point/15th Street Harbor Side

4.3.1 Water Quality

Over the 2015 and 2016 beach seasons, turbidity averaged 12.8 FNU at the center of beach location, which is low relative to other beaches located within the St. Louis River Area of Concern. Overall, air and water temperature averaged 21.6°C and 19.3°C, respectively, with wind speed averaging 5.0 mph during the 2015 – 2016 beach seasons. Water quality parameters measured at each sampling location can be found in Table 21. Water quality can potentially change with strong currents and Lake Superior seiche, which can occur in extreme wind and weather. High variation was seen in wind speed and air temperature during the 2015 and 2016 beach seasons. Despite strong winds at times, the wave height measured at this beach was less than one-half foot on average over the entire project duration. The harbor side of Minnesota Point is more protected from wind-driven wave action than the Lake Superior side of the point.

Table 21. Average (± Standard deviation) Values of Water Quality Parameters Measured at each Minnesota 15th St. Sampling Location during the 2015 and 2016 Beach Seasons of the Bacterial Source Tracking Project. NM = Not Measured.

	MN15 (Center of Beach)	MN15-E (East End of Beach)	MN15 –ST (Culvert)	MN15-W (West End of Beach)
Air Temperature (°C)	21.6(5.5)	NM	NM	NM
Dissolved oxygen (mg/L)	9.1 (1.1)	9.1 (1.1)	9.9 (1.2)	9.6 (1.2)
Wave Height (ft.)	0.3 (0.4)	NM	NM	NM
Water Temperature (°C)	19.3 (3.2)	19.2 (3.1)	17.7 (2.5)	19.4 (3.3)
Turbidity (FNU)	12.8 (17.7)	10.2 (5.9)	10.1 (5.2)	11.5 (7.0)
pH	7.8 (0.3)	7.8 (0.3)	7.9 (0.4)	8.0 (0.4)
Specific Conductivity (µS/cm)	168.5 (17.3)	169.0 (17.5)	160.3 (22.9)	168.2 (17.8)
Wind Speed (mph)	5.0 (4.0)	NM	NM	NM

4.3.2 *E. coli* Source Tracking Results and Discussion

During the 2015 and 2016 beach seasons, a total of 394 samples were collected from Minnesota Point - 15th St. locations. The *E. coli* concentration was greater >235 MPN/100 mL in 18% of samples collected (Table 22), the lowest combined average of the beaches sampled for this project. The center-of beach (MN-15) had the greatest frequency of *E. coli* exceedances at 22%, while the east end of the beach (MN15-E) had 17% exceedance frequencies and the west (MN15-W) end had 16% exceedance frequencies (Table 22). There appears to be a relationship between high *E. coli* concentrations, *E. coli* of human origin, and high-precipitation amount rain events. August 19, 2015 and July 13, 2016 were two of the highest precipitation rain events of this two-year investigation, and were the only two dates for which human DNA markers were detected in quantifiable concentrations. Human DNA markers (i.e., *Bacteroides* (HF183) or Human *Lachnospiraceae* (LACHNO2 or LACHNO3) were detected in ten of 21 samples analyzed via qPCR; half above the qPCR method's limit of quantification. All six genetic markers assayed were detected at this location (Table 23).

Table 22. Summary of *E. coli* Results for Sample Collected at the Minnesota 15th St. Location in the 2015 and 2016 Beach Seasons

	MN15	MN15-E	MN15-ST	MN15-W	All Sites
Total Number *E. coli* Analyses	126	129	11	128	394
Total Number of *E. coli* Exceedances (>235 MPN/100 mL)	28	22	0	21	71
Percent *E. coli* Exceedances >235 MPN/100 mL	22%	17%	0%	16%	18%
Minimum *E. coli* MPN/100 mL	8.6	14.8	21.3	7.5	7.5
Maximum *E. coli* MPN/100 mL	>2419.6	1553.1	112.6	1732.9	>2419.6
Average *E. coli* MPN/100 mL (±Std. Dev.)	185 (324)	172 (239)	55 (32)	175 (270)	174 (276)
Geometric Mean *E. coli* MPN/100 mL	98	101	49	97	102

Table 23. Summary of *E. coli* values and copy numbers (CN) for all Minnesota Point 15th St. samples sent for qPCR analysis. qPCR results for *E. coli* not included in this table.

Rain-fall (in)	Collection Date	Sample Site ID	*E. coli* (MPN/100 mL)		Human *Bacteroides* (CN/100 mL)	Human *Lachnospiraceae* (CN/100 mL)	Enterococcus (CN/100 mL)	Gull (CN/100 mL)	Ruminant (CN/100 mL)
0.6	6/30/2015	MN15		248.1	BLQ	BLQ	1762	1612	0
0	7/16/2015	MN15-E		248.9	0	BLQ	14643	0	0
0	7/28/2015	MN15-E		980.4	0	0	876772	742921	338
1.33	8/19/2015	MN15-E		307.6	884	299	25423	0	0
1.33	8/19/2015	MN15-W		122.3	BLQ	227	3009	0	0
0.23	6/29/2016	MN15-W		579.4	0	0	6679	1790	0
0	7/5/2016	MN15		488.4	0	0	3197	527	0
0	7/5/2016	MN15-E		488.4	0	0	2328	470	0
0	7/5/2016	MN15-W		410.6	0	0	1565	331	0
0.1	7/11/2016	MN15-W		1046.2	BLQ	BLQ	7666	0	0
2.97	7/12/2016	MN15		648.8	BLQ	BLQ	13486	1233	0
2.97	7/12/2016	MN15-E		344.8	BLQ	BLQ	11102	2229	0
3.13	7/13/2016	MN15		613.1	518	530	10107	944	0
3.13	7/13/2016	MN15-E		613.1	564	716	12954	1722	0
3.13	7/13/2016	MN15-W		648.8	860	763	11222	1961	0
0	7/20/2016	MN15	>	2419.6	0	0	2223	0	0
0	7/20/2016	MN15-E		488.4	0	0	586	0	0
0	7/20/2016	MN15-W		1732.9	0	0	2168	0	0
0	8/8/2016	MN15-W		920.8	0	0	45206	0	0
0	8/10/2016	MN15		579.4	0	0	3106	0	0

4.3.2.1 Minnesota Point/15th Street Harbor Side Beach

In order to address the primary objectives of this project, based on the 2015-2016 beach seasons:

1. The frequency of *E. coli* exceedances at (i.e., >235 MPN/100 mL) Minnesota Point/15th Street Harbor Side Beach (routine monitoring location only) was 22% (28 of the 126 samples collected), which is a moderately low frequency compared to the other beaches sampled for this project.
2. At the routine monitoring location, human genetic markers (*Bacteroides* and/or *Lachnospiraceae*) were detected in half of the six samples sent for qPCR analysis.

At the routine monitoring location, one of the three samples analyzed via qPCR was above the method's limit of quantification for human genetic markers. This sample was collected following 3.13 inches of precipitation in the previous 24-hour period. *Enterococcus* genetic marker, which is indicative of the presence of geese and the gull genetic marker were found most often in the samples collected from the center-of-beach location. Geese were observed much more frequently than gulls at this location; 3.3 geese were observed on average compared to 0.3 gull per day on average.

A multiple linear regression model was created to determine the most influential sanitary survey, climate, and flow parameters on the concentration of *E. coli* measured at the beach center (Table 24). Given the rare occurrence of observations of gulls, this parameter was not included in the model determination. The number of geese were included, but did not factor in as influencing the model. Without factoring in the *E. coli* concentration measured at the east and west sides of the beach, the combination of flow through the Duluth Entry (both one-week average and instantaneous flow), along-shore wind velocity, water clarity, and the amount of algae observed on the beach and in the nearshore area are what make up the multiple linear regression model that could be used to predict *E. coli* concentration at the beach center. The parameter having the largest influence on the model as a whole is one-week average flow rate through the Duluth Entry (United States Geological Survey station on Ariel Lift Bridge). The relationship between this parameter and the *E. coli* concentration at the beach center is shown in Figure 26. Positive flow values indicate water flowing from the St. Louis River Estuary into Lake Superior, while negative flow values indicate water flowing from Lake Superior into the St. Louis River Estuary. The relationship in Figure 26 indicates that when the water mass is mostly composed of Lake Superior water, *E. coli* concentrations are lower, however, when water is flowing from the estuary into Lake Superior, *E. coli* concentrations are higher.

Table 24. Multiple Linear Regression Model Showing Relative Influence of Sanitary Survey Parameters and Climate and Flow Data on the *E. coli* Concentration Measured in Samples Collected at the Beach Center.

Parameter	Relative Influence
One-Week Average Flow; Duluth Entry	37.55%
Along-Shore Wind Velocity	18.88%
Clarity (categorical)	13.34%
Amount of Algae on Beach (categorical)	12.46%
Instant Flow Rate; Duluth Entry	8.91%
Amount of Nearshore Algae (categorical)	8.86%

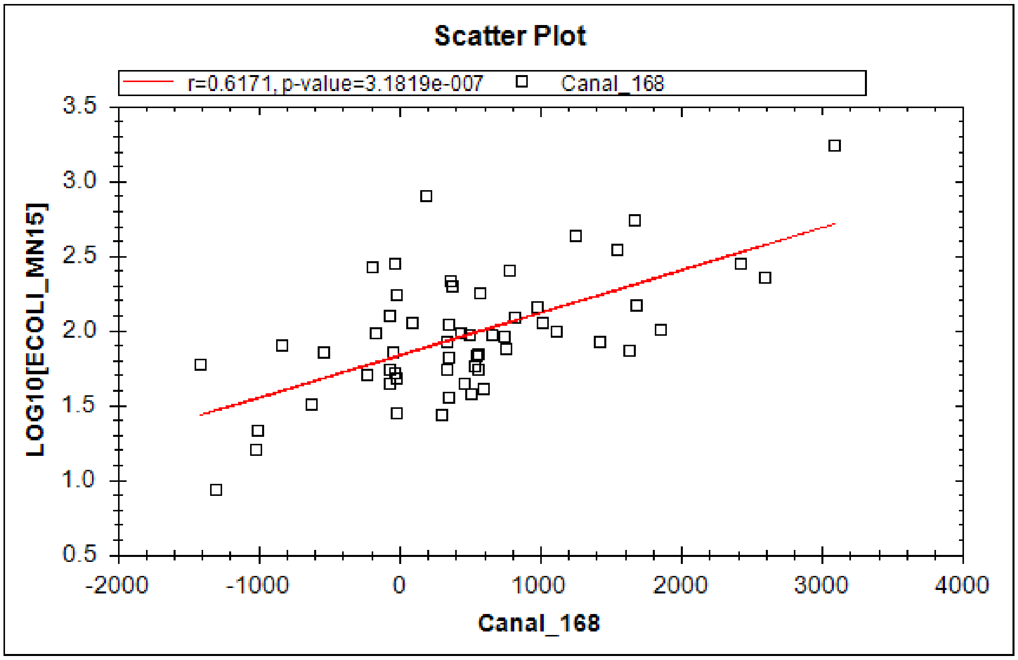

Figure 26. Scatter Plot of *E. coli* Concentration (log-transformed) at MN15 Beach Center and One-Week Average Discharge Through the Duluth Entry.

4.3.2.2 Determination of E. coli Contribution from Storm Water/Parking Lot Runoff

With the aim of determining if storm water runoff from the parking lots or near shore areas were contributing to high *E. coli* concentrations, samples were routinely collected not only from the center of beach location, but also from the northwest end (MN15-W) and the southeast end (MN15-E). A total of 21 exceedances occurred (>235 MPN/100mL *E. coli*) in the 128 samples that were collected from MN15-W, the beach closest to the Duluth Boat Club parking lot. Seven filters, from MN15-W were sent for qPCR analysis and three of the samples (43%) had human genetic markers detected, one of which was below the limit of quantification (Table 24). In 2015, during rain events, samples were also collected from the culvert (Figure 27; MN15-ST) off shore of the west end parking lot, but none of the eleven samples were exceedances so this sampling location was abandoned in 2016 in order to put sampling efforts elsewhere.

Figure 27 Culvert (MN15-ST) off Duluth boat club parking lot on west end of Minnesota 15th St. beach.

The east side of the beach, near the parking lot for the United States Army Reserve Center, showed very similar results to the west side. With the frequency of exceedances at 17 percent, eight of the 22 exceedances were sent for qPCR analysis. Four of the eight had human genetic markers detected in them: two below the limit of quantification. The overall results do not give a clear indication of directionality of the *E. coli* contributing to the center-of-beach location. Transect sampling, discussed in more detail below, indicated that overall, the near-shore samples (ankle depth) had significantly higher *E. coli* concentrations than the waist depth samples, suggesting that a nearshore source (i.e., storm water or parking lot runoff) may be contributing to the high *E. coli* concentrations at the center of beach location. Results do not indicate however that the *E. coli* or a human source in particular, was coming from one end of the beach or the other. Enterococcus genetic markers were found in relatively high abundancies in all of the Minnesota 15th St samples which may be an indication that geese are a contributing factor to the *E. coli* at the beach. Geese were often observed in or near the Duluth Boat Club parking lot (Figure 28).

Figure 28 Geese often observed near the Duluth boat club parking lot off the west side of the Minnesota Point 15th St. Beach

A second multiple linear regression model was created that combined the parameters from the first model (i.e., sanitary survey, climate, flow) with the *E. coli* concentration measured at the east and west side of the beach in order to determine the combination of all parameters measured having the most influence on the *E. coli* concentration at the beach center (Table 25). The combination of *E. coli* concentration on the east side of the beach, one-week average discharge through the Duluth Entry, along-shore wind velocity, amount of nearshore algae, and previous rainfall (24- and 48-hours prior) is what makes up the model that could be used to predict *E. coli* concentration at the beach center. Interestingly, it is only the east side of the beach that factors into the model, with a very high relative influence. When considering the influence of flow into and out of the Duluth Entry, this result is not unexpected, as when water from the estuary is flowing into the Lake that mass of water would be going from east to west along the beach. In this case, flow from the Duluth Entry could be considered a proxy for rainfall and its effect on the St. Louis River watershed as a whole. When the watershed experiences a high-volume input during a high-precipitation rain event, the discharge from tributaries within the estuary would result in a high flow rate of river water out of the Duluth Entry.

Table 25. Multiple Linear Regression Model Showing Relative Influence of *E. coli* Concentration at Nearby Sampling Locations, Sanitary Survey Parameters, Climate Data, and Flow Data on Measured *E. coli* Concentration at MN15 Beach Center.

Parameter	Relative Influence

E. coli Concentration East Side MN15 Beach	51.75%
One-Week Average Flow; Duluth Entry	24.77%
Along-Shore Wind Velocity	9.93%
Nearshore Algae Amount	6.66%
Previous 48-Hour Rainfall Total	4.50%
Previous 24-Hour Rainfall Total	2.39%

4.3.2.3 Determination of E. coli Contribution from Recreational Activities

The number of bathers (both in and out of the water) and the total number of people recreating at this beach was relatively rare. On average, there were 0.78 bathers observed per day and of these there were 0.02 bathers on average per day in the water. During the 2015-2016 beach seasons, there were an average of 0.46 people per day observed recreating. There was also less than one dog observed per day on average during the two-year investigation. These events were all too rare to be included in the multiple linear regression model determination, and were not included in the list of sanitary survey parameters used to inform the model. Based upon observational data alone, recreational activities do not appear to contribute to *E. coli* concentration measured at the beach center.

4.3.2.4 Determination of E. coli Contribution from Surrounding Ports

In order to determine if there was an off-shore contribution to *E. coli* concentrations, transect sampling was done during the 2016 beach season. Samples were collect at three depths (waist (3), knee (2), and ankle (1)) in the center of beach location as well as the west and east ends of the beach. Analysis of Variance (ANOVA) tests comparing *E. coli* concentrations at each depth for each sampling location (center, East side, West side) was completed and no statistically significant difference was found between the depths at each location. However, when the beach was looked at as a whole, there was a statistically significant difference in *E. coli* concentrations between depth 1 (ankle deep) and depth 3 (waist deep), with higher numbers in the shallower depth indicating that that source of *E. coli* was more likely a nearshore contributor rather than off-shore. The multiple linear regression model suggests that river water, rather than Lake Superior water, influences the *E. coli* concentration at the beach center (Table 25). No direct correlation can be made to port activity, although the majority of the port activity within the Duluth-Superior harbor happens within the estuary.

4.3.3 LSRI Recommendations

In terms of the St. Louis River Area of Concern removal target, the *E. coli* measured at Minnesota Point 15th St. beach does not appear to be from a "controllable" source (i.e., *E. coli* is not of human origin). Although there does appear to be some human signal showing up approximately half of the time it was not detected at levels above the benchmark for human sewage detection and may be miniscule in the actual contribution of *E. coli* leading to beach advisories or closures. This beach may be lower priority for further investigation. Investigating the relationship between *E. coli* values at this beach and near the local wastewater treatment facilities may be helpful. Applying hydrodynamic modeling at this beach may also be useful as water flow from the St. Louis River estuary to the Duluth entry may be a contributor to high *E. coli* concentrations, particularly during large rain events.

With the elevated *Enterococcus* marker detection frequency, it is recommended that goose deterrents be placed near the parking lot at each end of the beach to deter waterfowl from congregating in the area near the beach.

4.4 Barker's Island Inner

4.4.1 Water Quality

Water quality parameters measured at each Barker's Island sampling location during the 2015 and 2016 beach seasons are summarized in Table 26. The water within the bay sits relatively stagnant during dry, calm weather. Over the two beach seasons, at the main beach location, turbidity averaged 15.0 FNU, which is moderate relative to other beaches located within the St. Louis River Area of Concern. Water quality can potentially change with the strong currents that can occur in extreme wind and weather. Overall, air and water temperature averaged 22.4°C and 21.2°C, respectively, with wind speed averaging 3.7 mph during the 2015 – 2016 beach seasons.

Table 26 Average (± Standard deviation) Values of Water Quality Parameters Measured at each Barker's Island Inner Beach Sampling Locations during the 2015 and 2016 Beach Seasons of the Bacterial Source Tracking Project. NM = Not Measured.

	BARK	BARK-BL	BARK-FP	BARK-PP	BARK-PP243	BARK-PP246	BARK-PP251	BARK-ST	All Sites
Air Temperature (°C)	22.4 (5.6)	NM	NM	NM	NM	NM	NM	NM	22.4 (5.6)
Wind Speed (mph)	3.7 (3.3)	NM	NM	NM	NM	NM	NM	NM	3.7 (3.3)
Wave Height (ft)	0.13 (0.42)	NM	NM	NM	NM	NM	NM	NM	0.13 (0.42)
Water Temperature (°C)	21.2 (3.1)	20.4 (2.8)	19.9 (2.6)	20.5 (4.1)	17.3 (2.9)	17.2 (2.9)	18.6 (3.8)	19.7 (3.1)	19.8 (3.5)
Turbidity (NTU)	15.0 (12.5)	7.3 (3.4)	12.0 (6.2)	14.9 (17.9)	4.5 (5.1)	24.7 (78.7)	15.5 (23.1)	24.6 (29.2)	16.7 (31.6)

pH	7.8 (0.4)	7.8 (0.5)	7.8 (0.4)	7.5 (0.5)	7.6 (0.2)	7.7 (0.3)	7.8 (0.2)	7.7 (0.3)	7.7 (0.4)
Specific conductivity (µS/cm)	182.9 (25.6)	186.3 (15.7)	187.2 (19.6)	188.3 (14.2)	524.6 (207.9)	1754.0 (891.6)	356.5 (142.0)	232.4 (70.8)	394.1 (562.2)
Dissolved oxygen (mg/L)	8.7 (1.4)	8.7 (1.4)	8.3 (1.1)	7.5 (1.1)	9.2 (0.7)	8.5 (1.7)	7.6 (1.8)	8.5 (1.2)	8.3 (4.1)

4.4.2 *E. coli* Source Tracking Results and Discussion

Overall, 45% of the 370 water samples, collected from the ten Barker's Island Inner beach sampling locations during the 2015 and 2016 beach seasons, had greater than 235 MPN/100 mL *E. coli* and were considered exceedances. A summary of the *E. coli* analyses for each sampling location can be found in Table 27. Of the 78 samples sent for qPCR analysis, human DNA markers were detected in 41 of the samples (Table 28).

Table 27. Summary of E. coli Results for Sample Collected at the Barker's Island Inner Beach Locations During the 2015 and 2016 Beach Seasons.

	BARK	BARK-BL	BARK-FP	BARK-PP	BARK-PP235	BARK-PP243	BARK-PP246	BARK-PP251	BARK-ST	BARK-SW	All Sites
Total Number *E. coli* Analyses	71	36	22	45	21	23	30	30	70	22	370
Number of *E. coli* Exceedances (>235 MPN/100mL)	30	8	8	18	11	11	19	15	23	22	165
Percent Exceedances >235 MPN/100mL	42%	22%	36%	40%	52%	48%	63%	50%	33%	100%	45%
Minimum MPN/100mL	26.2	4.1	18.7	23.3	8.5	5.2	17.3	20.1	3.1	770.1	3.1
Maximum MPN/100mL	>2420	>2420	1987	1733	>2420	>2420	>2420	11,199	>2420	>24,196	>24,196
Average MPN/100mL	450 (619)	281 (585)	408 (576)	285 (429)	817 (986)	552 (800)	674 (751)	1137 (2142)	502 (840)	17,134 (9264)	1514 (4602)
Geometric Mean MPN/100 mL	243	70	188	124	268	175	322	297	129	12,627	346

4.4.2.1 Barker's Island Inner Beach E. coli Exceedance Frequency and Origin

The Barker's Island Inner Beach monitoring location had a total of 30 *E. coli* exceedances (42% of samples collected) over the two beach seasons (Table 28). Seven of the twelve samples sent for qPCR analysis detected human markers, two of which were above the benchmark used to detect the presence of human fecal contamination (Table 28). Four of the samples had human markers detected but BLQ.

Relating back to the primary objectives of this research, the frequency of *E. coli* exceedances at Barker's Island Inner beach was 42% in 2015 – 2016, which is a substantially high rate of exceedances. Although the data suggests there may be numerous sources contributing to the high rate of exceedances at this beach (i.e. enterococcus, gulls), contribution by a human source makes this an AOC issue.

Table 28. Summary of *E. coli* (MPN/100mL) and qPCR Results (CN) for samples from Barker's Island Inner Beach (BARK) sent for qPCR analysis. Bold numbers exceed the benchmark for the detection of human fecal contamination.

Rainfall (in)	Collection Date	LSRI *E. coli* (MPN/100mL)	Human *Bacteroides* (CN/100mL)	Human *Lachnospiraceae* (CN/100mL)	Enterococcus (CN/100mL)	*E. coli* (CN/100mL)	Gull2 (CN/100mL)
0	8/6/2015	435.2	0	0	6965	615	0
1.33	8/19/2015	488.4	**5175**	**9372**	14925	1026	0
1.77	8/20/2015	517.2	**4049**	**3114**	7149	313	881
0.05	8/31/2015	248.9	BLQ	BLQ	28560	1085	0
1.86	9/24/2015	1553.1	402	402	48830	1036	0
2.97	7/12/2016	2419.6	BLQ	BLQ	18307	1107	1347
0.53	7/21/2016	1413.6	0	0	4001	1565	0
0.22	8/4/2016	435.2	BLQ	BLQ	1151	0	0
0.42	8/11/2016	1046.2	0	BLQ	7000	1443	1064
1.18	8/16/2016	920.8	0	0	1817	0	0
1.7	9/6/2016	436	0	0	1961	0	0
0.57	9/8/2016	1046.2	0	0	7416	444	579

4.4.2.2 Contribution of Storm Water Runoff to E. coli Concentration Measured at Barker's Island Inner Beach

The samples from Barker's Island Inner beach having *E. coli* that was confirmed to be of human origin were all collected in 2015 and all samples were collected following significant rain events (8/19/15, 8/20/15, 9/24/15). The presence of human markers in the samples collected at the mouth of Faxon Creek, Pickle Pond, on those same dates indicate a likely contribution of *E. coli* of human origin from these locations to the beach. The presence of human markers in samples collected from the end of the fishing pier and its location relative to the beach supports this theory. In 2016, a sanitary sewer overflow occurred on 8/2/16, along Hill Avenue across from Norwood Avenue by Faxon Creek. 75,000 gallons overflowed in 8 hours; caused by a rain event that morning and equipment failure. Low levels of human markers were detected in the Faxon Creek mouth (BARK-ST) on this day, and at the main beach (BARK) on 8/4/16 supporting a possible delayed human contribution at the main beach (Table 29).

Table 29. Summary of *E. coli* values and copy numbers (CN) for all Barker's Island Inner Beach Samples Sent for qPCR Analysis. qPCR results for *E. coli* not included in this table.

Rain-fall (in)	Collection Date	Sample Site ID	*E. coli* (MPN/100 mL)	Human *Bacteroides* (CN/100mL)	Human *Lachnospiraceae* (CN/100mL)	Enterococcus (CN/100mL)	Gull2 (CN/100mL)	Ruminant (CN/100mL)
1.87	7/7/2015	BARK-FP	816.4					
1.87	7/7/2015	BARK-ST	727	0	0	3635	10329	0
0	8/6/2015	BARK	435.2	0	0	6965	0	0
0	8/6/2015	BARK-BL	248.9	0	0	1812	0	0
0.83	8/7/2015	BARK-PP	290.9	0	0	388330	0	0
1.33	8/19/2015	BARK	488.4	5175	9372	14925	0	0
1.33	8/19/2015	BARK-FP	387.3	4246	5932	8070	0	0
1.33	8/19/2015	BARK-PP	410.6	2521	3083	6628	0	0
1.33	8/19/2015	BARK-ST	> 2419.6	412	1085	267473	8278	0
1.77	8/20/2015	BARK	517.2	4049	3114	7149	881	0
1.77	8/20/2015	BARK-ST	272.3	34144	21494	22042	0	0
1.77	8/20/2015	BARK-ST	218.7	26150	13548	15285	0	0
0.05	8/31/2015	BARK	248.9	BLQ	BLQ	28560	1085	0
0.74	9/2/2015	BARK-BL	2419.6	BLQ	0	6625	0	0
0.84	9/18/2015	BARK-BL	> 2419.6	488	BLQ	1111262	0	0
0.84	9/18/2015	BARK-PP	325.5	400	572	3882	0	0
0.84	9/18/2015	BARK-ST	1046.2					
1.86	9/24/2015	BARK	1553.1	402	402	48830	0	0
1.86	9/24/2015	BARK-BL	727	0	0	7134	0	0
1.86	9/24/2015	BARK-FP	1986.3	1069	1526	9390	0	0
1.86	9/24/2015	BARK-PP	1732.9	1815	1495	67111	0	0
1.86	9/24/2015	BARK-ST	> 2419.6	BLQ	1177	135951	0	0
1.86	9/24/2015	BARK-ST	> 2419.6	521	1719			0
0	6/9/2016	BARK-PP243	290.9	0	0			311
0.18	6/23/2016	BARK-PP235	1203.3	0	BLQ	81683	0	0
0.18	6/23/2016	BARK-PP246	1732.9	0	BLQ	58888	0	0
0.18	6/23/2016	BARK-PP251	1203.3	0	0	34119	0	0
0	6/30/2016	BARK-PP235	> 2419.6	11644	22287	3093329	164963	0
0	6/30/2016	BARK-PP243	> 2419.6	1710	12309	2106966	32870	0
0	6/30/2016	BARK-PP246	> 2419.6	2126	9487	1953996	20358	0
0	6/30/2016	BARK-PP251	1732.9	0	1341	330273	2882	0
2.97	7/12/2016	BARK	2419.6	BLQ	BLQ	18307	1347	0
2.97	7/12/2016	BARK-FP	1986.3	763	1594	42126	2061	0
2.97	7/12/2016	BARK-PP	1732.9	601	886	36433	1522	0
2.97	7/12/2016	BARK-PP235	> 2419.6	0	0	69499	0	0
2.97	7/12/2016	BARK-PP243	> 2419.6	0	0	7369	0	0
2.97	7/12/2016	BARK-PP246	1732.9	0	0	93737	1821	0
2.97	7/12/2016	BARK-PP251	> 2419.6	BLQ	573	118215	1308	0
2.97	7/12/2016	BARK-ST	> 2419.6	BLQ	1691	135834	2670	0
0.53	7/21/2016	BARK	1413.6	0	0	4001	0	0
0.53	7/21/2016	BARK-PP235	> 2419.6	0	0	318192	0	0
0.53	7/21/2016	BARK-PP251	> 2419.6	0	0	70532	0	0
0.18	7/28/2016	BARK-PP243	613.1	0	0	33936	0	0
0.53	8/2/2016	BARK-FP	579.4	0	0	1153	0	0

Rain-fall (in)	Collection Date	Sample Site ID	*E. coli* (MPN/100 mL)	Human *Bacteroides* (CN/100mL)	Human *Lachnospiraceae* (CN/100mL)	Enterococcus (CN/100mL)	Gull2 (CN/100mL)	Ruminant (CN/100mL)
0.53	8/2/2016	BARK-PP	387.3	0	0	2210	474	0
0.53	8/2/2016	BARK-PP235	> 2419.6	475	0	654977	0	0
0.53	8/2/2016	BARK-PP243	325.5	0	0	20409	0	0
0.53	8/2/2016	BARK-PP246	> 2419.6	BLQ	0	810741	0	0
0.53	8/2/2016	BARK-PP251	> 2419.6	0	0	135425	0	0
0.53	8/2/2016	BARK-ST	> 2419.6	BLQ	0	121572	2176	0
0.22	8/4/2016	BARK	435.2	BLQ	BLQ	1151	0	0
0.22	8/4/2016	BARK-PP235	1274	0	0	44794	0	0
0.22	8/4/2016	BARK-PP243	960	BLQ	147	21613	0	0
0.22	8/4/2016	BARK-PP246	1187	0	0	206136	0	0
0.22	8/4/2016	BARK-PP251	3448	BLQ	0	58476	0	0
0.22	8/4/2016	BARK-ST	1119.9	BLQ	547	8279	0	0
0.42	8/11/2016	BARK	1046.2	0	BLQ	7000	1064	0
0.42	8/11/2016	BARK-PP243	> 2419.6	0	0	2627	1106	0
0.42	8/11/2016	BARK-PP246	686.7	0	0	10832	0	0
0.42	8/11/2016	BARK-PP251	920.8	0	0	8559	0	0
1.18	8/16/2016	BARK	920.8	0	0	1817	0	0
1.18	8/16/2016	BARK-PP235	2419.6	0	707	33510	0	0
1.18	8/16/2016	BARK-PP243	920.8	0	0	3014	0	0
1.18	8/16/2016	BARK-PP246	579.4	BLQ	0	56739	0	0
1.18	8/16/2016	BARK-PP251	> 2419.6	0	0	22856	0	0
0	8/18/2016	BARK-PP243	959	0	0	21856	0	0
0	8/18/2016	BARK-PP246	933	0	0	18153	0	0
1.7	9/6/2016	BARK	436	0	0	1961	0	0
1.7	9/6/2016	BARK-PP	344.8	0	0	845	0	0
1.7	9/6/2016	BARK-PP246	2098	0	0	2325	0	0
1.7	9/6/2016	BARK-PP251	11199	0	0	0	0	0
0.57	9/8/2016	BARK	1046.2	0	0	7416	579	0
0.57	9/8/2016	BARK-FP	1046.2	0	0	3784	0	0
0.57	9/8/2016	BARK-PP	727	0	0	6432	0	0
0.57	9/8/2016	BARK-PP235	703	0	0	7196	0	0
0.57	9/8/2016	BARK-PP246	620	0	BLQ	9600	0	0
0.57	9/8/2016	BARK-PP251	1918	0	0	38263	0	0
0.57	9/8/2016	BARK-ST	> 2419.6	0	BLQ	120785	8004	0

BLQ - qPCR detects marker, but CN is too low to quantify.

A multiple linear regression model was used to determine the relative influence of sanitary survey parameters plus water quality and climate data on measured *E. coli* concentrations at Barker's Island Inner beach (Table 30). While precipitation was among the parameters influencing the model, the flow of the Nemadji River and wind speed were both positively correlated to *E. coli* concentrations at the main beach. This indicates the potential for wind activity to transfer *E. coli* from Faxon Creek and Pickle Pond to the beach at Baker's Island Inner. As outlined in the following sections, storm water runoff may contribute to *E. coli* measured at Faxon Creek and Pickle Pond, affecting the beach at Barker's Island indirectly through wind action.

Table 30 Relative Influence of Sanitary Survey Parameters and Climate Data on Measured *E. coli* Concentrations at Barker's Island Inner Beach in a Multiple Linear Regression Model

Parameter	Relative Influence
Nemadji River Flow (7-Day Average)	23.76%
Number of Ducks	19.71%
Wind Speed (mph)	19.67%
Turbidity (NTU)	11.56%
Number of Geese	9.93%
Water Temperature (°C)	8.69%
Radar-Estimated 24-HR Rainfall Total	6.68%

4.4.2.3 Contribution from Faxon Creek (BARK-ST)

Thirty-three percent of the samples collected in 2015 and 2016 from the mouth of Faxon Creek were exceedances. Human signal was detected in ten of the eleven samples sent for qPCR analysis and four of them were above the benchmark used to indicate the presence of human fecal contamination (Table 29). Throughout the two seasons, only five exceedances occurred on non-rain event days; all but one of the samples sent for qPCR analysis were rain event samples. This sample did occur after a rain event and the sewer overflow event mentioned above. An additional multiple linear regression model was also used to determine the relative influence Faxon Creek *E. coli* concentrations, in combination with sanitary survey parameters plus water quality and climate data, had on measured *E. coli* concentrations at Barker's Island Inner beach (Table 31). This model indicates that a combination of Faxon Creek *E. coli* concentrations and Nemadji River flow, along with wind speed and water temperature had a relatively high influence on the *E. coli* concentration at the Barker's Island Inner beach. The Nemadji River was not sampled as part of this investigation, therefore, no conclusions can be made about whether the Nemadji River is a source of *E. coli* at Barker's Island Inner beach. The model suggests that increased weekly streamflow due to increased precipitation within the Nemadji River watershed, is correlated to increased *E. coli* concentration at Barker's Island Inner beach. Without additional *E. coli* data from Nemadji River, it cannot be determined whether this effect is due to precipitation alone or due to an *E. coli* contribution from Nemadji River. These initial modeling results indicate that an exploratory sampling at Nemadji River may provide data to determine whether there may be contribution of *E. coli* from the river itself.

Table 31. Relative Influence of Faxon creek *E. coli* concentrations, Sanitary Survey Parameters and Climate Data on Measured *E. coli* Concentrations at Barker's Island Inner Beach in a Multiple Linear Regression Model

Parameter	Relative Influence
Wind Speed (mph)	23.19%
Water Temperature (°C)	18.97%
Faxon Creek *E. coli* Concentration (MPN/100 mL)	18.37%
Nemadji River Flow (7-Day Average)	16.90%
Number of Bathers Out of Water	14.01%
Nemadji River Flow (24-HR Min)	8.54%

4.4.2.4 Contribution from Pickle Pond (BARK-PP)

In 2015, 60% of the samples collected at Pickle Pond (all during rain events) were exceedances. Three of the four 2015 Pickle Pond Samples (BARK-PP) sent for qPCR analysis had human markers present, and it was decided that more baseline data were needed to determine if Pickle Pond was a contributing source of *E. coli* to the Barker's Island Inner beach. In 2016, 30% of the samples collected at Pickle Pond were exceedances and all but three fell on rain event days. In the 2016 beach season, additional routine sampling locations were selected at the four accessible outfalls that drain into Pickle Pond. Measured *E. coli* concentration at these four outfalls ranged in exceedance frequency (>235 MPN/100 mL) from 48% to 63% of the times sample were collected. On one occasion (30 June 2016) it was noted that the water flowing from all four of these outfalls contained "black sludge." BARK-PP235, BARK-PP243, and BARK-PP246 all had *E. coli* concentrations >2,419.6 MPN/100 mL and BARK-PP251 had 1,733 MPN/100mL *E. coli* present. The main beach location, the Faxon Creek Storm Drain and the Pickle Pond sample all had fairly low *E. coli* values (<150 MPN/100 mL) and were not exceedances on this day. There was only a low flow of water that was moving out of Pickle Pond when the sample was collected and there was no detectable longshore current at the main beach, indicating that mixing of water from the main beach and the surrounding locations (i.e., Pickle Pond) may have been minimal. The four Pickle Pond outfall samples were sent for qPCR analysis and the one with the lowest *E. coli* number (BARK-PP251) had a human *Lachnospiraceae* (Lachno2) value of 1,341 CN/100 mL on this day, which is below the benchmark used to indicate the presence of human fecal contamination. Human markers of the other three Pickle Pond outfalls were positive for the detection of human fecal contamination. The 30 June 2016 result from the PP235 sample had human marker concentrations high enough to indicate a 1:100 dilution of untreated sewage with non-sewage contaminated water.

4.4.2.5 Contribution from Boat Landing (BARK-BL)

The boat landing was a routine sampling location in 2015 only. Overall, frequency of exceedances were the lowest (22%) at this sampling location. Human *Bacteroides* was quantifiable at 488 CN/100mL in one of the four qPCR samples analyzed (collected 9-18-15, Table 29). At this time, the longshore current was flowing from the beach toward the boat landing (Southeast) making it unlikely that the boat landing was a contributing factor to controllable sources of *E. coli* at the main beach. Given that the percentage of *E. coli* exceedances at this location was much lower than the percentage of *E. coli* exceedances at Barker's Island Beach, it is not likely that this location is a contributing source of *E. coli* at the beach.

4.4.2.6 Contribution of Waterfowl to E. coli Concentration Measured at Baker's Island Inner Beach

Of the parameters measured, ducks had the second-greatest influence on *E. coli* concentration at Barker's Island Inner beach according to the multiple linear regression model (Table 30) with a high-degree of linear correlation (Figure 29). High numbers of the *Enterococcus* marker, and gull markers found during the qPCR analysis are also indicators that birds are a large contributing factor to *E. coli* exceedances at this location.

Restoration efforts to eliminate standing water on the shore, and to deter geese and ducks are recommended in order to reduce potential pathogen sources at the swimming beach.

At the boat launch in 2015, the *Enterococcus* marker was generally high, ranging as high as 1,111,262 CN/100 mL. High copy numbers for Enterococci bacteria, such as this, can be correlated with high numbers of geese. Observations of the copious goose feces on the dock at the boat landing, which would wash into the water during rain events, is supportive of the premise that the high numbers of at the boat landing were likely coming from geese.

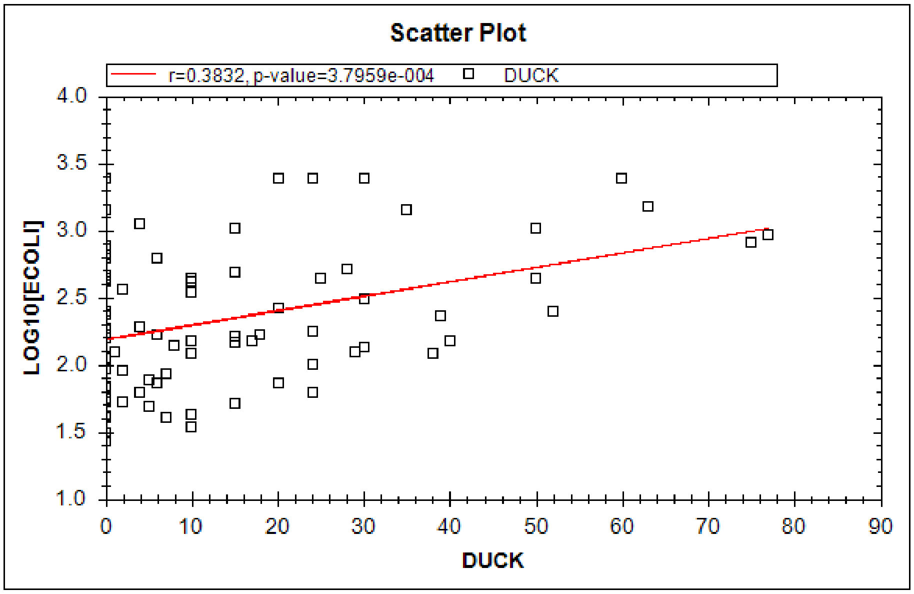

Figure 29. Relationship between *E. coli* Measured at Barker's Island Inner Beach and the Number of Ducks Counted during Sanitary Surveys.

4.4.2.7 Contribution of Recreational Activities to E. coli Concentration Measured at Barker's Island Inner Beach

In 2015, 22% of the samples collected from the boat launch near the marina were above the regulatory standard for recreational water. As mentioned above, human signal was detected in one of the samples analyzed via the qPCR method. Barker's Island Marina was not thought to be a source of *E. coli* of human origin at the beach, given the results from the 2015 beach season. Therefore, samples were not collected from this location in 2016.

Results from multiple linear regression analysis of the sanitary survey parameters indicated that the number of bathers out of the water have an influence on the measured concentration of *E. coli* at Barker's Island Inner beach (Table 31). There is a negative correlation between the numbers of bathers out of the water (i.e., the more bathers out of the water the lower the measured *E. coli* concentration). The number of people observed recreating in and around the beach did not influence measured *E. coli* concentration, which indicates that bathers actively swimming at the beach is the type of recreational activity that leads to higher *E. coli* concentrations measured at the beach. This could be due to mixing of the stagnant water from bathers exiting and entering the water at the beach area.

4.4.2.8 Contribution of Lack of Infiltration/Standing Water on Beach to E. coli Concentration Measured at Barker's Island Inner Beach

The highest frequency (100%) of exceedances came from the standing water samples whose concentration of *E. coli* averaged more than 24,196 MPN/100 mL. Given the water fowl observations and counts on the sanitary surveys, an assumption was made that these high numbers are a result of the large amount of water fowl feces present along the foreshore.

4.4.3 LSRI Recommendations

In conclusion, when combining all Barker's Island Inner Beach sampling locations, fifty-three percent of the samples sent for qPCR analysis had human markers detected in them. Of the 78 samples positive for human marker, 25 samples had quantifiable human marker concentrations (i.e., above the limit of quantification) and 14 were above the benchmark used to indicate the presence of human fecal contamination. This is sufficient to conclude that there is contribution of *E. coli* of human origin at the main beach, and sources should be controlled or mitigated before the beneficial use impairment is removed. It is apparent that storm water run-off via the mouth of Faxon Creek and the Pickle Pond outfalls, is contributing to a detectable human source at the Barker's Island Inner Beach. Additional investigation into determining the sources directly contributing to the elevated *E. coli* counts further up these storm water outfalls is recommended in order to determine what type of remediation should be done. It is also recommended that the beach at Barker's Island be re-designed to prevent standing water from accumulating on the beach. Rain gardens with vegetation that detract waterfowl from hanging out near the swimming beach and signs to discourage citizens from feeding the ducks would likely reduce the number of exceedances at Barker's Island Inner Beach.

4.5 Wisconsin Point

4.5.1 Water Quality

The water quality at WP3, WP2, and WP1 is influenced by precipitation and wind and can vary substantially. The Nemadji River, a nearby tributary with very high total suspended solids due to clay-rich suspended sediment enters Lake Superior at the northwestern end of Wisconsin Point. Precipitation and winds out of the north or northwest result in characteristic red clay colored water at the Wisconsin Point beaches with high turbidity. Without this influence from the Nemadji River, these Wisconsin Point beaches have water quality similar to open Lake Superior, which has much higher clarity and much lower turbidity. Selected water quality parameters measured at WP3, the mouth of Dutchman Creek (WP3-DC), WP2, and WP1 during the 2015 and 2016 beach seasons are summarized in Table 32. Over the two beach seasons, average turbidity was higher than any of the other beaches sampled within the St. Louis River Area of Concern for this project. Overall, air and water temperature averaged 21.7°C and 17.6°C, respectively, with wind speed averaging between 5.6 mph for the Wisconsin Point southeast beaches during the 2015 – 2016 beach seasons.

Table 32 Average (± Standard Deviation) of Selected Water Quality and Climate Parameters Measured at the Southeast Wisconsin Point Beaches and Mouth of Dutchman Creek.

Parameter	WP1	WP2	WP2-CR (2015)	WP2-OF (2015)	WP3	WP3-DC	All WP Sites
Air Temperature (°C)	22.0 (5.5)	21.5 (6.1)	NM	NM	21.6 (5.5)	NM	21.7 (5.7)
Wind Speed (mph)	5.7 (3.5)	6.1 (3.8)	NM	NM	5.2 (3.8)	NM	5.6 (3.7)

Wave Height (ft.)	1.2 (1.9)	1.0 (1.2)	NM	NM	0.9 (1.1)	NM	1.0 (1.4)
Water Temperature (°C)	17.1 (3.4)	17.2 (3.4)	18.6 (3.8)	15.9 (2.8)	17.6 (3.8)	18.5 (3.1)	17.6 (3.5)
Turbidity (FNU)	56.6 (131.2)	101.2 (200.7)	18.2 (47.9)	317.0 (392.2)	199.1 (358.4)	99.4 (122.2)	109.6 (226.9)
pH	7.8 (0.2	7.8 (0.4	6.8 (0.3	7.8 (0.3	7.8 (0.3	7.6 (0.3	7.7 (0.4)
Specific Conductivity (µS/cm)	117.2 (8.1)	118.1 (8.6)	210.2 (26.4)	122.2 (4.3)	132.4 (38.6)	235.2 (101.4)	156.5 (71.4)
Dissolved Oxygen (mg/L)	11.5 (14.8)	9.7 (0.9)	3.3 (1.5)	9.8 (0.8)	9.5 (1.0)	7.2 (1.8)	8.8 (7.3)

4.5.2 *E. coli* and qPCR analysis Results and Discussion

During the 2015 and 2016 beach seasons, a total of 304 samples were collected from the project's Wisconsin Point sampling locations. The *E. coli* concentration was greater than >235 MPN/100 mL in 28% of samples collected. Among the sampling locations, Wisconsin Point 2 beach had the greatest frequency of regulatory exceedances at 41%, followed by Dutchman Creek (31%), and Wisconsin Point 1 beach (20%) (Table 22). Human DNA (i.e., *Bacteroides* (HF183) or Human *Lachnospiraceae* (LACHNO2 or LACHNO3) was detected in four of 34 samples analyzed via qPCR and only from Wisconsin Point 2 beach or Dutchman Creek. Only one of the four samples was above the qPCR method's limit of quantification (sample collected from Dutchman Creek) and none of the samples were above the benchmark used to indicate the presence of fecal contamination. All six genetic markers assayed were detected at this location, however, ruminant marker was detected in only one of the samples (Dutchman creek), which occurred during the largest rain event of nearly three inches of rain in 24 hours (Table 34).

Table 33. Summary of *E. Coli* Results for Sample Collected at Wisconsin Point During the 2015 and 2016 Beach Seasons.

	WP1	WP2	WP2-CR	WP2-OF	WP3	WP3-DC	All WP Sites
Total Number Analyses	66	64	34	10	65	65	304
Total Number of Exceedances (>235 MPN/100 mL)	13	26	9	5	12	20	85
Percent *E. coli* Exceedances >235 MPN/100 mL	20%	41%	26%	50%	18%	31%	28%
Minimum MPN/100 mL	<1	1	13.5	24.1	2	2	<1
Maximum MPN/100 mL	1986	>2420	2420	>2420	>2420	>2420	>2420
Average (± Std. Dev) MPN/100 mL	188 (346)	515 (767)	303 (543)	721 (936)	207 (490)	347 (607)	325 (595)
Geometric Mean of MPN/100 mL	64	165	107	357	63	110	169

Table 34. Summary of *E. coli* values and copy numbers (CN) for all Wisconsin Point samples sent for qPCR analysis. qPCR results for *E. coli* not included in this table.

Rain-fall (in)	Collection Date	Sample Site ID	*E. coli* (MPN/100 mL)	Human *Bacteroides* (CN/100mL)	Human *Lachnospiraceae* (CN/100mL)	Enterococcus (CN/100mL)	Gull2 (CN/100mL)	Ruminant (CN/100mL)
0.02	6/16/2015	WP2	478.6	0	0	8453	52617	0
0.02	6/16/2015	WP2-OF	410.6	0	0	5072	19670	0
0.08	6/25/2015	WP2	1413.6	0	0	37665	138997	0
0.6	6/30/2015	WP1	1986.3	0	0	10857	3416	0
0	7/2/2015	WP2-CR	1299.7	0	0	58803	249766	0
0.94	7/14/2015	WP2-OF	> 2419.6	0	0	49923	185324	0
0	7/16/2015	WP3-DC	235.9	BLQ	0	5428	0	0
0	7/21/2015	WP3	285.1	0	0	3393	7684	0
0.83	8/7/2015	WP1	461.1	0	0	3128	0	0
0.83	8/7/2015	WP1	325.5	0	0			0
0.83	8/7/2015	WP2	461.1	BLQ	BLQ	14748	0	0
0.83	8/7/2015	WP2-OF	547.5	0	0	37389	0	0
0.83	8/7/2015	WP3-DC	488.4	0	0	1898	0	0
1.33	8/19/2015	WP3-DC	866.4	BLQ	0	2610	0	0
1.77	8/20/2015	WP3	1119.9	0	0	30151	7716	0
1.86	9/24/2015	WP1	275.5	0	0	2177	0	0
1.86	9/24/2015	WP2	396.8	0	0	3153	0	0
1.86	9/24/2015	WP3	435.2	0	0	1177	0	0
1.86	9/24/2015	WP3-DC	2419.6	0	0	73133	0	0
0	6/9/2016	WP1	344.8	0	0	5439	17017	0
0	7/5/2016	WP2	648.8	0	0	12646	59879	0
2.97	7/12/2016	WP3-DC	> 2419.6	BLQ	772	196169	0	8595
0.53	7/21/2016	WP3	> 2419.6	0	0	16252	0	0
0.53	7/21/2016	WP3-DC	1046.2	0	0	12738	1251	0
0.05	7/26/2016	WP2	1119.9	0	0	4562	4415	0
0.18	7/28/2016	WP1	325.5	0	0	0	0	0
0.53	8/2/2016	WP1	307.6	0	0	5138	5904	0
0.22	8/4/2016	WP2	980.4	0	0	2675	2687	0
0.22	8/4/2016	WP3	272.3	0	0	797	0	0
0.22	8/4/2016	WP3-DC	461.1	0	0	0	0	0
1.18	8/16/2016	WP3	248.1	0	0	600	282	0
1.7	9/6/2016	WP3-DC	920.8	0	0	2422	0	0
0.57	9/8/2016	WP3	488.4	0	0			0
0.57	9/8/2016	WP3-DC	435.2	0	0			0

4.5.2.1 Wisconsin Point 3 (WP3), Wisconsin Point 2 (WP2), and Wisconsin Point 1 (WP1) Beaches

For the purposes of this investigation, Wisconsin Point's southeast beaches are considered one beach (results composited) even though this stretch of beach is divided into three for monitoring purposes (Figure 30). To address the primary objectives of this investigation, based upon the data from the 2015 – 2016 beach seasons:

1. The frequency of *E. coli* exceedances (i.e., >235 MPN/100 mL) at Wisconsin Point's southeast beaches (routine monitoring locations only) was 28% (51 of the 195 samples collected), which is a moderate frequency compared to the other beaches sampled for this project.
2. At the routine monitoring locations, human genetic markers (*Bacteroides* and/or *Lachnospiraceae*) were detected in only one (WP2) of the 21 samples sent for qPCR analysis.

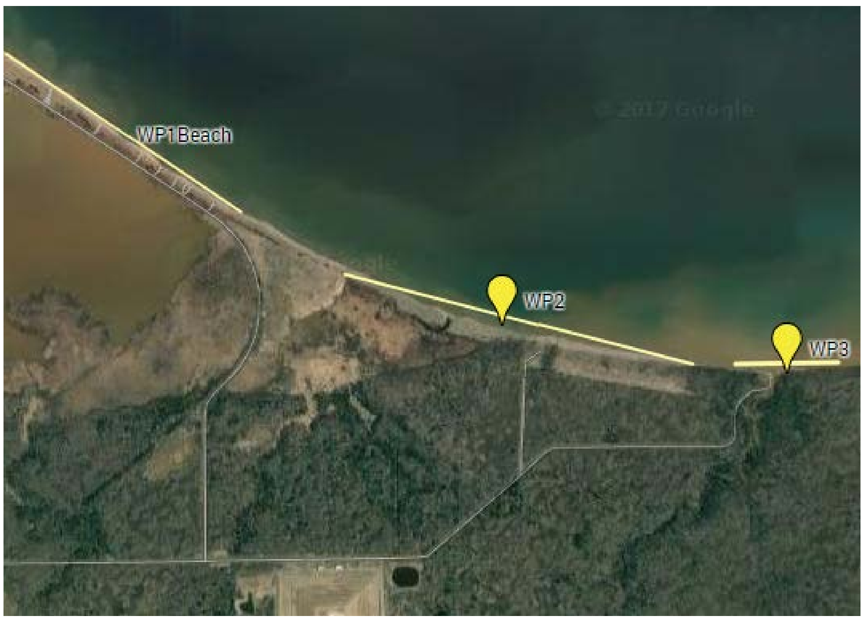

Figure 30. Wisconsin Point Southeast Stretch of Beach.

Table 35 shows the multiple linear regression model for each of the three routine monitoring locations, consisting of the combination of sanitary survey parameters and climate data that have the greatest influence on the *E. coli* concentration measured at each location. The *E. coli* concentration of other surrounding sampling locations within the southeast portion of Wisconsin Point were not factored into these models. On average, water temperature and cloud cover have the highest relative influence on the multiple linear regression model; these two parameters were also common to all three beach monitoring locations. Water clarity also ranked highly on average, but was common to only WP1 and WP3. Total 48-hour rainfall ranked highly as well, but was common to only WP1 and WP2.

Table 35. Multiple Linear Regression Models Showing Relative Influence of Sanitary Survey Parameters on Wisconsin Point's Southeast Beaches.

Parameter	Relative Influence at WP1	Relative Influence at WP2	Relative Influence at WP3	Average Relative Influence
Water Temperature (°C)	13.45%	24.65%	7.56%	15.22%
Cloud Cover	7.50%	20.33%	12.70%	13.51%
Clarity	18.36%	---	18.25%	12.20%
Radar-Estimated 48-HR Rainfall Total	17.14%	14.55%	---	10.56%
Current Speed	---	15.35%	10.68%	8.68%
Wave Height (ft)	23.67%	---	---	7.89%
Previous 48-HR Rainfall	---	---	22.26%	7.42%
Wave Height (ft) x Number of Gulls	---	---	18.00%	6.00%
On-Shore Wind Velocity	---	12.86%	---	4.29%
Along-Shore Wind Velocity	---	12.26%	---	4.09%
Floatables	---	---	10.55%	3.52%
Radar-Estimated 24-HR Rainfall Total	10.42%	---	---	3.47%
Wind Speed (MPH)	9.46%	---	---	3.15%

4.5.2.2 Determination of E. coli Contribution from Landfill Drainage

The multiple linear regression models for WP1 and WP2 both have 48-hour rainfall total as relatively high influences to the model. Indirectly, this positive correlation could indicate the potential for landfill leachate to transport *E. coli* to WP1 and WP2 beaches during heavy precipitation events, however, there is no direct evidence to support this theory (i.e., landfill leachate or adjacent small creeks were not sampled as part of this investigation). The drainage located at WP2beach was never observed to breach the beach, even during heavy rain events. There were a total of 34 samples collected from the drainage in 2015, and 26% of those samples (WP2-CR) were in exceedance of the regulatory standard for recreational water, which was a much lower frequency of exceedances than the WP2 beach monitoring location. Although only one sample was sent for qPCR analysis in 2015, it did not have human DNA present. The relatively low exceedance rate and the lack of human DNA triggered this sampling location to be abandoned in 2016.

4.5.2.3 Determination of E. coli Contribution from Seabirds and Waterfowl

There was a persistent and substantial gull presence at Wisconsin Point's southeast beaches during the 2015 and 2016 beach seasons, especially at WP2, which had an average of 275 gulls observed on sample collection days. Comparatively, WP1 had 82 gulls on average per sampling day and WP3 had 62 gulls on average per sampling day. The presence of other waterfowl was much less pronounced, less than one goose or duck on average per sampling day at all three monitoring locations. Given the rarity of this observation, the number of geese and ducks were left out of the dataset used to develop the multiple linear regression models for each beach. At the WP2 monitoring location, gull DNA marker was detected in five of seven samples sent for qPCR analysis. The average concentration of this marker was much higher at WP2 than either WP1 or WP3, which had gull marker detected in two of six and three of nine samples, respectively. The multiple linear regression models do not indicate a direct correlation with the number of gulls observed and the *E. coli* concentration, however, the observational data paired with the qPCR data indicate a relationship (although not statistically significant). The WP2 monitoring location had 41% exceedances, which is twice the frequency of WP1 and WP3. The WP2 monitoring location also had over four times the number of gulls compared to WP1 and WP3. This presence of gulls at WP2 seems to have an influence on rate of *E. coli* exceedances, although not directly correlated, this conclusion is easily made through comparison of observational data and *E. coli* concentration.

4.5.2.4 Determination of E. coli Contribution from Recreational Beach Use

At the southeast end of Wisconsin Point, recreational beach use is not often observed. Of the three monitoring locations, WP1 was the most popular for bathers with an average of two bathers per sampling day. This may be due to easier access from Wisconsin Point Road at this location. The number of bathers observed in the water was less than one per sampling day on average at WP1. Access to WP2 is restricted due to this beach's designation as a Piping Plover nesting habitat; the public is not able to access the beach until mid-July and this is reflected in the observation of bathers, which was less than one bather out of and in the water on average per sampling day. There was also less than one person on average per sampling day observed recreating at this location. Although access is not restricted to WP3, this beach is located furthest to the southeast off of Moccasin Mike Road, which is away from the majority of the beaches on Wisconsin Point that are located off of Wisconsin Point Road. Debris, which is an indicator of recreational beach use, was a sanitary survey parameter included in the development of multiple linear regression models for these beaches, and was not found to be an influence on the model in any case. Due to the rarity of beach recreational use at Wisconsin Point's southeast beaches, recreational use is not considered to be a contributing source of *E. coli*.

4.5.2.5 Determination of E. coli Contribution from Dutchman Creek

A second set of multiple linear regression models were developed for Wisconsin Point's southeast beaches, which included the sanitary survey parameters plus the *E. coli* concentration measured at all sampling points for which two years of data were available. The results for WP1, WP2, and WP3 were averaged and are shown in Table 36. Although on average, the *E. coli* concentration at WP1 had the highest relative influence on the multiple linear regression model, the *E. coli* concentration at Dutchman Creek by far had the highest relative influence on the model for WP3. There is a very strong positive correlation between *E. coli* concentration measured at WP3 and *E. coli* concentration measured at Dutchman Creek as shown in Figure 31. This correlation, in combination with a trend of higher *E. coli* concentration in Dutchman Creek after high-precipitation rain events, indicates a potential for Dutchman Creek to be a source of *E. coli* at WP3 beach. Although Dutchman Creek does not appear to be a source of *E. coli* of human origin, it is nonetheless a source of *E. coli* during larger rain events. The influence of Dutchman Creek on the beach monitoring locations decreases with distance; the creek did not factor into the model for WP1 at all and had a moderate influence on the model for WP2. This could be the result of a dilution effect as *E. coli* is transported out of the creek mouth and northwest along Wisconsin Point. When looking at the average of the models, it is clear that a relationship exists between *E. coli* measured at all three of Wisconsin Point's beaches and at Dutchman Creek, however, wave height and clarity again factor in as a relatively high influence on the model. Sanitary survey parameters such as on-shore wind velocity, presence of floatables, number of gulls, water temperature, and 24-hour rainfall total as influence the overall model. For WP1, the 7-day average flow of the Nemadji River also had a relatively high influence on the model for *E. coli* concentration. This tributary was not sampled as part of this investigation, however, these initial modeling results indicate that an exploratory sampling effort may be warranted.

Table 36. Multiple Linear Regression Models Showing the Influence of Sanitary Survey Parameters and *E. coli* Concentration Measured at Surrounding Sample Locations on the Southeast Portion of Wisconsin Point.

Parameter	Relative Influence at WP1	Relative Influence at WP2	Relative Influence at WP3	Average Relative Influence
WP1 *E. coli* Concentration (MPN/100 mL)	N/A	26.39%	23.43%	24.91%
WP2 *E. coli* Concentration (MPN/100 mL)	20.67%	N/A	19.87%	20.27%
Dutchman Creek *E. coli* Concentration (MPN/100 mL)	---	20.85%	29.19%	16.68%
WP3 *E. coli* Concentration (MPN/100 mL)	5.38%	25.98%	N/A	15.61%
Wave Height (ft)	28.75%	---	16.10%	14.95%
Clarity	15.84%	9.49%	---	8.44%
Nemadji River, 7-Day Average Flow	17.45%	---	---	5.82%
On-Shore Wind Velocity	11.91%	---	---	3.97%
Floatables	---	10.52%	---	3.51%
Number of Gulls	---	---	9.46%	3.15%
Water Temperature (°C)		6.77%	---	2.26%
Radar-Estimated 24-HR Rainfall Total	---	---	1.95%	0.65%

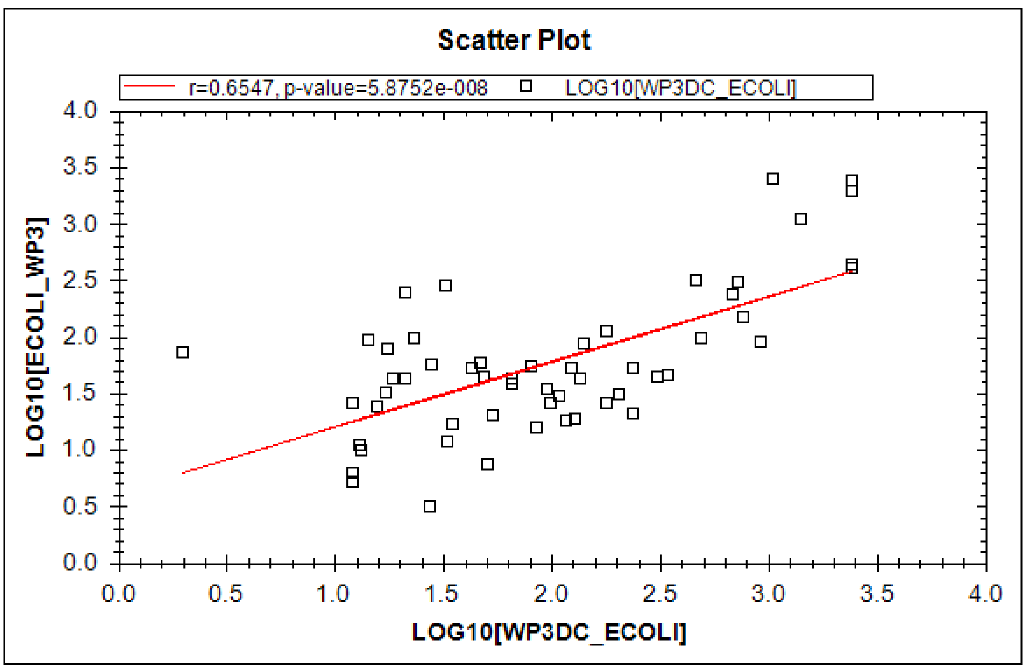

Figure 31. Scatter Plot Showing Relationship Between *E. coli* Measured at WP3 and *E. coli* Measured at Dutchman Creek (WP3DC).

4.5.3 LSRI Recommendations

In terms of the St. Louis River Area of Concern removal target, the *E. coli* measured at Wisconsin Point's southeast beaches does not appear to be of human origin or of "controllable" source. At the beach monitoring locations, there was only one sample in two years that had human DNA detected (WP2) and this detection was below the limit of quantification for the analytical method. It is clear, however, that all three beaches and Dutchman Creek have a high frequency of *E. coli* exceedances. Within Dutchman Creek, there was one sample that had quantifiable human DNA marker, which was coincident with a very heavy rain event and may indicate possible transport of *E. coli* from the watershed. The rural drainage area of the creek is prone to septic system issues due to the high clay content of the soils is a likely source of the human signal. A watershed approach through an appropriate state or federal regulatory program would be necessary to address this source. Additional research is recommended for Dutchman Creek, including sampling in upstream areas during/following heavy rain events, especially given the modeling results for WP3 that indicate a very high influence of *E. coli* measured at Dutchman Creek on *E. coli* measured at the beach monitoring location. The model for WP1 also indicated that the flow out of Nemadji River may have some influence on *E. coli* measured at this monitoring location. Therefore, exploratory sampling at this tributary is recommended. Based on the observational data, it is clear that there is a continual and substantial gull presence, particularly at WP2. Given that WP2 had the highest frequency of *E. coli* exceedances and by far the highest number of gulls, there is a clear relationship between the two. Since WP2 is not accessible to the public until mid-July and since the number of bathers and people using the beach for recreational purposes is very low, it is recommended that the City of Superior considering closing this beach to the public permanently to reduce exposure to potential pathogens. The gulls are attracted to both the active and closed landfills, and deterring their presence at WP2 beach and the closed landfill would not be feasible until the Moccasin Mike landfill is no longer active.

*

5 Conclusions

Based on the evidence of his two-year investigation, two of the six impaired beaches should be considered high priority for further investigation as an AOC issue. Sources at Leif Erikson Park and Barker's Island Inner should be controlled or mitigated before the beneficial use impairments are removed. At Leif Erikson Park, Human Bacteroides or Human Lachnospiraceae (Lachno2) values were above the benchmark used to detect the presence of human fecal contamination in 28% of samples collected, indicating a human contamination source is coming from both the storm drain and Chester Creek. Continued rain event monitoring of Leif Erikson Park beach and the mouth of Chester Creek is recommended; for monitoring purposes priority to should be given to dates having greater than or equal to one-inch of precipitation within a 48-hour period. Monitoring should include qPCR analysis in order to gather more data regarding origin of *E. coli*. Exploratory sampling upstream of the mouth of Chester Creek is highly recommended. For the Barker's Island Inner Beach sampling locations, 53% of the samples sent for qPCR analysis had human markers detected in them and 18% were above the benchmark used to indicate the presence of human fecal contamination. It is apparent that storm water run-off via the mouth of Faxon Creek and the Pickle Pond outfalls is contributing to a detectable human source at the Barker's Island Inner Beach. Continued rain event monitoring of Barker's Island Inner beach, Pickle Pond, and the mouth of Faxon Creek is recommended; for monitoring purposes priority should be given to dates having greater than or equal to one-inch of precipitation within a 48-hour period. Exploratory sampling upstream of the mouth of Faxon Creek is highly recommended (sampling effort underway in summer of 2017). Monitoring and exploratory sampling should include qPCR analysis in order to gather more data regarding origin of measured *E. coli*. Exploratory sampling is also recommended at the mouth of the Nemadji River (i.e., at least three baseline and three rain event samples over the course of a single beach season). Additional investigation into determining the sources directly contributing to the elevated *E. coli* counts further up Faxon Creek and the Pickle Pond storm water outfalls is recommended in order to determine what type of remediation should be done. To further reduce *E. coli* exceedances, it is also recommended that the beach at Barker's Island be re-designed to prevent standing water from accumulating on and near the beach. Rain gardens with vegetation that detract waterfowl from hanging out near the swimming beach and signs to discourage citizens from feeding the ducks would also likely reduce the number of exceedances at Barker's Island Inner Beach.

Although there is no sufficient evidence to support measured *E. coli* of human origin at the remaining beaches, there are several recommended actions that can be taken to reduce the frequency of *E. coli* exceedances. There is also additional exploratory sampling that could provide data to help determine the source of *E. coli* exceedances at these beaches, given the public health concern from a high frequency of *E. coli* exceedances (regardless of origin). At Clyde Ave boat launch, it is recommended that the portable toilet be located further away from the water. Second, placing trash receptacles that are emptied on a regular schedule at the boat launch is recommended. Investigating the relationship between *E. coli* values at the Minnesota 15th St. beach and near the local wastewater treatment facilities may be helpful. Applying hydrodynamic modeling at this beach, and others, may also be useful as water flow from the St. Louis River estuary to the Duluth Entry may be a contributor to high *E. coli* concentrations, particularly during large rain events. Finally, it is recommended that a deterrent for waterfowl be installed near each beach, and that signage be placed in a highly-visible location to discourage feeding of birds. Exploratory rain event sampling is suggested at both the boat launch and the mouth of Stewart Creek, with priority given to samples collected within 48 hours of a rain event totaling greater than or equal to one inch (within a 48-hour period). In addition, exploratory sampling further upstream of the 2015-2016 Stewart Creek sampling location (both baseline and rain event) is recommended given that data from this project suggests a potential upstream source. Measures taken at Minnesota Point/15th Street (harbor side) to reduce or deter geese and waterfowl from congregating on the beach would likely reduce the number of E. coli exceedances. Hydrodynamic modeling may be a useful tool to explore the flow from the St. Louis River estuary through the Duluth Entry and the potential impact from wastewater treatment facilities located within the estuary on the beach. Exploratory rain event sampling (i.e., within 48 hours of greater than one-inch of precipitation) including qPCR analysis will provide additional data on origin of measured *E. coli* during high precipitation periods. Wisconsin Point from Dutchman's Creek to Lot 1 does not appear to be impacted by E. coli of human origin, however, the frequency of E. coli exceedances at the three beaches in this project was high. Additional exploratory sampling in Dutchman's Creek is recommended, including sampling in upstream areas during/following heavy rains (and including qPCR analysis). Based on the observational data, it is clear that there is a continual and substantial gull presence, particularly at WP2. Given that WP2 had the highest frequency of *E. coli* exceedances and by far the highest number of gulls, there is a clear relationship between the two. Since WP2 is not accessible to the public until mid-July and since the number of bathers and people using the beach for recreational purposes is very low, it is recommended that the City of Superior considering closing this beach to the public permanently to reduce exposure to potential pathogens. The gulls are attracted to both the active and closed landfills, and deterring their presence at WP2 beach and the closed landfill would not be feasible until the Moccasin Mike landfill is no longer active.

6 References

WDNR *Bacterial Source Tracking at Impaired Beaches in the St. Louis River Area of Concern Quality Assurance Project Plan (QAPP)* Prihoda, K.R., Saillard, H.J.

Newton, R.J., Vandewalle, J.L., Borchardt, M.A., Gorelick, M.H., and McLellan, S.L. (2011) *Lachnospiraceae* and *Bacteroidales* Alternative Fecal Indicators Reveal Chronic Human Sewage Contamination in an Urban Harbor. Appl Environ Microbiology77: 6972–6981

USEPA (U.S. Environmental Protection Agency). *Great Lakes Beach Sanitary Survey User Manual*—May 2008 _Office of Water 4305 - EPA-823-B-06-001

7 APPENDIX 1

Results of Quality Control Data for *E. coli* Analyses

QC Activity	Frequency	Method or Procedure	Acceptance Criteria (Data Quality Indicator)	Corrective Action	Number Samples Analyzed	Number Samples Within Acceptance Criteria	Number Samples Failed
Method Blank	1 per sampling event	Sterilized Harbor Water analyzed using same analysis methods as samples.	Non-detect (Bias)	None	63	61	1
Field Blank	1 per sampling event	Sterilized DI water is poured into a sample bottle while in the field, transported to the LSRI Microbiology Lab, and analyzed.	Non-detect (Bias)	None	62	62	0 0
Positive Control	Weekly	Sterilized DI water is spiked with *E. coli* and analyzed.	>1 MPN/100 mL	None	49	49	0
Negative Control	Weekly	Sterilized DI water is spiked with non-coliform bacteria and analyzed.	Non-detect	None	47	47	0
IDEXX QC Standard	1 IDEXX-QC kit; 3 analyses monthly	Quantitative standard that consists of one non-coliform bacteria, one coliform (non-*E. coli*) bacteria, and one *E. coli* in certified concentrations.	Accuracy within limits set by manufacturer.	None	8	8	0
Laboratory Duplicate	1 per sampling event	Two representative aliquots from one homogenous sample are analyzed.	≤30% RPD (Precision)	Recommend Revising Acceptance Criteria*	103; Average RPD was 20.0	85	18
Field Duplicate	1 per sampling event	Two independent samples are collected simultaneously by one member of the sampling team, transported to the LSRI Microbiology Laboratory, and analyzed.	≤35% RPD (Precision)	Recommend Revising Acceptance Criteria*	131; Average RPD was 23.2	98	33
QA Count	10% of the samples analyzed	A second microbiologist counts the number of positive wells on each IDEXX tray that has already been counted.	≤20% RPD (Bias)	None	259 Average RPD was 0.67	259	0