4-2020

Senior Project: An Investigative Study into the Bacterial Contamination of Little Black Creek

Margaret I. Brenneman
Grand Valley State University

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Margaret I. Brenneman

Frederik Meijer Honors College, Grand Valley State University

HNR 499, Senior Project

Dr. Richard Rediske, Annis Water Resource Institute

April 1, 2020

Abstract

An investigation of *Escherichia coli* concentrations in a west Michigan stream was conducted to determine sources of fecal contamination that impact water quality. Little Black Creek (LBC) is located in Muskegon County and discharges into Lake Michigan at the P.J. Hoffmaster Campground beach. Often referred to as an "indicator bacteria," water contaminated with *E. coli* has a high probability to contain other enteric pathogens as well. Beach water testing in 2020 using Colilert-18 methods revealed *E. coli* levels of 579 cfu/100mL in the creek discharge area that exceeded total body contact criteria of 300 cfu/100mL. A follow-up study of the creek found concentrations of *E. coli* exceeding the total body contact criteria at multiple locations. Samples collected after a rain event found *E. coli* levels > 2,400 cfu/100mL in the mouth of LBC. Further investigation into sites of LBC nearest to the campground's sanitary facilities found *E. coli* levels of 860 cfu/100mL where a drainage pipe empties into LBC. Spatial and temporal trends of microbial data will be discussed for the beach and the creek. Results of quantitative polymerase chain reaction (qPCR) using the human marker, HF183 were negative, suggesting the bacterial contamination was from wildlife sources.

Acknowledgements

I was blessed with the privilege to work as a summer intern at the Robert B. Annis Water Resource Institute (AWRI) from June to August of 2020, where I began my research for my Honors Senior Project. I was granted the opportunity to continue my internship into the academic year, and it's been my genuine pleasure to intern for Dr. Rick Rediske. I am so excited to continue my internship with him until the end of my undergraduate career in December 2021. I am beyond grateful to everyone at AWRI who's welcomed me, guided me, and believed in me. Thank you to Rick for your attention to detail, giving me the chance to jump in on groundbreaking opportunities, and for working with me so patiently to formulate and perfect my presentation of my research and senior project paper; thank you to Brian Scull for creating a wonderful atmosphere where learning is paramount and failure is okay; thank you to Matthew Allen and Kurt Thompson for your assistance with GIS; and thank you to Molly Lane and Alexis Porter, my beach monitoring partners, for being wonderful scientists, gracious teachers, and dear friends who are just a joy to work with and be around.

Introduction

Michigan is known for the beauty and majesty of the surrounding Great Lakes, and the beaches that border them. As time has passed, Michiganders have learned the hard way the importance of fiercely protecting those lakes that make up the crowning jewel of the midwest. While the recreational and drinking waters are protected by the Clean Water Act, Safe Drinking Water Act, and BEACH Act, the water can still be contaminated by a variety of different sources (United States Environmental Protection Agency, 2021). If the water becomes contaminated with fecal matter and its associated bacteria, it could become an established "Area of Concern," by the International Joint Commission under the Great Lakes Water Quality Agreement (Nevers et al., 2018). If consistent contamination is found, it may cause a beach beneficial use impairment (BUI). A BUI refers to the environmental and biological changes in the area surrounding the Great Lakes that restrict or inhibit the utility of the resources (Staley and Edge, 2016). Consistent beach closures due to the ongoing problem of bacterial contamination contribute to the BUI of West Michigan Beaches.

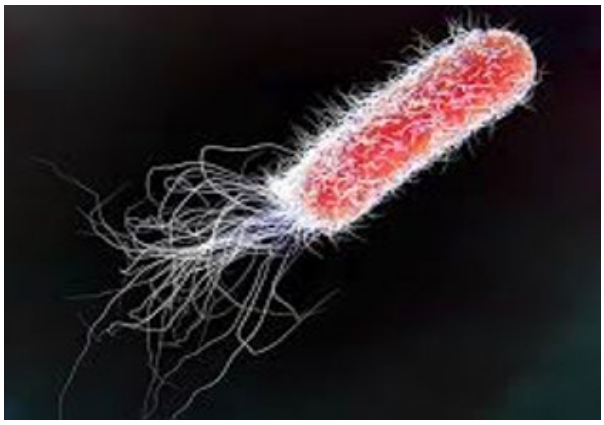

Figure 1. *Escherichia coli* bacillus.

Beach closures can be devastating to coastal communities whose quality of life depends on the environmental, economic, and recreational uses of Lake Michigan. The bacterial contamination is monitored by testing the lake water for the presence of *Escherichia coli* (Figure 1). While the bacterium can be harmless, some strains of *E. coli* are pathogenic, like the Shiga-toxin-producing strains (Seyfried et al., 1985 (II)). These are more uncommon, so any *E. coli* outbreaks in America are most likely caused by one of these less common pathogenic strains.

Scientifically, *E. coli* is known as an "indicator bacteria" because its presence in a water supply is often indicative of the presence of other potentially pathogenic enteric bacteria as well (Cabelli et al., 1982). There are other fecal indicator bacteria like *Enterococci* and fecal coliforms, but there have been studies showing that *E. coli* is a better predictor of GI illness in freshwater than *Enterococci*, so it is routinely used for water quality assessment (Lavender and Kinzelman, 2009). The presence of enteric pathogens in a water supply can result in an infection causing ear and respiratory infections, skin irritations, and aggressive gastrointestinal illness (Seyfried et al., 1985 (I)). Moreover, the economic impact from an outbreak can be detrimental to a coastal community like West Michigan because of loss of tourism spending. Contaminated water can often be introduced into Lake Michigan through tributaries that empty into the lake. This is the case of the beach at the PJ Hoffmaster Campground located in Muskegon County, where Little Black Creek discharges into Lake Michigan. This is why beach monitoring is so important: to make sure the Great Lakes and their tributaries are not impacted by pathogens that affect recreational use.

AWRI has been surveying the Hoffmaster Campground beach and monitoring water quality since 2001. In 2020, due to erosion and debris from record high water levels, the campground began directing patrons to a new area of the shoreline to swim. The new beach was located at the mouth of Little Black Creek, where it discharges into Lake Michigan. Routine monitoring of the PJ Hoffmaster Campground beach yielded consistently high bacterial counts at the sampling site nearest to the mouth of Little Black Creek. This was cause for concern because the campground is a popular place for vacationing families with children, and exposure to contaminated water puts them at risk for contracting a swimming-associated illness. Since young children are among the highest risk population for gastrointestinal illness (Wade et al., 2008), it

was important that the cause of contamination be identified and addressed. The goal of this project was to investigate the spatial distribution of *E. coli* in Little Black Creek and identify the source of contamination. We used traditional culture-based techniques to measure *E. coli* and quantitative Polymerase Chain Reaction (qPCR) methods to identify the human fecal marker, HF183. The presence of HF183 would indicate contamination from septic systems or sewage. We also used Geographical Information Systems (GIS) to examine area land use to locate potential bacterial sources.

Methods

Site Description

Six sites along Little Black Creek (Figure 2) were identified and monitored beginning on July 22, 2020. The site descriptions are listed in Table 1. The Lake Michigan beach at the Hoffmaster Campground and the mouth of Little Black Creek that discharges into that beach had already been sampled several times beginning on July 6, 2020.

Table 1. Little Black Creek Sampling Locations.

Site Number	Site Name	Site Coordinates	Dates Sampled
1	Black Lake Road	43.126008°N, -86.252914°W	7/22-8/19
2	Hoffmaster State Park	43.132442°N, -86.266728°W	7/22-8/19
3	Hoffmaster Campground Mouth	43.137007°N, -86.281919°W	7/6-8/24
4	Hoffmaster Campground Trail	43.137767°N, -86.279666°W	7/22-8/24
5	Hoffmaster Campground Entrance	43.137328°N, -86.272874°W	7/22-8/24
6	Lake Harbor Road	43.140530°N, -86.271276°W	7/22-8/19

Figure 2. Little Black Creek Sampling Locations (2020).

Site 1 was sampled from a bridge near where the creek diverges from Black Lake. Site 2 is inside the Hoffmaster State Park, which has a separate entrance from the campground. There is a stretch of golf courses in the area between Sites 1 and 2, but additional testing of this point of the creek did not yield high results for *E. coli*, so it was ruled out as a routine sampling site. Site 3 is the mouth of the creek, where it discharges into Lake Michigan at the Hoffmaster Campground beach. Site 4 is a bend of the creek along one of the trails in the campground where the water is around five feet deep. Site 5 is a shallow portion of the creek right at the entrance to the campground, and Site 6 is a separate branch of the creek that runs through a residential area and feeds into the main branch between Sites 4 and 5.

Little Black Creek gets its name from Black Lake because both bodies of water are known for their dark coloration from dissolved organic matter.. At the Hoffmaster Campground

beach, the creek displays a rusty red color that is believed to be influenced by tannins released from trees that have fallen into the creek. But despite its color, Little Black Creek was full of small children splashing around in many areas of the warm, shallow waters not just at the mouth, but all throughout the campground as well.

Figure 3 shows a comparison of the aerial view of Site 3, the mouth of the creek at two points throughout the summer. The image on the left is from July 6th, at the beginning of the study, where LBC is clearly flowing right into the “Center” part of the beach. The image on the right is from August 6th, one month later, and shows how the flow of the creek had changed direction since we began the study and is now flowing further south. The movement of the creek’s mouth was due to wind and wave action of Lake Michigan moving the beach sand and backing up the water flow.

Figure 3. Aerial Comparison of the Mouth of Little Black Creek Discharging into Lake Michigan at the Hoffmaster Campground Beach. (Left image, July 6, 2020. Right image, August 6, 2020).

It's important to consider that, like much of West Michigan, the greater area of the Black Lake watershed contained some areas of farmland, which could contribute animal wastes to the stream. The National Land Cover Data change from 2001 to 2016 showed that the agricultural land use in the watershed decreased over the years (Table 2).

Table 2. Black Lake Sub-Basin National Land Cover Data Change Analysis 2001 - 2016.

COUNT	NLCD_2001	ACRES	COUNT	NLCD_2016	ACRES	2001-2016 CHANGE
1031	Open Water	229.29	1074	Open Water	238.85	9.56
5186	Developed, Open Space	1153.34	5115	Developed, Open Space	1137.55	-15.79
3008	Developed, Low Intensity	668.96	3300	Developed, Low Intensity	733.90	64.94
1545	Developed, Medium Intensity	343.60	2208	Developed, Medium Intensity	491.05	147.45
980	Developed, High Intensity	217.95	1418	Developed, High Intensity	315.36	97.41
258	Barren Land (Rock/Sand/Clay)	57.38	248	Barren Land (Rock/Sand/Clay)	55.15	-2.22
6138	Deciduous Forest	1365.06	5610	Deciduous Forest	1247.63	-117.42
931	Evergreen Forest	207.05	780	Evergreen Forest	173.47	-33.58
2529	Mixed Forest	562.44	2425	Mixed Forest	539.31	-23.13
62	Shrub/Scrub	13.79	67	Shrub/Scrub	14.90	1.11
1318	Grassland/Herbaceous	293.12	1335	Grassland/Herbaceous	296.90	3.78
210	Hay/Pasture	46.70	145	Hay/Pasture	32.25	-14.46
1631	Cultivated Crops	362.73	1357	Row Crops	301.79	-60.94
5000	Woody Wetlands	1111.97	4812	Woody Wetlands	1070.16	-41.81
217	Emergent Herbaceous Wetlands	48.26	160	Emergent Herbaceous Wetlands	35.58	-12.68
	Total	6681.62		Total	6683.85	

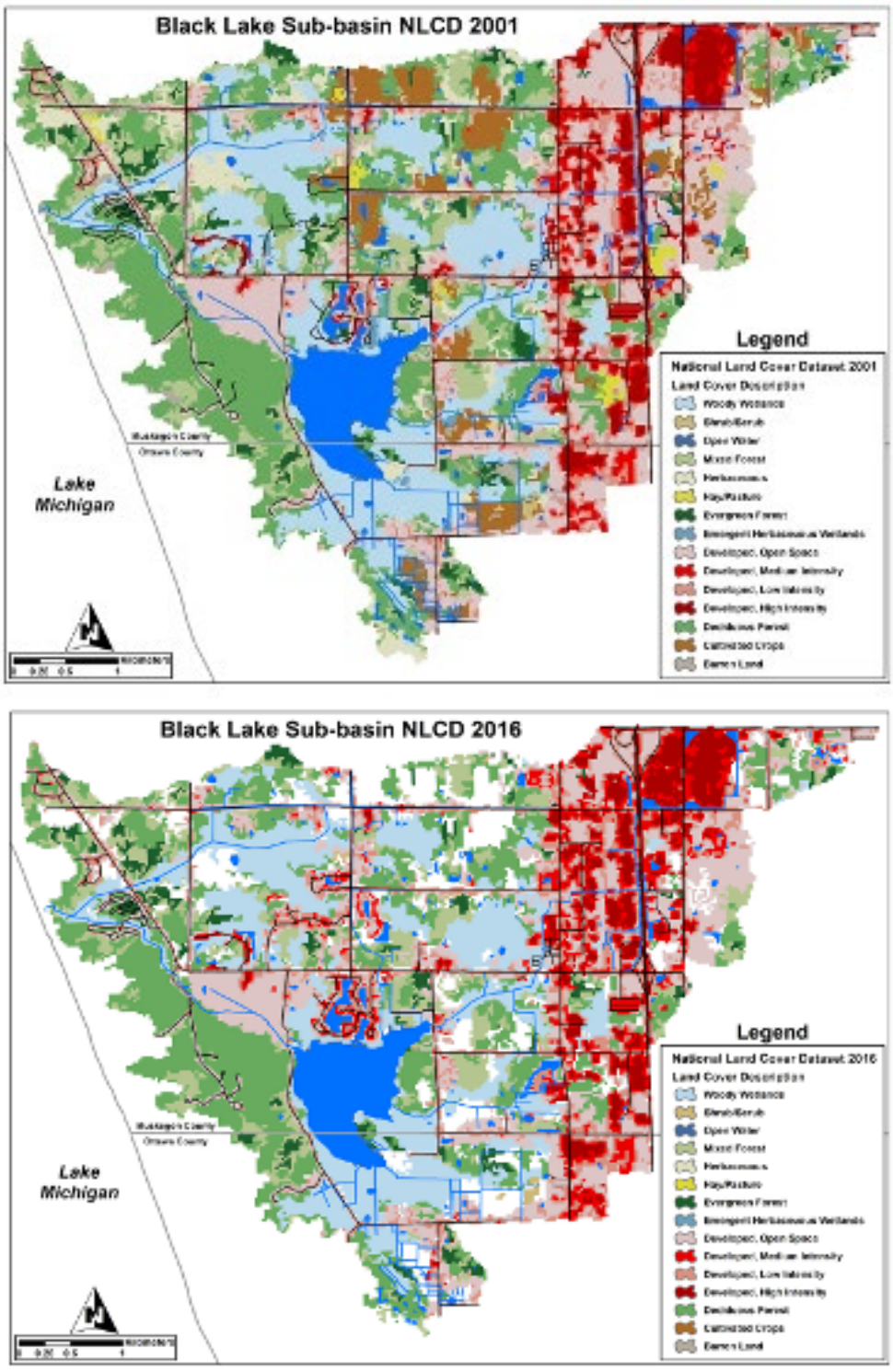

Figure 4. Comparison of National Land Cover Changes for Black Creek Watershed From 2001 to 2016.

The Land Cover map also showed that there was a significant increase in residential development in the Black Lake watershed area that could also be connected to the contamination (Figure 4). Much of the land cover in the area surrounding Little Black Creek consisted of various types or forests, wetlands, and developed spaces. Residential development and the campground can result in an increase of wildlife that feed off garbage such as rodents, raccoons, and opossums. These animals also can be a source of *E. coli*.

Sampling Methods

We sampled multiple Lake Michigan and inland lake beaches throughout the greater Muskegon area with the following technique: wade into knee-level water, and fully submerge a sterile bottle at the North, Center, and South sampling sites of the beach, collecting anywhere from 300-3000 mL of sample (Standard Operating Procedure, 2018). These three sites were either composited in the lab or used to calculate a geometric mean, depending on the beach. At each beach we also completed the Great Lakes Beaches Routine On-Site Sanitary Survey to record the conditions of the beach. at each beach. Including human and wildlife presence, record algae appearance in the water and on the shore, find the temperature and turbidity of the water, report beach litter, track the general climate of the beach environment, and take note of anomalies like black sand, flooding, and dead wildlife. The data from the Sanitary Surveys completed at each beach during the season were entered into the Michigan Beach Guard database for public viewing (https://www.egle.state.mi.us/beach/).

Colilert-18

The Colilert-18 Method was used to quantify *E. coli* concentrations in beach and stream water. Colilert-18 is a defined substrate method that measures the amount of culturable *E. coli* in a sample. It differs from a traditional nutrient-rich media that would allow the target organisms

(*Escherichia coli*) as well as other coliforms to grow, yielding inaccurate data and potential false positives. A 100 mL water sample was mixed with "MUG" (4-methyl- umbelliferyl β-D-glucuronide), a substrate with a fluorogenic compound that was converted to fluorogen specifically by the *E. coli* enzyme, β-glucuronidase (Kinzelman et al., 2005). This was contrasted in Figure 5 with the mechanism for the fluorescent reaction for a general coliform that does not have the β-glucuronidase enzyme, meaning that no other coliforms can display fluorescence in the presence of this substrate specific to *E. coli* (IDEXX, 2019).

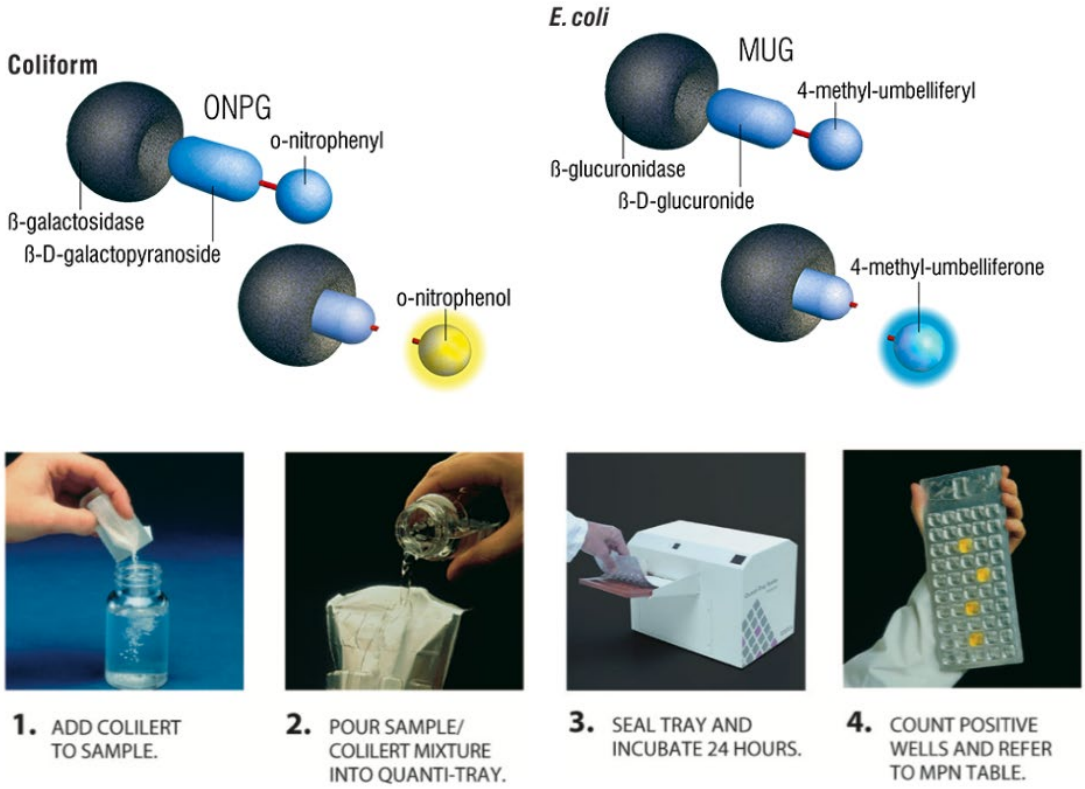

Figure 5. A Visual Explanation of the Science Behind the Colilert-18 Method and the Steps of the Method.

The sample-substrate mixture was poured into a Quanti-Tray, heat-sealed, incubated for 18 hours at 35°C, and then assessed the next day. Using ultraviolet light, the number of fluorescent wells were read and used to calculate the MPN, or "most probable number" of *E. coli*

coliforms in that sample. This was reported as the number of colony forming units per 100 mL of water, or cfu/100mL. The advantage of the Colilert-18 Method was that it required a simple setup with few materials, and provided accurate, easy to interpret results. The disadvantage of Colilert was that results were not available until the next day, which means results from a beach with potentially unsafe conditions cannot be reported until the following day, after countless beachgoers could have been exposed to contaminated waters.

Figure 6 shows a partially fluorescent Colilert plate (left), compared with a fully fluorescent plate (center) both from the Hoffmaster Campground mouth, and a completely non-fluorescent plate containing water from the lab blank (right). The plate on the left is of further interest because the bright neon wells were the ones counted toward the MPN, but the wells that were not illuminated were yellow. This looks different from the non-fluorescent wells of the lab blank on the right. This was because the Colilert-18 substrate turns all coliforms yellow, and only illuminates the ones that specifically have *E. coli* coliforms (IDEXX, 2019), indicating that there was more than just one species present in that water sample.

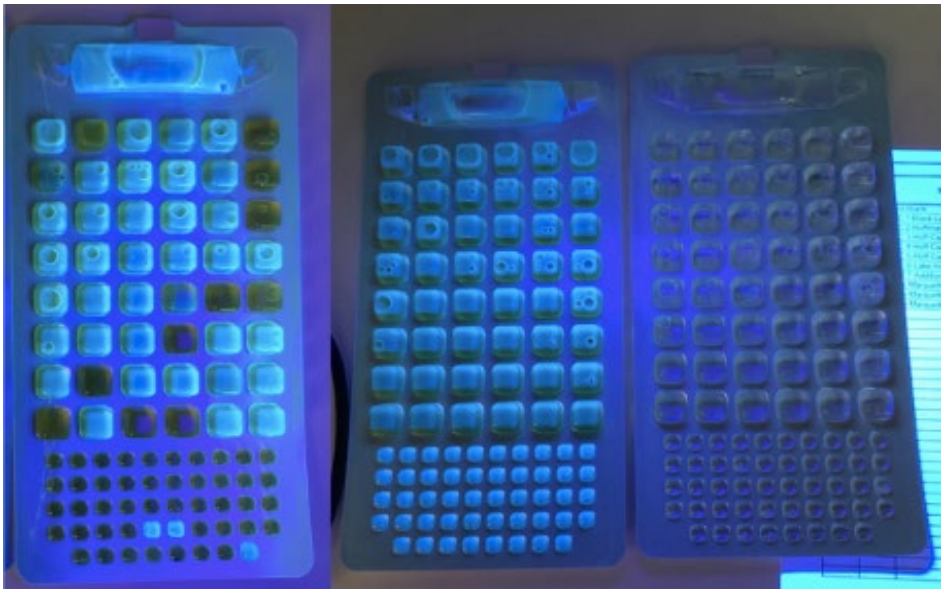

Figure 6. Example of Colilert-19 Sample Trays with Many Fluorescent Wells (Left) and All Wells Fluorescent (Center) and One Blank Tray with No Fluorescent Wells (Right).

If the MPN for the geometric mean of the North, Center, and South samples was above 300 cfu/100mL, the data were reported to the Public Health Department, and they issued an advisory at that particular beach. A beach advisory discourages swimming and contact with the water, but does not prohibit beachgoers from accessing it if they so choose. If the MPN was above 1000 cfu/100mL, the Public Health Department closes the beach to the public for that day (E.P.A., 2004). If either threshold-was reached, it was routine to sample the water again the next day to monitor the state of the contamination. It's important to note that these thresholds only apply to beaches, so even though the creek exceeds these thresholds, no immediate action was taken to restrict access.

qPCR Methods

It's often said that the future of beach monitoring is in qPCR. Quantitative Polymerase Chain Reaction, or qPCR, exponentially amplifies the amount of a specific type of DNA in a sample and measures the amount of DNA amplification cycles (out of 40) that it takes for the concentration of amplified DNA to surpass the threshold of 0.03 (Figure 7). This is the point at which the fluorescence being measured by the instrument is due to the bacteria in the sample, and not background noise. qPCR using draft Method C is a quicker way to test water quality, and offers same-day results, though it still takes roughly 2-3 hours to obtain and analyze results (USA, Environmental Protection Agency, 2014). The cycle at which the fluorescence surpasses that 0.03 threshold gives a “CT” value, or threshold cycle, which can also be used to suggest beach closures, similar to the MPN that Colilert gives. For this study, qPCR technology was used for the purposes of microbial source tracking, or MST, to see if the source of the water’s contamination was human. This was done following the guidelines of the United States Environmental Protection Agency’s Method 1696, which uses *Bacteroides* HF183, a human-

specific bacterial marker that has a limited ability to survive in the environment. In contrast, *E. coli* is found naturally in the gut of warm-blooded animals, but can still grow outside of the gut in the environment, even in a water supply (Van Elsas et al., 2011). This method will determine if the high bacterial levels were from human sources.

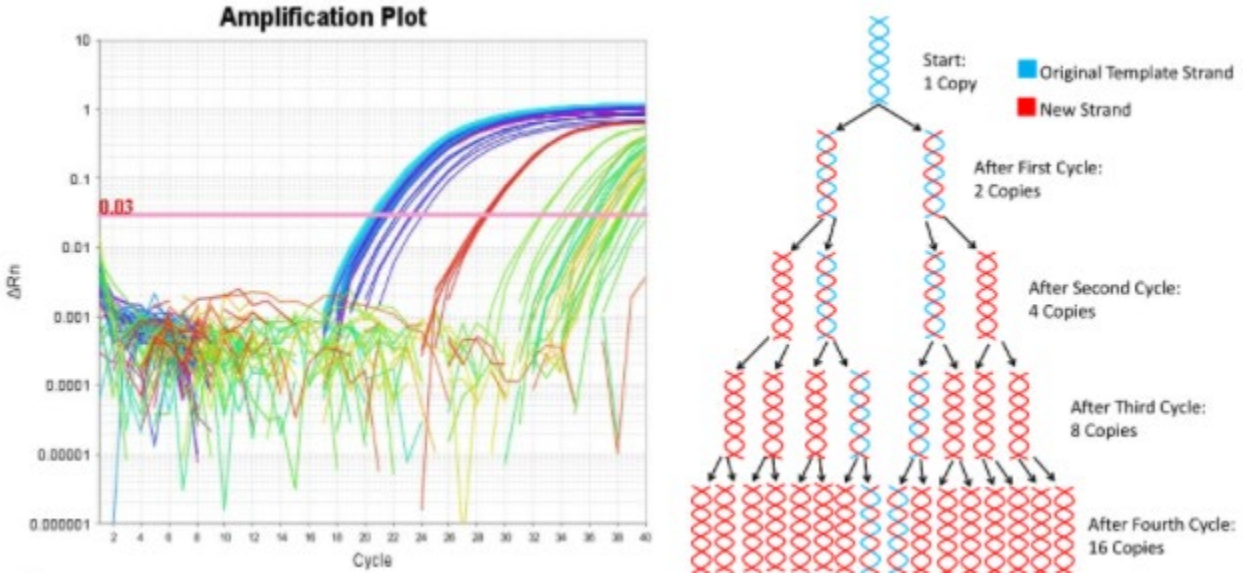

Figure 7. An Example of the Amplification Plot Given by the Applied Biosystems Instrument After a qPCR Test is Complete, and a Depiction of the qPCR DNA Amplification Process.

The process of qPCR Method 1696 is far more involved than that of Colilert. First, the 100 mL water sample from the creek sample was run through a sterile membrane filter, followed by phosphate buffered saline as a wash. When finished, the filter was folded into a small triangular shape and placed into a 2mL tube containing glass beads. If the analysis is not being done the same day, the filter tubes can be stored in a -80°C freezer until ready to analyze if needed. To perform the analysis, the fluid in the filter must be extracted. We added extraction fluid spiked with "Sketa" or salmon DNA, to be used as a sample control upon reading the results. The tubes were homogenized, allowing the glass beats to beat the substances on the filter into solution, then filtered and centrifuged several times in a sequential purifying process to eventually isolate the final pure supernatant, which is what was used for analysis. This was all

done in a laminar flow hood. We used two laminar flow hoods for sample preparation. One hood was used for sample processing and the second was used to prepare the qPCR plate. These precautions were taken to ensure a sterile working environment and to minimize the chances of cross-contamination from the raw sample. The 96 well plate was loaded in the clean hood with the TaqMan agent, or *Thermus aquaticus*, a reagent which contains DNA with exonuclease activity (Holland et al., 1991), as well as "master mix" which is a combination of the specific mixture of polymerase, primers, and probes necessary for the HF183 test. For this method, that included the HF183 *Bacteroides* qPCR assay (United States Environmental Protection Agency, 2018). The plate was then sealed and put into the Applied Biosystems qPCR instrument.

While qPCR can provide molecular identification of gene fragments related to the target organism, the results are not directly comparable to culture based methods. The qPCR method involves the replication of both the viable and nonviable DNA in the sample, which may not be an accurate representation of the number of living organisms in the sample. Additionally, since the instrument measures fluorescence, interferences from bubbles in the wells, or organic material in the sample can alter the reading. All samples were analyzed in triplicate to assess the precision of preparation and the samples were spiked with salmon DNA to check for inhibition from organic material.

Results

Colilert-18 Results

The Colilert-18 data from the Hoffmaster Campground beach revealed *E. coli* levels of 579 cfu/100mL, or colony forming units per 100 milliliters of water sample, in the creek discharge area that exceeded the state of Michigan's full-body contact criteria of 300 cfu/100mL. A follow-up study of the six creek sample sites found concentrations of *E. coli* consistently

exceeding the total body contact criteria at 5 of the 6 locations (Table 3). Despite the high counts at the Center and South sampling points of the beach, the North sample was so low that it balanced out the geometric mean to only 99.2 cfu/100mL. Since potential beach advisories and closures are based on the value of the geometric mean, the data left us unable to take any action at the beach as a whole, despite the clear contamination of the Center and South portions. This was cause for concern, and prompted the launch of an investigative study to take a closer look into what could be causing this contamination.

Table 3. Colilert-18 Data From July 6, 2020.

Site ID	Large wells	Small wells	Total # cfu/100mL
Hoffmaster Camp North	3	1	**4**
Hoffmaster Camp Center	49	29	**579**
Hoffmaster Camp South	49	23	**411**
Little Black Creek	49	34	**770**

The MPN of contamination in the creek at these sites found concentrations of *E. coli* consistently exceeding the total body contact criteria at Sites 2, 3, 4, 5, and 6, and exceeding it only once at Site 1. The data for all six sites of the creek are presented in Figure 7. The royal blue lines represent the average value for each site. The orange line indicates the criteria for full-body contact at the level 300 cfu/100mL, which would call for a beach advisory. The red line is at the level 1000 cfu/100mL, the criteria for partial body contact, which would call for a beach closure, and the black line indicates the upper limit of quantification offered by the Colilert method, 2419.6 cfu/100mL. Samples collected after rain events on July 29th and August 11th found *E. coli* levels above the upper level of quantification of our Colilert-18 Method at Sites 3 and 4 in the Hoffmaster Campground. The upper level of quantification was exceeded at Site 6

as well on August 11th. This means the number of *E. coli* coliforms on these dates was too numerous to count, and could be exponentially higher than 2419.6 cfu/100mL but unfortunately, the exact concentration on these dates is unknown.

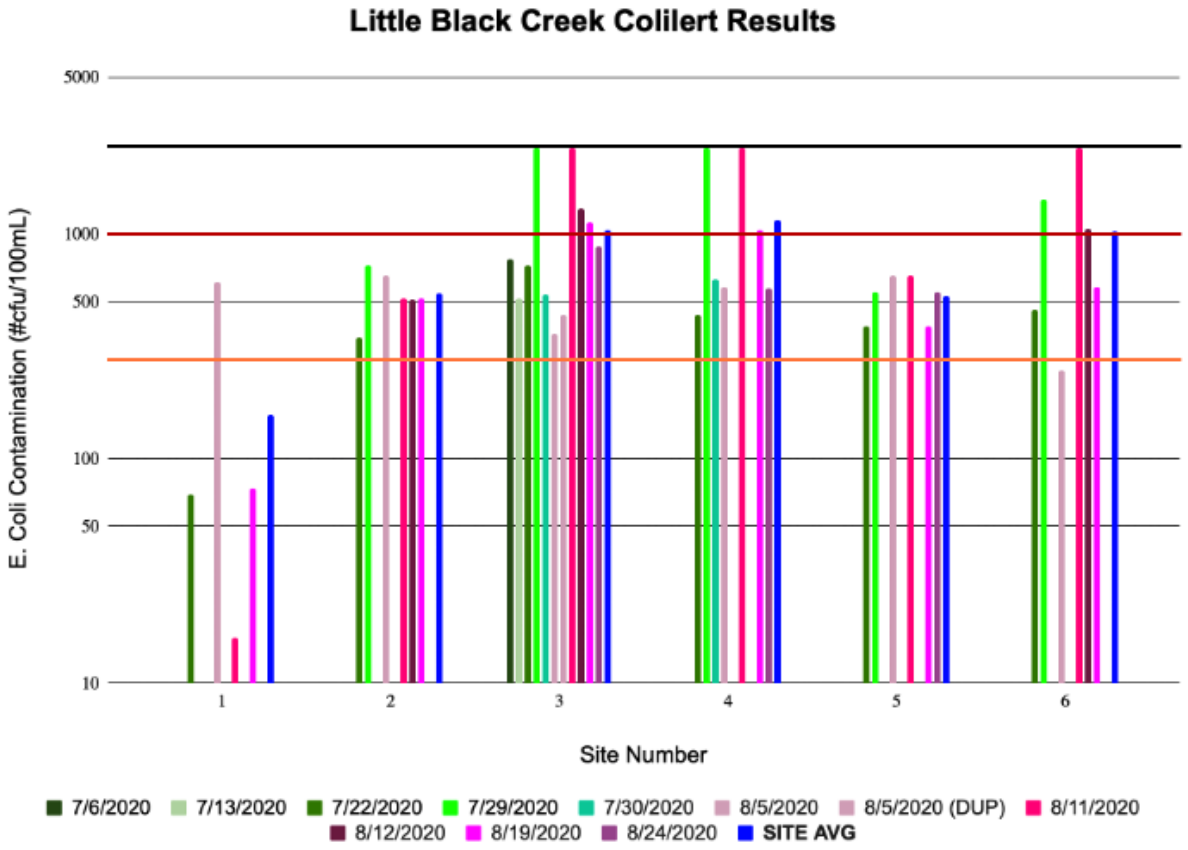

Figure 7. A Graphical Representation of the Escherichia coli Concentrations Measured in 2020 Little Black Creek Samples.

The data for Site 3 are shown in Figure 8. This was the site at which LBC discharges into Lake Michigan at the Hoffmaster Campground beach, where dozens of children were playing on each sample date. At Site 3, all Colilert data reported concentrations above the criteria for total body contact, with the lowest value of 361 cfu/100mL being recorded on August 5th.

Several of the samples were above 1000 cfu/100mL, the criteria for partial body contact. The results were so severe that further action had to be taken. The next step was investigating the

cause of this contamination through microbial source tracking using quantitative polymerase chain reaction.

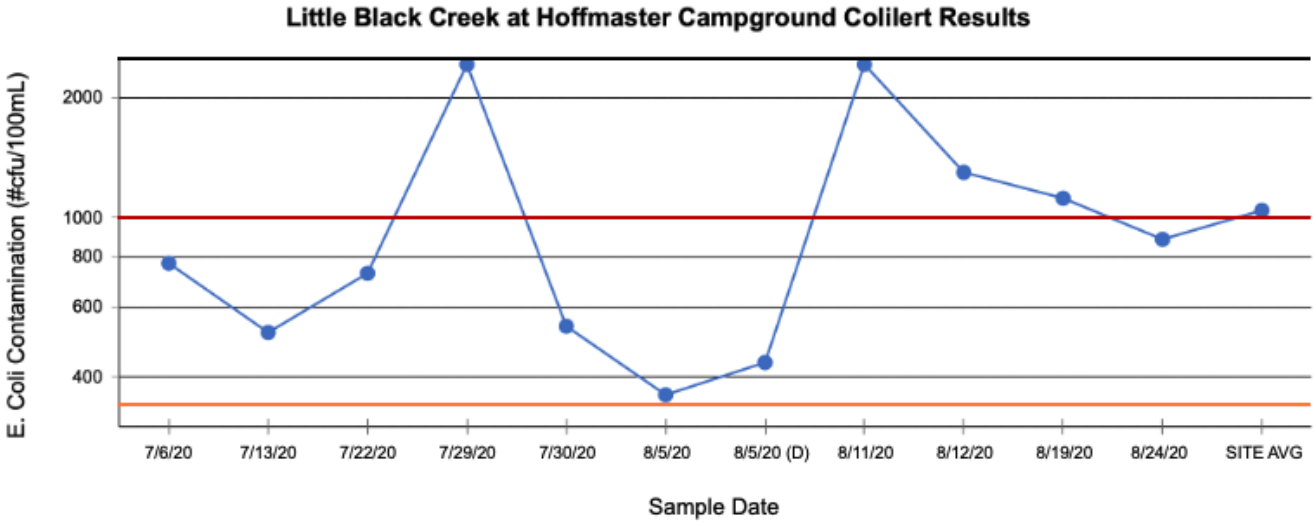

Figure 8. A Graphical Representation of the Escherichia coli Concentrations at Little Black Creek Site 3.

qPCR Results

The raw qPCR data were evaluated using an Excel workbook specific to Method 1696, which completed the necessary calculations and provided interpretable results (Sivaganesan et al., 2019). All of the creek samples presented Cq values that were below the lower level of quantification (LLOQ) for the HF183 assay. Cq is the data value that refers to the quantification cycle used to make the concentration estimate for that sample.

Discussion

It's important to understand that the Muskegon County Public Health guidelines for water quality only apply to closures and advisories for beaches, not tributaries. But there are guidelines that apply to full (involving potential submersion of the head) and partial contact with contaminated water (Wu et al., 2018), and those do apply to Little Black Creek. Because the Colilert-18 data exceeded full and partial body contact guidelines on every day the creek was

sampled, for all sites except Site 1, the creek water was so contaminated that if it were a beach, the Muskegon County Public Health Department would issue a beach advisory or closure, and declare the water unsafe for full-body submersion. At Site 3, the water quality exceeded the full-body contact criteria on every sampling date, and exceeded the partial body contact criteria on several of the dates. This means that on every single sample date, the creek water was either not safe for swimming, or any contact with the water, despite all the children that were seen splashing around in the warm water each week.

Additionally, because of the negative results for the HF183 qPCR assay test, the high levels of bacterial contamination found in the creek all summer were not from a human source. These results were unexpected as there seemed to be several signs pointing to human contamination of the creek. First, LBC flows mainly through either residential areas or the Hoffmaster Campground. Sites 1 and 6 were in residential areas, Site 2 was in the Hoffmaster State Park, and Sites 3, 4, and 5 were in the Hoffmaster Campground. Second, the campground beach was very popular for families and their young children. Even dirty diapers can contribute to the fecal contamination of a water source. Lastly, when discussing my concerns about the creek with the State Park administrative staff, one of the Park Rangers informed me that there had been maintenance done on the Campground's septic system in December of 2019 through a manhole located just off the bank of the creek. A separate investigation into additional sites of LBC nearest to that manhole and the campground's sanitary facilities found *E. coli* levels of 860 cfu/100mL where a drainage pipe empties into the creek, as seen in Table 4. All signs seemed to point to contamination of the water due to human waste. The creek was sampled at several sites moving downstream at about 20-50 foot intervals near the sanitation facilities of the campground, the lift station, and the manhole that the State Park Ranger said had been moved in

December 2019. These sites seemed to have relatively higher counts, but nothing overly significant compared to the data we had seen all summer.

Table 4. Additional Sampling Sites of LBC in the Hoffmaster Campground.

Site Name	**#cfu/100mL**
LBC Mouth (Site 3)	884
LBC Trail (Site 4)	573
LBC Upstream Trail L Side	857
LBC Upstream Trail R Side	86
LBC Lift Station/Site 24	644
*LBC Manhole Bridge L	759
*LBC Manhole Bridge R	776
*LBC Manhole Outlet	860
LBC Camp Entrance (Site 5)	548

Critical locations were the LBC Manhole Bridge L and R, and the Manhole Outlet. The manhole outlet is the image shown to the left, a small drainage pipe with water flowing into the creek. The sample taken from the center of the creek near this pipe yielded a high number Colilert result, 860 cfu/100mL. The two sampling sites about 20 and 40 feet downstream from this pipe, Manhole Bridge L and Manhole Bridge R also yielded high results, as well as the Lift Station Site a little further downstream. These results also seemed to indicate that the contamination was likely to be from human fecal matter. But the HF183 human marker was not

present in any significant quantities, which required a different approach to determine the source of the contamination of Little Black Creek.

Conclusion

Further Action

Regardless of the source of the contamination, the source of contamination needs to be identified and controlled. Dr. Rediske previously worked with the Muskegon County Public Health Department to get a sign posted at Meinert Beach warning of the bacterial contamination of Little Flower Creek. An image of the sign is included in Figure 9.

Figure 9. An Example of the Public Health Advisory Notice of the Bacterial Contamination of Little Flower Creek Posted at Meinert Beach.

My next goal is to work to get a similar sign posted at the Hoffmaster Campground as well, at the Lake Michigan beach and near the entrance of the campground. While it is a possibility that the State Park Rangers may have cleared the debris and are planning to resume directing patrons to the original beach for the summer of 2021, the contamination of Little Black Creek is still an issue. Not only do children play in the creek all throughout the campground, but there have been important changes in the geography of that area that will continue to be a problem, even if the campground resumes use of their original beach site. As shown in Figure 4, the flow of the creek had been steered further south by the formation of sandbars in the nearshore water from heavy rain events last summer. Since the original beach is south of the new beach, the creek flow could be

contaminating the water of the new beach and the original beach as well with its new southward flow.

Additional Investigation

This summer we will be sampling the original six sites in addition to more areas both inside and outside the campground, to create a better map of where in the creek the contamination is highest. We will look further into the area around Site 6, which was the only sampling site on that separate branch of the creek. The contamination of Site 6 was consistently high throughout the summer and that branch of the creek feeds into the main branch before Sites 3 and 4, which were also consistently high. Therefore, investigating the area upstream of Site 6, and its surrounding landscape will be part of the additional investigation being conducted on the creek this summer, in search of the source of the contamination.

At this point in the study, our next step is to test the samples for various animal markers. The campground would tend to attract raccoons and other small mammals that feed on human refuse. Deer may inhabit the forests in the park and the wetland surrounding Black Lake could attract birds. This is consistent with the findings in the Finger Lakes region in western New York where geese were identified as the primary source of *E. coli* contamination in the watershed, followed by cows, deer, and humans (Somarelli et al., 2007). Since West Michigan has no shortage of geese, they could be contributing to the contamination in Little Black Creek as well. The residential areas around Site 6 could be contaminated by the runoff of pet waste into the stream, which sits at a much lower elevation. AWRI has been granted the funds and supplies to test the 2020 samples for bird and dog markers, and moving forward with the study, we are hopeful that we'll get the resources to test the summer 2021 samples for other markers like deer,

cow, pig, or raccoon. We're also planning to broaden the scope of the investigation and sample the Black Lake wetlands and the surrounding open water.

Testing the samples for the HF183 human fecal marker made it clear that the cause of the contamination must be coming from elsewhere. Moving forward, we have to look deeper into the geography of the Black Lake watershed to develop a better understanding of the area, and search for clues that could hint to the source of the bacterial contamination. Dr. Rediske and I have plans to finally discover the source this summer. We will continue to communicate with the Hoffmaster State Park Campground to figure out just why the concentrations are so high in the campground. We plan to continue advocating for a sign to be posted at the Hoffmaster Campground beach, to hopefully protect the little ones from getting sick. We will solve this problem this summer, to protect beachgoers from illness and preserve the natural resources that are so special to West Michigan.

Bibliography

(1) Beaches Environmental Assessment and Coastal Health Act of 2000. (2000). 106th Congress. *Public Law 106–284,GPO*. https://www.congress.gov/106/plaws/publ284/PLAW-106publ284.pdf.

(2) Cabelli, V. J., Dufour, A. P., McCabe, L. J. and Levin, M. A. (1982). "Swimming-Associated Gastroenteritis and Water Quality." *American Journal of Epidemiology* 115(4), 606–616. https://doi.org/10.1093/oxfordjournals.aje.a113342.

(3) Colford, J.M., Wade, T.J., Schiff, K.C., Wright, C.C., Griffith, J.F., Sandhu, S.K., Burns, S., Sobsey, M., Lovelace, G. and Weisberg, S.B. (2006). "Water Quality Indicators and the Risk of Illness at Beachers with Nonpoint Sources of Fecal Contamination." *Epidemiology* 18, 27-35.

(4) E. P. A. (2004). Water Quality Standards for Coastal and Great Lakes Recreation Waters. *Federal Register*, 69(220), 62219–62221. https://www.federalregister.gov/documents/2004/11/16/04-25303/water-quality-standards-for-coastal-and-great-lakes-recreation-waters.

(5) Holland, P. M., Abramson, R. D., Watson, R., and Gelfand, D. H. (1991). "Detection of Specific Polymerase Chain Reaction Product by Utilizing the 5'----3' Exonuclease Activity of Thermus Aquaticus DNA Polymerase." *Proceedings of the National Academy of Sciences* 88(16), 7276-7280.

(6) IDEXX, Laboratories Inc. (2019). *IDEXX Colilert-18/Quanti-Tray.* IDEXX US. https://www.idexx.com/en/water/water-products-services/colilert-18/.

(7) Kinzelman, J. L., Singh, A., Ng, C., Pond, K. R., Bagley, R. C. and Gradus, S. (2005). "Use of IDEXX Colilert-18® and Quanti-Tray/2000 as a Rapid and Simple Enumeration Method for the Implementation of Recreational Water Monitoring and Notification Programs." *Lake and Reservoir Management* 21(1), 73-77.

(8) Lane, M.J. (2019). *The Implementation of qPCR Beach Monitoring Methods: Analysis of a Multi Lab Validation Study and the Role of Environmental Parameters on a Comparison of Colilert and qPCR Methods* [Unpublished master's thesis]. Grand Valley State University.

(9) Lavender, J.S. and Kinzelman, J.L. (2009). "A Cross Comparison of qPCR to Agar-Based or Defined Substrate Test Methods for the Determination of *Escherichia coli* and *Enterococci* in Municipal Water Quality Monitoring Programs." *Water Research* 43(19), 4967-4979.

(10) Nevers, M. B., Byappanahalli, M. N., Shively, D., Buszka, P. M, Jackson, P. R. and M. S. Phanikumar. (2018). "Identifying and Eliminating Sources of Recreational Water Quality Degradation Along an Urban Coast." *Journal of Environmental Quality: Microbial Water Quality—Monitoring and Modeling.*

(11) Preparations and Standard Operating Procedure (SOP) for 2018 Beach Season: Protocol Updates for Method C. (2018). Pre-seasonSOP_042718.

(12) Seyfried, P. L., Tobin, R. S., Brown, N. E. and Ness, P. F. (1985). "A Prospective Study of Swimming Related Illness. I. Swimming-Associated Health Risk." *American Journal of Public Health* 75(9), 1068–1070. https://doi.org/10.2105/AJPH.75.9.1068.

(13) Seyfried, P. L., Tobin, R. S., Brown, N. E. and Ness, P. F. (1985). "A Prospective Study of

Swimming Related Illness. II. Morbidity and the Microbiological Quality of Water." *American Journal of Public Health* 75(9), 1071–1075. https://doi.org/10.2105/AJPH.75.9.1071.

(14) Shanks, O., and Korajkic, A. (2019). "Microbial Source Tracking: Characterization of Human Fecal Pollution in Environmental Waters with HF183 Quantitative Real-Time PCR." *Microbial Forensics* 3, 71–87. Academic Press Incorporated. https://cfpub.epa.gov/si/si_public_record_report.cfm?Lab=NRMRL&dirEntryId=347630.

(15) Sivaganesan, M., Aw, T., Briggs, S. , Dreelin, E., Aslan, A., Dorevitch, S., Shrestha, A., Isaacs, N., Kinzelman, J., Kleinheinz, G., Noble, R., Rediske, R., Scull, B., Rosenberg, S., Weberman, B., Sivy, T., Southwell, B., Siefring, S., Oshima, K. and Haugland, R. (2019). "Standardized Data Quality Acceptance Criteria for a Rapid *Escherichia coli* qPCR Method (Draft Method C) for Water Quality Monitoring at Recreational Beaches." *Water Research*. Elsevier Science Ltd, New York, NY, 156, 456-464. https://doi.org/10.1016/j.watres.2019.03.011.

(16) Somarelli, J. A., Makarewicza, J.C., Siab, R. C. and Simonc, R. (2007). "Wildlife Identified as Major Source of *Escherichia coli* in Agriculturally Dominated Watersheds by BOX A1R-derived Genetic Fingerprints." *Journal of Environmental Management* 82, 60–65.

(17) Staley, Z. R. and Edge, T. A. (2016). "Comparative Microbial Source Tracking Methods for Identification of Fecal Contamination Sources at Sunnyside Beach in the Toronto Region Area of Concern." *Journal of Water and Health.* IWA Publishing.

(18) United States Environmental Protection Agency. (2018). "Method 1696: Characterization of Human Fecal Pollution in Water by HF183/BacR287 TaqMan Quantitative Polymerase Chain Reaction (qPCR) Assay."

(19) United States Environmental Protection Agency. (2021) "Recreational Water Quality Criteria and Methods." *Office of Water* 820-D-11-002. http://water.epa.gov/scitech/swguidance/standards/criteria/health/recreation/upload/recreation_document_draft.pdf.

(20) USA, Environmental Protection Agency, Office of Water. (2014). "Method C: *Escherichia coli* in Water by Taqman® Reaction (qPCR) Quantitative Polymerase Chain."

(21) Van Elsas, J.D., Semenov, A.V., Costa, R. and Trevors, J.T. (2011). "Survival of *Escherichia coli* in the Environment: Fundamental and Public Health Aspects." *ISME Journal* 5(2), 173-183.

(22) Wade, T., Calderon, R., Brenner, K., Sams, E., Beach, M., Haugland, R., Wymer, L., Dufour, A. (2008). "High Sensitivity of Children to Swimming-Associated Gastrointestinal Illness: Results Using a Rapid Assay of Recreational Water Quality." *Epidemiology,* 19(3), 375-383.

(23) Wu, H., Oun, A., Kline-Robach, R. and Xagoraraki, I. (2018)."Microbial Pollution Characterization at a TMDL Site in Michigan: Source Identification." *Journal of Great Lakes Research* 44, 412-420.