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ASSESSING CONTRIBUTION OF ENTERIC BACTERIA TO INLAND LAKES AFTER A MAJOR RAIN EVENT IN LEELANAU COUNTY

FINAL REPORT

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INTRODUCTION

Public health officials have been testing recreational waters for enteric bacteria contamination for decades. The following, taken from "Bacterial Water Quality Standards for Recreational Waters Status Report" (U.S. Environmental Protection Agency Office of Water, 2003), provides an excellent short background summary of water testing in the U.S. during the early years.

Water Quality Standards Background

In response to widespread public concern about the condition of our nation's waters, the United States Congress enacted landmark legislation in 1972. This statute, the Federal Water Pollution Control Act Amendments of 1972 (referred to as the Clean Water Act of 1972, or CWA), expanded and built upon existing laws designed to control and prevent water pollution. Successive amendments to the 1972 CWA (the Clean Water Act of 1977 and the Water Quality Act of 1987) have continued to strengthen the law to better protect our nation's waters.

Water quality standards are the cornerstone of a state's water quality management program. States, territories, and Indian tribes set water quality standards for waters within their jurisdictions. Water quality standards define a use for a waterbody and describe the specific water quality criteria to achieve that use. The water quality standards also contain antidegradation policies to protect existing water quality. These are the goals by which success is ultimately gauged for a given waterbody or watershed.

The water quality standards program is administered by the U.S. Environmental Protection Agency (EPA). Congress has mandated that the EPA is responsible for providing water quality criteria recommendations; approving state-adopted standards for waters of the United States; evaluating adherence to the standards; and overseeing enforcement of standards compliance. Guidance for the development of standards by individual states, tribes, and territories is contained in the EPA documents Water Quality Standards Handbook, Second Edition (1983) and Ambient Water Quality Criteria for Bacteria (1986).

Fecal bacteria have been used as an indicator of the presence of gastrointestinal pathogens in surface and drinking waters for many years. Their presence in water is known to relate to the risk of developing gastrointestinal disease, based on epidemiological evidence of gastrointestinal disorders from ingestion of contaminated surface water or raw shellfish. Contact with fecal contaminated water can lead to ear or skin infections, and inhalation of contaminated water can cause respiratory diseases. The pathogens responsible for these diseases can be bacteria, viruses, protozoans, fungi, or parasites that live in the gastrointestinal tract and are shed in the feces of warm-blooded animals.

However, because of the difficulties in analyzing for and detecting the many possible pathogens or parasites, indicators of the presence of fecal bacteria, such as fecal coliforms, *Enterococcus sp.*, and *Escherichia coli*, are used as the primary indicators of fecal contamination. The latter two indicators are considered to have a higher degree of association with outbreaks of certain gastrointestinal diseases than fecal coliforms and were recommended as the basis for bacterial water quality standards in the 1986 Ambient Water Quality Criteria for Bacteria document (both for fresh waters, enterococci for marine waters). The standards are defined as a concentration of the indicator above which the health risk from waterborne disease is unacceptably high.

Prior to the 1986 revision to the National criterion, there were recommendations in the report of the National Technical Advisory Committee to the Secretary of the Interior, Water Quality Criteria (1967) and by EPA in Quality Criteria for Water (1976). Both of these documents were based on fecal coliforms and recommended that maximum densities not exceed geometric means of 200 organisms per 100 ml in recreational waters.

Since publication of this status report in 2003, advances have been made to decrease the time needed for results and increase the specificity of target organisms. A complete summary can be found in "Recreational Water Quality Criteria", a 2012 EPA document. These advances are important for several reasons. Recent research has revealed that *Escherichia coli*, the ubiquitous bacterium found in most vertebrate host digestive systems, has adapted to freshwater ecosystems and can not only live but also successfully reproduce in aquatic systems outside a vertebrate host (Zhi et al., 2019, 2016). This complicates standards established for *E. coli* due to confounding data that may be produced. For this reason, many water monitoring programs have moved to assess enterococci as an indicator of fecal pollution. The presence of enterococci correlates strongly with the presence of fecal bacteria. Enterococci is generally not able to replicate within the environment, but it persists longer than *E. coli*, making it a more appealing indicator organism. Moreover, there are a number of studies that have linked gastrointestinal illness and febrile respiratory illness with enterococci levels in water (Kay et al., 1994; Fleisher et al., 1996; Wade et al., 2003, 2010; Heaney et al., 2012, 2014). This relationship is stronger between illness and enterococci than it is for *E. coli* (Borchardt et al., 2003; Risebro et al., 2012).

Monitoring for enterococci can be accomplished by culture-based techniques or via quantitative polymerase chain reaction (qPCR). Since qPCR methods are more sensitive and allow for assessment of multiple targets from a single water sample, this approach is preferable for monitoring programs. The EPA approved Method 1611 (*Enterococci in Water by TaqMan Quantitative Polymerase Chain Reaction (qPCR) Assay*) in 2012 as an alternative to more conventional culture-based methods that require many hours of incubation time. This ultimately reduces beach closure days due to rapid analysis.

METHODS

We followed EPA Method 1611 to assess the impact of a significant rain event with respect to increased enteric bacteria on four lakes in Leelanau County, Michigan in 2019. Triplicate 50ml water samples were collected at water inlet sites on each lake after an extended period of no measurable precipitation and again after a significant rain event. Samples from each site were combined and a 45ml subsample was drawn and suction filtered through a 0.5 µm Pall filter (FMFNL 1050). The filter paper was cut in half and one half preserved in 95% ethanol for processing at the University of Alberta. The other half was used for DNA extraction at our Lime Lake field laboratory. EPA Method 1611 was followed to assess enterococcus levels for each sample using the qPCR assay. All extracted DNA samples were validated on a core qPCR machine at the University of Alberta. All samples exceeding EPA Beach Action Values for enterococcus were source tracked at the University of Alberta core lab for human (HF183), bovine, and goose enteric bacteria contribution.

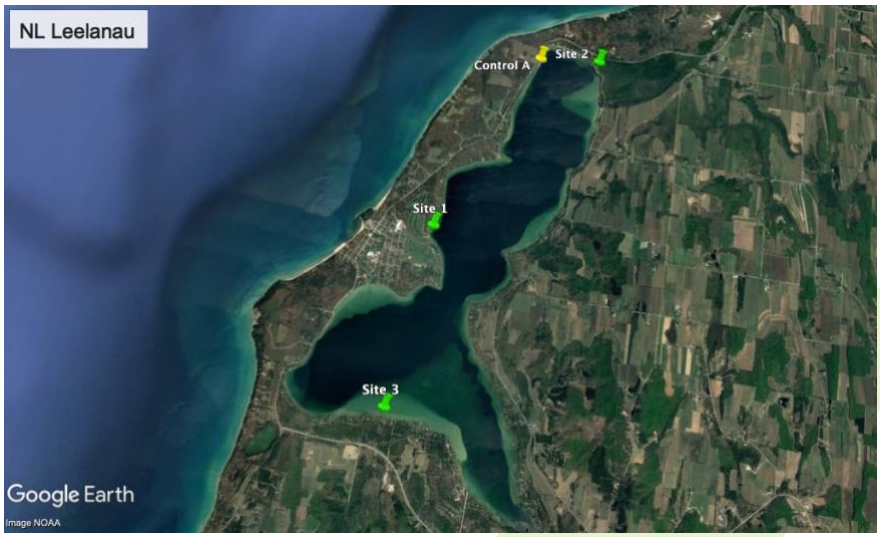
To distinguish between inlet water and general lake water, three distinct sites were sampled near each inlet. One triplicate sample was drawn in the inlet stream right at the mouth, and other triplicate samples alongshore ~50m from the inlet in both directions.

RESULTS

Water samples were drawn by trained volunteers on 10 July and again on 16 July. The last measurable precipitation in the region prior to 10 July occurred on 2 July (0.4 inch) and only an additional 0.1 inch had been recorded since 26 June. Therefore, conditions were dry for the 10 July sampling. A significant rain event occurred on the morning of 15 July (1.4 inch in ~3 hours), prompting our second round of sampling on the morning of 16 July, when inlets were full and runoff was obvious.

The following maps illustrate inlet sampling sites and control sites on all lakes.


Lime Lake
Control H
Control G
Control F
Site 1
Control A
Control B
Site 4
Control C
Control D
Control E
Site 3
Site 2
Google Earth


NL Leelanau
Site 2
Control A
Site 1
Site 3
Google Earth
Image NOAA


SL Leelanau
Control B
Site 6
Site 4
Site 5
Google Earth

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Lake Inlet Research				
Lake	Inlet	Pre-rain	Post-rain	Difference
Glen	Site 1	79.2	0.0	-79.2
Glen	Right	0.0	708.6	708.6
Glen	Left	0.0	61.6	61.6
Glen	Site 2	76.9	765.7	688.8
Glen	Right	52.3	0.0	-52.3
Glen	Left	0.0	0.0	0.0
Glen	Site 3	140.8	1091.6	950.9
Glen	Right	0.0	0.0	0.0
Glen	Left	0.0	0.0	0.0
Glen	Site 4	0.0	0.0	0.0
Glen	Right	161.5	1299.9	1138.4
Glen	Left	0.0	1068.9	1068.9
Glen	Site 5	0.0	2842.9	2842.9
Glen	Right	0.0	803.8	803.8
Glen	Left	0.0	149.8	149.8
Glen	Site 6	2320.4	4862.3	2541.9
Glen	Right	0.0	0.0	0.0
Glen	Left	0.0	677.3	677.3
Lime	Site 1	0.0	460.8	460.8
Lime	Right	0.0	0.0	0.0
Lime	Left	0.0	0.0	0.0
Lime	Site 2	776.7	2101.4	1324.6
Lime	Right	0.0	0.0	0.0
Lime	Left	0.0	0.0	0.0
Lime	Site 3	0.0	2014.3	2014.3
Lime	Right	0.0	0.0	0.0
Lime	Left	0.0	0.0	0.0
Lime	Site 4	0.0	4365.5	4365.5
Lime	Right	0.0	0.0	0.0
Lime	Left	0.0	0.0	0.0
LT Lake	Site 1	27.5	2466.0	2438.4
LT Lake	Right	20.1	0.0	-20.1
LT Lake	Left	50.6	0.0	-50.6
LT Lake	Site 2	16.2	0.0	-16.2
LT Lake	Right	0.0	0.0	0.0
LT Lake	Left	14.5	0.0	-14.5
LT Lake	Site 3	0.0	2402.9	2402.9
LT Lake	Right	0.0	8244.6	8244.6
LT Lake	Left	17.8	623.6	605.7
LT Lake	Site 4	357.0	3712.4	3355.4
LT Lake	Right	0.0	0.0	0.0
LT Lake	Left	421.8	0.0	-421.8
Leelanau	Site 1	0.0	196.9	196.9
Leelanau	Right	0.0	0.0	0.0
Leelanau	Left	0.0	44.4	44.4
Leelanau	Site 2	196.9	142.8	-54.1
Leelanau	Right	0.0	0.0	0.0
Leelanau	Left	20.3	0.0	-20.3
Leelanau	Site 3	92.8	241.8	149.0
Leelanau	Right	11.1	83.4	72.3
Leelanau	Left	0.0	0.0	0.0
Leelanau	Site 4	76.8	258.9	182.2
Leelanau	Right	0.0	0.0	0.0
Leelanau	Left	0.1	0.0	-0.1
Leelanau	Site 5	68.4	222.2	153.7
Leelanau	Right	0.0	11.2	11.2
Leelanau	Left	0.0	14.4	14.4
Leelanau	Site 6	0.0	28.7	28.7
Leelanau	Right	0.0	0.0	0.0
Leelanau	Left	0.0	66.6	66.6
Totals		5000.0	42035.2	37035.2
		Percent Increase		741%

The following tables show levels of enterococcus as Genome Equivalents (GE/100ml) measured on 10 July (dry period) and 16 July (after rain event) at each inlet (plus ~50m left and right of inlet) and at the control sites. Red color fills signify EPA suggested Beach Action Values (BAV) as shown in the diagram below. Exceeding the 1280 GE limit would normally trigger a source tracking study and possibly close a beach. If the source is found to not be human or cow, then the 1280 limit no longer applies and is raised to 6400 GE, which would lead to beach closure even if the source was 100% goose. These actions are based on risk assessment studies that model campylobacter from bird as the main bacterial risk, campylobacter and *E. coli* 0157 from cattle, and various bacterial and viral risks from humans.

EPA 1611 Beach Action Values (BAV)
>1280 GE/100ml (beach closure, source tracking)
>6400 GE/100ml (beach closure, regardless source)

Lake Controls				
Lake	Control	Pre-rain	Post-rain	Difference
Glen	Control A	0.00	0.00	0.00
Glen	Control B	0.00	325.75	325.75
Lime	Control A	0.00	0.00	0.00
Lime	Control B	0.00	0.00	0.00
Lime	Control C	263.30	202.19	-61.11
Lime	Control D	203.46	0.00	-203.46
Lime	Control E	0.00	0.00	0.00
Lime	Control F	0.00	1785.85	1785.85
Lime	Control G	863.43	0.00	-863.43
Lime	Control H	0.00	1339.64	1339.64
LT Lake	Control A	0.00	0.00	0.00
LT Lake	Control B	30.35	0.00	-30.35
LT Lake	Control C	0.00	0.00	0.00
LT Lake	Control D	0.00	0.00	0.00
LT Lake	Control E	0.00	0.00	0.00
LT Lake	Control F	0.00	0.00	0.00
LT Lake	Control G	0.00	1410.58	1410.58
LT Lake	Control H	416.14	0.00	-416.14
LT Lake	Control I	0.00	0.00	0.00
LT Lake	Control J	0.00	0.00	0.00
LT Lake	Control K	0.00	0.00	0.00
LT Lake	Control L	0.00	0.00	0.00
Leelanau	Control A	0.00	18.55	18.55
Leelanau	Control B	0.00	21.78	21.78
Totals		1776.7	5104.3	3327.7
		Percent Increase		187%

This table compares total enterococcus values (GE/100ml) for all lakes participating is this research.

Whole-Lake Comparisons				
Lake	Pre-rain	Post-rain	Difference	% Increase
Glen	2831.2	14332.3	11501.1	406%
Lime	776.7	8942.0	8165.2	1051%
LT Lake	925.7	17449.4	16523.8	1785%
Leelanau	466.4	1311.5	845.1	181%

This table shows the identified source of the enterococcus found in positive samples exceeding EPA BAV values.

Lake	Inlet	Pre-rain	Post-rain	Source
Glen	Site 4 Right	161.5	1299.9	Unknown
Glen	Site 5 Inlet	0.0	2842.0	Unknown
Glen	Site 6 Inlet	2320.4	4862.3	Trace Human
Lime	Site 2 Inlet	776.7	2101.4	Trace Human
Lime	Site 3 Inlet	0.0	2014.3	Unknown
Lime	Site 4 Inlet	0.0	4365.5	Unknown
L.Traverse	Site 1 Inlet	27.5	2466.0	Unknown
L.Traverse	Site 3 Inlet	0.0	2402.9	Unknown
L.Traverse	Site 3 Right	0.0	8244.6	Trace Human
L.Traverse	Site 4 Inlet	357.0	3712.4	Unknown
Lime	Control F	0.0	1785.9	Unknown
Lime	Control H	0.0	1339.6	Trace Human
L.Traverse	Control G	0.0	1410.6	Trace Human

DISCUSSION

Caution should be used when drawing conclusions from this research. Sampling occurred on only one day during a dry period and one day right after a rain event. While the data show dramatic increases in enteric bacteria after a rain event, more routine and extensive collection and analysis would need to occur after more rain events in order to draw more definitive conclusions.

From this limited study, we can say enteric bacteria sharply increases after a rain event at water inlets on the lakes studied. Since these sites are primarily chosen to measure input from streams, the values measured are likely most impacted by the stream and not the lake. The two smaller lakes (Lime, Little Traverse) show the greatest percent increase. Lake Leelanau appears to be least affected by enteric bacteria entering the lake, since they had no sites exceeding EPA guidelines before or after the rain.

Results from source tracking show that human contamination from these point sources is low, meaning that sources other than humans are likely the most significant fecal contributors at these

sites. This is not unexpected, since human fecal contamination is often sporadic in these aquatic environments. Thus, it may be worthwhile undertaking a more frequent sampling schedule to determine where and when human fecal contamination is found. It is also not uncommon to have detectable enterococcus values, but not have a determinable source. The target bacteria differ for each test, and enterococcus is known to be more abundant in environmental matrices. This is one of the reasons why it makes a good enteric indicator test.

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