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Enteric Bacteria Monitoring Research

Year 2 Data Summary Report

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Submitted by

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*This report was prepared for the associations of Glen Lake, Lime Lake, and Little Traverse Lake, all Leelanau County, Michigan 501 (c) (3) non-profit organizations.

Summary

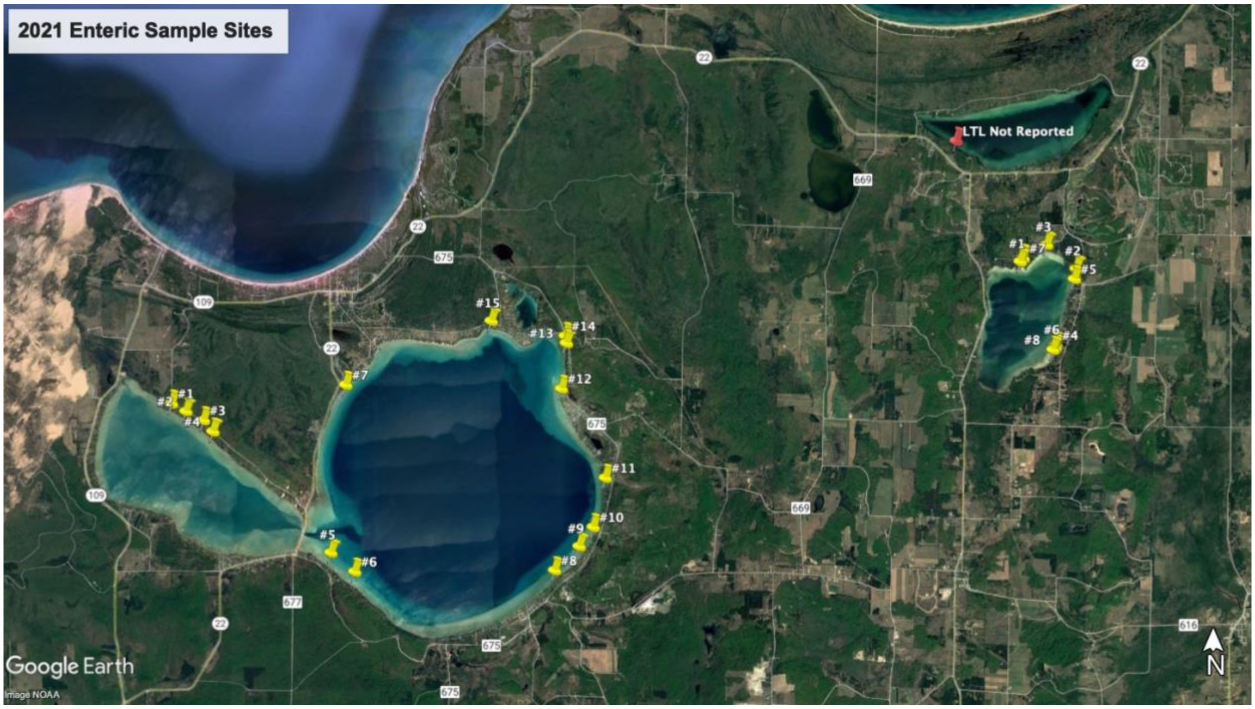
The data contained in this summary report represent the second year of a 3-year research initiative aimed at evaluating the potential for using newer molecular biology (qPCR) and drone surveillance (IR imaging) technology to assess the contribution of riparian septic system effluent to our lake waters. This new initiative comes after two years of research on lake ecosystem enteric bacteria analysis: 2018 showed evidence of human fecal bacteria in about 25% of lake surface water samples around the lakes in Leelanau County, and 2019 showed significant increases in enteric bacteria via inlet streams after a rain event on the same lakes. Our primary goals with this initiative include (a) increasing enteric bacteria baseline data for both surface and ground water around recreational lakes, (b) archiving water samples for both surface and drinking water, (c) assessing changes over time during the high-use summer season, and (d) determining correlation between IR imaging of drain fields using new drone technology and enteric bacteria in surface and ground water.

Well water and lake surface water samples were collected and analyzed from 32 residences around Glen Lake, Lime Lake, and Little Traverse Lake (Leelanau County, MI) in June, July, and August, 2021. Samples were collected using an aseptic protocol, immediately refrigerated and returned to the lab where they were processed in <6 hours. DNA extracts were analyzed for *Enterococcus* (general fecal bacteria) in the FWS lab and then shipped overnight to the University of Alberta where they were analyzed for the *Bacteroides* HF183 marker (unique to humans). Archived samples are currently stored at -80C at the University of Alberta.

Nighttime drone infrared (IR) imaging of corresponding drain fields was conducted at all locations from both 2020 and 2021. Images were captured using multiple color palettes at varying heights above each drain field as trees and wires would allow. Images showing unusual heat signatures will be ground-truthed in the field using septic probes and augers in 2022. In addition, selected fields will be photographed during the daylight hours to assess heat contribution from plants and other possible sources.

2021 Sample Sites

Sample sites were selected by representatives from each lake association and were the result of riparians who responded positively to a call for volunteers. All sites had water frontage, either lake or stream, within the Glen Lake/Crystal River and Good Harbor Bay Watersheds. Sample sites are only identified by number and general location for this report to insure privacy for the volunteers. A more detailed description (name, address, GPS coordinates) of each site is provided to each lake association board representative upon request.



qPCR Sample Analysis

Each sample was analyzed for *Enterococcus* (general bacteria) and *Bacteroides* HF183 (unique to humans). *Enterococcus* values are reported as Genome Equivalents (GE)/100ml). All samples were run for the human *Bacteroides* (HF183). This year, no positive samples for the human marker were detected, and therefore those data have been omitted in the qPCR Sample Analysis tables below. The *Enterococcus* qPCR test uses the exact same qPCR primers and probe as United States EPA method 1611. The protocol is modified, but we assume a generally accepted recreational water guidelines of 1280 (cell calibrator equivalents) CCE/100ml is reflected in the modified GE method used here, which assumes a genome copy number of four for the target gene of the qPCR test. Thus, exceeding a GE limit of 1280 for *Enterococcus* in a recreational water sample, which would normally trigger a follow-up source tracking study, is also used for the purposes of flagging samples that are prioritized for HF183 presence or absence analysis. However, since most values for the samples of this study fall below the 1280 GE/100ml threshold, we assessed *every* sample for the human *Bacteroides* HF183. Inhibition of qPCR was assessed using the 1611 salmon sperm technique as published in United States EPA method 1611. Any samples that displayed inhibition were diluted and reanalyzed, or not used for analysis. Surface water samples over the US EPA Beach Action Value for *Enterococcus* (1280 GE/100ml) were also assessed for Gull fecal contamination using the LeeGull qPCR assay. None of them were positive.

Quantitative PCR is incredibly sensitive and is able to detect as little as one *Enterococcus* bacterium in one mL of water. There are limits, however, to the ability of qPCR to quantify very low concentrations of *Enterococcus*. We are confident in any values that are 100 GE/100mL or greater. Any samples that are below 100 GE/100mL fall below our test limit of detection and are reported as BLD (below limit of detection). These samples yielded an *Enterococcus* detection, but the qPCR values were low enough that we are unable to provide a quantitative result.

It is important to note that well water samples would not normally be assessed against recreational water quality guidelines. United States EPA Method 1611 is not used for assessing drinking water contamination by *Enterococcus*. However, United States groundwater guidelines, such as the EPA Ground Water Rule, identify a variety of approved water quality tests that target *Enterococcus*. In general, detection of *any* fecal contamination in well water used for drinking is cause for concern. More information can be found at: https://www.epa.gov/privatewells/protect-your-homes-water#welltestanchor.

Residence Use Logs

Each participating volunteer was asked to log the number of people sleeping at their residence each night from June 1 until the last sample date in August. These data estimate the amount of septic system use for each residence and are cataloged cumulatively with the assumption septic tanks and drain fields will increase in levels and saturation as the summer progresses, especially for seasonal residences. This data is now being assessed within the context of the qPCR results presented in the report. An important next step of this project is to determine the impact that use (as measured by logs and drone imaging) has, if any, on detection of surface and well fecal pollution.

IR Imaging

The drain field for each participant was visually examined and its GPS location recorded in 2021. Nighttime IR images were obtained for each site for both 2020 and 2021 (Covid-related supply chain issues delayed imagining in 2020). Images will be analyzed over the coming months and irregularities further explored during the 2022 field season.

qPCR Sample Analysis – Glen Lake

Site	Jun Well Ent 1	Jun Well Ent 2	Jun Surf Ent 1	Jun Surf Ent 2	Jul Well Ent 1	Jul Well Ent 2	Jul Surf Ent 1	Jul Surf Ent 2	Aug Well Ent 1	Aug Well Ent 2	Aug Surf Ent 1	Aug Surf Ent 2
GL 1	0	BLD	0	398.67	0	0	0	BLD	860.77	0	0	0
GL 2	0	BLD	0	0	0	BLD	0	BLD	BLD	BLD	603.1	802.02
GL 3	0	287.06	0	0	0	0	0	0	0	0	229.69	BLD
GL 4			0	0	361.2	BLD	102.24	BLD	BLD	0	3,646.94	526.62
GL 5	0	BLD	BLD	0	0	BLD	0	667.61	BLD	0	BLD	850.41
GL 6	BLD	0	0	0	0	0	BLD	0	0	0	433.58	239.37
GL 7	0	0	0	577.97	0	0	BLD	0	BLD	0	0	140.31
GL 8	BLD	502.71	0	0	0	0	0	BLD	0	0	0	0
GL 9	0	BLD	BLD	0	0	BLD	0	BLD	0	0	BLD	788.61
GL 10	0	0	0	0	0	0	0	0	0	0	BLD	0
GL 11	BLD	0	0	0	0	0	0	0	105.67	0	0	BLD
GL 12	0	0	BLD	0	0	0	BLD	0	0	BLD	236.07	BLD
GL 13	112.14	0	BLD	0	0	0	425.37	BLD	0	0	BLD	0
GL 14	0	0	0	0	0	0	0	0	0	0	BLD	BLD
GL 15	BLD	BLD	0	0	0	0	0	0	BLD	0	BLD	0

qPCR Sample Analysis – Lime Lake

Site	Jun Well Ent 1	Jun Well Ent 2	Jun Surf Ent 1	Jun Surf Ent 2	Jul Well Ent 1	Jul Well Ent 2	Jul Surf Ent 1	Jul Surf Ent 2	Aug Well Ent 1	Aug Well Ent 2	Aug Surf Ent 1	Aug Surf Ent 2
LL 1	0	0	BLD	0	BLD	BLD	158.72	BLD	0	BLD	BLD	0
LL 2	0	0	0	0	BLD	BLD	0	0	0	BLD	0	BLD
LL 3	BLD	0	1,117.79	BLD	0	BLD	0	154.13	0	533.18	151.41	407.91
LL 4	0	0	0	BLD	0	0	0	BLD	BLD	0	BLD	BLD
LL 5	0	0	0	0	BLD	BLD	BLD	0	0	0	263.25	BLD
LL 6	BLD	0	0	7,867.83	BLD	168.17	0	BLD	BLD	0	351.05	873.34
LL 7	BLD	BLD	0	438.32	BLD	0	298.87	BLD	BLD	BLD	507.25	610.61
LL 8	0	BLD	0	0	0	0	0	0	0	191.93	0	BLD

qPCR Sample Analysis – Little Traverse Lake

Site	Jun Well Ent 1	Jun Well Ent 2	Jun Surf Ent 1	Jun Surf Ent 2	Jul Well Ent 1	Jul Well Ent 2	Jul Surf Ent 1	Jul Surf Ent 2	Aug Well Ent 1	Aug Well Ent 2	Aug Surf Ent 1	Aug Surf Ent 2
LT 10	0	0	0	0	0	0	0	0	160.19	BLD	BLD	BLD
LT 11	0	0	0	0	0	BLD	BLD	BLD	BLD	0	0	BLD
LT 12	0	0	0	0	0	BLD	0	224.89	792.54	530.64	BLD	BLD
LT 13	0	0	123.74	0	220.47	BLD	0	BLD	0	0	BLD	16,049.11
LT 14	0	BLD	0	0	464.8	BLD	BLD	BLD	0	0	0	BLD
LT 15	0	BLD	992.85	0	BLD	0	0	0	0	0	584.97	0
LT 16	0	0	1,099.13	2,526.15	BLD	BLD	BLD	1,142.85	0	0	BLD	0
LT 17	0	BLD	0	0	BLD	0	BLD	113.39	0	0	0	BLD

Year 2 Observations

1. *Enterococcus* values were generally low again in 2021, with a few exceptions. This is good, as it indicates that most surface water sites fall within acceptable recreational water quality parameters. Some well water samples returned a positive result when assessed for enterococcus. As was the case last year, it may be worthwhile assessing these locations using the Michigan standard well water test, which assesses culturable *E. coli* or for the presence of fecal coliforms. As mentioned, it is generally not acceptable to detect any fecal contamination in drinking water.

2. Year 2 yielded no positive samples for the *Bacteroides* bacteria unique to humans (HF 183) in well or surface water. This is not unusual, as *Bacteroides* does not survive long within the environment, and it has been shown that the HF183 target has a half-life in lake water of ~24 hours. Thus, this test is best at detecting recent human fecal pollution, which is typically rare in surface water (however, that is why our 2018 positive results were surprising). These data will be compared to use logs and septic system activity (drone imaging) in the coming months. As part of the third year of this project, we will also be assessing (using septic dye) the residual time that sewage spends within a septic system as a way to provide additional context for the HF183 part of this study.

3. Many samples returned a result of BLD – below limit of detection. This means that while there was an *Enterococcus* detection, it was below 100GE/100mL. These samples are very likely positive, but we are not confident in providing a quantitative value on the *Enterococcus* levels.

4. Little Traverse Lake Association collected additional samples from inlet streams in 2021, as well as duplicate samples for conventional *E. coli* analysis for all surface water samples. The results from those samples will be reported separately and possibly combined with data from another similar study on Crystal Lake currently being prepared for publication.

5. Some locations and times of the year appear to have increased likelihood of being positive for *Enterococcus*. This will be an issue we investigate further statistically.

Appendix

The following data come from water samples provided by LTLA volunteers and were additional to the water samples collected as prescribed in the 3-year Enteric Bacteria Monitoring Research. FWS was only provided with site numbers and descriptors as reported.

Site	Jun Well Ent 1	Jun Well Ent 2	Jun Well HF 1	Jun Well HF 2	Jun Surf Ent 1	Jun Surf Ent 2	Jun Surf HF 1	Jun Surf HF 2	Jul Well Ent 1	Jul Well Ent 2	Jul Well HF 1	Jul Well HF 2	Jul Surf Ent 1	Jul Surf Ent 2	Jul Surf HF 1	Jul Surf HF 2	Aug Well Ent 1	Aug Well Ent 2	Aug Well HF 1	Aug Well HF 2	Aug Surf Ent 1	Aug Surf Ent 2	Aug Surf HF 1	Aug Surf HF 2
LT 18					0	0	Negative	Negative					1,172.38	0	Negative	Negative					0	0	Negative	Negative
LT 19					0	0	Negative	Negative					BLD	BLD	Negative	Negative					439.84	BLD	Negative	Negative
LT 20					0	0	Negative	Negative					0	0	Negative	Negative					BLD	0	Negative	Negative
LT 21					0	0	Negative	Negative					686.25	1,255.91	Negative	Negative					BLD	0	Negative	Negative
LT 22					0	0	Negative	Negative					BLD	BLD	Negative	Negative					0	BLD	Negative	Negative
LT 3	0	0	Negative	Negative					328.98	BLD	Negative	Negative					0	BLD	Negative	Negative				
LT 7	0	0	Negative	Negative					0	BLD	Negative	Negative					0	0	Negative	Negative				
LT 8	BLD	0	Negative	Negative	0	0	Negative	Negative	BLD	0	Negative	Negative	BLD	0	Negative	Negative	BLD	0	Negative	Negative	BLD	0	Negative	Negative