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Enteric Bacteria Monitoring Research

Year 3 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 third 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 came 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 non-human enteric bacteria via inlet streams after a rain event on the same lakes. Our primary goals with this initiative were (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 groundwater.

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, 2022. 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) and *Bacteroides* HF183 marker (unique to humans) in the University of Alberta laboratory by a FWS scientist. 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 new locations in 2022. Images were captured using multiple color palettes at varying heights above each drain field as trees and wires would allow. All fields were also photographed during the daylight hours to assess heat contribution from plants and other possible sources.

2022 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. No map of Little Traverse collection sites is included. A more detailed description (name, address, GPS coordinates) of each site is provided to each lake association board representative upon request.

Figure 1: Lime Lake collection sites 2022

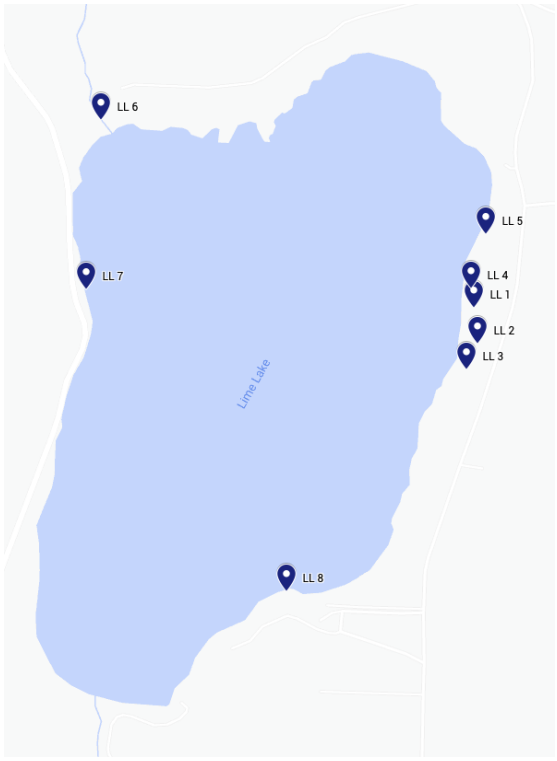
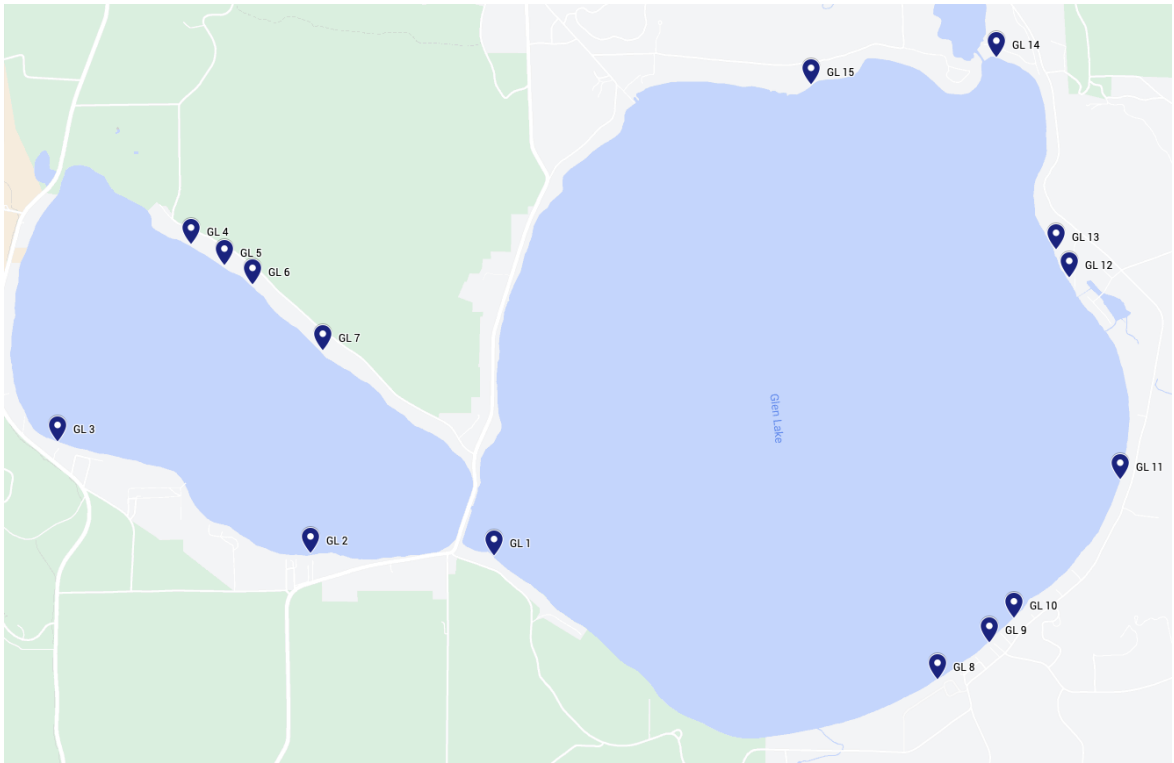


Figure 2: Glen Lake collection sites 2022



qPCR Sample Collection & Analysis

Duplicate samples were collected at all well and surface water sites by lake volunteers who had been trained by FWS scientists. Samples on Lime Lake occurred on 21 June, 18 July and 2 August. Little Traverse samples were taken on 22 June, 14 July and 3 August. Because of the number of samples collected on Glen Lake, collection sites were split up into two separate days each month and were collected on 21 & 22 June, 14 & 18 July and 2 & 3 August.

Samples were transported to the FWS lab on ice and filtered within 6 hours of collection. Filters were then frozen until DNA extraction could occur. Once DNA was extracted, each sample was analyzed for *Enterococcus* (general bacteria) and *Bacteroides* HF183 (unique to humans). All samples were run at the core laboratory at University of Alberta by Kelsey Froelich.

Enterococcus values are reported as Genome Equivalents (GE/100ml). 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.

A value reported as DNQ for enterococcus represents 'detected, not quantified.' This means that one of the two duplicates in the qPCR run came back positive, while the other was negative, which is sometimes the case for samples that return low positivity. All DNQ results were rerun to validate results. HF183 results are reported as negative, possible positive, or positive. A negative result is just as it sounds - no DNA was amplified during qPCR. Possible positive results mean that one of the two duplicate runs in qPCR came back positive. This generally would signify a very low level of target DNA in the sample. Positive samples represent a test that came back positive for both duplicates.

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 2022. Daytime RGB and nighttime IR images were obtained for each site in 2022. Images will be analyzed over the coming months.

Results

Below you will find the raw data from 2022 for the three study lakes. Numbers with red text exceed the 1,280 GE/ 100 mL threshold. Boxes that are filled in orange have a possible positive result for HF183 and those with red have a positive result for HF183. There were two samples taken at each site (surface and well) for each date. These are shown as 1 and 2 in the data tables.

Figure 3: 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	0	DNQ	0	574.05	50.6	0	DNQ	DNQ	277.94	0	86.03
GL 2	111.42	DNQ	0	0	DNQ	63.01	1125.18	0	0	402.74	777.85	441.35
GL 3	0	0	205.49	0	0	0	0	573.5	DNQ	DNQ	336.88	DNQ
GL 4	0	0	0	0	0	0	415.93	728.14	248.38	285.24	707.75	DNQ
GL 5	0	709.69	178.52	994.51	0	0	DNQ	863.82	289.63	DNQ	315.18	DNQ
GL 6	DNQ	349.55	DNQ	0	0	0	DNQ	157.71	0	0	150.5	0
GL 7	75.04	0	0	DNQ	DNQ	DNQ	DNQ	DNQ	0	0	300.1	DNQ
GL 8	DNQ	275.48	183.76	DNQ	0	DNQ	0	0	1777.98	0	110.65	DNQ
GL 9	267.55	863.56	208.64	203.32	DNQ	DNQ	DNQ	402.4	0	0	413.96	522.31
GL 10	0	0	963.97	999.92	0	0	211.85	142.55	DNQ	0	428.99	415.25
GL 11			66.8	243.5	0	0	0	DNQ	0	DNQ	504.43	170.52
GL 12	DNQ	DNQ	668.39	268.02	0	235.14	530.27	1219.21	DNQ	328.98	2235.62	2666.18
GL 13	DNQ	0	2203.04	DNQ	0	DNQ	762.58	889.64	0	DNQ	2620.66	2084.93
GL 14	155.47	116.81	58.52	134.76	0	349.49	DNQ	DNQ	0	DNQ	0	673.49
GL 15	0	DNQ	137.95	393.7	0	0	741.58	DNQ	0	DNQ	161.48	DNQ

Figure 4: 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	DNQ	DNQ	DNQ	148.41	225.17	0	332.6	0	0	0	232.19	DNQ
LL 2	0	DNQ	128.27	DNQ	0	0	222.51	0	0	0	647.3	327.69
LL 3	85.07	153.19	DNQ	112.36	0	0	DNQ	507.21	0	0	0	437.09
LL 4	DNQ	76.92	102.13	99.9	DNQ	DNQ	DNQ	231.18	0	DNQ	126.5	646.11
LL 5	DNQ	0	DNQ	DNQ	0	0	DNQ	0	0	190.04	DNQ	
LL 6	0	DNQ	296.11	0	0	DNQ	536.98	1006.56	0	DNQ	797.76	601.94
LL 7	0	0	191.17	DNQ	747.65	0	0	DNQ	0	0	DNQ	263.34
LL 8	0	DNQ	DNQ	0	0	0	DNQ	0	0	DNQ	DNQ	573.28

Figure 5: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 1	237.64	0	DNQ	272.19	0	0	414.03	DNQ	0	DNQ	1673.55	970.77
LT 9					0	DNQ	270.46	DNQ				
LT 23	0	0	1705.11	0	0	0	0	DNQ	0	DNQ	521.42	293.89
LT 24	0	0	DNQ	DNQ	DNQ	184.78	DNQ	DNQ	0	0	199.57	466.8
LT 25	0	0	DNQ	1263.1	DNQ	0	775.21	0	DNQ	DNQ	434.26	0
LT 26	60.42	0	0	DNQ	0	0	0	0	0	DNQ	376.94	0
LT 27	DNQ	0	DNQ	0	181.57	DNQ	355.75	DNQ	DNQ	DNQ	0	0
LT 28	0	0	DNQ	DNQ	133.2	0	DNQ	DNQ	DNQ	0	584.93	950.1
LT 29	0	0	DNQ	0	DNQ	615.59	0	0	0	DNQ	DNQ	DNQ

Year 3 Observations

1. For the third year in a row, *Enterococcus* values were generally low. 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 previously, 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 3 resulted in nine possible positives and one positive result for HF183. Although a 'possible positive' result indicates low levels of HF183, we report it as such because the bacteria we are testing for (*Bacteroides*) does not persist long in the environment, so any positive is generally an indication of some sort of human fecal pollution. Three of the HF183 possible positive results were found in well water and we encourage these riparians to have their water tested for culturable *E. coli*.

3. Many samples returned a result of DNQ- detectable, not quantifiable for *Enterococcus*. This means that while there was an *Enterococcus* detection, it only showed in one of the duplicate qPCR runs. These samples are very likely positive, but we are not confident in providing a quantitative value on the *Enterococcus* levels.

4. In the winter of 2022-23, we will be formally analyzing and preparing this data for publication. We hope to compare many factors such as use logs, heat signatures from drone images, age of septic, depth of well, etc. to the *Enterococcus* and HF183 values.