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Enteric Bacteria Monitoring Research

Year 1 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 first 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 bacteria analysis: 2018 showed evidence of human 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, 2020. Samples were collected using an aseptic protocol, immediately refrigerated and returned to the lab where they were processed in <6 hours. DNA extracts were shipped overnight to the University of Alberta where they were analyzed for *Enterococcus* (general fecal bacteria) and the *Bacteroides* HF183 marker (unique to humans). Archived samples are currently stored at -80C at the University of Alberta.

Unfortunately, nighttime drone infrared (IR) imaging of corresponding drain fields was delayed until 2021 due to the unavailability of obtaining an IR camera. This was due to the Covid-19 pandemic putting unusual pressure on manufacturers to produce IR equipment used to measure body temperature. The 2020 drain fields will be analyzed along with the 2021 sites next summer. The camera has now been delivered and encouraging progress is being made by testing compromised septic systems in Benzie and Leelanau County.

2020 Sample Sites

Most 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.

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 and source-tracking of *Bacteroides* HF183 reported as "positive" or "negative". 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 recreational water guidelines of 1280 (cell calibrator equivalents) CCE/100ml are generally 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 analyzed for HF183 presence or absence. 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.

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 detect very low concentrations of *Enterococcus*. We are confident in any values that are 100GE/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 were low enough to question whether the sample was truly positive.

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 approved for use in 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 reported cumulatively with the assumption septic tanks and drain fields will increase in levels and saturation as the summer progresses, especially for seasonal residences. Note: data for sites not listed were not reported to us for analysis.

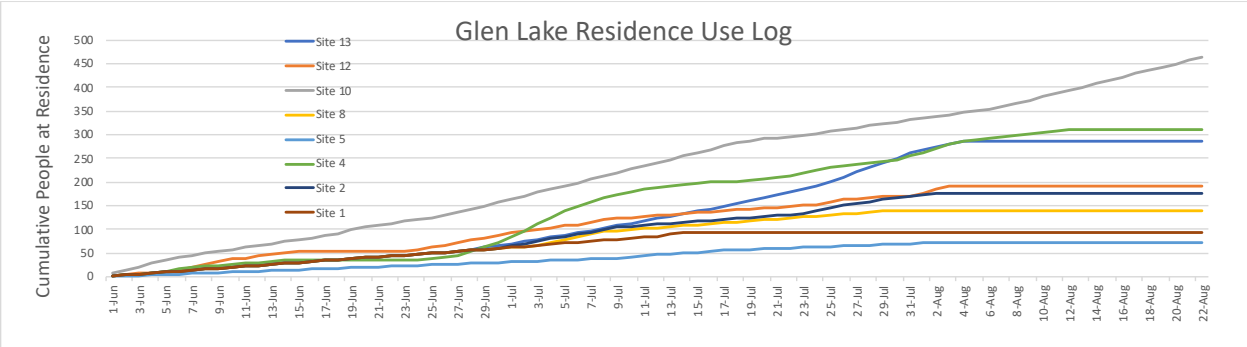
IR Imaging

The drain field for each participant was visually examined and its GPS location recorded in 2020. A nighttime IR image will be obtained in 2021 with the assumption the residence will have similar use to 2020. Results will be reported as an addendum to this report.

qPCR Sample Analysis – Glen Lake

Site	Jun Well Ent	Jun Well HF	Jun Surf Ent	Jun Surf HF	Jul Well Ent	Jul Well HF	Jul Surf Ent	Jul Surf HF	Aug Well Ent	Aug Well HF	Aug Surf Ent	Aug Surf HF
GL1	BLD	Negative	BLD	Negative	0.00	Negative	BLD	Negative	0.00	Negative	302.07	Negative
GL2	BLD	Negative	BLD	Negative	BLD	Negative	110.84	Negative	125.99	Negative	186.79	Negative
GL3	0.00	Negative	BLD	Negative	0.00	Negative	0.00	Negative	BLD	Negative	104.70	Negative
GL4	BLD	Negative	BLD	Negative	BLD	Positive	BLD	Negative	0.00	Negative	BLD	Negative
GL5	BLD	Negative	BLD	Negative	0.00	Negative	BLD	Positive	0.00	Negative	BLD	Negative
GL6	BLD	Negative	102.56	Negative	BLD	Negative	0.00	Negative	BLD	Positive	0.00	Negative
GL7	0.00	Negative	BLD	Negative	BLD	Negative	BLD	Negative	BLD	Negative	0.00	Negative
GL8	BLD	Negative	BLD	Negative	BLD	Negative	BLD	Negative	0.00	Negative	BLD	Negative
GL9	0.00	Negative	BLD	Negative	BLD	Negative	BLD	Negative	BLD	Negative	BLD	Negative
GL10	BLD	Positive	BLD	Negative	0.00	Negative	0.00	Negative	BLD	Negative	0.00	Negative
GL11	BLD	Negative	105.15	Negative	0.00	Negative	BLD	Negative	297.44	Negative	468.89	Negative
GL12	BLD	Negative	BLD	Negative	0.00	Negative	BLD	Negative	109.17	Negative	130.33	Negative
GL13	BLD	Negative	BLD	Positive	0.00	Negative	BLD	Negative	0.00	Negative	406.11	Negative
GL14	0.00	Negative	146.69	Negative	BLD	Negative	0.00	Positive	379.31	Negative	151.63	Negative
GL15	0.00	Negative	BLD	Negative	0.00	Negative	BLD	Negative	BLD	Negative	408.60	Negative

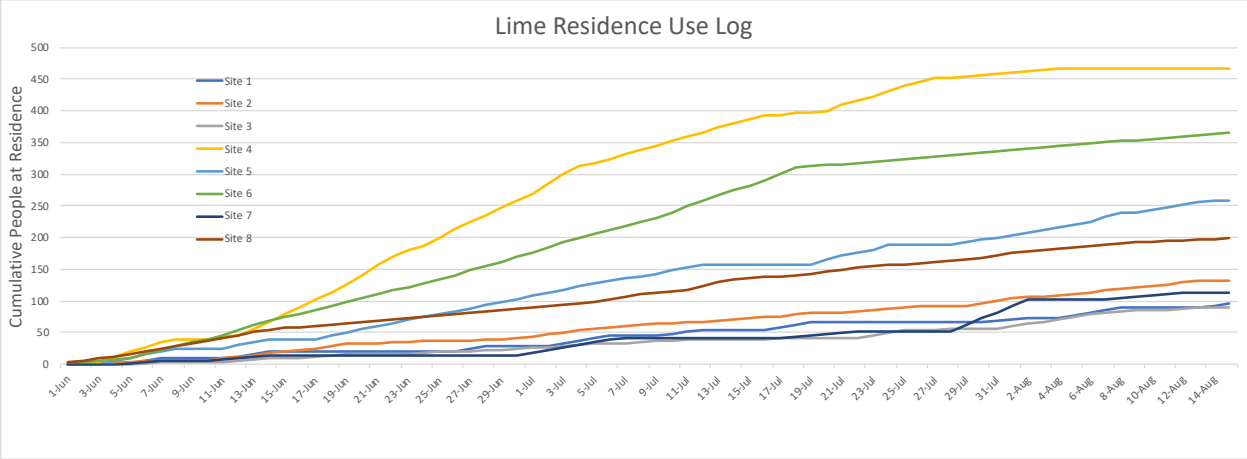
Residence Use Log – Glen Lake



qPCR Sample Analysis – Lime Lake

Site	Jun Well Ent	Jun Well HF	Jun Surf Ent	Jun Surf HF	Jul Well Ent	Jul Well HF	Jul Surf Ent	Jul Surf HF	Aug Well Ent	Aug Well HF	Aug Surf Ent	Aug Surf HF
LL1	0.00	Negative	161.83	Negative	BLD	Negative	0.00	Negative	BLD	Negative	BLD	Negative
LL2	BLD	Negative	211.16	Negative	BLD	Negative	BLD	Negative	235.86	Negative	1460.00	Negative
LL3	BLD	Negative	272.44	Negative	0.00	Negative	0.00	Negative	BLD	Negative	325.89	Negative
LL4	BLD	Negative	0.00	Negative	BLD	Negative	BLD	Negative	266.32	Negative	107.51	Negative
LL5	127.95	Positive	116.33	Negative	BLD	Negative	BLD	Negative	BLD	Negative	0.00	Negative
LL6	BLD	Negative	189.89	Negative	325.04	Negative	0.00	Negative	BLD	Negative	BLD	Positive
LL7	0.00	Negative	135.65	Negative	0.00	Negative	BLD	Negative	BLD	Negative	BLD	Negative
LL8	195.90	Negative	BLD	Negative	BLD	Negative	BLD	Negative	BLD	Negative	191.86	Negative

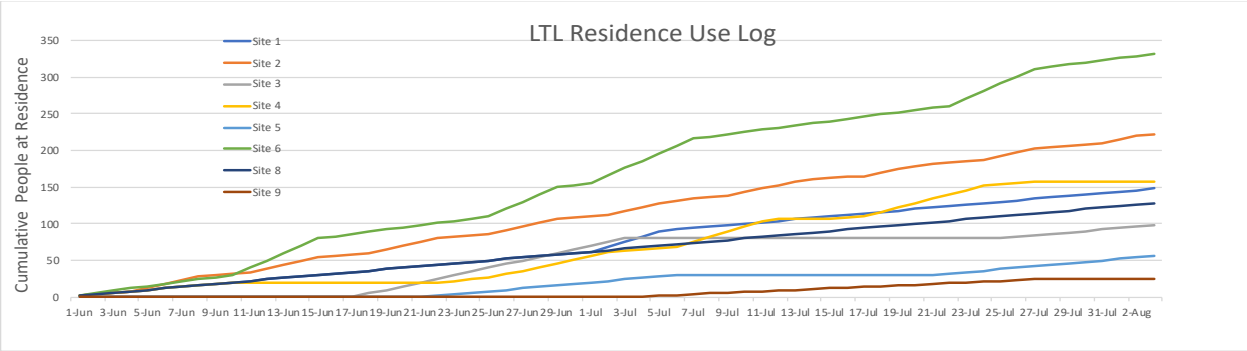
Residence Use Log – Lime Lake



qPCR Sample Analysis – Little Traverse Lake

Site	Jun Well Ent	Jun Well HF	Jun Surf Ent	Jun Surf HF	Jul Well Ent	Jul Well HF	Jul Surf Ent	Jul Surf HF	Aug Well Ent	Aug Well HF	Aug Surf Ent	Aug Surf HF
LTL1	BLD	Negative	BLD	Negative	127.99	Positive	0.00	Negative	121.20	Negative	0.00	Negative
LTL2	BLD	Negative	BLD	Negative	BLD	Negative	BLD	Negative	0.00	Negative	BLD	Negative
LTL3	0.00	Negative	BLD	Negative	BLD	Negative	0.00	Negative	488.41	Negative	BLD	Negative
LTL4	BLD	Negative	0.00	Negative	BLD	Negative	BLD	Negative	BLD	Negative	BLD	Negative
LTL5	BLD	Negative	BLD	Negative	BLD	Negative	0.00	Negative	BLD	Negative	BLD	Negative
LTL6	331.95	Negative	BLD	Negative	BLD	Negative	0.00	Negative	BLD	Negative	0.00	Negative
LTL7	270.08	Negative	0.00	Negative	BLD	Negative	BLD	Negative	BLD	Negative	BLD	Negative
LTL8	BLD	Negative	BLD	Negative	0.00	Negative	BLD	Negative	BLD	Positive	BLD	Negative
LTL9	NO SAMPLE	NO SAMPLE	NO SAMPLE	NO SAMPLE	BLD	Negative	BLD	Negative	BLD	Negative	165.15	Negative

Residence Use Log – Little Traverse Lake



Year 1 Observations

1. *Enterococcus* values were generally low, with the exception of Lime Lake Site 2 August surface water sample. This is good, as it indicates that general fecal contamination of the water at sampled sites is low. We would anticipate that surface water samples would present higher *Enterococcus* values than would well water samples because the fecal contributors to surface waters are more numerous. We also know that run off during and immediately after rainfall events can significantly elevate *Enterococcus* values.
2. There were infrequent HF183-positive samples from well and surface water sites, as is expected. All sites positive for HF183 warrant retesting at least once in 2021, particularly those samples that were collected from wells, as this may be indicative of septic contamination. From only a single year of sampling, we are not able to pinpoint the source or severity of fecal contamination.
3. Based on our initial Year 1 data, there does not seem to be a correlation between usage at a property and those sites that test higher for fecal contamination. However, additional sampling may resolve questions related to use and fecal contamination. This may be impacted by age of a septic system, age of surrounding septic systems, or hydrology of an area.
4. 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 low enough that we are not confident enough in the result to indicate the sample was positive. However, we report the result as BLD because some of these samples are positive for HF183, which suggests that the low level of fecal contamination may be of human origin.