Enteric Bacteria Assessment Research

Glen Lake Association, Lime Lake Association, Little Traverse Lake Association, Lake Charlevoix Association

20 November 2018

Submitted by

Ron Reimink, M.A.
Freshwater Solutions, LLC (FWS)
&
Patrick Hanington, Ph.D.
University of Alberta (UA)

Table of Contents

Introduction

Older or poorly maintained septic systems near recreational waters may introduce excess nutrients and microbial contaminants capable of causing human disease into aquatic ecosystems. Recent studies have shown that runoff passing through septic fields, even ones that are properly functioning, will lead to spikes in enteric bacteria being monitored as well. Historically, *Cladophora* sp., a macrophytic alga that thrives in conditions of high nutrients and hard substrate, was used as an indicator of nutrient loading. In fact, many lake associations conduct annual shoreline surveys, either by boat or more recently using drone technologies, to identify *Cladophora* sp. and subsequent nutrient loading.

Recently, many new PCR-based methods for microbial source tracking (MST) have been developed in an effort to characterize the sources of fecal pollution and nutrient loading in recreational waters. Numerous quantitative (q)PCR-based tests have been validated for the detection of fecal bacteria that is specific to single source animals; human, cow, goose are some examples. This assessment research tested for human waste contamination in water samples from Glen Lake, Lime Lake, Little Traverse Lake, and Lake Charlevoix in NW Michigan by amplifying the human-specific HF183 16S rRNA marker that is specific to *Bacteriodes* bacteria found in human waste. Many state and federal monitoring agencies in the US have adopted this test for their routine testing of recreational beaches.

Benefits to lake associations of this newer molecular metric include:
1. Establishment of whole-lake baseline data for reference in coming years.
2. Identification of potential failing septic systems or issues with runoff.
3. Archived samples for research purposes in years to come.
4. A data set that may be used to drive legislation of septic system inspections.
5. Advancement of mobile qPCR technology which will allow in-house monitoring in the very near future.

FWS offered to collaborate with two high school students participating as GIS Mapping Interns through the GRACE Project run by Eastern Michigan University. Cecelia Denton (The Leelanau School) and Adam Tuthill (Homeschool) helped with data collection and completed their GIS mapping project using the results we obtained from the lakes in Leelanau County.

Methods

Two 50ml water samples were collected on Leelanau County lakes (Glen, Lime, Little Traverse) every ~150m and at 20 locations on Lake Charlevoix. Samples were collected within 20 cm of the surface exactly 15m from shore as measured using a high-precision rangefinder (+/-0.1m). Water samples were stored in a cooler and transported to the lab for analysis. Collections occurred on 9 July (Lime), 16 July (Little Glen), 16-17 July (Big Glen), 17 July (Little Traverse), and 18 July (Lake Charlevoix).

Additional physical characteristics were recorded at each site. Those included GPS coordinates, time, water temperature, water depth, wind speed, wind direction for all lakes and Hydrolab data (dissolved oxygen, pH, conductivity, turbidity, percent O_2 saturation) on Glen and Lime Lakes.

All samples were individually suction-filtered through 0.4um filter discs and the DNA from all organisms extracted from each sample using a standard bead-beating method. Three aliquots of each DNA extract were then used to run triplicate qPCR analyses on a Chai portable qPCR machine and the average from all three tests reported. Additional duplicate qPCR analyses were also run on all samples using a core qPCR machine at the UA to validate results.

Results

Of the 231 samples collected and analyzed using the portable Chai Open qPCR machine, only one (Site 36 Little Glen) amplified the HF183 marker indicating the presence of human enteric bacteria. Five samples were collected again from Site 36, one at the site and two on either side approximately 45m (150ft) and 75m (250ft) down the shoreline in either direction. All five subsequent samples were negative.

Select samples at river inflows were analyzed for an ungulate marker (hooved mammals) at the following locations: Lime Lake Sites #1, 24, 27; Glen Lake Site #65; Little Traverse Lake Site #19. All samples were negative. Gull enteric bacteria markers were assessed at the following locations: Lime Lake Sites #5, 23; Glen Lake Sites #9, 34, 65, 123; Little Traverse Lake Sites #3, 27. All samples were negative. Additionally, four water wells were sampled and evaluated around Little Traverse Lake for the human HF183 marker. All were negative.

All extracted DNA samples from 2018 were sent to the UA for validation. Due to increased sensitivity of the core machine, validation results revealed low levels of the *Bacteriodes* HF183 marker in a small number of samples from each lake. These values were below the limit of detection for the Chai Open qPCR machine, which explains why they were not detected in the initial assessment. The validated results are reported below. Enteric bacteria levels were statistically compared to each metadata category individually to evaluate correlation. None was found.

Resampling at Site 36 on Little Glen Lake a few days later showed bacteria levels at 19.7. None of the other four collections (two on either side, 45m and 75m) were positive. Of the four water wells sampled from Little Traverse Lake, only one (Well #2) showed positive enteric bacteria, also at 19.7. None of the ungulate or gull samples were positive upon validation with the core machine.

Validated results showing positive human *Bacteriodes* HF183 are highlighted in YELLOW (1-99) and RED (>100).

Table 1 – Lime Lake Data

Site	Date	Time	Latitude	Longitude	Bacteria Level
1	7/9/18	8:27 AM	44.89457	-85.83191	0.0
2	7/9/18	8:47 AM	44.89609	-85.83160	0.0
3	7/9/18	8:53 AM	44.89755	-85.83113	0.0
4	7/9/18	9:02 AM	44.89861	-85.83091	0.0
5	7/9/18	9:12 AM	44.89968	-85.83017	0.0
6	7/9/18	9:25 AM	44.90080	-85.83088	0.0
7	7/9/18	9:38 AM	44.90357	-85.83335	0.0
8	7/9/18	9:50 AM	44.90383	-85.83480	0.0
9	7/9/18	9:59 AM	44.90373	-85.83658	0.0
10	7/9/18	10:09 AM	44.90301	-85.83807	22.7
11	7/9/18	10:24 AM	44.90218	-85.83941	0.0
12	7/9/18	10:35 AM	44.90143	-85.84131	43.9
13	7/9/18	10:46 AM	44.90133	-85.84342	0.0
14	7/9/18	11:00 AM	44.90134	-85.84517	43.0
15	7/9/18	11:11 AM	44.90173	-85.84704	0.0
16	7/9/18	11:21 AM	44.90064	-85.84891	0.0
17	7/9/18	11:34 AM	44.89556	-85.84870	0.0
18	7/9/18	11:49 AM	44.89567	-85.84849	0.0
19	7/9/18	12:00 PM	44.89042	-85.85015	0.0
20	7/9/18	12:11 PM	44.88911	-85.85057	0.0
21	7/9/18	12:31 PM	44.88799	-85.85094	0.0
22	7/9/18	12:26 PM	44.88640	-85.85081	0.0
23	7/9/18	12:39 PM	44.88591	-85.85118	0.0
24	7/9/18	12:51 PM	44.88585	-85.85109	0.0
25	7/9/18	1:00 PM	44.88588	-85.85106	0.0
26	7/9/18	1:16 PM	44.88595	-85.85114	25.0
27	7/9/18	1:31 PM	44.88609	-85.83707	17.3
28	7/9/18	1:39 PM	44.88610	-85.83706	21.0
29	7/9/18	1:48 PM	44.88648	-85.83564	0.0
30	7/9/18	1:55 PM	44.88679	-85.83522	4.5
31	7/9/18	2:03 PM	44.88939	-85.83361	0.0
32	7/9/18	2:11 PM	44.89060	-85.83305	68.3
33	7/9/18	2:22 PM	44.89182	-85.83098	0.0
34	7/9/18	2:32 PM	44.89364	-85.83117	0.0
35	7/9/18	2:47 PM	44.89499	-85.84389	0.0

Diagram 1 – Lime Lake Bacteria Heat Map

Lime Lake Enteric Bacteria
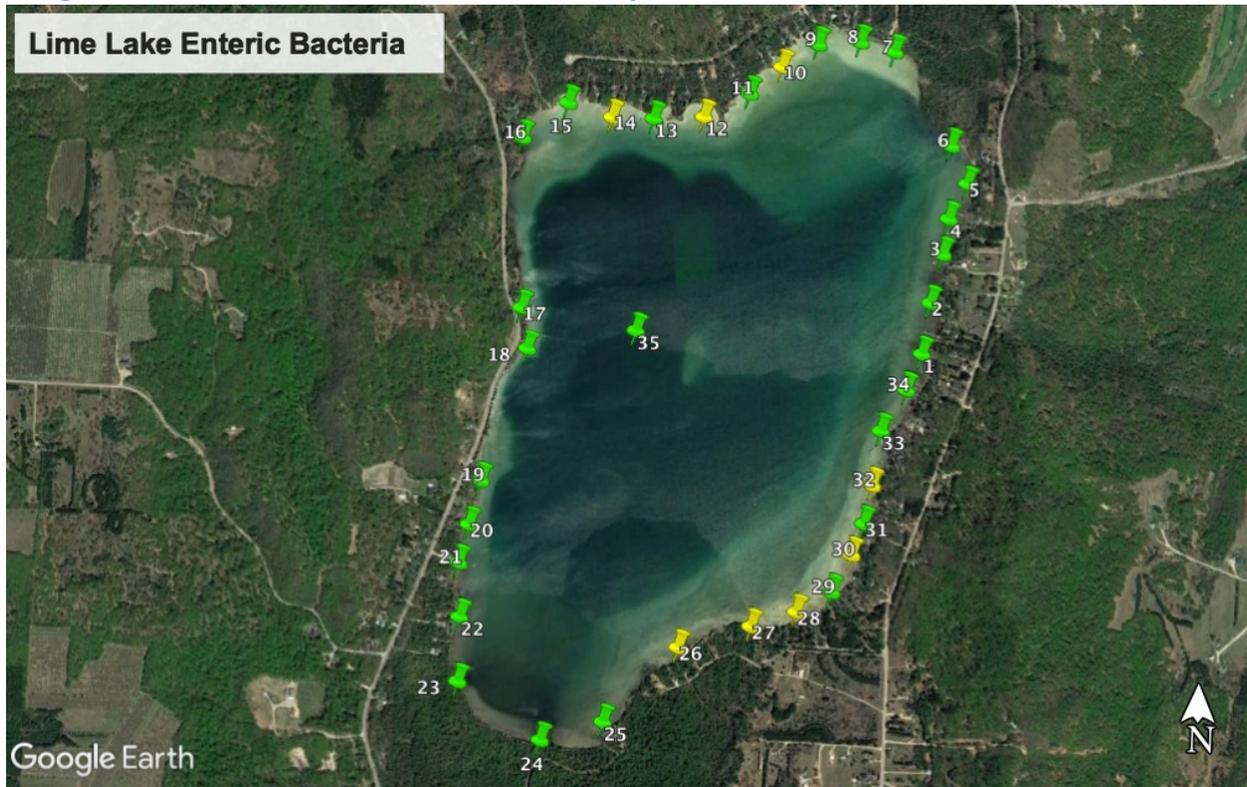
Table 2 – Little Glen Lake Data

Site	Date	Time	Latitude	Longitude	Bacteria Level
1	7/16/18	8:43 AM	44.86675	-86.00205	0.0
2	7/16/18	8:49 AM	44.86761	-86.00377	475.7
3	7/16/18	8:55 AM	44.86873	-86.00511	0.0
4	7/16/18	8:59 AM	44.86956	-86.00671	0.0
5	7/16/18	9:03 AM	44.87048	-86.00866	17.1
6	7/16/18	9:07 AM	44.87112	-86.01034	0.0
7	7/16/18	9:12 AM	44.87222	-86.01175	0.0
8	7/16/18	9:15 AM	44.87344	-86.01369	0.0
9	7/16/18	9:21 AM	44.87426	-86.01544	0.0
10	7/16/18	9:25 AM	44.87529	-86.01680	0.0
11	7/16/18	9:30 AM	44.87616	-86.01820	0.0
12	7/16/18	9:34 AM	44.87682	-86.01956	17.4
13	7/16/18	9:39 AM	44.87744	-86.02117	14.7
14	7/16/18	9:45 AM	44.87757	-86.02177	50.6
15	7/16/18	9:51 AM	44.87899	-86.02482	0.0
16	7/16/18	9:55 AM	44.87944	-86.02613	0.0
17	7/16/18	10:01 AM	44.88050	-86.02745	76.3
18	7/16/18	10:21 AM	44.88219	-86.03564	53.9
19	7/16/18	10:26 AM	44.88120	-86.03723	6.1
20	7/16/18	10:30 AM	44.88044	-86.03754	46.1
21	7/16/18	10:36 AM	44.87913	-86.03836	46.6
22	7/16/18	10:42 AM	44.87774	-86.03929	242.3
23	7/16/18	10:49 AM	44.87637	-86.03984	0.0
24	7/16/18	10:55 AM	44.87486	-86.04002	75.9
25	7/16/18	11:01 AM	44.87320	-86.04021	91.9
26	7/16/18	11:21 AM	44.87132	-86.04027	0.0
27	7/16/18	11:30 AM	44.86931	-86.04018	46.1
28	7/16/18	11:35 AM	44.86823	-86.03961	47.1
29	7/16/18	11:39 AM	44.86706	-86.03891	0.0
30	7/16/18	11:43 AM	44.86581	-86.03681	22.9
31	7/16/18	11:49 AM	44.86542	-86.03425	0.0
32	7/16/18	11:51 AM	44.86491	-86.03280	0.0
33	7/16/18	11:55 AM	44.86464	-86.03081	0.0
34	7/16/18	11:58 AM	44.86477	-86.02915	0.0
35	7/16/18	12:02 PM	44.86407	-86.02682	45.7
36	7/16/18	12:05 PM	44.86384	-86.02657	17.8
37	7/16/18	12:09 PM	44.86361	-86.02491	0.0
38	7/16/18	12:15 PM	44.86048	-86.02136	2.5
39	7/16/18	12:20 PM	44.86110	-86.02252	0.0
40	7/16/18	12:24 PM	44.85863	-86.01706	0.0

41	7/16/18	12:30 PM	44.85833	-86.01655	0.0
42	7/16/18	12:33 PM	44.85794	-86.01445	0.0
43	7/16/18	12:39 PM	44.85780	-86.01109	0.0
44	7/16/18	12:41 PM	44.85797	-86.00810	0.0
45	7/16/18	12:45 PM	44.85763	-86.00453	0.0
46	7/16/18	12:49 PM	44.85775	-86.00301	23.9
47	7/16/18	12:52 PM	44.85800	-86.00237	0.0
48	7/16/18	1:00 PM	44.85775	-86.00246	0.0
49	7/16/18	1:04 PM	44.85785	-86.00047	7.2
50	7/16/18	1:09 PM	44.85952	-85.99724	0.0
51	7/16/18	1:13 PM	44.86310	-85.99680	0.0
52	7/16/18	1:17 PM	44.86323	-85.99628	0.0
53	7/16/18	1:22 PM	44.86486	-85.99818	0.0

## Diagram 2 – Little Glen Lake Bacteria Heat Map
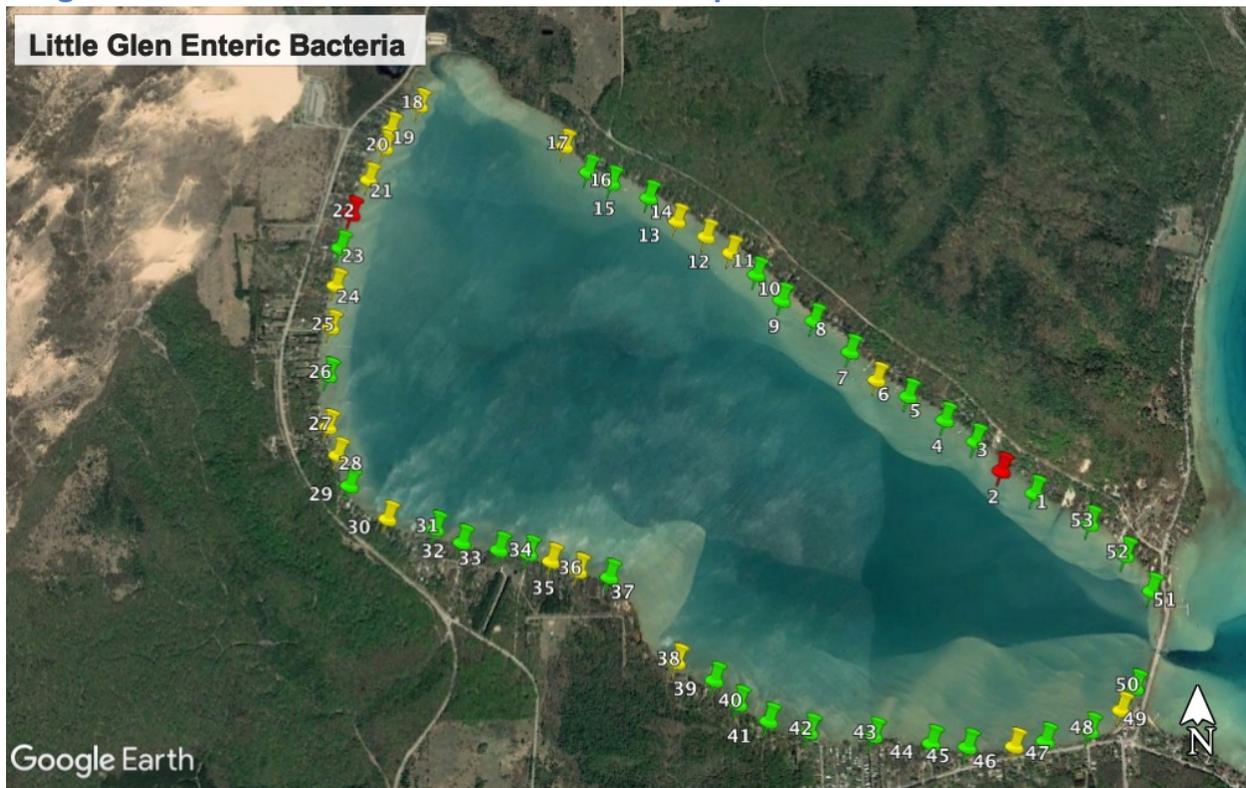
Table 3 – Big Glen Lake Data

*Note: The site numbers jump from 87 to 101 showing a different date of collection. No sites are missing.

Site	Date	Time	Latitude	Longitude	Bacteria Level
54	7/16/18	1:47 PM	44.85838	-85.99560	0.0
55	7/16/18	2:05 PM	44.85711	-85.99304	0.0
56	7/16/18	2:11 PM	44.85634	-85.99108	0.0
57	7/16/18	2:16 PM	44.85559	-85.98958	34.5
58	7/16/18	2:22 PM	44.85415	-85.98712	0.0
59	7/16/18	2:27 PM	44.85305	-85.98574	0.0
60	7/16/18	2:32 PM	44.85216	-85.98383	0.0
61	7/16/18	2:36 PM	44.84844	-85.97788	0.0
62	7/16/18	2:42 PM	44.84491	-85.96824	0.0
63	7/16/18	2:47 PM	44.84516	-85.96622	0.0
64	7/16/18	2:51 PM	44.84528	-85.96382	6.3
65	7/16/18	2:54 PM	44.84556	-85.96191	32.6
66	7/16/18	2:58 PM	44.84623	-85.95926	0.0
67	7/16/18	3:03 PM	44.84666	-85.95710	24.6
68	7/16/18	3:06 PM	44.84737	-85.95466	0.0
69	7/16/18	3:10 PM	44.84796	-85.95296	0.0
70	7/16/18	3:14 PM	44.84865	-85.95122	0.0
71	7/16/18	3:18 PM	44.84948	-85.94939	0.0
72	7/16/18	3:22 PM	44.85042	-85.94792	0.0
73	7/16/18	3:26 PM	44.85128	-85.94694	0.0
74	7/16/18	3:30 PM	44.85232	-85.94419	0.0
75	7/16/18	3:35 PM	44.85339	-85.94261	0.0
76	7/16/18	3:39 PM	44.85435	-85.94158	0.0
77	7/16/18	3:44 PM	44.85528	-85.93972	0.0
78	7/16/18	3:52 PM	44.85649	-85.93785	0.0
79	7/16/18	3:57 PM	44.85744	-85.93671	0.0
80	7/16/18	4:01 PM	44.85816	-85.93546	0.0
81	7/16/18	4:06 PM	44.85935	-85.93446	0.0
82	7/16/18	4:09 PM	44.86049	-85.93361	0.0
83	7/16/18	4:12 PM	44.86158	-85.93305	0.0
84	7/16/18	4:16 PM	44.86330	-85.93287	0.0
85	7/16/18	4:19 PM	44.86501	-85.93165	17.7
86	7/16/18	4:22 PM	44.86665	-85.93164	0.0
87	7/16/18	4:24 PM	44.86786	-85.93166	0.0
101	7/17/18	2:13 PM	44.86305	-85.99499	17.9
102	7/17/18	2:17 PM	44.86450	-85.99472	0.0
103	7/17/18	2:22 PM	44.86602	-85.99234	17.9
104	7/17/18	2:30 PM	44.86754	-85.99298	17.9

105	7/17/18	2:34 PM	44.86916	-85.99315	17.7
106	7/17/18	2:38 PM	44.87089	-85.99308	0.0
107	7/17/18	2:42 PM	44.87276	-85.99273	138.0
108	7/17/18	2:46 PM	44.87432	-85.99242	29.5
109	7/17/18	2:49 PM	44.87621	-85.99151	0.0
110	7/17/18	2:52 PM	44.87759	-85.99072	0.0
111	7/17/18	2:55 PM	44.87907	-85.99008	37.1
112	7/17/18	3:00 PM	44.88051	-85.98935	0.0
113	7/17/18	3:04 PM	44.88185	-85.98788	91.4
114	7/17/18	3:08 PM	44.88342	-85.98681	0.0
115	7/17/18	3:12 PM	44.88470	-85.98473	0.0
116	7/17/18	3:16 PM	44.88570	-85.98312	0.0
117	7/17/18	3:21 PM	44.88695	-85.98103	0.0
118	7/17/18	3:25 PM	44.88801	-85.98062	28.6
119	7/17/18	3:30 PM	44.88962	-85.97918	0.0
120	7/17/18	3:34 PM	44.89005	-85.97705	0.0
121	7/17/18	3:38 PM	44.89015	-85.97493	0.0
122	7/17/18	3:52 PM	44.89003	-85.97287	33.7
123	7/17/18	3:56 PM	44.88987	-85.97060	0.0
124	7/17/18	4:00 PM	44.88967	-85.96833	0.0
125	7/17/18	4:05 PM	44.88948	-85.96606	16.6
126	7/17/18	4:11 PM	44.88931	-85.96429	0.0
127	7/17/18	4:19 PM	44.89036	-85.96187	0.0
128	7/17/18	4:24 PM	44.89049	-85.95977	0.0
129	7/17/18	4:29 PM	44.89110	-85.95783	0.0
130	7/17/18	4:33 PM	44.89190	-85.95623	0.0
131	7/17/18	4:36 PM	44.89163	-85.95394	18.7
132	7/17/18	4:39 PM	44.89094	-85.95162	0.0
133	7/17/18	4:42 PM	44.88947	-85.94934	0.0
134	7/17/18	4:46 PM	44.88973	-85.94665	0.0
135	7/17/18	4:49 PM	44.89153	-85.94579	15.2
136	7/17/18	4:52 PM	44.89165	-85.94342	0.0
137	7/17/18	4:55 PM	44.89101	-85.94171	0.0
138	7/17/18	4:58 PM	44.88868	-85.94010	0.0
139	7/17/18	5:02 PM	44.88560	-85.93982	0.0
140	7/17/18	5:05 PM	44.88431	-85.94034	0.0
141	7/17/18	5:11 PM	44.88284	-85.94116	0.0
142	7/17/18	5:17 PM	44.87815	-85.93893	4.9
143	7/17/18	5:22 PM	44.87603	-85.93702	0.0
144	7/17/18	5:26 PM	44.87387	-85.93508	44.9
145	7/17/18	5:31 PM	44.87264	-85.93393	0.0
146	7/17/18	5:34 PM	44.87033	-85.93225	0.0

Diagram 3 – Big Glen Lake Bacteria Heat Map

Big Glen Enteric Bacteria
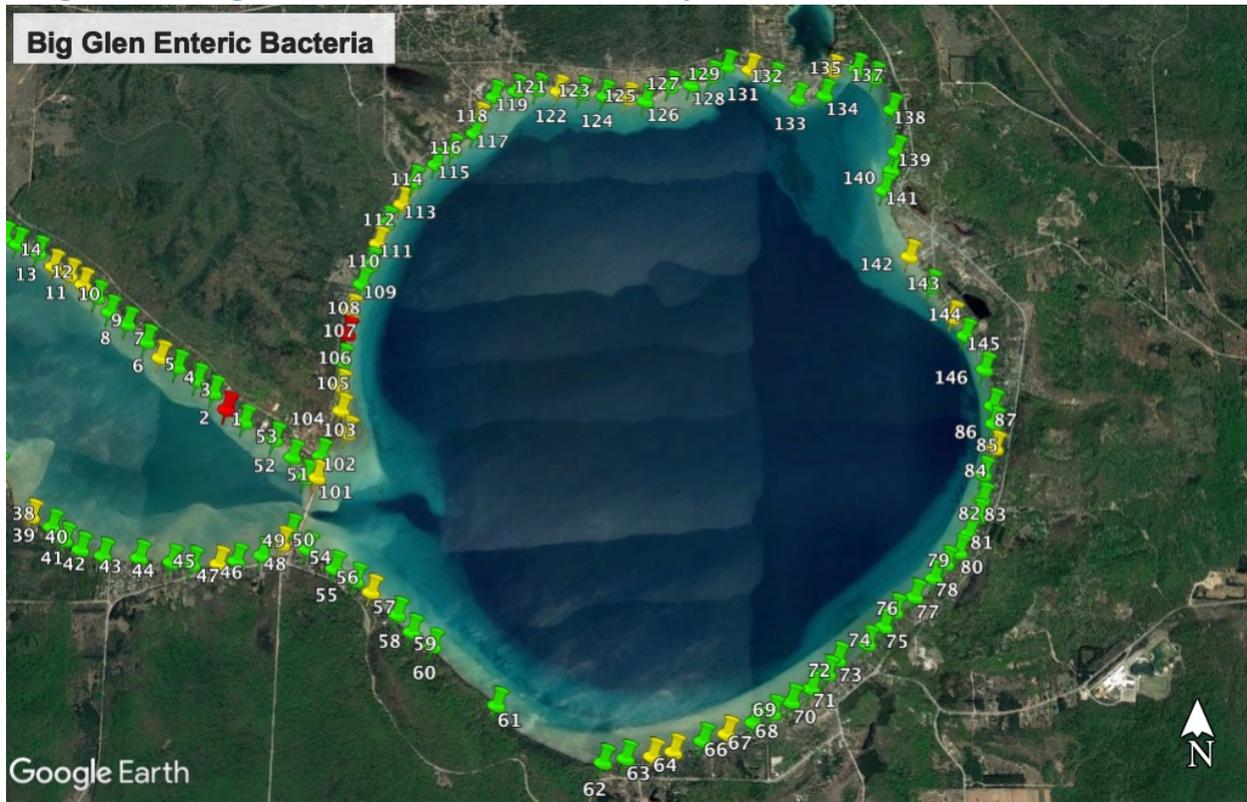
Table 4 – Little Traverse Lake Data

Site	Date	Time	Latitude	Longitude	Bacteria Level
1	7/17/18	8:51 AM	44.92486	-85.86133	0.0
2	7/17/18	9:03 AM	44.92493	-85.86280	6.1
3	7/17/18	9:05 AM	44.92433	-85.86313	0.0
4	7/17/18	9:11 AM	44.92314	-85.86241	45.9
5	7/17/18	9:14 AM	44.92238	-85.86099	0.0
6	7/17/18	9:20 AM	44.92208	-85.85996	20.3
7	7/17/18	9:25 AM	44.92144	-85.85847	0.0
8	7/17/18	9:30 AM	44.92035	-85.85575	0.0
9	7/17/18	9:33 AM	44.91952	-85.85450	0.0
10	7/17/18	9:39 AM	44.91875	-85.85304	0.0
11	7/17/18	9:44 AM	44.91729	-85.84974	0.0
12	7/17/18	9:51 AM	44.91664	-85.84676	0.0
13	7/17/18	9:56 AM	44.91657	-85.84412	0.0
14	7/17/18	9:59 AM	44.91706	-85.84248	0.0
15	7/17/18	10:02 AM	44.91741	-85.84064	0.0
16	7/17/18	10:06 AM	44.91771	-85.83884	0.0
17	7/17/18	10:11 AM	44.91788	-85.83717	19.1
18	7/17/18	10:14 AM	44.91829	-85.83507	0.0
19	7/17/18	10:20 AM	44.91859	-85.83254	0.0
20	7/17/18	10:23 AM	44.91964	-85.83125	13.4
21	7/17/18	10:27 AM	44.92153	-85.82873	0.0
22	7/17/18	10:33 AM	44.92513	-85.82562	0.0
23	7/17/18	10:37 AM	44.92686	-85.82282	6.5
24	7/17/18	10:42 AM	44.92792	-85.82217	19.6
25	7/17/18	10:47 AM	44.93037	-85.82433	0.0
26	7/17/18	10:55 AM	44.92974	-85.82609	7.6
27	7/17/18	11:05 AM	44.92901	-85.82762	0.0
28	7/17/18	11:09 AM	44.92835	-85.82945	0.0
29	7/17/18	11:13 AM	44.92832	-85.83111	0.0
30	7/17/18	11:18 AM	44.92775	-85.83318	0.0
31	7/17/18	11:22 AM	44.92740	-85.83539	0.0
32	7/17/18	11:27 AM	44.92679	-85.83763	0.0
33	7/17/18	11:31 AM	44.92655	-85.84032	0.0
34	7/17/18	11:35 AM	44.92639	-85.84237	0.0
35	7/17/18	11:38 AM	44.92618	-85.84456	0.0
36	7/17/18	11:41 AM	44.92577	-85.84671	0.0
37	7/17/18	11:47 AM	44.92516	-85.84854	0.0
38	7/17/18	11:50 AM	44.92508	-85.85058	0.0
39	7/17/18	11:53 AM	44.92497	-85.85215	0.0
40	7/17/18	11:57 AM	44.92473	-85.85404	17.9

41	7/17/18	12:01 PM	44.92469	-85.85554	20.7
42	7/17/18	12:08 PM	44.92453	-85.85718	0.0
43	7/17/18	12:10 PM	44.92443	-85.85857	63.5

Diagram 4 – Little Traverse Lake Bacteria Heat Map
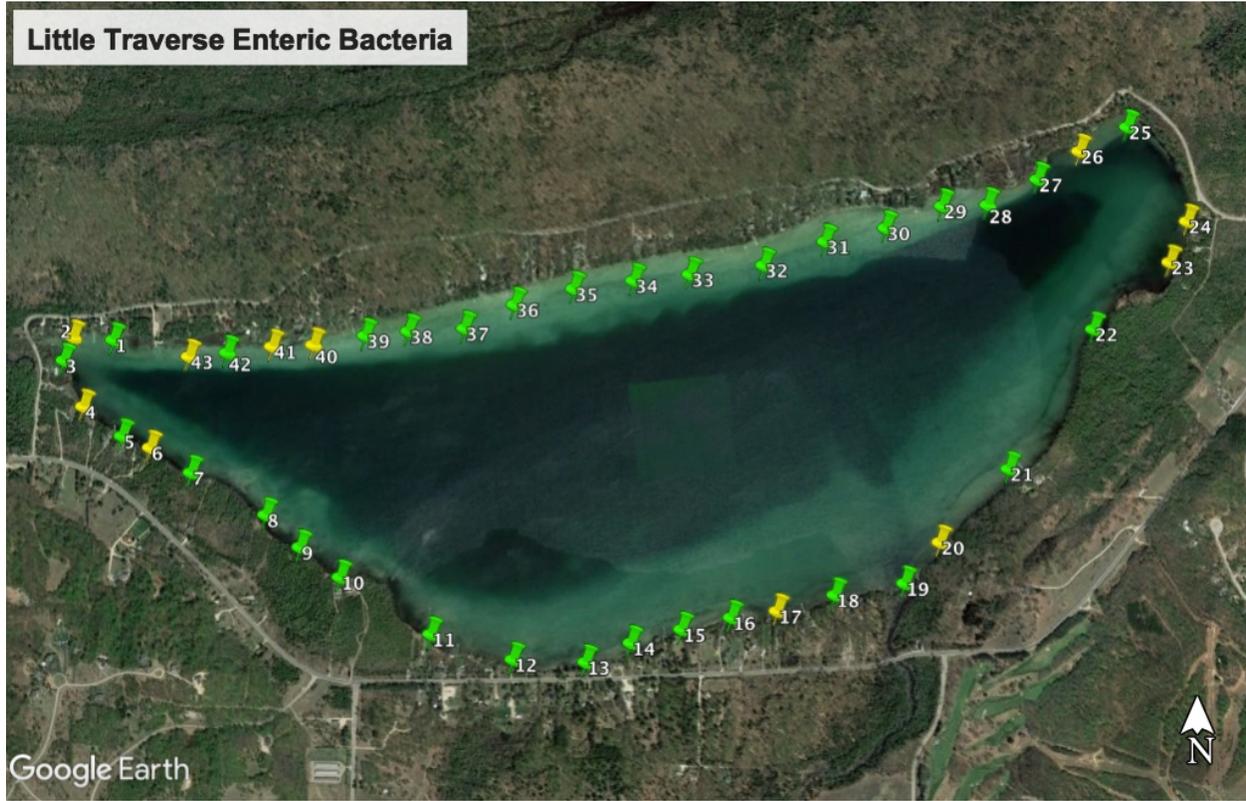
Table 5 – Lake Charlevoix Data

Site	Date	Time	Latitude	Longitude	Bacteria Level
1	7/7/18	8:30 AM	45.22560	-85.03420	0.0
2	7/7/18	8:48 AM	45.23710	-85.06160	0.0
3	7/7/18	9:07 AM	45.28190	-85.08660	19.4
4	7/7/18	9:17 AM	45.27840	-85.12180	19.4
5	7/7/18	9:35 AM	45.29060	-85.17430	0.0
6	7/7/18	9:49 AM	45.32240	-85.20930	0.0
7	7/7/18	10:05 AM	45.30290	-85.24760	0.0
8	7/7/18	10:16 AM	45.28740	-85.23290	0.0
9	7/7/18	10:31 AM	45.26890	-85.19720	0.0
10	7/7/18	10:55 AM	45.23140	-85.16750	0.0
11	7/7/18	11:10 AM	45.19790	-85.16140	0.0
12	7/7/18	11:20 AM	45.17780	-85.16250	13.1
13	7/7/18	11:30 AM	45.15720	-85.14040	0.0
14	7/7/18	11:42 AM	45.19120	-85.14970	0.0
15	7/7/18	11:55 AM	45.23170	-85.15750	18.4
16	7/7/18	12:18 PM	45.26640	-85.17360	0.0
17	7/7/18	12:36 PM	45.25080	-85.11150	0.0
18	7/7/18	12:48 PM	45.23230	-85.08660	326.9
19	7/7/18	12:58 PM	45.21670	-85.06560	0.0
20	7/7/18	1:14 PM	45.21210	-85.01860	0.0

Diagram 5 – Lake Charlevoix Bacteria Heat Map

Lake Charlevoix Enteric Bacteria
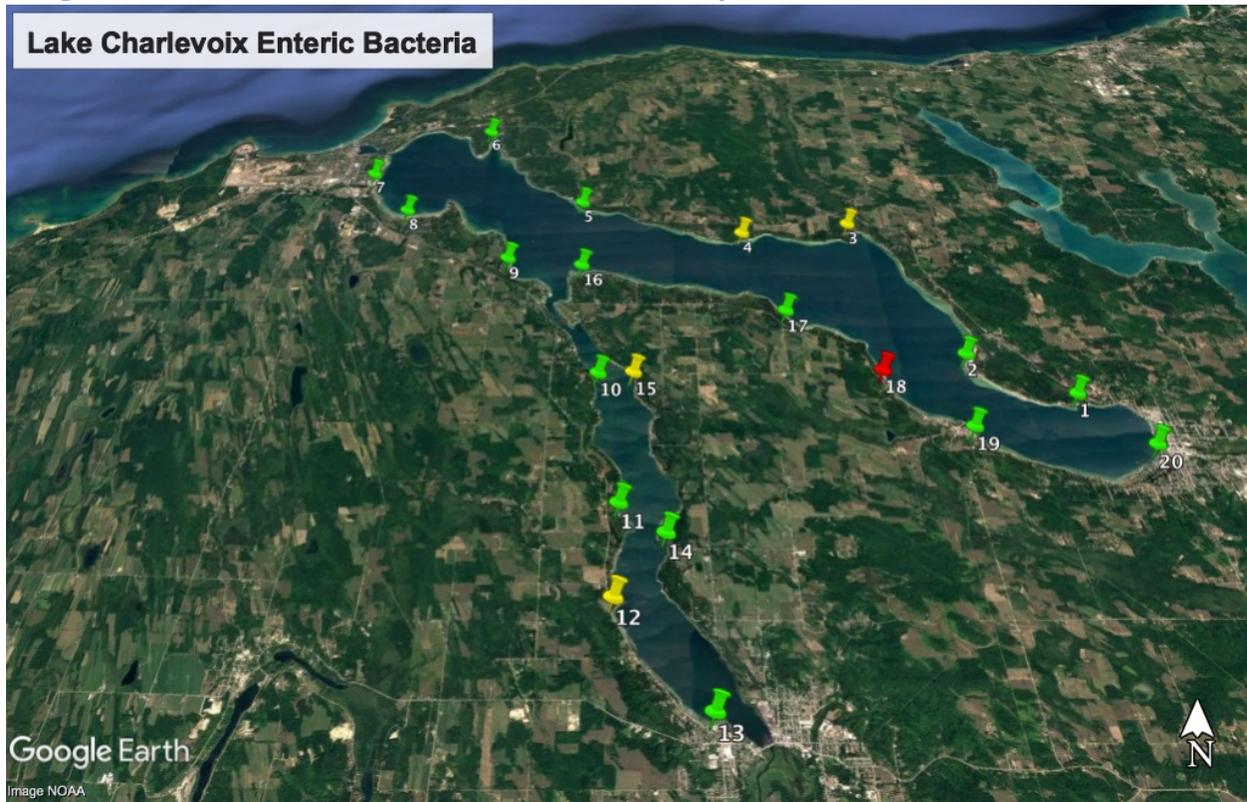
Discussion

The mean number of copies of the target sequence yielding a probability of detection of 0.95 is called limit of detection or LOD95%. The Chai qPCR machines have a limit of detection 95 (LOD_{95}) of ~50 gene copies. HF183 contamination levels run at the dilution required for qPCR testing typically ranges from 8-25 copies, far below the LOD_{95} of the Chai qPCR. This discrepancy is easily remedied when using the Chai by increasing the initial water sample volume. For example, instead of collecting 100ml of lake water as we did in 2018, the volume could easily be increased to 500ml or even 1L. Processing and qPCR analysis using a portable Chai machine would then detect these lower levels of bacteria.

Bacteria levels are reported in bacterial genome equivalents. A bacterial genome equivalent represents approximately one bacterial cell. qPCR results are reported in this way because qPCR measures both live and dead bacteria. This is one advantage of qPCR monitoring versus cell culture assessment, since levels of live bacteria typically decrease throughout the day. Therefore, qPCR provides a more reliable number of contaminations throughout a single day.

The main objective of this study was to identify possible sites of human contamination, not to assess health risks for water recreation. We cannot directly compare non-specific *E. coli* levels to *Bacteroides* levels, and the HF183 marker should not be used to predict human health impacts. Numerous studies have found the HF183 marker to be a very reliable marker for human contamination, but it does not correlate well with human health effects.

Drone and/or manual shoreline surveys locating *Cladophora* sp. algae could now be compared to bacterial discoveries reported here to check for correlation. Additionally, septic system age, obtained through local government agencies, may also show correlation to these reported bacteria findings.

Lake associations may want to consider including tests for nitrogen and phosphorous, along with qPCR analysis for the enteric HF183 marker, to give a more accurate assessment of actual nutrient loading. Testing before and after a measureable rain event to gauge the impact of runoff through septic fields may also provide valuable information about human influence.

Acknowledgments