2019 SURVEY of TORCH LAKE and LAKE BELLAIRE for SWIMMER'S ITCH CECERIAE, and HUMAN ENTERIC BACTERIA USING qPCR METHODOLOGY

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Executive Summary

To determine if the qPCR methodology could be used by high school students to generate reliable water quality data, TLA's 2019 summer interns collected 60 water samples, which were analyzed for enteric bacteria and swimmer's itch cercariae content using qPCR methodology. The enteric bacteria test results showed near-shore locations around Torch and Bellaire lakes with values that exceeded and that did not exceed the EPA's levels for follow up action. Similarly, swimmer's itch cercariae were detected in locations where swimmers had reported developing swimmer's itch symptoms. Therefore, this study demonstrated that students can utilize qPCR methodology to generate reliable water quality data.

Introduction

Sitting in the northwest corner of Northern Michigan are two beautiful freshwater lakes, Lake Bellaire and Torch Lake. Both Lake Bellaire and Torch Lake are part of the Elk River Chain of Lakes, and are enjoyed by many locals and tourists year round. Lake Bellaire sits to the southwest of the town of Bellaire. Lake Bellaire has an average depth of 43 feet, with a maximum depth of around 101 feet. The irregular shape of the lake, a distorted "S" shape, is a contributing factor to the lengthy shoreline, at about 11.5 miles. The lake is approximately 4.5 miles long, and approximately 1.3 miles at its widest point. The surface area of the lake is 1,793 acres.[6] Water from Lake Bellaire flows through Grass River, Clam Lake and Clam River into Torch Lake. Torch Lake sits East and just inland from Lake Michigan and, at its southern end, Elk Lake. Torch Lake runs from the town of Eastport in the North, to Rapid City in the South. The lake is 18 miles long, making it Michigan's longest inland lake. While the lake is long, it is very narrow, only being approximately 2 miles wide at its widest point. The lake has an average depth of 111 feet, with a maximum depth of around 302 feet. Torch Lake has an even lengthier shoreline, at 41 miles. With a surface area of roughly 18,770 acres, Torch Lake is Michigan's second largest inland lake.[7]

Cercarial Dermatitis, commonly referred to as Swimmer's Itch, is an allergic reaction caused by the cercarial stage of several species of schistosomes. In humans, it causes the creation of pruritic bumps that generally persist for no greater than three weeks. The life cycle of these schistosomes takes place through several hosts, as well as spending periods in open water. This cycle begins when the egg of a schistosome is immersed in water, after which a miracidium emerges.[4] While short-lived, these miracidia seek out their desired hosts: snails. Following this infection, the miracidium develops into a mother sporocyst. Through Asexual reproduction, this mother sporocyst produces a number of daughter sporocysts. In turn, these daughter sporocysts produce large numbers of cercariae, which are then shed from the snail when exposed to a natural day-night cycle.[4] The shed cercariae then move along the surface of the water in search of their definitive host. Generally, ducks are the preferred host. However, during this larval stage, cercariae may penetrate the skin of a swimmer. While duck hosts allow the life cycle of schistosomes to continue, the penetration of a swimmer's skin results in the death of the larva. In turn, the human body activates an allergic immune reaction to the foreign body, leading to the formation of the pruritic bumps that are characteristic of cercarial dermatitis. While infection is possible whenever cercariae are present in the water, it is most prominent on sunny mornings, when the day-night cycle causes the greatest shedding of cercariae from snails.[4] As a result, contact risk decreases throughout the day, due to the fragile cercariae being broken along the shore and declining levels of snail shedding. While the most simple solution to avoid contact may be to confine swimming to post-peak times, other methods may also be employed to limit miracidium populations. In Michigan, governments had previously used copper sulfate as a molluscicide in an attempt to hinder the life cycle of schistosomes. However, this strategy was generally unsuccessful, as well as damaging to the natural order of the lakes on which it was used. Currently, studies have shown that the use of anti-parasitic drugs are effective in controlling the infection of host mollusks, limiting the ability of the parasitic life cycle to continue. Furthermore, surface level barriers, filter systems, and micron netting have also proved effective at corralling and destroying host-seeking cercariae.[4]

Enteric Bacteria are bacteria that reside in animal and human intestines. Enterococci are an indicator of the presence of fecal material in water and of the possible presence of disease-causing bacteria, viruses, and protozoa.[5] The purpose of the Enterococci and Enteric bacteria studies are to locate the entry point. Some ways the Enterococcal and Enteric Bacteria enter the waterways are by recreational waters include sewage, agricultural and urban runoff, storm water, septic tanks, pausters, direct input by animals via defecation, plant debris, polluted groundwater, and contaminated streams.[2] A new standard method for measuring Enterococci in water was developed by the United States Environmental Protection Agency (USEPA), which uses quantitative polymerase chain reaction (qPCR) in conjunction with a hydrolysis probe.[5] This new method of measuring Enterococci (qPCR) was used in this study.

qPCR, also known as quantitative Polymerase Chain Reaction; is a methodology to measure the relative quantities of a known DNA sequence in a sample. For the purpose of this study, qPCR was used to measure the quantity of Cercarial DNA in samples. qPCR first requires a genetic probe specific to the DNA sequence of the organism you are testing for. This probe is then mixed with the water sample and put into a fluorometer. This machine causes target DNA to replicate many times. This is measured by the binding of fluorescent molecules to replicated DNA sequences, creating a fluorescent effect. The more DNA that is in the initial sample, the greater the ending fluorescence. The intensity of the fluorescence is directly proportional to the content of the original sample and is measured by the fluorometer.

Methods

Methods for Cercarial Sampling

Swimmers itch samples were taken on Lake Bellaire and Torch Lake approximately 1.5 miles apart. The sample collecting steps include, scooping water, straining water, spraying of ethanol,and putting the sample in the 50ml test tube. Samples were obtained by scooping 25 1L scoops of water and dumping them through a filter, either off of a dock or in a horseshoe pattern in waist deep water. As water is collected it strains through a 20 micron plankton net held vertically, that captures the cercariae. The water volume is reduced by filtration, and 95% ethanol is used to preserve the sample. The solution is poured into a labeled and sterile 50mL collection tube and is placed in a cooler before being delivered to the lab to be analyzed.

Methods For Enterococcus Lake Sampling

Over the course of the study, forty water samples in total were collected along Torch Lake and Lake Bellaire for enterococcus bacteria. Twenty-seven water samples were collected along Torch Lake, and twelve samples were collected along Lake Bellaire. To assist with time management for sampling, Torch Lake was split into two halves: North Torch and South Torch. The sampling sites were spaced out about a mile and a half from each other. At a measured 15 meters from the shoreline, three samples per site location were collected in sterile 50mL vials, one each from the front, one side and the back of a boat. Each sample vial was lowered by hand and collected 6-12 inches below the water surface. To avoid contamination, fingers or any material containing human DNA were to not be placed in the vials or touch them on the inside of

the cap. Additionally, water temperature was recorded in celsius using a floating thermometer, average wind speed was taken in kilometers per hour using an anemometer; the general wind direction and the last known date of a major rain event were all logged on a data collection sheet. After each sample was individually taken, samples were held on ice in a cooler until delivery to the lab. The environmental water samples had to be filtered and have the DNA extracted as soon as possible after collection, and could not be held for more than 4-6 hours in between the collection and the start of the filtration process. Samples were to be analyzed by qPCR for enterococcus (EPA method 1611) and for bacteroides (HF183).

Methods For Enterococcus Stream Sampling

Enterococcus sampling was conducted in tributary streams before and after major rain events. This allowed for a more accurate identification of any Enterococci contamination in the selected tributaries that might flow into surrounding lakes. Stream samples were collected by aseptic technique into pre-labeled sterile 100 ml bacteriology sample bottles and kept on ice in a cooler until delivered to the laboratory. E. coli samples were analyzed by standard culture by SOS Analytical in Traverse City. Enteric bacteria samples were analyzed for Enterococcus by qPCR at Freshwater Solutions in Cedar, Michigan and for Bacteroides HF183 by qPCR at the University of Alberta in Canada.

Methods For Enterococcus Sandbar Sampling

Enterococcus sampling was conducted on the Torch Lake Sandbar during low occupancy (July 2) and again during a high occupancy (July 4). These samples were also collected using an aseptic technique, and collected using pre-labeled sterile 100ml bacteriology bottles, and kept on ice in a cooler until delivered to the laboratory. Sandbar samples were analyzed using the qPCR methodology at Freshwater Solutions in Cedar, Michigan.

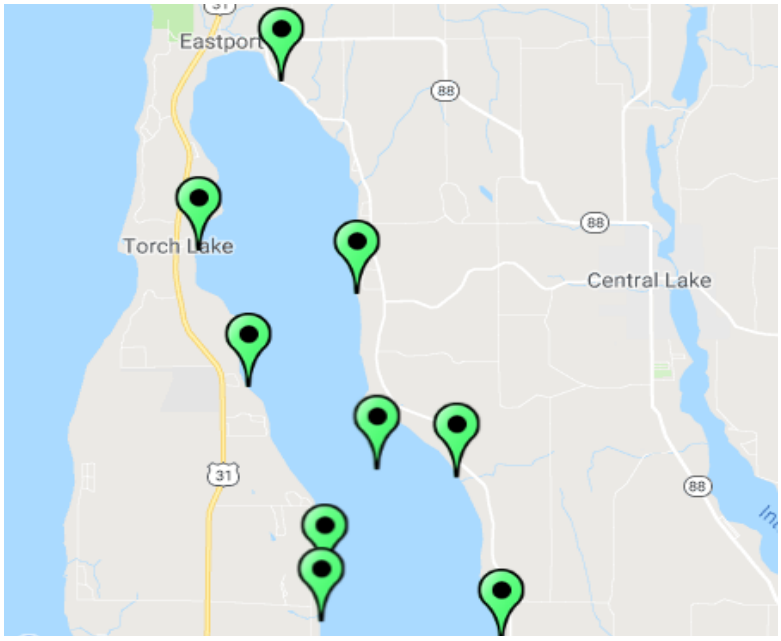
Results

Swimmer's Itch

Over the course of the study, water samples were taken out of nine different sampling sites at the north end of Torch Lake for the purpose of testing for swimmer's itch related organisms. Our sampling on Torch Lake was limited to this area because sampling for a more comprehensive evaluation of the entire lake was being done by others. All nine of the samples returned negative for cercarial DNA (Table 6). This indicates that there were no swimmer's itch related schistosomes in the water samples.

Ten water samples were taken out of Lake Bellaire and showed a few areas of medium to high concern of encountering swimmer's itch related cercariae. One of the ten water samples showed a medium concern level, while three other samples showed very high levels of cercarial DNA in the samples taken (Table 7), indicating a substantial risk of getting swimmer's itch. Two high concern locations were located in a cluster on the western shoreline, while the third was located among a low concern cluster on the eastern shoreline.

Fig 1. North Torch Lake Swimmer's Itch findings



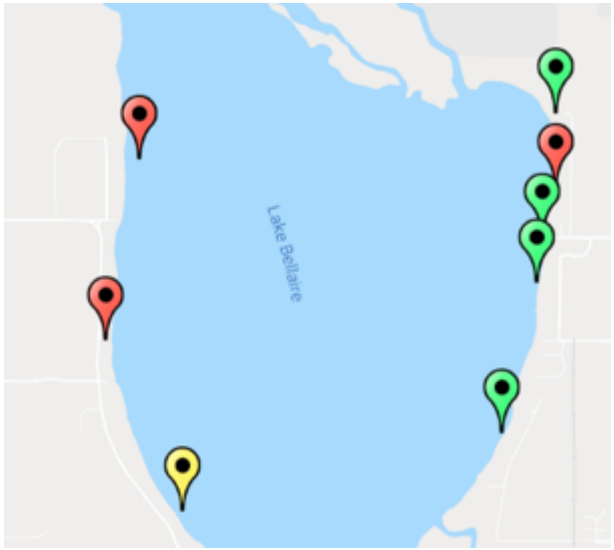


Fig 2. Lake Bellaire Swimmer's Itch Locations

Fig 3. Lake Bellaire Swimmer's Itch

Lake Bellaire Swimmer's Itch Findings		
Location	Date	Ave/25L
West (G15)	7/30	15.75
West (F12)	7/30	554
West (F9)	7/30	368
East (L9)	7/30	6819

Enterococcus

Enterococcus samples tested positive in 20 of 27 samples taken on Torch Lake. Of those 20 samples, 16 tested at a level that could indicate possible contamination in the future. Four of the samples that tested positive all had values of 640 CCE or higher, which requires action, per EPA standards. All enterococcus samples taken on Torch Lake tested negative for human assays (HF183). Twelve samples for enterococcus were taken on Lake Bellaire, and four of them tested positive, but not at a level that would require any action to be taken. Again, all of the samples on Lake Bellaire tested negative for human assays (HF183).

Four tributary streams were sampled for enterococcus bacteria. After a heavy rainfall, three streams tested negative for enterococcus bacteria, and one tested positive; Eastport Creek tested at a level of moderate concern. At that time, all the creeks tested negative for human assays (HF183). After a relatively dry period, the same four creeks were sampled again, and of the four creeks, two tested at a level of moderate concern, Grass Creek and The Creek, and one creek that tested positive at a level of high concern, Eastport Creek. All four creeks tested negative for human assays (HF183) at the time of relatively dry sampling.

The Sandbar on Torch Lake was sampled for enterococcus during a time of low occupancy and during a time of high occupancy. At the time of low occupancy, there were traces of enterococcus bacteria found, but not at a level where action was required. When the Sandbar was tested again at a time of high occupancy, a moderate level of enterococcus bacteria was found, which could require action to be taken. During the time of both high and low occupancy, the Sandbar samples tested negative for human assays (HF183).

Fig. 4 Lake Bellaire Enterococcus Locations

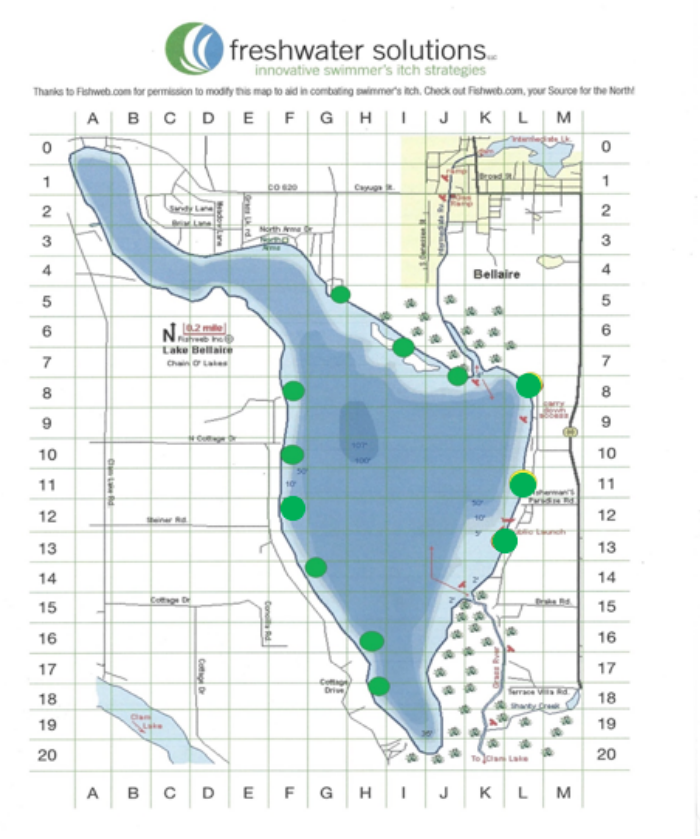


Fig. 5 Torch Lake Enterococcus Locations

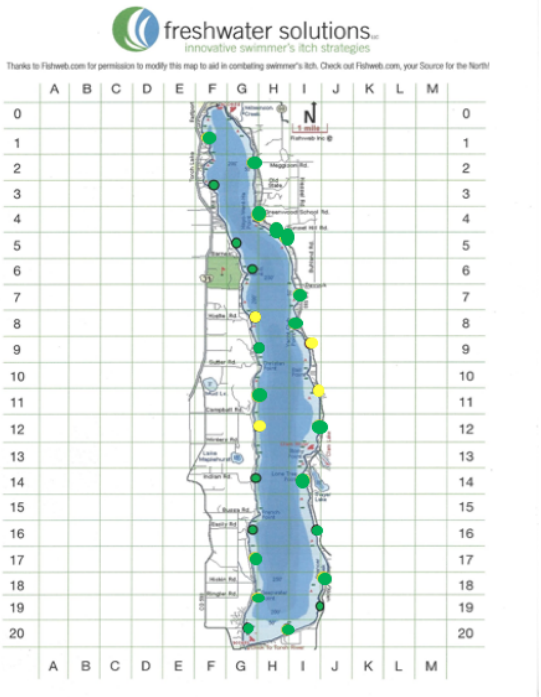


Fig. 6 Torch Lake Enterococcus Results

Torch Lake Enterococcus Samples				
Sample	**Date**	**Location**	**Enterococcus**	**Human (HF183)**
1	6-25-19	G 20	0	Negative
2	6-25-19	G 19	94	Negative
3	6-25-19	G 17	50	Negative
4	6-25-19	G 16	0	Negative
5	6-25-19	G 14	0	Negative
6	6-25-19	I 14	47	Negative
7	6-25-19	I 16	443	Negative
8	6-25-19	J 18	125	Negative
9	6-25-19	I 19	0	Negative
10	6-25-19	H 20	35	Negative
11	7-9-19	G 12	678	Negative
12	7-9-19	G 11	163	Negative
13	7-9-19	G 9	446	Negative
14	7-9-19	G 8	824	Negative
15	7-9-19	I 8	108	Negative
16	7-9-19	I 9	1195	Negative
17	7-9-19	I 11	1093	Negative
18	7-9-19	I 12	156	Negative
19	7-19-19	H 5	156	Negative
20	8-6-19	H 4	280	Negative
21	8-6-19	H 5	109	Negative
22	8-6-19	I 7	44	Negative
23	8-6-19	G 6	0	Negative
24	8-6-19	G 5	0	Negative
25	8-6-19	F13	0	Negative
26	8-6-19	F 1	121	Negative
27	8-6-19	G 2	50	Negative

Fig. 7 Lake Bellaire Enterococcus results

Lake Bellaire Enterococcus Samples				
Sample	**Date**	**Location**	**Enterococcus**	**Human (HF183)**
1	7/23/19	K13	179	Negative
2	7/23/19	H18	0	Negative
3	7/23/19	H16	0	Negative
4	7/23/19	G14	0	Negative
5	7/23/19	F12	23	Negative
6	7/23/19	F10	0	Negative
7	7/23/19	F8	0	Negative
8	7/23/19	G5	0	Negative
9	7/23/19	I7	0	Negative
10	7/23/19	J8	0	Negative
11	7/23/19	L8	31	Negative
12	7/23/19	L11	279	Negative

Fig. 8 Stream Enterococcus Locations

Fig. 9 Stream Enterococcus Results

Tributaries					
Heavy Rain					
Sample	**Date**	**Location**	**E-Coli (CFU)**	**Enterococcus (CCE)**	**Human (HF183)**
1	6/13/19	Grass Creek	139	0	Negative
2	6/13/19	Wilkinson Creek	1548	0	Negative
3	6/13/19	Eastport Creek	823	991	Negative
4	6/13/19	The Creek	316	0	Negative
Relatively Dry					
1	7/2/19	Grass Creek	47	670	Negative
2	7/2/19	Wilkinson Creek	61	76	Negative
3	7/2/19	Eastport Creek	159	1308	Negative
4	7/2/19	The Creek	211	766	Negative

Fig. 10 Sandbar Enterococcus Results

Sandbar Findings During Low Occupancy (7/2/2019)				
Sample	Date	Location	Enterococcus	Human (HF183)
1	7/2/19	Torch Lake Sandbar	182.77	Negative
Sandbar Findings During High Occupancy (74/2019)				
2	7/4/19	Torch Lake Sandbar	680.31	Negative

Discussion

In conclusion cercariae were not found along the Northern portion of Torch Lake. Cercariae were found in Lake Bellaire. There were multiple locations on the westside with high or moderate levels of cercariae, with only one location on the east side with a high level of cercariae. During the study more water fowl were observed on Torch Lake than on Lake Bellaire. Time of day and weather conditions are known to affect the cercarial density and these may have affected the results. There is no obvious factor that caused us to find more cercariae on Lake Bellaire than on the north end of Torch Lake.

In conclusion, Torch Lake itself only had a few areas of minor concern of enterococcus. In total four samples had a minor enterococcus count, and all of them were negative for human bacteria. In addition, the enterococcus results for Lake Bellaire were not of concern, meaning that there were no medium or high enterococcus counts in any of the sampling sites.

When sampling the tributary streams during a relatively dry period, two of the four streams had a medium enterococcus count, meaning that they were 640 CCE or above while one had a major level of concern for enterococcus bacteria, meaning that it had a level of 1280 CCE or above. However when the same tributary streams were sampled during a heavy rain period, only one came back with any enterococcus counts (table 4). Based on our limited data set, the E. coli culture results showed poor correlation with the enterococcus qPCR results. Due to the limiting sampling locations in the lakes, no correlation can be made between the stream results and lake results. The Torch Lake Sandbar during low occupancy did not have a high or medium concerning level. The samples taken during a high occupancy on the 4th of July had a medium concerning level for enterococci, but were negative for human markers. Overall, it was determined that qPCR is an appropriate methodology to analyze water quality within water samples.

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