

ENTERIC BACTERIA TRACING RESEARCH IN THE COLD CREEK WATERSHED USING qPCR ANALYTICAL TECHNOLOGY

Introduction

In the summers of 2021 and 2022 the CLWA and Benzie Conservation District (BCD) conducted a research study focused on enteric bacteria within the three tributaries of Cold Creek watershed, Village of Beulah storm water drains, and four other major tributaries of Crystal Lake. The purpose of the study was to better understand the sources and types of bacteria that emanate from these sub-watersheds that may eventually enter Crystal Lake.

Concerns over fecal contamination at sample locations in the Cold Creek region of Beulah near its mouth have existed for years. The CLWA and BCD have invested resources to collect and analyze water samples for enteric bacteria annually since 2013. Before that, samples were analyzed periodically by the state. Bacterial analytical data was performed by culture test for E.coli. Whereas this provided a means of detecting live bacteria in the branches of the Cold Creek system, it did not provide data on the source and types of bacteria.

The recently developed analytical method of quantitative polymerase chain reaction (qPCR) provides a means of detecting DNA in the water systems that can indicate the source of bacteria, from either specific animals or human source. It is being widely used for microbial source tracking in aquatic systems. In addition, it is the most reliable way to detect and measure human fecal pollution by targeting the HF183 marker in Bacteroides. Our goal of this study was to identify areas of high fecal bacteria pollution and source track samples for a human fecal marker (HF183) and animal fecal markers.

Weekly sampling was performed in 2021 and 2022 at critical points within the Cold Creek sub-watershed. Samples were analyzed using qPCR targeting *enterococcus*, followed by source tracking for human, dog, and Canada goose fecal contamination (HF183, DG3, and CG0F1 markers). In 2021, samples were taken each week from 6/30 to 8/11 from eleven sites, for a total of 87 samples. In 2022, samples were taken every other week from 6/1 to 8/24 from ten sites for a total of 105 samples. The 2022 total also included 28 samples (seven each) of four tributaries to the lake.

Cold Creek Watershed Sampling Points

Figure 1 shows an aerial view of the Cold Creek watershed along the eastern shore. Note the North and East directional arrows. The three branches of the Cold Creek system are identified as the North, Middle, and South Branches. Sampling sites are identified based on the coding system shown in the figure. The North Branch extends to the southeast behind the businesses on US31. This slow flowing creek accepts drainage from an extensive watershed that

encompasses all of the conservation district land and terrain further up a steep slope beyond Eldridge Road to the Crystal Lake Golf Course. The Middle Branch runs along Narrow-Gauge Road on both sides and accepts water from this steep valley topography. The middle branch comprises a north and south section to the middle branch along the road. The South Branch extends from the higher ground along the Betsie Valley Trail and Case Road where it eventually enters the Conservation Land and passes under US31 where it joins with the other branches and enters the settling pond. The settling pond outflow goes directly to the mouth of Cold Creek into the lake.

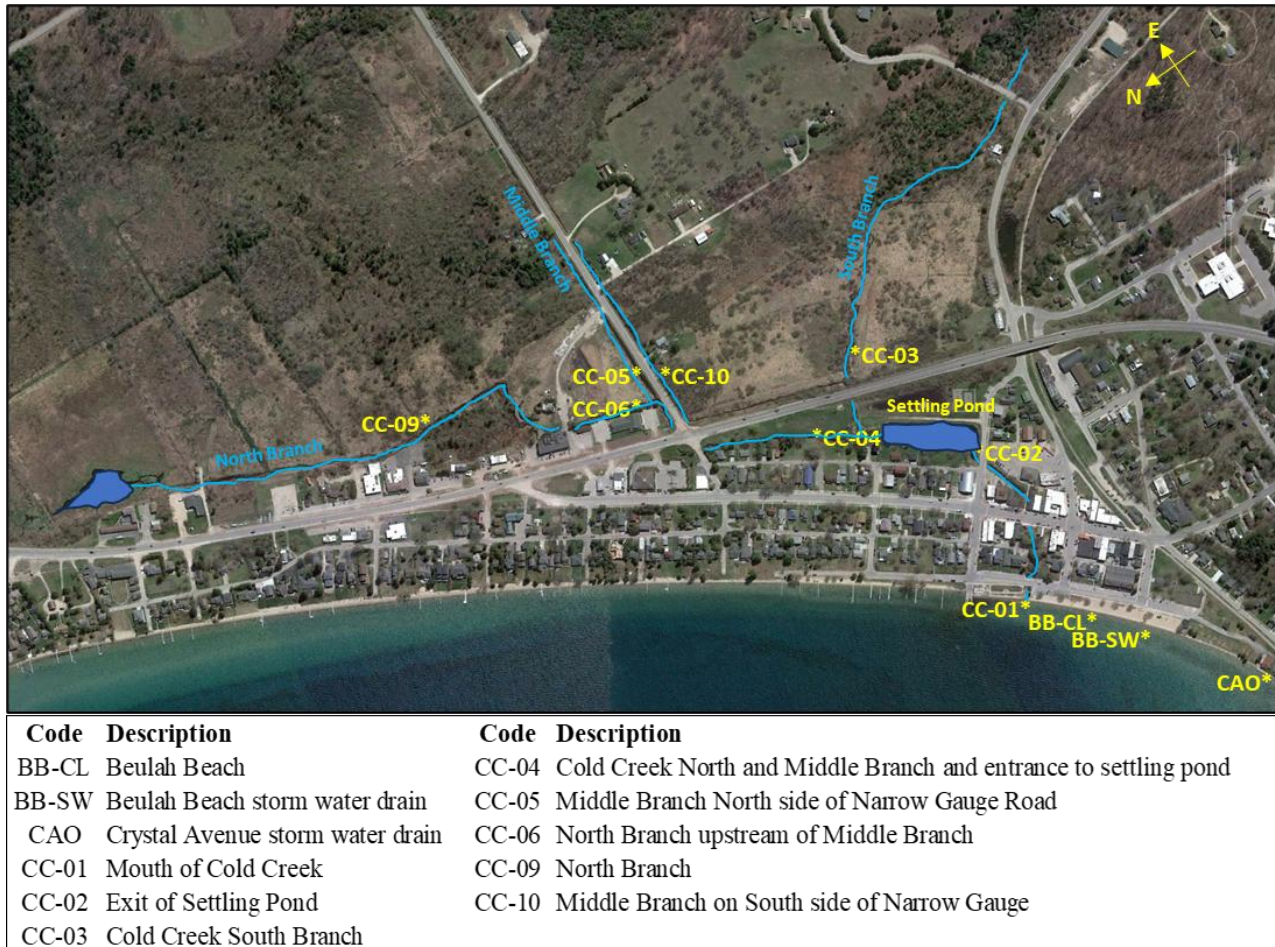


Figure 1. Aerial photograph of the Village of Beulah, the Cold Creek Tributaries, with the location of sampling sites.

Crystal Lake Tributary Sampling Points

Figure 2 shows the map of Crystal Lake with the designation of the four sampling sites performed at the mouths of the respective lake tributaries in 2022. The abbreviations are used

in the results section. Also shown are the inlet from Cold Creek and two major storm water inlets in Beulah.



Figure 2: Sampling sites at the mouths of four tributaries outside plus the Cold Creek sub-watershed

Results from the Two- Year study of Enteric Bacteria in the Cold Creek Sub-Watershed

Examination of the qPCR results for *Enterococcus* bacteria DNA for the years 2021 and 2022 generally showed high levels. 47% of samples showed an *Enterococcus* level that is high enough to be regarded as a risk and to warrant source tracking. 33% were considered contaminated enough to warrant a beach closing or posting. Though the purpose of the study was not to test for health-related levels, it was felt that examination of the data using these parameters provided a suitable means of gauging the extent of contamination. It also provided a means of ranking the sampling sites.

Table 1 shows the number of samples that registered at levels above which is considered safe for human contact.

Total of enteric bacteria above safe levels		2021	2022	Total
Middle Branch, south of Narrow Gauge Road	CC-10	10	12	22
Entry to Settling Pond	CC-04	12	8	20
Middle Branch, north of Narrow Gauge Road	CC-05	10	7	17
Mouth of Cold Creek	CC-01	10	7	17
North Branch Upstream of Middle Branch	CC-06	10	4	14
Beulah Beach storm water drain	BB-SW	7	1	8
Crystal Avenue Storm water drain	CAO	6	1	7
South Branch of Cold Creek	CC-03	6	1	7
Exit of Settling Pond	CC-02		4	4
Beulah Beach	BB-CL	1	0	1
Harris Creek	HC		4	
Glen Rhoda Creek	GR		0	
Shadko Creek	SC		0	
Bellows Creek	BC	8	0	

Table 1: Number of sampling sites that showed *Enterococcus* levels above safe for human contact.

It is apparent that the levels for most of the sites had decreased considerably in 2022. It is known that rainfall volume per hour and relative to sampling date can result in the variation in detected levels. However, this could not be verified as the cause for the apparent decrease. In spite of the yearly differences, it is worth noting that several sites remained a concern. One is the middle branch (CC-05 and CC-10), which did not show a decrease. Both the north and south sides of Narrow Gauge Road frequently showed high levels for *enterococcus* bacteria. In addition, the entry point to the Settling Pond (CC-04) maintained a high level, this is also the inflow culmination from all three branches in the watershed. Though the mouth of Cold Creek showed a decrease, any high levels of bacterial contamination pose a risk in this area. As far as the tributaries, Bellows Creek inlet showed eight high samples in 2021, but was not repeated in 2022. And Harris Creek inlet remains a site of concern based on the 2022 data.

Human source bacteria detection (HF183) in the Cold Creek Sub-Watershed

Figure 3 shows a satellite view of the east end of the lake and the Cold Creek watershed. Sampling locations that showed two or more positive hits for HF183 are delineated by a red square. The sampling labels and their geographic positions are shown in Figure 1. Focusing on the 4 and 3 positive hits for HF183, we can see that the Mouth of Cold Creek and the Beulah storm water drains are inlets of great concern. Another area of concern is the Middle Branch creeks along Narrow Gauge. This area may be a contributing source of human based bacteria. It is known that HF183 bacteria DNA has a relatively short life span in aquatic systems, thus any site producing a positive identification for HF183 is of significant concern.

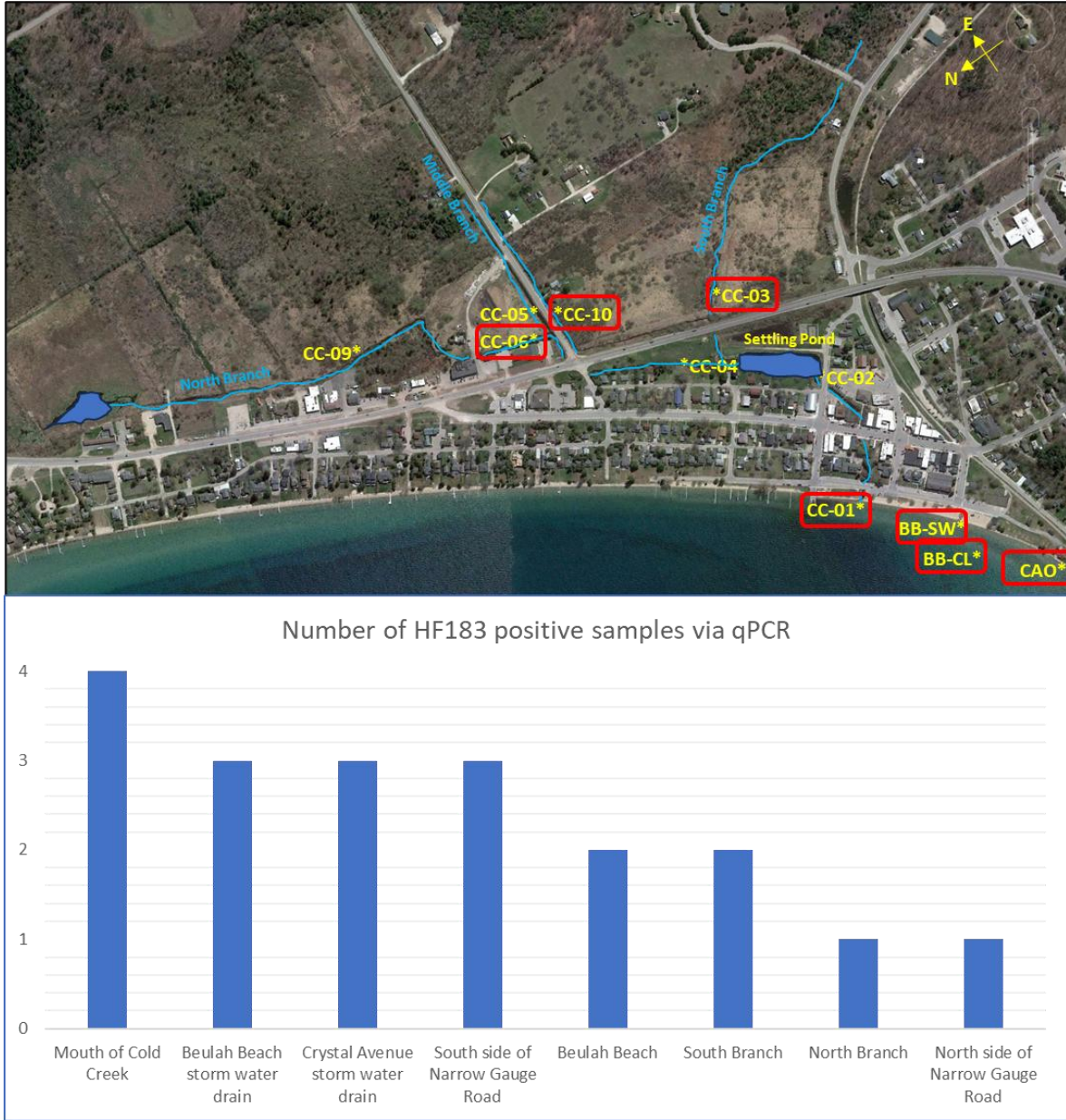


Figure 3: Number of HF183 positive bacteroid on the tributaries of Cold Creek over the 2021 and 2022 two-summer period.

Dog and Goose Bacteria Detection in the Cold Creek Sub-watershed

One of the advantages of using qPCR technology to identify the source of bacteria in a watershed system is the ability to reanalyze samples for specific animal sources. Newly developed markers for general dog and goose were applied in the qPCR analysis of samples from the 2021 and 2022 study. Figure 4 shows the number of samples showing positive results for six points in the watershed. All other sampling points showed no detection of dog fecal contamination. It is apparent that the area of the mouth of Cold Creek, the two major Beulah storm water drains, and the beach itself produced a high number of positive identifications.

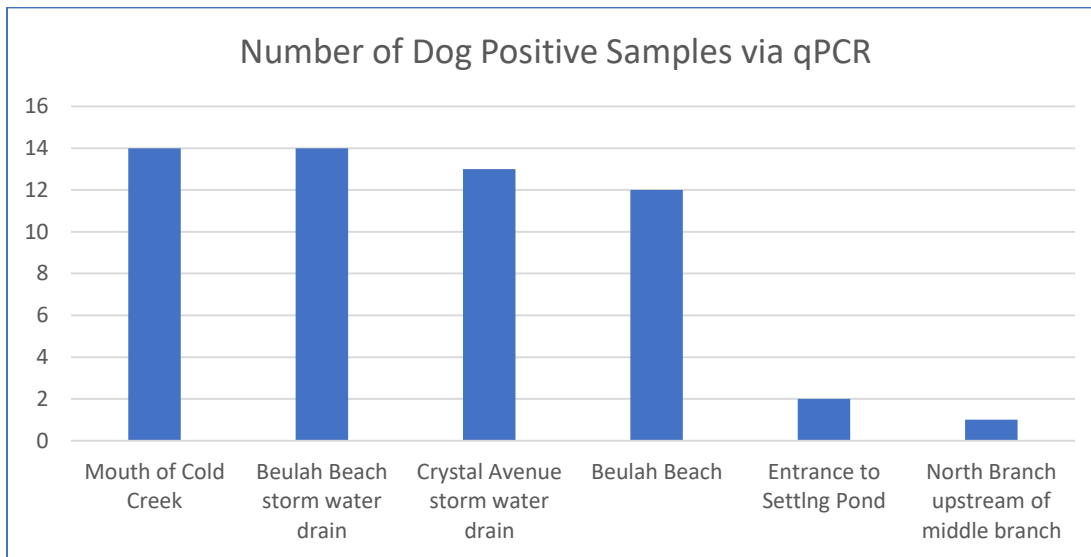


Figure 4: The number of dog positive samples identified by qPCR markers over the two-year sampling period.

Figure 5 shows the number of positive samples for the identification of goose bacteria. Bellows Creek and Harris Creek inlets to the lake showed high levels of bacterial tracking from this source. The north branch of cold creek also showed a significant level and this area has been observed as a significant goose habitat, especially in migration periods.

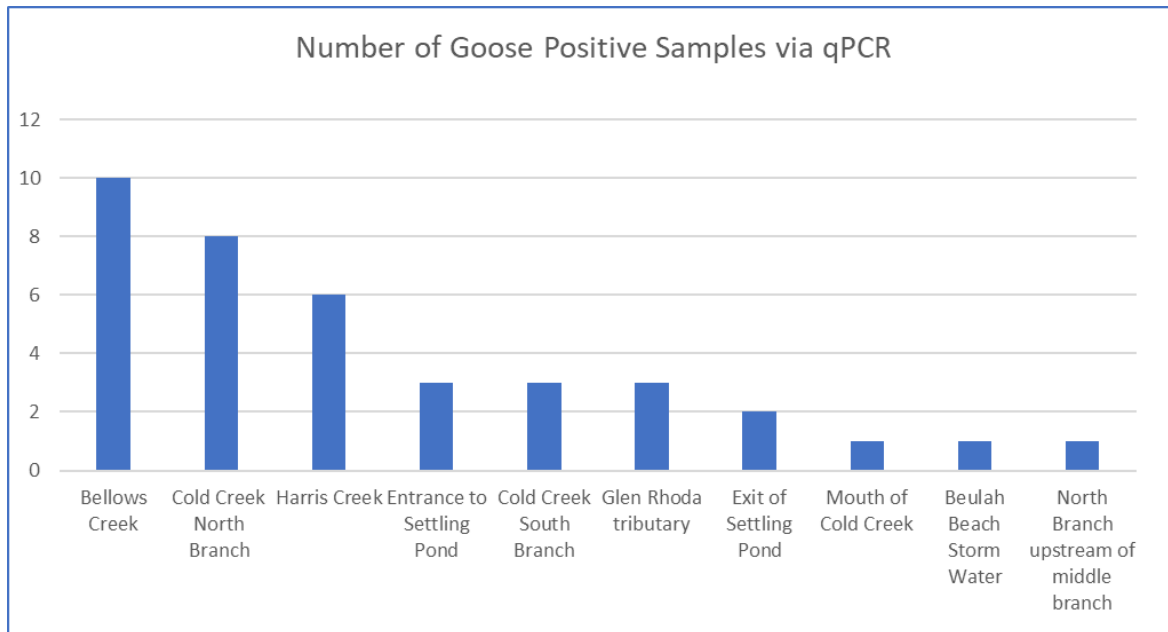


Figure 5: Number of goose positive samples identified by qPCR markers over the two-year sampling period

Conclusions:

For years the CLWA and the Village of Beulah has been concerned about the effluent that enters the lake from Cold Creek in terms of nutrients, sediment, and bacterial contamination. It is felt that this study provided a more detailed examination of bacterial contamination and provided greater knowledge as a result of a more detailed examination to watershed regions. The following are specific and general conclusions from this study:

- Cold Creek (CC-01) is a major source of bacterial contamination for the lake. It provides enterococcus, human, and dog bacteria to the lake and beach.
- The major contributors to the Mouth of Cold Creek appear to be the North and Middle Branches.
- There exists a significant concern that the Beulah Storm water drains (BB-SW and CAO) showed the detection of the human bacteria, HF183.
- The Middle branch of Cold Creek on both sides of Narrow Gauge Road is a significant contributor of *enterococcus* bacteria and HF183. The south side is of special concern (CC-10).
- Though contamination from goose bacteria is of limited control, the presence of dog bacteria at the storm drains and mouth of Cold Creek is potentially more controllable.

- The four additional tributaries showed relatively low bacterial influx, except for Harris Creek. This area requires continuous monitoring and potential microbial source tracking.

*-- Bruce Gerhart, CLWA Water Quality Committee
(October 2, 2023)*