Septic Leachate Assessment of Fish Lake, Indiana

Fish Lake Chain

LaPorte County, Indiana

December, 2018

Fish Lake Conservancy District

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Interpretation of Results

Results of the Fish Lake septic leachate assessment indicate that no widespread, concentrated, or localized human bacterial contamination is occurring in the Fish Lake Chain. Treated septic leachate is likely entering the lake through shallow groundwater flow, however, there is no evidence of any measurable impacts to water quality. To support this conclusion, data were collected during 2 sample events; one in July and one in October of 2018. Data collection focused on indicators of septic leachate including: Optical Brightening Agents (OBAs), organic content, nutrients (nitrate and phosphorus), bacteria (*E. coli* and DNA source tracking), and conductivity.

Water Sampling

Combined results from both sampling events demonstrate that each indicator validates conclusions. Results are also consistent with historically good water quality conditions in the lakes. Sampling events are summarized below; Figure 1 through Figure 4 depicts key indicators relied upon to draw conclusions:

1. Fluorometery combined with Dissolved Organic Content (DOC) was used to detect and assess the relative presence of OBAs, common in household cleaners; fluorometry is measured in the form of Relative Fluorometric Values (RFV). DOC was used to correct RFV values for dissolved organic matter, presented as a fluorometry/DOC ratio (F/DOC). Figure 1 depicts the F/DOC ratios for both sampling events.
    a. The average RFV was 93 with higher values observed at locations exhibiting higher DOC. Maximum values of 280 and 264 occurred in Mud Lake; the study reference site at the North End of Upper Fish Lake near Mill Creek and Mill Creek also generated results in the upper range of values.
    b. Corrected for DOC, no one lake sample indicated the presence of septic leachate in the form of OBMs. The Mill Creek sample did slightly exceed a threshold value indicating the potential for leachate, however, other indicators did not align to provide validation. Further investigation is needed to determine if septic leachate is entering the lake from external sources.
    c. Measurements were consistent across both sampling events and serve to validate assessment conclusions.

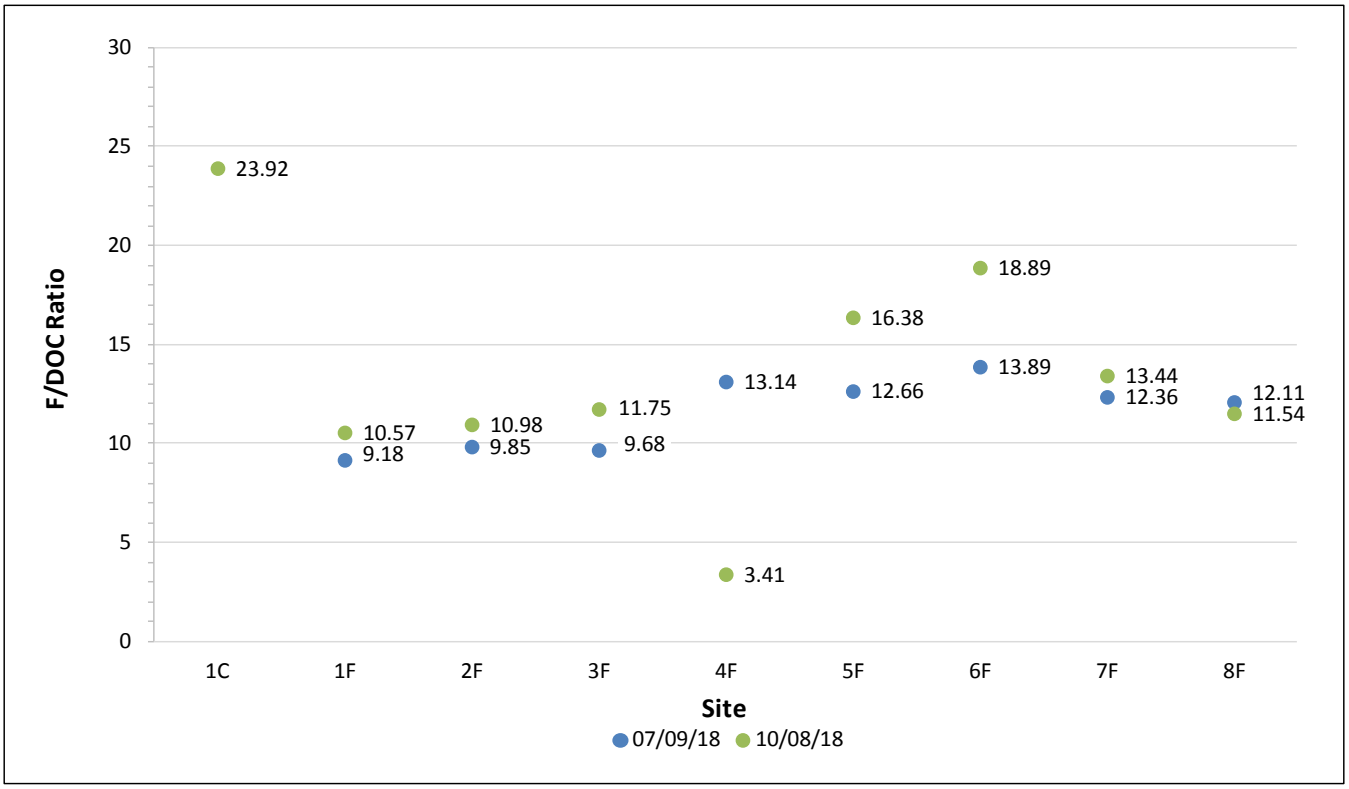

Figure 1 - Fluorometry/DOC Ratio

2. High concentrations of nutrients, such as nitrogen and phosphorus, can indicate the presence of septic leachate.
    a. Of the 17 water samples analyzed for nitrate and total phosphorus, only 1 resulted in a concentration above laboratory detection limits. This one sample was for nitrate at a concentration of 0.744 mg/L. These results also validate assessment conclusions.
3. Bacteria, specifically *E. coli,* are found in human and animal feces and are an indicator of potential septic pollution. To isolate the human bacteria component associated with septic leachate, specialized testing was performed on a total of 13 samples to distinguish between human and animal (goose) DNA biomarkers.
    a. The average *E. coli* concentration measured over 2 sampling events from a total of 18 water samples was 47.6 MPN/100 mL. *E. coli* is measured using a table of most probable numbers to estimate the coliform content of the sample and reported in MPN/100mL (Curtis and Koopal, 2012). The maximum result of 365 MPN/100 mL was observed during the July sampling event at the north end of the channel between Upper and Lower Fish Lake (Figure 2). Subsequent testing determined the presence of goose DNA in this sample; no human DNA was detected. Human DNA was detected at the study reference site at the north end of Upper Fish Lake near Mill Creek in July. The location was sampled again in October and no human DNA biomarkers were identified. Mill Creek was sampled to determine if results from the reference site could have been influenced by external loading. Although this sample recorded the second highest *E. coli* concentration of 260 MPN/100 mL, no human or goose DNA was detected. The October sampling event did not yield any detections of human or goose DNA biomarkers.
    b. Results from the bacteria analysis support assessment conclusions, indicating no measurable impact from septic systems. The primary sources of *E. coli* bacteria are now better understood

and are likely the result of animals that inhabit the lake and the surrounding watershed. Human and geese are not likely the primary sources of *E.coli* in the lake.

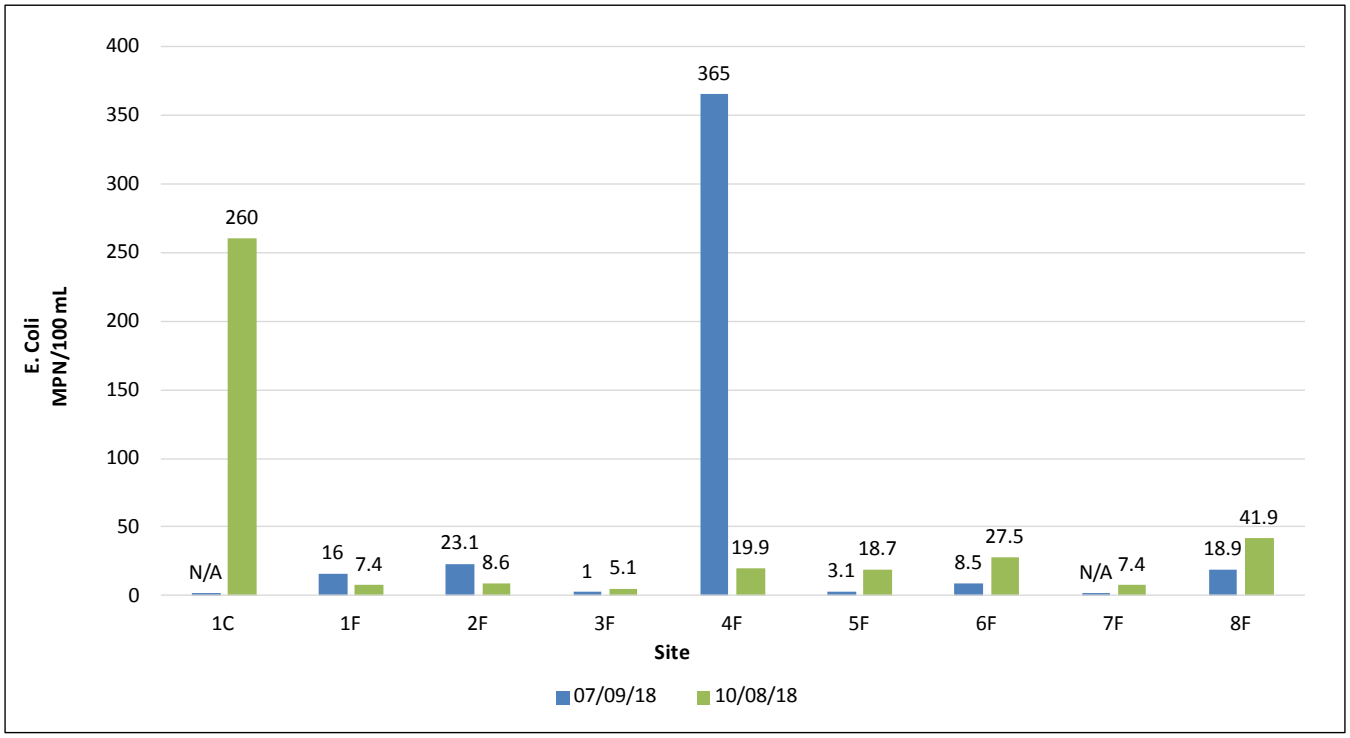

Figure 2 - E.coli Results

4. Septic or sewage effluent can raise the conductivity of the water because of the presence of chloride, phosphate, and nitrate it contributes to the water (Curtis and Koopal, 2012).
    a. Average conductivity over both sampling events was 393.75 µs/cm with the maximum value exceeding 500 µs/cm. Overall conductivity was stable and consistent, also validating study conclusions when combined with other indicators of septic leachate.
5. A substantial baseline of data now exists for the Lake Chain. If desired, similar future studies can be performed in a more cost-effective manner if concerns of septic systems' impacts persist or to track changes over time.

Outlet Lower Fish Lake – Sample Site 1F

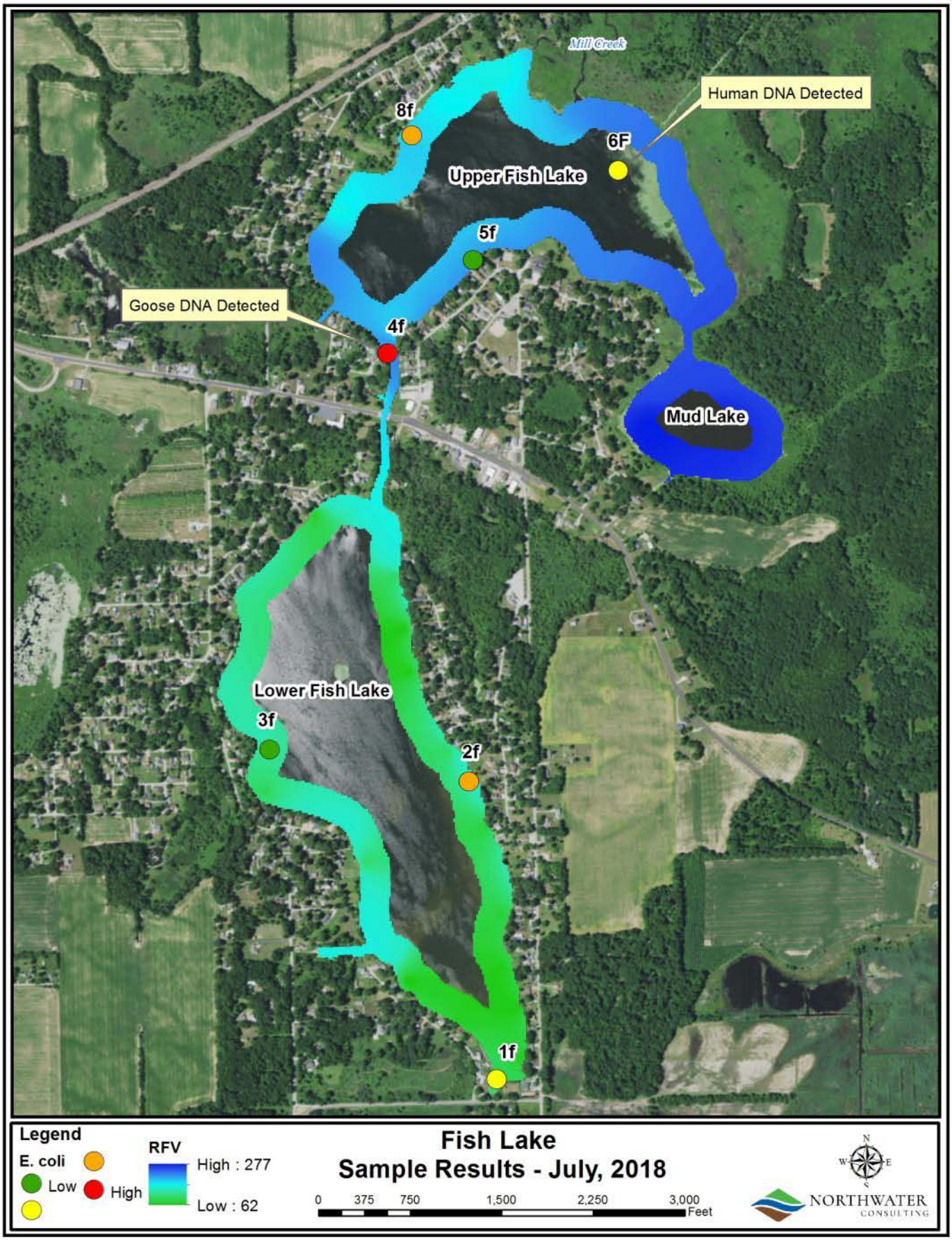

Figure 3 - E.coli & Fluorometry Results - July, 2018

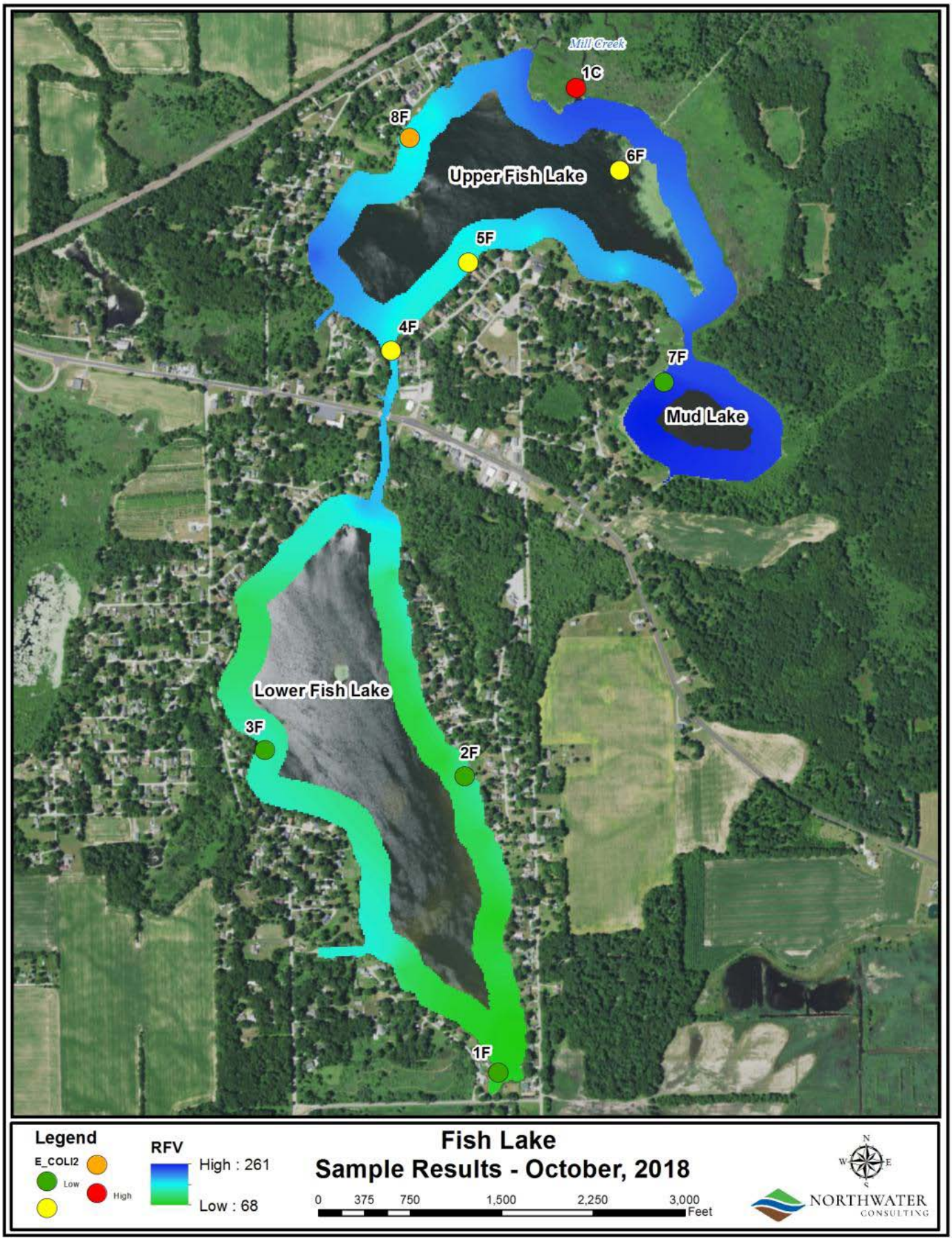

Figure 4 - E.coli & Fluorometry Results - October, 2018

1.0 Introduction

1.1 Purpose

The purpose of this study is to assess the occurrence and extent of septic leachate to the Fish Lake chain. Septic leachate is the liquid that remains after wastewater drains through septic solids. The remaining liquid contains elevated concentrations of bacteria and organic compounds from waste, detergents, and other household materials (Curtis and Koopal, 2012). This concise study was designed to assess whether or not septic systems are negatively impacting the Fish Lake chain and its water quality. The results provide the necessary information to address resident concerns relating to septic system usage surrounding the lake chain.

Existing datasets and prior studies have not indicated septic leachate to be an issue, however, no such studies have directly focused on the subject. This assessment has been prompted by recent resident concerns and the desire to better understand whether or not septic system infrastructure is negatively impacting the water quality of the lake.

This assessment builds on complementary studies in the watershed and lake chain, and applies similar technology and methods as other assessments with similar circumstances.

1.2 Study Area

Fish Lake is located in the east-central section of LaPorte County, Indiana, and is comprised of Upper Fish Lake, Mud Lake (139 acres), and Lower Fish Lake (134 acres). The Fish Lake chain is within Township 36N Range 1W, Sections 17, 20, and 36. The lake is approximately 23 feet at its deepest point in Upper Fish Lake (Figure 5). The 5.7 miles of shoreline is mostly developed with lakeside single family homes, all of which have individual septic systems; the highest density of homes is on Lower Fish Lake. The lake chain is fed through a combination of groundwater and two major tributaries; Mill and Fish Creek, with a watershed area of 6,490 acres. Soils surrounding the lake system are variable; Histosols (highly organic, poorly drained soils) are predominant around Upper Fish Lake and Mud Lake, while the Tracy series (well drained, coarse-loamy, mixed soils) is predominant around Lower Fish Lake.

The 273-acre lake chain provides a variety of recreational uses for area residents and visitors, including fishing and boating. Fish Lake is an unincorporated community surrounding the lakes; the Fish Lake Conservancy District (FLCD) is the entity responsible for lake maintenance and improvements.

The State of Indiana 2018 impaired waters list classifies Upper Fish Lake as Category 5A and impaired for phosphorus; Lower Fish Lake is classified as Category 5B for polychlorinated biphenyl or PCBs (Fish Tissue). These impairments have remained since 2008 and are based on a very limited set of data. The Fish Lake Chain Watershed Diagnostic Study (Northwater Consulting, 2015) results present water quality and sediment data that does not corroborate with the regulatory impairments. Furthermore, the septic leachate assessment sampling program found, with only one exception, no exceedances in state water quality standards. The one exception was for *E. coli* bacteria and it was determined, through DNA testing, that the bacteria originated from geese.

The map depicted in Figure 1 is copyrighted by the Indiana Department of Natural Resources (IDNR). Permission is granted for reprint and usage with credit given to IDNR.

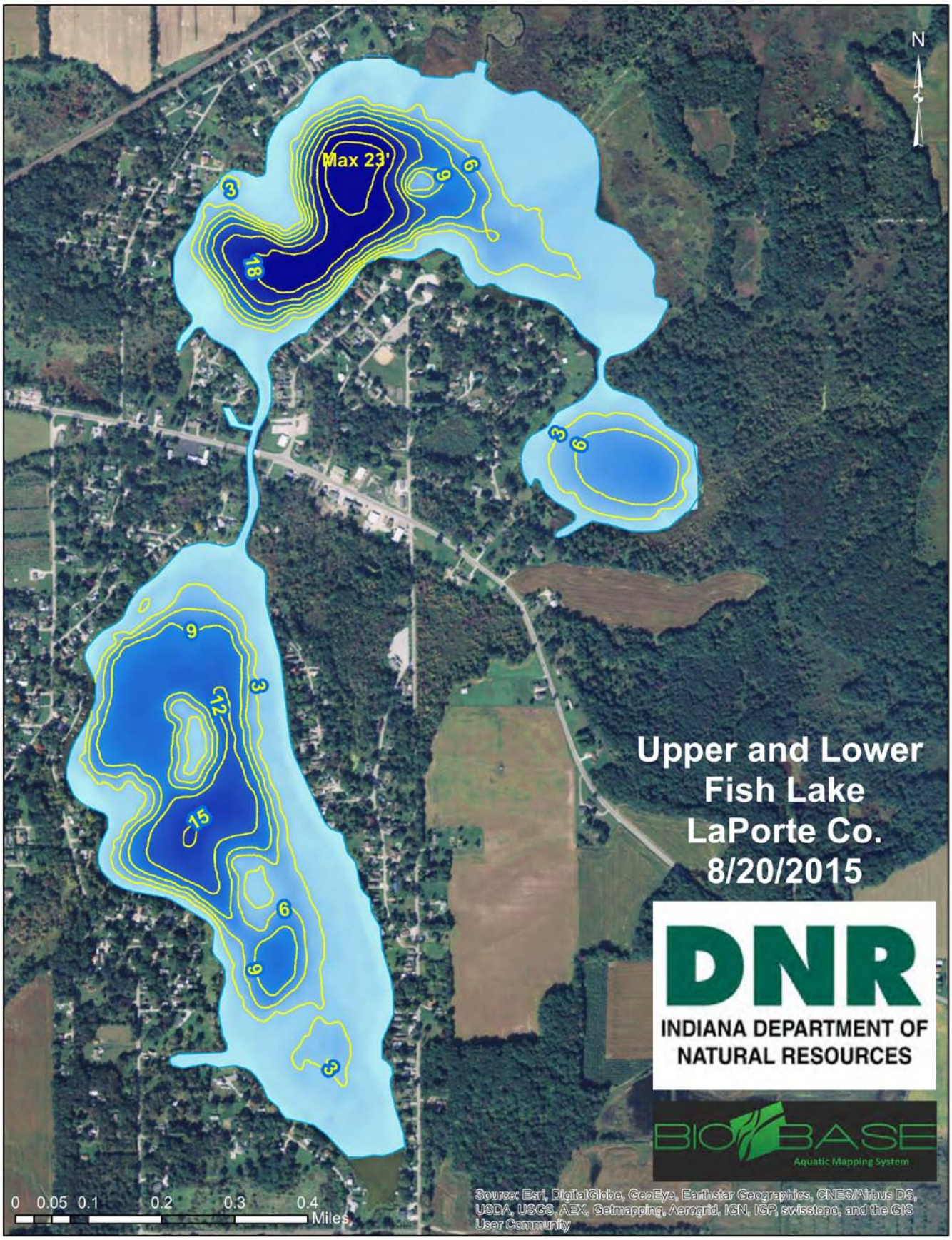

Figure 5 - Fish Lake Chain Bathymetry

1.3 Septic Systems

Septic systems are designed to collect household waste in a tank and then filter wastewater and pollutants through leach fields. Functioning leach fields break down and neutralize contaminants before they enter ground or surface water systems (Curtis and Koopal, 2012). In the United States, septic tank systems are a major residential wastewater treatment option. Almost one in five households in the United States depends on individual or small community septic systems to treat their wastewater (U.S. Environmental Protection Agency, 2018).

Decomposition of waste begins in the septic tank where wastewater separates into layers. The solids that settle to the bottom of the tank are digested by naturally occurring bacteria that transform up to 50% of the solids into liquids and gasses. Once the wastewater leaves the tank and enters the drainfield, further digestion of organic matter occurs. Wastewater is processed chemically, physically, and biologically. Chemical treatment occurs when wastewater comes into contact with soil. Nutrients adsorb soil particles preventing them from moving into groundwater. Physical treatment occurs as wastewater moves through pores in the soil which act as a filter removing particulate contaminants (solids). Finally, biological treatment occurs as microorganisms feed on the wastewater. Every square inch of soil contains millions of naturally occurring beneficial microscopic organisms which complete the wastewater treatment process by killing disease-causing organisms in the sewage and by removing excess nutrients (Hart et al., 2006).

Modern septic systems can be cost-effective options for wastewater treatment; however, poor septic performance or even system failure can arise from a number of scenarios, including improper initial system design, impermeability of soil, improper soil drainage class, improper vertical distance between the absorption field and the water table, and improper slope. For instance, an absorption field must be located below the frost line, within a biologically active zone, and above the seasonal water table. Low permeability of soil may force effluent toward the surface. Shallow or coarse soils may be too permeable, allowing effluent to move laterally or downward too quickly for sufficient decomposition, potentially transporting untreated or improperly treated effluent into groundwater, tributaries, or the lake (Curtis and Koopal, 2012).

Health and surface water quality concerns arising from septic systems can include bacteria and nutrient loading, synthetic detergents, chlorides, and other contaminants.

Lower Fish Lake

2.0 Study Design & Methodology

The study methods and approach are adapted from Curtis and Koopal (2012); this informative publication details an investigation of septic leachate into Whitefish Lake, Montana. Excerpts directly from the Curtis and Koopal report are included in subsequent sections for context and to provide background information necessary to understand each major study element.

2.1 Sampling Frequency, Location, & Techniques

Sampling occurred on two occasions throughout the lake chain system. The first sample event occurred on July 8 and 9, 2018. It was timed to capture the busiest time of the year at Fish Lake where residents and visitors celebrated the July 4 holiday; a time of the year when septic systems would more likely receive greater inputs. The second sampling event occurred on October 7 and 8, 2018; this period was selected to represent a more subdued time of the year where visitors to the lake are at a minimum.

Septic leachates are known to contain elevated concentrations of both organic and inorganic compounds (Canter and Knox, 1985). Water samples were therefore analyzed using a combination of techniques including: fluorometry, dissolved organic carbon (DOC), fluorometry/DOC ratio (F/DOC), *E. coli* enumeration, human and goose DNA biomarkers, conductivity, total dissolved solids (TDS), total phosphorus (TP), and nitrate (N).

Station 3F

A total of 48 screening sites were selected and evaluated for fluorometry in July; 47 (including 1 stream site) were evaluated in October (Figure 6). Water quality (DOC, E. coli, human and goose DNA biomarkers, TP, and N) samples were taken at 8 full analysis locations in July and 9 in October.

Fluorometry was evaluated on the first day and results were used to screen for full analysis water quality stations on the second day. A reference site at the north end of Upper Fish Lake was selected prior to the first sampling event. Based on results from the first sampling event in July, one additional full analysis site was added in Mill Creek to isolate the potential for bacteria from the watershed.

The position of each sample location was recorded using a GPS receiver. At each, metadata and field water quality data was collected which included: sample times, water depth, temperature (°C), conductivity (µs/cm), TDS, pH, dissolved oxygen (DO in mg/L), and DO % saturation. An Oakton PTTestr 35 pen and YSI Pro 1020 handheld meter were used for field water quality data collection and recorded on field data sheets. Conductivity results were translated from TDS values using a conversion factor of 0.7.

Van Dorn Sampler

For all assessment locations, grab samples were taken using an opaque horizontal Van Dorn self closing sampler which was rinsed with 10% hydrochloric acid (HCL) solution prior to each trip and rinsed once with sample site water at each sample location. Where water quality samples were taken, the Van Dorn sampler was rinsed with 10% HCL solution at each station. All samples were collected at a maximum of one foot above the lake sediments or bottom.

Fluorometric values were analyzed at each site with an Aquafluor™ portable Fluorometer (Turner Designs, Sunnyvale, California) set by the manufacturer to detect the specific light spectrum emitted from long wavelength Optical Brightener Agents (OBAs) found in domestic cleaning products. Fluorometric calibration was conducted using a solution of 1% household detergent containing a known whitener compound (Tide, Procter and Gamble, Cincinnati, Ohio), and a deionized water base. Fluorometer results are reported in Relative Fluorescent Values (RFVs) (Curtis and Koopal, 2012).

Cuvettes (3.5 mL) were filled using a bulb syringe dipped into a single water sample. Disposable cuvettes were used one time at each sample site; the bulb syringe was rinsed with sample water at each site. *E. coli*, DOC, TP, and N water samples were collected using sealed laboratory bottles, iced after collection, and delivered within 12 hours to PDC Laboratories in Springfield, Illinois. A subset of water sample duplicates were collected at two locations during both sample events and provided to PDC Laboratories for quality assurance and quality control (QA/QC) purposes.

Water samples for goose and human Bacteroidetes DNA biomarkers were collected at 7 locations during the first sample event and 9 on the second using the Van Dorn sampler and transferred to sealed 1,000 mL bottles provided by Source Molecular. Samples were placed on ice and sent via overnight courier to the Source Molecular laboratory in Florida. Samples were frozen and stored by the laboratory until *E. coli* results were received. Human and goose Bacteroidetes DNA biomarker presence/absence analysis was prioritized at select stations based on a review of *E. coli* and RFV results and the spatial representation of the lake system. Due to the relative consistency in fluorometric values across the lakes, these data were not strictly used to guide the location of water quality sampling for bacteria and other parameters. All laboratory reports are included in Appendix A.

Sample Site 8F

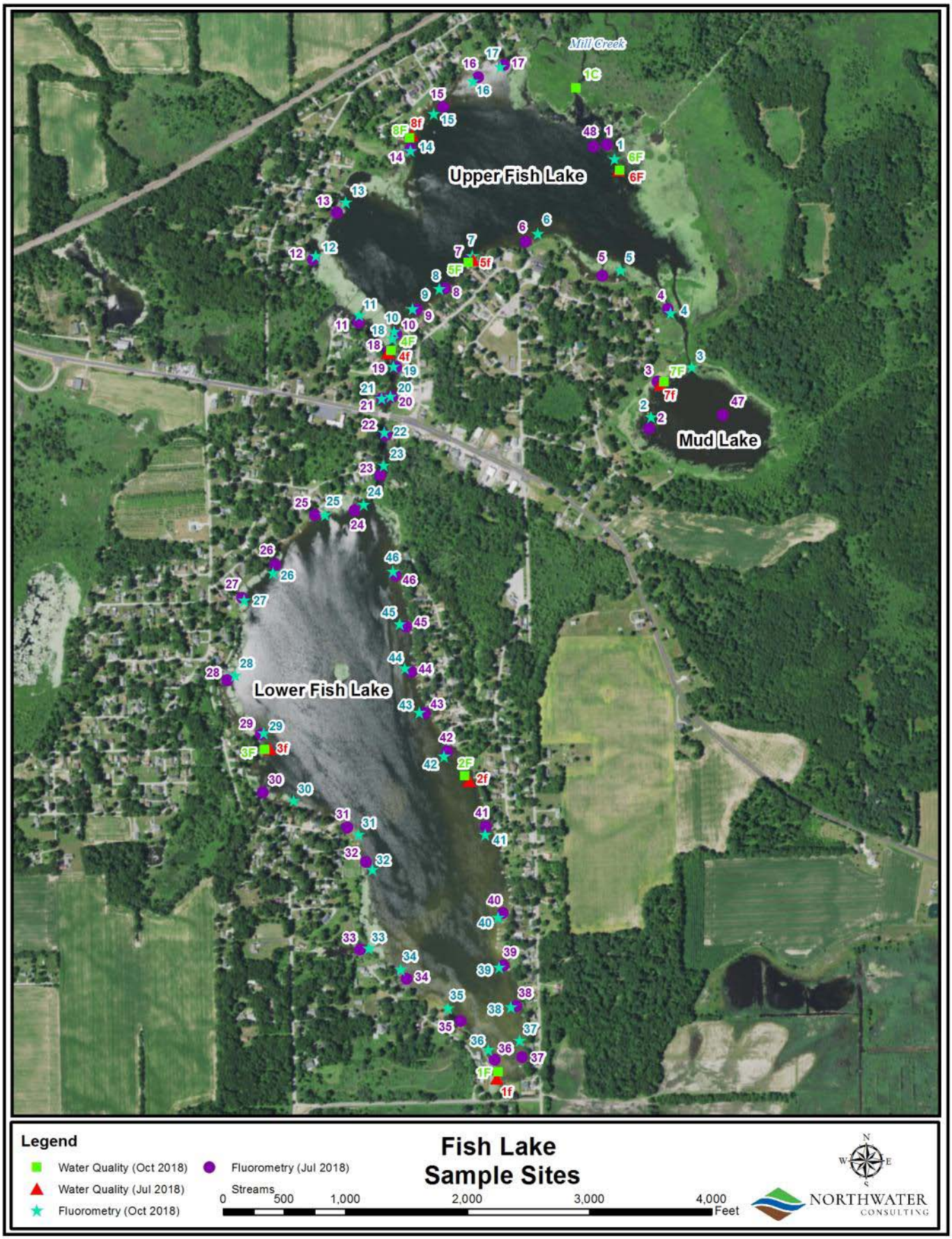

Figure 6 – Fish Lake Chain Sample sites

2.2 Study Elements & Analytes

This section provides background information on each element of the study and a description of those analytes used to draw conclusions.

Much of the narrative below has been summarized directly from Curtis and Koopal (2012) and they are credited for clearly and concisely describing the topics.

2.2.1 Fluorometry (Relative Fluorescent Values)

Optical Brightener Agents found in domestic cleaning products are activated by near-ultraviolet (UV) range wavelengths and then emit light in the blue range. Studies have shown that wastewater effluent contains near-UV fluorescent organics from OBAs (Kerfoot and Brainard, 1978, Kerfoot & Skinner, 1981, Hagedorn et al., 2005, Hartel et al., 2007). While fluorometric readings alone may indicate the presence of OBAs, they can also indicate the presence of naturally occurring DOC from humic and fulvic compounds. The major organic components of soil (humus) are made up of substances produced by the biodegradation of organic matter. These humic compounds produce fluorescence, but generally at much lower RFVs than OBAs. The lower fluorescent value is a result of the concentration of materials being lower in humic compounds than in OBAs (Thurman and Malcolm, 1981; Stanford et al., 1985).

2.2.2 Dissolved Organic Carbon (DOC)

Dissolved organic content describes dissolved material found in water from organic matter such as decomposed plant matter. DOC is known to emit a similar, though generally far lower magnitude light spectrum as whitener compounds detected by fluorometry and, therefore, was collected and measured separately to describe dissolved material. DOC in streams, seeps, and areas with heavy influences of organic matter can fluoresce in the higher output ranges. DOC results were therefore also used as a component in developing an F/DOC ratio. DOC is measured in mg/L.

2.2.3 Fluorometric to Dissolved Organic Carbon Ratio (F/DOC)

This technique involves using a similar F/DOC ratio as developed by Jourdonnais & Stanford (1985). The F/DOC ratio was developed in an effort to distinguish optical brightener-emitted fluorescent compounds from fluorescent compounds naturally present in uncontaminated water measured as DOC. Using this F/DOC ratio method, the background fluorescence from DOC can be reduced from the final F/DOC values. F/DOC is a more robust measurement than fluorometry alone, particularly in streams and seeps where DOC is typically elevated.

Curtis and Koopal (2012) determined that an F/DOC ratio in excess of 22.7 would indicate septic leachate.

2.2.4 Conductivity

Conductivity is a measure of the ability of water to pass an electrical current, and it is affected by the presence of inorganic dissolved solids. Conductivity in seeps, streams, and rivers is influenced primarily by the bedrock geology and mineral composition of the sediments through which the water flows. Water that flows through more inert materials that do not dissolve into ionic components will have a lower conductivity. Water that flows through soils with compounds that are ionized have a higher conductivity. Septic or sewage effluent would raise

the conductivity of the water because of the presence of chloride, phosphate, and nitrate it contributes to the water.

2.2.5 Escherichia coli (E. coli) Enumeration

E. coli are bacteria found in human and animal feces. Because *E. coli* are generally not found growing and reproducing in the environment, they are considered to be the best species of coliform bacteria to indicate warm-blooded fecal pollution and the possible presence of pathogenic (disease-causing) bacteria and viruses. The United States Environmental Protection Agency (USEPA) recommends *E. coli* as the best indicator of health risk from sewage contamination in recreational waters (USEPA, 1986). *E. coli* is measured using a table of most probable numbers to estimate the coliform content of the sample and reported in MPN/100mL (Curtis and Koopal, 2012).

2.2.6 Human & Goose Bacteroidetes ID

Three tests were performed by Source Molecular on all submitted samples: Human Bacteroidetes ID: Dorei, Human Bacteroidetes ID: EPA and Goose Bacteroidetes ID: Target 1. Each test is described below (Source Molecular, 2018).

The Human Bacteroidetes ID™ Species*: B. dorei* service targets the species *Bacteroides dorei*. *B. dorei* is an anaerobe that is frequently shed from the gastrointestinal tract and isolated from human feces worldwide. It is a newly discovered species that is widely distributed in the United States. The human-associated marker DNA sequence is located on the 16S rRNA gene of *B. dorei*. The marker is the microbial source tracking (MST) marker of choice for detecting human fecal pollution due to its exceptional sensitivity and specificity. Internal validations have been conducted on hundreds of sewage, septic, human, and animal host fecal samples collected from throughout the U.S. and archived in the Source Molecular fecal bank. The marker has also been evaluated in both inland and coastal waters. A recent, comprehensive, multi-laboratory MST method evaluation study, exploring the performance of current MST methods, concluded the *B. dorei qPCR* assay to be the top performing human-associated assay amongst those tested. The success and consistency of this marker in numerous studies around the world makes the Human Bacteroidetes ID™ Species*: B. dorei* service the primary service for identifying human fecal pollution at Source Molecular.

The Human Bacteroidetes ID™: EPA Developed Assay service is designed around the principle that fecal *Bacteroidales*-like bacteria are found in large quantities in feces of warm-blooded animals. Furthermore, certain strains have been shown to be associated with humans. As such, these bacterial strains can be used as indicators of human fecal contamination. An advantage of the Human Bacteroidetes ID™ service is that the entire portion of water sampled is filtered to concentrate bacteria. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates. This is an advantage for highly contaminated water systems with, potentially, multiple sources of fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique *B. dorei* DNA sequence. Through a heating process called thermal cycling, the double- stranded DNA is denatured, hybridized to the complementary primers and amplified to create as many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA

fragment that is specific to the *B. dorei* DNA sequence, then billions of copies of the DNA fragment will be available, detected and quantified.

The Canada Goose Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals. Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in Canada geese. Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in Canada geese. As such, these bacterial strains can be used as indicators of Canada geese fecal contamination. One of the advantages of the Canada Goose Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with, potentially, multiple sources of fecal contamination.

Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected. Through a heating process called thermal cycling, the double-stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times, ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available, detected and quantified

2.2.7 Nutrients: Total Phosphorus & Nitrate

Phosphorus - Phosphorus is a major cellular component of organisms. Phosphorus can be found in dissolved and sediment-bound forms. However, phosphorus is often locked up in living biota, primarily algae. In the watershed, phosphorus is found in fertilizers and in human and animal wastes. The availability of phosphorus determines the growth and production of algae and makes it the limiting nutrient in the system.

Nitrate (NO3) – Nitrate is a compound of nitrogen and oxygen which occurs in trace quantities in groundwater and sometimes reaches high levels in surface water. Nitrate travels easily through soil carried by water into surface waterbodies and groundwater. Sources of nitrates include wastewater treatment plants, runoff from fertilized lawns and cropland, failing on-site septic systems, runoff from animal manure storage areas, and industrial discharges that contain corrosion inhibitors. Nitrates from land sources end up in rivers and streams more quickly than other nutrients like phosphorus. This is because they dissolve in water more readily than phosphates, which have an attraction for soil particles. As a result, nitrates serve as a better indicator of the possibility of a source of sewage or manure pollution during dry weather (USEPA, 2018).

Sample Site 6F – Reference Site

3.0 Results

Results from the 2018 assessment are presented in this section and organized by sample date and analysis parameter.

3.1 July 2018 Event

The first sampling event occurred July 7 - 8, 2018, following the 4th of July events at Fish Lake. Fluorometry was collected at 48 screening sites throughout the day between 10:15am and 2:33pm on July 7 (Table 1). Conductivity, DO, pH, TDS, and water temperature were also measured at each site.

Water chemistry sampling occurred on July 8 (Table 2). Samples were collected at 8 full analysis stations and sent to PDC Laboratories for N, TP, *E. coli*, and DOC analysis. Upon returning to the sites, field water quality was collected again which included: fluorometry, conductivity, DO, pH, TDS, and water temperature. Only 7 samples were iced and sent via overnight courier to Source Molecular in Florida where they were flash frozen and stored until analysis; the Mud Lake site was excluded due to its mostly undeveloped shoreline and coverage from the reference site located in close proximity. Based on an analysis of water quality results from PDC Laboratories, Source Molecular Laboratories was then instructed to run human and goose DNA analysis on 5 of the 7 samples.

3.1.1 Fluorometry

Fluorometric values were measured and recorded immediately on the survey boat. Of the 48 screening sites, the highest reading of the study was 280 RFV at site 2 in Mud Lake. Comparatively high values were also recorded at the outlet of Mud Lake and at the reference site located at the north end of Upper Fish Lake near Mill Creek.

The three lowest RFV values all occurred in Lower Fish Lake, with the lowest value of 62 RFV at screening site 34, near the outlet of Lower Fish Lake. The average RFV value across all sites was 91 and 42% of sites exceeded the average. Overall, RFV values were consistent throughout; higher recorded values were associated with more organically enriched locations in the lake confirmed by DOC results described in Section 3.1.2 below. No one Fluorometric measurement indicated a direct concern for septic leachate and the consistency of all results further validate the conclusion that no measureable impacts to lake water quality are occurring.

3.1.2 Dissolved Organic Carbon

DOC samples were collected at all 8 full analysis stations and analyzed by PDC Laboratories. The highest reading of 21.1 mg/L was recorded at 7F (Mud Lake); the second highest reading of 9.64 was recorded at 6F (reference site – Upper Fish Lake). The elevated DOC values at these stations correlate with the high flourometric values measured at the same locations. The average DOC value for all 8 stations was 10 mg/L with the lowest value of 8.35 mg/L recoded at both 5F (east side Upper Fish Lake) and 8F (west side Upper Fish Lake).

3.1.3 F/DOC Ratio

An F/DOC ratio was calculated using fluorometric and DOC results. The two highest F/DOC ratios occurred at full analysis station 6F (13.89 mg/L at reference site – Upper Fish Lake) and 4F (13.1 mg/L at upper end of the channel between Upper and Lower Fish Lake). The lowest ratio was 9.1 at 1F (outlet of Lower Fish Lake). The average ratio for all 8 full analysis stations was 11.61 mg/L. These results also further validate the assessment

conclusions as all results are below the 22.7 threshold that would indicate septic leachate as described by Curtis and Koopal (2012).

3.1.4 *E. coli* Enumeration

Fecal coliform bacteria were analyzed at all full analysis stations except for 7F (Mud Lake). *E. coli* results ranged from 1 MPN/100 mL at 3F (west side Lower Fish Lake) to a high of 365 MPN/100 mL at 4F (upper end of the channel between Upper and Lower Fish Lake). All other full analysis stations recorded values below 24 MPN/100 mL. With the one exception at 4F, *E. coli* concentrations were found to be well below the minimum state standard of 235 MPN/100 mL across all stations. Overall, results did not indicate any concern with bacteria in the Fish Lake chain at the time of sampling; coupled with the bacteria DNA analysis, this supports the conclusion that septic systems are not having a measurable impact on lake water quality.

3.1.5 Human & Goose DNA Biomarkers

A selection of water samples were analyzed for human and goose DNA biomarkers. Of the 5 samples analyzed, there was only one affirmative human DNA result, and one goose DNA biomarker result. The laboratory noted low concentrations of biomarkers in both cases. The positive goose DNA result was found at full analysis station 4F (upper end of the channel between Upper and Lower Fish Lake) and corresponds with the high *E. coli* result at that station. The positive human DNA biomarker was found at station 6F (reference site – Upper Fish Lake near Mill Creek). As a result of this, a stream station was established in Mill Creek in an attempt to isolate contamination that might be originating from the watershed; Mill Creek was evaluated during the second sampling event in October.

3.1.6 Conductivity

Conductivity of the water was generally stable across all screening sites, with a slightly elevated value in the channel between Upper and Lower Fish Lake and some lower values in Mud Lake. The highest recorded value of 521 µs/cm occurred at site 21. Average conductivity across all sites was 397 µs/cm. Conductivity can be used to indicate concentrations of dissolved solids by using a correction factor. Conductivity results did not indicate any concerns related to septic leachate and further validate the conclusions presented in this report.

3.1.7 Nutrients: Nitrate & Total Phosphorus

Samples were collected at 6 of the 8 full analysis stations and analyzed for total phosphorus and nitrate. Stations 5F (east side Upper Fish Lake) and 7F (Mud Lake) were not analyzed for nutrients. All stations returned undetectable results or results below the laboratory detection limits.

Table 1 – July Screening Site Results

Site #	Location	Fluorometric values (RFV)	Temp °C	pH	Conductivity (µs/cm)	TDS (mg/L)	DO (mg/L)	DO % Saturation
1	Upper Fish Lake (reference site)	122	26.6	88	426	298	7.73	94.4
2	Mud Lake	280	27.1	8.4	240	168	2.09	26.9
3	Mud Lake	169	27.1	8.1	240	168	4.5	57
4	Mud Lake Channel	128	25.8	8.6	424	297	10.06	124.4

Site #	Location	Fluorometric values (RFV)	Temp °C	pH	Conductivity (µs/cm)	TDS (mg/L)	DO (mg/L)	DO % Saturation
5	Upper Fish Lake	106	26.9	8.6	403	282	9.65	121
6	Upper Fish Lake	97	27.6	8.7	416	291	8.62	110.6
7	Upper Fish Lake	97	28	8.6	419	293	7.34	94.9
8	Upper Fish Lake	97	27.3	8.6	423	296	7.54	96.4
9	Upper Fish Lake	96	27.8	8.6	423	296	7.59	99.5
10	Upper Fish Lake	92	27.8	8.5	424	297	7.78	99.9
11	Upper Fish Lake	96	27.5	8.3	420	294	6	76
12	Upper Fish Lake	105	26.5	8.2	424	297	6.11	80.7
13	Upper Fish Lake	89	27.9	8.9	426	298	7.86	100.3
14	Upper Fish Lake	94	27.2	8.5	424	297	7.3	84.6
15	Upper Fish Lake	91	28	8.6	414	290	9.05	115.2
16	Upper Fish Lake	90	28.1	8.4	424	297	7.45	95.8
17	Upper Fish Lake	88	27.9	8.8	419	293	7.32	93.8
18	Channel between Upper and Lower	103	28.8	8.4	421	295	8.48	109.3
19	Channel between Upper and Lower	101	28.9	8.5	421	295	7.7	100.1
20	Channel between Upper and Lower	96	28.7	8.5	429	300	6.93	89.5
21	Channel between Upper and Lower	91	28.8	8.5	521	365	7.12	92.6
22	Channel between Upper and Lower	93	28.7	8.4	430	301	7.07	91
23	Channel between Upper and Lower	90	27.3	8.5	430	301	7.01	91.2
24	Lower Fish Lake	68	29.2	8.6	424	297	7.32	95.9
25	Lower Fish Lake	63	29.6	8.8	394	276	8.5	111.1
26	Lower Fish Lake	67	29	8.7	390	273	7.68	100.9
27	Lower Fish Lake	66	29.7	8.8	390	273	8.26	109.7
28	Lower Fish Lake	67	29	8.6	393	275	7.42	100
29	Lower Fish Lake	68	29.5	8.7	390	273	8.9	116.6
30	Lower Fish Lake	64	31.1	8.7	391	274	8.27	107.4
31	Lower Fish Lake	71	28.8	8.7	383	268	8.24	107.8
32	Lower Fish Lake	65	29.6	8.7	390	273	8.04	107.1
33	Lower Fish Lake	82	28	8.4	396	277	6.7	89.9
34	Lower Fish Lake	62	29.2	8.6	387	271	7.31	95.7
35	Lower Fish Lake	63	30	8.7	376	263	8.06	105.5
36	Lower Fish Lake	64	28.6	8.6	380	266	7.96	105
37	Lower Fish Lake	63	28.8	8.7	364	255	8.17	106.8
38	Lower Fish Lake	62	29.2	8.5	367	257	8.4	109.6
39	Lower Fish Lake	63	29.4	8.8	386	270	7.34	95.4
40	Lower Fish Lake	63	29.3	8.7	390	273	7.86	103.8
41	Lower Fish Lake	63	29.4	8.8	399	279	7.91	105.4
42	Lower Fish Lake	65	30	8.6	397	278	7.98	105.3

Site #	Location	Fluorometric values (RFV)	Temp °C	pH	Conductivity (µs/cm)	TDS (mg/L)	DO (mg/L)	DO % Saturation
43	Lower Fish Lake	66	29.2	8.8	397	278	8.48	111.6
44	Lower Fish Lake	62	30	8.6	391	274	7.34	97
45	Lower Fish Lake	62	29.7	8.8	390	273	7.57	100.4
46	Lower Fish Lake	65	30.2	8.6	396	277	7.76	108.7
47	Center Mud Lake	239	27.2	8.2	239	167	3.96	49.1
48	Upper Fish Lake (near reference site)	89	29.6	8.4	423	296	8.12	105.9

Table 2 – July Full Analysis Site Results

Site #	Location	RFV values	Temp °C	pH	Cond (µs/cm)	TDS (mg/L)	DO (mg/L)	DO % Sat	N[1,3] (mg/L)	TP[2,3] (mg/L)	DOC (mg/L)	E. Coli (MPN/ 100 mL)	Human DNA	Goose DNA[3]	F/DOC Ratio
1F	Outlet Lower Fish Lake	82	26.4	8.6	310	217	7.12	88	U	U	8.92	16	U	U	9.18
2F	East Side Lower Fish Lake	88	27.5	8.3	386	270	6.25	79	U	U	8.96	23.1	U	U	9.85
3F	West Side Lower Fish Lake	84	28	8.3	403	282	7.07	93.6	U	U	8.72	1	-	-	9.68
4F	Upper End of Channel Upper/ Lower Fish Lake	111	26.3	8.2	426	298	5.89	79.1	U	U	8.44	365	DNQ	Detect low conc.	13.14
5F	East Side Upper Fish Lake	106	27.1	8.3	429	300	7.18	94	-	-	8.35	3.1	-	-	12.66
6F	North End Upper Fish Lake (reference site)	134	27.2	8.3	424	297	7.57	95.4	U	U	9.64	8.5	DNQ	U	13.89
7F	Mud Lake	261	27.2	7.3	269	188	3.2	38.8	-	-	21.1	-	-	-	12.36
8F	West Side Upper Fish Lake	101	27	8.3	424	297	8.14	102.7	U	U	8.35	18.9	U	U	12.11

1 – Nitrate, 2 – Total Phosphorus, ND – Not Detected, DNQ – Detected, not quantified

3.2 October 2018 Event

The second sampling event occurred October 7 - 8, 2018, following several days of light rain in the watershed. Fluorometry was collected at 47 screening sites throughout the day between 9:41am and 12:12pm on October 7 (Table 3). Conductivity, DO, pH, TDS, and water temperature were also measured at each site.

Water chemistry sampling occurred on October (Table 4). Samples were collected at 9 full analysis stations, including one stream site on Mill Creek immediately prior to entering the lake. All samples were sent to PDC laboratories for N, TP, *E. coli*, and DOC analysis. Upon returning to the sites, field water quality was collected again which included: Fluorometry, conductivity, DO, pH, TDS, and water temperature. Nine samples were iced and sent via overnight courier to Source Molecular in Florida where they were flash frozen and stored until

analysis. Based on an analysis of water quality results from PDC Laboratories, Source Molecular Laboratories was then instructed to run human and goose DNA analysis on 8 of the 9 samples.

3.2.1 Fluorometry

Fluorometric values were measured and recorded immediately on the survey boat. Of the 47 screening sites, the highest reading of the study was 263 RFV at site 2 in Mud Lake. Comparatively high values were also recorded at the outlet of Mud Lake and at the steam site in Mill Creek prior to entering Upper Fish Lake.

The lowest value of 68 was recorded at site 37, near the outlet of Lower Fish Lake. The second and third lowest values were also recorded in Lower Fish Lake. The average RFV across all sites was 96 and 34% of sites exceeded the average.

3.2.2 Dissolved Organic Carbon

Dissolved organic content samples were taken at all 9 full analysis stations and analyzed by PDC Laboratories. The highest reading of 28 mg/L was recorded at 4F (upper end of the channel between Upper and Lower Fish Lake); the second highest reading of 19 mg/L was recorded at 7F (Mud Lake). The elevated DOC values at these stations correlate with the high fluorometric values measured at the same locations. The average DOC value for all 9 stations was 10.18 mg/L with the lowest value of 5.7 recorded at 6F (reference site – Upper Fish Lake).

3.2.3 F/DOC Ratio

An F/DOC ratio was calculated using fluorometric and DOC results. The two highest overall F/DOC ratios (23.92 and 18.89) occurred at full analysis station 1C (Mill Creek) and 6F (reference site – Upper Fish Lake), respectively. The lowest ratio was 3.41 occurred at 4F (upper end of the channel between Upper and Lower Fish Lake). The average ratio for all 9 full analysis stations was 13.34. These results also further validate the assessment conclusions as all results from samples taken in the lake are below the 22.7 threshold that would indicate septic leachate as described by Curtis and Koopal (2012). The result from Mill Creek exceeds the threshold indicating the potential for septic leachate from external sources. This is not conclusive due to a lack of validation from other indicators, specifically, the lack of human DNA biomarkers; further investigation is required to make a determination on septic contamination from the watershed.

3.2.4 *E. coli* Enumeration

Fecal coliform bacteria were analyzed at all full analysis stations. *E. coli* results ranged from 5.1 MPN/100 mL at 3F (west side Lower Fish Lake) to a high of 260 MPN/100 mL at 1C (Mill Creek). All other full analysis stations recorded values below 42 MPN/100 mL.

3.2.5 Human & Goose DNA Biomarkers

A selection of water samples were analyzed and reported by Source Molecular Laboratories in Florida. Of the 8 full analysis stations analyzed, no affirmative human or goose DNA biomarker results were detected.

3.2.6 Conductivity

Conductivity of the water was generally stable across all screening sites, with a slightly elevated value in the channel between Upper and Lower Fish Lake and some lower values in Mud Lake. The highest recorded value of

440 µs/cm occurred at site 21. Average conductivity across all sites was 405 µs/cm. Conductivity can be used to indicate concentrations of dissolved solids by using a correction factor.

3.2.7 Nutrients: Nitrate & Total Phosphorus

Samples were collected at all 9 full analysis stations and analyzed for TP and N. Only one station returned a detectable result – 0.744 mg/L of N at 1C (Mill Creek). All other stations returned undetectable results.

Table 3 - October 7th Sample Results

Site #	Location	Fluorometric values (RFV)	Temp °C	pH	Conductivity (µs/cm)	TDS (mg/L)	DO (mg/L)	DO % Saturation
1	Upper Fish Lake (reference site)	132	18	8.3	421	295	5.5	55.1
2	Mud Lake	264	18.4	8.4	293	205	6.33	67.5
3	Mud Lake	171	17.9	8.5	360	252	6.97	76.3
4	Mud Lake	114	18	8.6	416	291	6.2	64.9
5	Upper Fish Lake	96	18.3	8.5	423	296	7.33	78.2
6	Upper Fish Lake	95	18.5	8.7	429	300	7.83	83.5
7	Upper Fish Lake	93	18.5	8.7	433	303	7.58	81.4
8	Upper Fish Lake	93	18.5	8.7	434	304	8.09	86.3
9	Upper Fish Lake	93	18.6	8.8	434	304	8.01	85.5
10	Upper Fish Lake	94	18.6	8.8	433	303	7.73	82.8
11	Upper Fish Lake	102	18.5	8.4	433	303	7.25	76.5
12	Upper Fish Lake	138	18	8.6	431	302	7.4	78.6
13	Upper Fish Lake	98	18.3	8.8	436	305	7.83	83.8
14	Upper Fish Lake	94	18.3	8.7	436	305	7.9	84
15	Upper Fish Lake	94	18.4	8.7	434	304	7.77	82.7
16	Upper Fish Lake	106	18.1	8.7	434	304	7.34	77.8
17	Upper Fish Lake	111	18.2	8.7	427	299	6.6	70
18	Channel between Upper and Lower	95	18.3	8.7	434	304	7.78	83.1
19	Channel between Upper and Lower	97	18.3	8.8	436	305	7.85	83.7
20	Channel between Upper and Lower	97	18.3	8.7	436	305	7.5	80.2
21	Channel between Upper and Lower	106	18.3	8.7	440	308	6.5	69.8
22	Channel between Upper and Lower	101	18.3	8.8	439	307	7.83	83.4
23	Channel between Upper and Lower	100	18.3	8.7	437	306	7.69	81.9
24	Lower Fish Lake	104	18.3	8.7	436	305	6.38	68.7
25	Lower Fish Lake	82	18.6	8.7	404	283	6.64	71.3
26	Lower Fish Lake	78	18.7	8.9	393	275	7.65	83.7
27	Lower Fish Lake	75	18.6	8.8	391	274	7.86	84.7
28	Lower Fish Lake	76	18.8	8.8	389	272	7.9	85.1
29	Lower Fish Lake	79	18.8	8.8	389	272	7.6	81.9

Site #	Location	Fluorometric values (RFV)	Temp °C	pH	Conductivity (µs/cm)	TDS (mg/L)	DO (mg/L)	DO % Saturation
30	Lower Fish Lake	79	18.8	8.8	386	270	8	86.3
31	Lower Fish Lake	78	18.8	8.8	387	271	8.09	87.1
32	Lower Fish Lake	79	18.8	8.9	384	269	8.28	88.8
33	Lower Fish Lake	88	18.4	8.6	381	267	7.11	76.3
34	Lower Fish Lake	74	18.5	8.8	381	267	8.03	85.5
35	Lower Fish Lake	72	18.2	8.6	381	267	7.52	80.2
36	Lower Fish Lake	70	18.3	8.8	377	264	8.07	85.6
37	Lower Fish Lake	68	18.4	8.8	369	258	7.93	84.1
38	Lower Fish Lake	70	18.5	8.8	371	260	8.08	86.5
39	Lower Fish Lake	74	18.5	8.8	376	263	7.55	80.6
40	Lower Fish Lake	71	18.7	8.8	379	265	7.97	85.4
41	Lower Fish Lake	74	18.7	8.8	377	264	8.12	87.4
42	Lower Fish Lake	73	18.8	8.8	383	268	7.55	81.3
43	Lower Fish Lake	73	18.9	8.8	389	272	7.6	82.2
44	Lower Fish Lake	74	18.9	8.8	387	271	7.98	86
45	Lower Fish Lake	76	18.7	8.8	390	273	7.83	84.5
46	Lower Fish Lake	75	18.8	8.8	391	274	8.4	90.6
1C	Mill Creek	183	16.7	8.6	421	295	4.56	47

Table 4 - October 8th Sample Results

Site #	Location	RFV values	Temp °C	pH	Cond (µs/ cm)	TDS (mg/L)	DO (mg/L)	DO % Sat	N[1,3] (mg/L)	TP[2,3] (mg/L)	DOC (mg/L)	E. Coli (MPN/ 100 mL)	Human DNA	Goose DNA[3]	F/DOC Ratio
1C	Mill Creek	179.4	18.5	7.8	4.43	3.1	4.41	46.5	0.74	U	7.5	260	U	U	23.92
1F	Outlet Lower Fish Lake	70.82	18.4	8.6	382.86	268	6.5	69.5	U	U	6.7	7.4	U	U	10.57
2F	East Side Lower Fish Lake	73.54	18.7	7.3	420.00	294	6.1	65.5	U	U	6.7	8.6	U	U	10.98
3F	West Side Lower Fish Lake	77.57	18.7	8.6	391.43	274	6.89	73.6	U	U	6.6	5.1	U	U	11.75
4F	Upper End of Channel Upper/ Lower Fish Lake	95.48	18.5	8.5	437.14	306	7.07	75.6	U	U	28	19.9	U	U	3.41
5F	East Side Upper Fish Lake	95.03	18.6	8.3	444.29	311	7.46	79.5	U	U	5.8	18.7	U	U	16.38
6F	North End Upper Fish Lake (reference site)	107.7	18.6	8.6	435.71	305	6.65	70	U	U	5.7	27.5	U	U	18.89
7F	Mud Lake	255.4	19.3	8.3	294.29	206	7.3	79	U	U	19	7.4	U	U	13.44
8F	West Side Upper Fish Lake	88.82	19.2	7.7	458.57	321	7.4	79.8	U	U	7.7	41.9	U	U	11.54

4.0 Conclusions

The purpose of the study is to assess the occurrence and extent of septic leachate to the Fish Lake chain. The following conclusions are based on the results of this assessment which included two sampling events.

1. Screening the lake for OBAs did not identify any hotspots to suggest discrete septic leaching to the lake. Fluorometry results maintained a stable and consistent pattern during both sampling events which did not correlate to *E. coli* or DNA biomarkers. Higher RFV results were observed in areas with increased organic enrichment (i.e., Mud Lake), and are not believed to be associated with anthropogenic sources.
2. The screening for OBAs and analysis of septic pollution water quality indicators suggest that any inputs to the lake are a diffuse and background condition and do not correlate to measureable impacts to lake water quality. There were no correlations between bacteria concentrations, fluorometry results, and other indicators.
3. The one positive sample for human bacteria occurred in July at the reference site at the north end of Upper Fish Lake near the outlet of Mill Creek. No homes are present along the shoreline in this area, and it is possible the source of human bacteria is from the Mill Creek watershed. Mill Creek was subsequently sampled during the October event, and although *E. coli* bacteria was high, neither human or goose DNA was detected at that site or at the other 7 full analysis sites in the lake. Considering the concentration from the July event was very low, and there was only one detection out of 13 samples over two sampling events, we do not believe that human bacterial contamination to be an issue for the lake.
4. The one positive sample for goose bacteria occurred in July at the northern end of the channel between Upper and Lower Fish Lake, this location corresponded to a high *E. coli* bacteria concentration. Widespread bacterial contamination from geese does not appear to be an issue for the Lake based on the assessment results.
5. The primary sources of *E. coli* bacteria are better understood based on this assessment, and are likely the result of animals that inhabit the lake and the surrounding watershed. We determined that human and geese are not likely the primary sources of *E.coli* in the lake.
6. There is no indication that widespread, concentrated, or localized human bacterial contamination is occurring from septic leachate in the Fish Lake Chain. Treated septic leachate is likely entering the lake through shallow groundwater flow, however, there is no evidence of any measurable impacts to water quality.

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APPENDIX A: Laboratory Results & Chain of Custody Forms

PDC Laboratories, Inc.

Monday, July 16, 2018

Jeff Boeckler

Northwater Consulting
960 Clocktower Drive, Suite F
Springfield, IL 62704

TEL: (217) 725-3181

FAX: NA

RE: Fish Lake PDC WO: 18G0095

PDC Laboratories, Inc. received 9 sample(s) on 7/9/2018 for the analyses presented in the following report.

All applicable quality control procedures met method specific acceptance criteria unless otherwise noted.

If you have any questions, please feel free to contact me at (224) 253-1348.

Respectfully submitted,

Christina E. Pierce
Project Manager

Certifications: NELAP/NELAC - IL #100323

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake **Lab Order:** 18G0095
Client Sample ID: 1F **Lab ID:** 18G0095-01
Collection Date: 7/9/18 8:21 **Matrix:** Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	7/10/18 11:10	7/10/18 11:17	EPA300.0 R2.	ZZZ
Conventional Chemistry Parameters									
*Phosphorus	U	0.0500		mg/L	2	7/16/18 11:33	7/16/18 14:58	SM4500P-E	CDM
Dissolved Conventional Chemistry Parameters									
***Total Organic Carbon**	**8.92**	1.00		mg/L	1	7/12/18 8:00	7/12/18 18:53	SM5310C 20(	DMS
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS
E. Coli	**16.0**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS

Client Sample ID: 2F **Lab ID:** 18G0095-02
Collection Date: 7/9/18 8:32 **Matrix:** Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	7/10/18 11:10	7/10/18 12:20	EPA300.0 R2.	ZZZ
Conventional Chemistry Parameters									
*Phosphorus	U	0.0500		mg/L	2	7/16/18 11:33	7/16/18 14:58	SM4500P-E	CDM
Dissolved Conventional Chemistry Parameters									
***Total Organic Carbon**	**8.96**	1.00		mg/L	1	7/12/18 8:00	7/12/18 18:53	SM5310C 20(	DMS
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS
E. Coli	**23.1**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake **Lab Order:** 18G0095
Client Sample ID: 3F **Lab ID:** 18G0095-03
Collection Date: 7/9/18 8:40 **Matrix:** Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	7/10/18 11:10	7/10/18 13:22	EPA300.0 R2.	ZZZ
Conventional Chemistry Parameters									
*Phosphorus	U	0.0500		mg/L	2	7/16/18 11:33	7/16/18 14:58	SM4500P-E	CDM
Dissolved Conventional Chemistry Parameters									
***Total Organic Carbon**	**8.72**	1.00		mg/L	1	7/12/18 8:00	7/12/18 18:53	SM5310C 20(	DMS
Microbiological Parameters									
Total Coliforms	**1990**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS
E. Coli	**1.00**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS

Client Sample ID: 4F **Lab ID:** 18G0095-04
Collection Date: 7/9/18 8:52 **Matrix:** Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	7/10/18 11:10	7/10/18 13:43	EPA300.0 R2.	ZZZ
Conventional Chemistry Parameters									
*Phosphorus	U	0.0500		mg/L	2	7/16/18 11:33	7/16/18 14:58	SM4500P-E	CDM
Dissolved Conventional Chemistry Parameters									
***Total Organic Carbon**	**8.44**	1.00		mg/L	1	7/12/18 8:00	7/12/18 18:53	SM5310C 20(	DMS
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS
E. Coli	**365**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake **Lab Order:** 18G0095
Client Sample ID: 5F **Lab ID:** 18G0095-05
Collection Date: 7/9/18 9:00 **Matrix:** Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Dissolved Conventional Chemistry Parameters									
***Total Organic Carbon**	**8.35**	1.00		mg/L	1	7/12/18 8:00	7/12/18 18:53	SM5310C 20(	DMS
Microbiological Parameters									
Total Coliforms	**1200**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS
E. Coli	**3.10**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS

Client Sample ID: 6F **Lab ID:** 18G0095-06
Collection Date: 7/9/18 9:10 **Matrix:** Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	7/10/18 11:10	7/10/18 15:49	EPA300.0 R2.	ZZZ
Conventional Chemistry Parameters									
*Phosphorus	U	0.0500		mg/L	2	7/16/18 11:33	7/16/18 14:58	SM4500P-E	CDM
Dissolved Conventional Chemistry Parameters									
***Total Organic Carbon**	**9.64**	1.00		mg/L	1	7/12/18 8:00	7/12/18 18:53	SM5310C 20(	DMS
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS
E. Coli	**8.50**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS

Client Sample ID: 7F **Lab ID:** 18G0095-07
Collection Date: 7/9/18 9:20 **Matrix:** Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Dissolved Conventional Chemistry Parameters									
***Total Organic Carbon**	**21.1**	2.00		mg/L	2	7/12/18 8:00	7/12/18 18:53	SM5310C 20(	DMS

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake **Lab Order:** 18G0095
Client Sample ID: 8F **Lab ID:** 18G0095-08
Collection Date: 7/9/18 9:30 **Matrix:** Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	7/10/18 11:10	7/10/18 16:11	EPA300.0 R2.	ZZZ
Conventional Chemistry Parameters									
*Phosphorus	U	0.0500		mg/L	2	7/16/18 11:33	7/16/18 14:58	SM4500P-E	CDM
Dissolved Conventional Chemistry Parameters									
***Total Organic Carbon**	**8.35**	1.00		mg/L	1	7/12/18 8:00	7/12/18 18:53	SM5310C 20(	DMS
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS
E. Coli	**18.9**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS

Client Sample ID: 9F **Lab ID:** 18G0095-09
Collection Date: 7/9/18 9:40 **Matrix:** Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	7/10/18 11:10	7/10/18 16:31	EPA300.0 R2.	ZZZ
Conventional Chemistry Parameters									
*Phosphorus	U	0.0500		mg/L	2	7/16/18 11:33	7/16/18 14:58	SM4500P-E	CDM
Dissolved Conventional Chemistry Parameters									
***Total Organic Carbon**	**8.05**	1.00		mg/L	1	7/12/18 8:00	7/12/18 18:53	SM5310C 20(	DMS
Microbiological Parameters									
Total Coliforms	**2420**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS
E. Coli	**7.40**	1.00		MPN/100 mL	1	7/9/18 16:48	7/10/18 10:51	SM9223B	DMS

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake **Lab Order:** 18G0095

Notes and Definitions

*	NELAC certified compound.
U	Analyte not detected (i.e. less than RL or MDL).

Chain of Custody Record

Central IL - 1210 Capital Airport Drive - Springfield, IL 62707-8490 - Phone (217) 753-1148 - Facsimile (217) 753-1152
Chicago IL Office - 9114 Virginia Rd., Ste 112 - Lake in the Hills, IL 60156 - Phone (847) 651-2604 - Facsimile (847) 458-9680
Central / Southern IL Contact - Phone (217) 414-7762 - Facsimile (217) 753-1152

Prairie Analytical Systems, INCORPORATED
www.prairieanalytical.com

Client	Northwater Consultany
Address	960 Clocktower Dr. Suite F
City, State, Zip Code	Springfield IL 62704
Phone / Facsimile	217 725 3181
Project Name / Number	Fish Lake
Project Location	Fish Lake, IN
P.O. # or Invoice To	Northwater Consultany
Contact Person	Jeff Boeckler

Sample Description	Sampling Date	Sampling Time	Matrix Code	Preserv Code	No. of Containers	Sample Type Comp	Sample Type Grab	Nitrate	E. Coli - Quantitray	Total Phosphorus	Dissolved Organic Carbon						Sampler Comments
1F	7/9/18	0821	A	0	4		X	X	X	X	X						
2F	7/9/18	0832	A	2	4		X	X	X	X	X						
3F	7/9/18	0840	A	2	4		X	X	X	X	X						
4F	7/9/18	0852	A	2	4		X	X	X	X	X						
5F	7/9/18	0900	A	0	2		X		X		X						
6F	7/9/18	0910	A	2	4		X	X	X	X	X						
7F	7/9/18	0920	A	0	1		X				X						
8F	7/9/18	0930	A	2	4		X	X	X	X	X						
9F	7/9/18	0940	A	2	4		X	X	X	X	X						

Reporting: TACO ☐ CCDD ☐ Residential ☐ Industrial / Commercial; CALM ☐ A ☐ B ☐ C ☐ D ☐ E ☐ F; RISC ☐ Residential ☐ Industrial

Matrix Code	A - Aqueous	DW - Drinking Water	GW - Ground Water	NA - Non-Aqueous Liquid	S - Solid	O - Oil	X - Other (Specify)
Preservative Code	0 - None	1 - HCl	2 - H2SO4	3 - HNO3	4 - NaOH	5 - 5035 Kit	X - Other (Specify)

Relinquished By	Date	Time	Received By	Date	Time	Method of Shipment
Jeff Boeckler	7/9/18	1557		7.9.18	1557	Hand

Special Instructions:

Turnaround Time: Standard ☐ Rush ☒
Date Required: 5 day

QC Level: 1 ☐ 2 ☒ 3 ☐ 4 ☐

On wet ice? ☐ Yes ☐ No

Temperature (°C): 5.3

Page 7 of 7

Copies: White - Client / Yellow - PAS, Inc. / Pink - Sampler
PAS COC - Chemical

Revision 4
February 20, 2017

Chain Of Custody Record

Revision 1.0
Effective Date 8/31/17

Source Molecular

Source Molecular Corporation
4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

Company Name	Northwater Consulting
Contact Name(s)	Jeff Boeckler
Send Results To	(email) jeff@northwaterco.com
Phone	217-725-3181
Address	960 Clocktower Dr. Suite F &D
City/State/Zip	Springfield IL 62704
Billing Info	PO#: [X] Will call with credit card

Sample ID	Analysis Requested (see pg. 2) Mark boxes with "X": Human qPCR Pore	Human qPCR Kept	Goose qPCR Target 1				Quantification (Yes or No)	Comments (i.e. special requests, # of containers)	Collection Date	Collection Time
1F	X	X	X				yes	Hold Samples Do Not run Marker	7/9/18	0821
2F	X	X	X				yes	Hold Sample Do not run Marker	7/9/18	0832
3F	X	X	X				yes	Hold Sample Do not run Marker	7/9/18	0840
4F	X	X	X				yes	Hold Sample Do not run Marker	7/9/18	0852
5F	X	X	X				yes	Hold Sample Do not Run Marker	7/9/18	0900
6F	X	X	X				yes	Hold Sampl do not Run Marker	7/9/18	0910
8F	X	X	X				yes	Hold Sample Do not run Marker	7/9/18	0936

Completed by Client:

Relinquished By: Jeff Boeckler
Signature:
Date/Time: 7/9/18 1030

To protect confidentiality, confirmation and results will only be sent to email address provided or authorized by contact provided. Signed form indicates agreement with the test limitations on the back of this form and the company's terms of use found here: sourcemolecular.com/about-sourcemolecular/privacy_statement.html.

Completed by Source Molecular:

Temperature: 6.0°C
Thermometer: 001
Cooler Number: BM025
Received/Filtered: Malik Rivas
Signature:
Date/Time: 7/10/18 11:04 AM

4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

ACCREDITED
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ISO 17025 Accredited
Testing Laboratory

Human Fecal Quantification ID

Detection and quantification of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Northwater Consulting
Date Received: July 10, 2018
Report Generated: July 23, 2018

ND: Not Detected
DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-8G10005	1F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8G10008	2F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8G10010	4F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8G10014	6F	Human Bacteroidetes ID: Dorei	DNQ	**Detected**
SM-8G10016	8F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8G10017	1F	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8G10018	2F	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8G10021	4F	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8G10023	6F	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8G10025	8F	Human Bacteroidetes ID: EPA	ND	Not Detected

Revision 1.2
Effective Date 11/2/17

4985 SW 74th Court, Miami, FL 33155 USA
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

ACCREDITED Certificate 4126.01
ISO 17025 Accredited Testing Laboratory

Preliminary Interpretation of Human Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Northwater Consulting
Date Received: July 10, 2018
Report Generated: July 23, 2018

Sample ID	INTERPRETATION	
	Concentration of Human Fecal Pollution in Sample	Comment
1F	Not Detected	Human fecal biomarker not detected
2F	Not Detected	Human fecal biomarker not detected
4F	Not Detected	Human fecal biomarker not detected
6F	**Low Concentration**	Low levels of Human fecal biomarker(s)
8F	Not Detected	Human fecal biomarker not detected

The opinions/interpretations identified/expressed in this report are outside the scope of this organization's A2LA Accreditation.

Laboratory Comments

Submitter: Northwater Consulting

Report Generated: July 23, 2018

Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Human Bacteroidetes ID™ Species*: B. dorei*

The **Human Bacteroidetes ID™ Species*: B. dorei*** service targets the species *Bacteroides dorei*. *B. dorei* is an anaerobe that is frequently shed from the gastrointestinal tract and isolated from human feces worldwide. It is a newly discovered species that is widely distributed in the USA.[1,2] The human-associated marker DNA sequence is located on the 16S rRNA gene of *B. dorei*.[3] The marker is the microbial source tracking (MST) marker of choice for detecting human fecal pollution due to its exceptional sensitivity and specificity. Internal validations have been conducted on hundreds of sewage, septage, human and animal host fecal samples collected from throughout the U.S and archived in the Source Molecular fecal bank. The marker has also been evaluated in both inland and coastal waters. A recent, comprehensive, multi-laboratory MST method evaluation study, exploring the performance of current MST methods, concluded the *B. dorei* qPCR assay to be the top performing human-associated assay amongst those tested. The success and consistency of this marker in numerous studies around the world[1,3,4] makes the **Human Bacteroidetes ID™ Species*: B. dorei*** service the primary service for identifying human fecal pollution at Source Molecular.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[5] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The Human Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[3,5,6,7,8] Furthermore, certain strains of *Bacteroidetes* have been found to be associated with humans.[3,6] As such, these bacterial strains can be used as indicators of human fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique *B. dorei* DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the *B. dorei* DNA sequence, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve by the qPCR software. The absence of an amplification curve indicates that the *B. dorei* gene biomarker is not detected in the water sample because it is either not present or present at concentrations below the analytical detection limit.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as
Human Bacteroidetes ID™ Species*: B. stercoris*,
Human Bacteroidetes ID™ Species*: B. fragilis,* and
Human Bacteroidetes ID™ Species*: B. thetaiotaomicron*.

[1]Boehm, A., Fuhrman, J., Mrse, R., Grant, S. **Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California**. Environ Sci Technol. 2003 37**:** 673–680.
[2]Bakir, M., Sakamoto, M., Kitahara, M., Matsumoto, M., Benno, Y. **Bacteroides dorei sp. nov., isolated from human faeces**. Int. J. Syst. Evol. Microbiol. 2006 56: 1639–1641.
[3] Bernhard, A., Field, K. **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Appl. Environ. Microbiol. 2000b 66: 4571-4574.
[4]Ahmed, w., Masters, N., Toze, S. **Consistency in the host specificity and host sensitivity of the Bacteroides HF183 marker for sewage pollution tracking. Lett. Appl. Microbiol. 2012 55: 283-289.**
[5] Scott, T., Rose, J., Jenkins, T., Farrah, S., Lukasik, J. **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. 2002 68: 5796-5803.
[6] Bernhard, A., Field, K. **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Appl. Environ. Microbiol. 2000a 66: 1587-1594.
[7] Fogarty, L., Voytek, M. **A Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species.** Appl. Environ. Microbiol. 2005 71: 5999-6007.
[8] Dick, L., Bernhard, A., Brodeur, T., Santo Domingo, J., *et al*. **Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification.** Appl. Environ. Microbiol. 2005 71: 3184-3191.

Human Bacteroidetes ID™: EPA Developed Assay

The **Human Bacteroidetes ID™: EPA Developed Assay** service targets a functional gene biomarker in *Bacteroidales*-like anaerobic bacteria that is present in high concentrations in the human gut. The U.S Environmental Protection Agency (U.S. EPA) was the first to target the biomarker using Polymerase Chain Reaction (PCR) technology in order to detect ground and surface waters impacted by human fecal pollution.[1] Since it's development, the assay has been used succesfully around the U.S to identify fecal pollution originating from human sources, such as sewage and septage wastewaters.

The U.S. EPA Developed assay has been shown to be highly associated with human fecal pollution. It has successfully been validated in multiple nationwide studies using at least 300 individual reference fecal material from 22 different animal species known to commonly contaminate environmental waters.[1,2] A reported 99.2% specificity to human fecal material makes this one of the leading assays to confirm the presence of fecal contamination that is of human origin.[1] The *Bacteroidales*-like bacteria is widely distributed. It was detected in 100% of hundreds of sewage and human reference fecal samples collected from more than 20 human populations, making it highly sensitive. Internal validations have also been conducted on hundreds of wastewater, human and animal host fecal samples archived in the Source Molecular fecal bank.

Fecal anaerobic bacteria are considered for several reasons an interesting alternative to more traditional fecal indicator organisms such as *E. coli* and *Enterococci*.[3] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems.[3] This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The **Human Bacteroidetes ID™: EPA Developed Assay** service is designed around the principle that fecal *Bacteroidales*-like bacteria are found in large quantities in feces of warm-blooded animals.[4,5] Furthermore, certain strains have been shown to be associated with humans.[4,5] As such, these bacterial strains can be used as indicators of human fecal contamination. An advantage of the Human Bacteroidetes ID™ service is that the entire portion of water sampled is filtered to concentrate bacteria. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates. This is an advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique B. dorei DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the B. dorei DNA sequence, then billions of copies of the DNA fragment will be available, detected and quantified.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as
Human Bacteroidetes ID™ Species*: B. dorei*,
Human Bacteroidetes ID™ Species*: B. fragilis,* and
Human Bacteroidetes ID™ Species*: B. stercoris*

[1] Shanks, O., Kelty, C., Sivaganesan, M., Varma, M. and Haugland, R. **Quantitative PCR for Genetic Markers of Human Fecal Pollution**. Appl. Environ. Microbiol. 2009 75: 5507-5513.
[2] Layton, B., Cao, Y., Ebentier, D., Hanley, K., Ballesté, E., Brandão, J., *et al*. **Performance of Human Fecal Anaerobe-Associated PCR-Based Assays in a Multi-Laboratory Method Evaluation Study.** Water Research. 2013 In Press.
[3] Scott, T., Rose, J., Jenkins, T., Farrah, S. and Lukasik, J. **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. 2002 68: 5796-5803.
[4] Bernhard, A., Field, K. **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Appl. Environ. Microbiol. 2000a 66: 1587-1594.
[5] Bernhard, A., Field, K. **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Appl. Environ. Microbiol. 2000b 66: 4571-4574.

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Testing Laboratory

Goose Fecal Quantification ID

Detection and quantification of the fecal associated Goose gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Northwater Consulting
Date Received: July 10, 2018
Report Generated: July 23, 2018

ND: Not Detected
DNQ: Detected Not Quantified

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-8G10026	1F	Goose Bacteroidetes ID: Target 1	ND	Not Detected
SM-8G10027	2F	Goose Bacteroidetes ID: Target 1	ND	Not Detected
SM-8G10029	4F	Goose Bacteroidetes ID: Target 1	DNQ	**Detected**
SM-8G10031	6F	Goose Bacteroidetes ID: Target 1	ND	Not Detected
SM-8G10032	8F	Goose Bacteroidetes ID: Target 1	ND	Not Detected

Revision 1.2
Effective Date 11/2/17

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ACCREDITED Certificate 4126.01
ISO 17025 Accredited Testing Laboratory

Preliminary Interpretation of Goose Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Goose gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Northwater Consulting
Date Received: July 10, 2018
Report Generated: July 23, 2018

Sample ID	INTERPRETATION	
	Concentration of Goose Fecal Pollution in Sample	Comment
1F	Not Detected	Goose fecal biomarker not detected
2F	Not Detected	Goose fecal biomarker not detected
4F	**Low Concentration**	Low levels of Goose fecal biomarker(s)
6F	Not Detected	Goose fecal biomarker not detected
8F	Not Detected	Goose fecal biomarker not detected

The opinions/interpretations identified/expressed in this report are outside the scope of this organization's A2LA Accreditation.

Laboratory Comments

Submitter: Northwater Consulting

Report Generated: July 23, 2018

Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Theory Explanation of Canada Goose Bacteroidetes "Quantification" ID™

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[1] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Canada Goose Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[2,3,4,5,6] Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in Canada geese.[7] Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in Canada geese.[7] As such, these bacterial strains can be used as indicators of Canada geese fecal contamination.

One of the advantages of the Canada Goose Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.
Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available for detection in real-time.

References

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[7] Fremaux, B., Boa, T., Yost, C. K. **Quantitative Real-Time PCR Asays for Sensitive Detection of Canada Goose-Specific Fecal Pollution in Water Sources.** Appl. Environ. Microbiol. 2010 76: 4886-4889.

PDC Laboratories, Inc.

Friday, October 19, 2018

Jeff Boeckler

Northwater Consulting
960 Clocktower Drive, Suite F
Springfield, IL 62704

TEL: (217) 725-3181

FAX: NA

RE: Fish Lake PDC WO: 18J0210

PDC Laboratories, Inc. received 10 sample(s) on 10/8/2018 for the analyses presented in the following report.

All applicable quality control procedures met method specific acceptance criteria unless otherwise noted.

If you have any questions, please feel free to contact me at (224) 253-1348.

Respectfully submitted,

Christina E. Pierce
Project Manager

Certifications: NELAP/NELAC - IL #100323

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake
Lab Order: 18J0210
Client Sample ID: 1C
Lab ID: 18J0210-01
Collection Date: 10/8/18 9:45
Matrix: Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
***Nitrate (as N)**	**0.744**	0.250		mg/L	10	10/9/18 8:56	10/9/18 19:59	EPA300.0 R2.	KSB
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
E. Coli	**260**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM

PDC Laboratories, Inc. - Peoria

General Chemistry									
TOC Soluble	**7.5**	0.50		mg/L	1	10/13/18 17:37	10/13/18 17:37	SM 5310C	SAH
Nutrients									
Phosphorus - total as P	U	0.10		mg/L	1	10/16/18 7:19	10/17/18 12:13	SM 4500-P F	TTH

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake
Lab Order: 18J0210
Client Sample ID: 1F
Lab ID: 18J0210-02
Collection Date: 10/8/18 8:23
Matrix: Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	10/9/18 8:56	10/9/18 20:20	EPA300.0 R2.	KSB
Microbiological Parameters									
Total Coliforms	**2420**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
E. Coli	**7.40**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
PDC Laboratories, Inc. - Peoria									
General Chemistry									
TOC Soluble	**6.7**	0.50		mg/L	1	10/13/18 18:01	10/13/18 18:01	SM 5310C	SAH
Nutrients									
Phosphorus - total as P	U	0.10		mg/L	1	10/16/18 7:19	10/17/18 12:25	SM 4500-P F	TTH

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake
Lab Order: 18J0210
Client Sample ID: 2F
Lab ID: 18J0210-03
Collection Date: 10/8/18 8:35
Matrix: Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	10/9/18 8:56	10/9/18 20:41	EPA300.0 R2.	KSB
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
E. Coli	**8.60**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM

PDC Laboratories, Inc. - Peoria

General Chemistry									
TOC Soluble	**6.7**	0.50		mg/L	1	10/13/18 18:25	10/13/18 18:25	SM 5310C	SAH
Nutrients									
Phosphorus - total as P	U	0.10		mg/L	1	10/16/18 7:19	10/17/18 12:49	SM 4500-P F	TTH

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake
Lab Order: 18J0210
Client Sample ID: 3F
Lab ID: 18J0210-04
Collection Date: 10/8/18 8:45
Matrix: Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	10/9/18 8:56	10/9/18 21:02	EPA300.0 R2.	KSB
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
E. Coli	**5.10**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM

PDC Laboratories, Inc. - Peoria

General Chemistry									
TOC Soluble	**6.6**	0.50		mg/L	1	10/13/18 18:49	10/13/18 18:49	SM 5310C	SAH
Nutrients									
Phosphorus - total as P	U	0.10		mg/L	1	10/16/18 7:19	10/17/18 12:42	SM 4500-P F	TTH

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake
Lab Order: 18J0210
Client Sample ID: 4F
Lab ID: 18J0210-05
Collection Date: 10/8/18 9:00
Matrix: Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	10/9/18 8:56	10/9/18 21:24	EPA300.0 R2.	KSB
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
E. Coli	**19.9**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM

PDC Laboratories, Inc. - Peoria

General Chemistry									
TOC Soluble	**28**	1.0		mg/L	2	10/16/18 23:28	10/16/18 23:28	SM 5310C	SAH
Nutrients									
Phosphorus - total as P	U	0.10		mg/L	1	10/16/18 7:19	10/17/18 12:48	SM 4500-P F	TTH

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake
Lab Order: 18J0210
Client Sample ID: 5F
Lab ID: 18J0210-06
Collection Date: 10/8/18 9:12
Matrix: Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	10/9/18 8:56	10/9/18 21:44	EPA300.0 R2.	KSB
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
E. Coli	**18.7**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM

PDC Laboratories, Inc. - Peoria

General Chemistry									
TOC Soluble	**5.8**	0.50		mg/L	1	10/13/18 19:35	10/13/18 19:35	SM 5310C	SAH
Nutrients									
Phosphorus - total as P	U	0.10		mg/L	1	10/16/18 7:19	10/17/18 12:53	SM 4500-P F	TTH

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake
Lab Order: 18J0210
Client Sample ID: 6F
Lab ID: 18J0210-07
Collection Date: 10/8/18 9:35
Matrix: Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	10/9/18 8:56	10/9/18 22:04	EPA300.0 R2.	KSB
Microbiological Parameters									
Total Coliforms	**2420**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
E. Coli	**27.5**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM

PDC Laboratories, Inc. - Peoria

General Chemistry									
TOC Soluble	**5.7**	0.50		mg/L	1	10/13/18 19:58	10/13/18 19:58	SM 5310C	SAH
Nutrients									
Phosphorus - total as P	U	0.10		mg/L	1	10/16/18 7:19	10/17/18 12:54	SM 4500-P F	TTH

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake
Lab Order: 18J0210
Client Sample ID: 7F
Lab ID: 18J0210-08
Collection Date: 10/8/18 9:22
Matrix: Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	10/9/18 8:56	10/9/18 22:25	EPA300.0 R2.	KSB
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
E. Coli	**7.40**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM

PDC Laboratories, Inc. - Peoria

General Chemistry									
TOC Soluble	**19**	0.50		mg/L	1	10/13/18 20:22	10/13/18 20:22	SM 5310C	SAH
Nutrients									
Phosphorus - total as P	U	0.10		mg/L	1	10/16/18 7:19	10/17/18 12:56	SM 4500-P F	TTH

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake
Lab Order: 18J0210
Client Sample ID: 8F
Lab ID: 18J0210-09
Collection Date: 10/8/18 9:55
Matrix: Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	10/9/18 8:56	10/9/18 23:48	EPA300.0 R2.	KSB
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
E. Coli	**41.9**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM

PDC Laboratories, Inc. - Peoria

General Chemistry									
TOC Soluble	**7.7**	0.50		mg/L	1	10/14/18 0:47	10/14/18 0:47	SM 5310C	SAH
Nutrients									
Phosphorus - total as P	U	0.10		mg/L	1	10/16/18 7:19	10/17/18 12:54	SM 4500-P F	TTH

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake
Client Sample ID: 9F
Collection Date: 10/8/18 9:05

Lab Order: 18J0210
Lab ID: 18J0210-10
Matrix: Water

Analyses	Result	Limit	Qual	Units	DF	Date Prepared	Date Analyzed	Method	Analyst
Anions by Ion Chromatography									
*Nitrate (as N)	U	0.250		mg/L	10	10/9/18 8:56	10/10/18 0:09	EPA300.0 R2.	KSB
Microbiological Parameters									
Total Coliforms	>2419.2	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM
E. Coli	**17.1**	1.00		MPN/100 mL	1	10/9/18 9:35	10/10/18 10:18	SM9223B	CDM

PDC Laboratories, Inc. - Peoria

General Chemistry									
TOC Soluble	**8.1**	1.0		mg/L	2	10/16/18 23:06	10/16/18 23:06	SM 5310C	SAH
Nutrients									
Phosphorus - total as P	U	0.10		mg/L	1	10/16/18 7:19	10/17/18 12:58	SM 4500-P F	TTH

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake **Lab Order:** 18J0210

Anions by Ion Chromatography - Quality Control

Analyte	Result	Reporting Limit	Units	Spike Level	Source Result	%REC	%REC Limits	RPD	RPD Limit	Notes
Batch B005219 - EPA300.0/SW9056A Anions										
Blank (B005219-BLK1)				Prepared & Analyzed: 10/09/2018						
Nitrate (as N)	U	0.0250	mg/L							
LCS (B005219-BS1)				Prepared & Analyzed: 10/09/2018						
Nitrate (as N)	0.124	0.0250	mg/L	0.11295		110	90-110			
Matrix Spike (B005219-MS1)		**Source: 18J0210-10**		Prepared: 10/09/2018 Analyzed: 10/10/2018						
Nitrate (as N)	1.36	0.263	mg/L	1.1889	0.124	104	90-110			
Matrix Spike (B005219-MS2)		**Source: 18J0237-01**		Prepared: 10/09/2018 Analyzed: 10/10/2018						
Nitrate (as N)	1.74	0.263	mg/L	1.1889	0.498	105	90-110			
Matrix Spike Dup (B005219-MSD1)		**Source: 18J0210-10**		Prepared: 10/09/2018 Analyzed: 10/10/2018						
Nitrate (as N)	1.34	0.263	mg/L	1.1889	0.124	102	90-110	2	20	
Matrix Spike Dup (B005219-MSD2)		**Source: 18J0237-01**		Prepared: 10/09/2018 Analyzed: 10/10/2018						
Nitrate (as N)	1.75	0.263	mg/L	1.1889	0.498	105	90-110	0.3	20	

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake **Lab Order:** 18J0210

Nutrients - Quality Control

Analyte	Result	Reporting Limit	Units	Spike Level	Source Result	%REC	%REC Limits	RPD	RPD Limit	Notes
Batch B821158 - No Prep										
Blank (B821158-BLK1)				Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	U	0.10	mg/L				-			
Blank (B821158-BLK2)				Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	U	0.10	mg/L				-			
Blank (B821158-BLK4)				Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	U	0.10	mg/L				-			
Blank (B821158-BLK5)				Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	U	0.10	mg/L				-			
LCS (B821158-BS1)				Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	2.13	0.10	mg/L	2.000		106	80-120			
LCS (B821158-BS2)				Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	2.06	0.10	mg/L	2.000		103	80-120			
LCS (B821158-BS4)				Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	2.01	0.10	mg/L	2.000		100	80-120			
LCS (B821158-BS5)				Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	2.12	0.10	mg/L	2.000		106	80-120			
Matrix Spike (B821158-MS1)		**Source: 8102090-02**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.18	0.10	mg/L	1.000	0.143	104	80-120			
Matrix Spike (B821158-MS2)		**Source: 8102120-01**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	4.90	0.10	mg/L	1.000	3.85	105	80-120			

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake **Lab Order:** 18J0210

Nutrients - Quality Control

Analyte	Result	Reporting Limit	Units	Spike Level	Source Result	%REC	%REC Limits	RPD	RPD Limit	Notes
Batch B821158 - No Prep										
Matrix Spike (B821158-MS3)		**Source: 8102404-01**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	2.71	0.10	mg/L	1.000	1.66	105	80-120			
Matrix Spike (B821158-MS4)		**Source: 8102091-02**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.25	0.10	mg/L	1.000	0.215	104	80-120			
Matrix Spike (B821158-MS5)		**Source: 18J0210-01**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.01	0.10	mg/L	1.000	ND	101	80-120			
Matrix Spike (B821158-MS6)		**Source: 18J0210-02**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	0.965	0.10	mg/L	1.000	ND	96	80-120			
Matrix Spike (B821158-MS7)		**Source: 18J0210-04**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.05	0.10	mg/L	1.000	ND	105	80-120			
Matrix Spike (B821158-MS8)		**Source: 18J0210-09**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.00	0.10	mg/L	1.000	ND	100	80-120			
Matrix Spike (B821158-MS9)		**Source: 8102613-01**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	3.75	0.10	mg/L	1.000	2.61	114	80-120			
Matrix Spike Dup (B821158-MSD1)		**Source: 8102090-02**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.21	0.10	mg/L	1.000	0.143	107	80-120	3	20	
Matrix Spike Dup (B821158-MSD2)		**Source: 8102120-01**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	5.07	0.10	mg/L	1.000	3.85	122	80-120	3	20	Q2
Matrix Spike Dup (B821158-MSD3)		**Source: 8102404-01**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	2.68	0.10	mg/L	1.000	1.66	102	80-120	1	20	

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake **Lab Order:** 18J0210

Nutrients - Quality Control

Analyte	Result	Reporting Limit	Units	Spike Level	Source Result	%REC	%REC Limits	RPD	RPD Limit	Notes
Batch B821158 - No Prep										
Matrix Spike Dup (B821158-MSD4)		**Source: 8102091-02**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.24	0.10	mg/L	1.000	0.215	102	80-120	0.8	20	
Matrix Spike Dup (B821158-MSD5)		**Source: 18J0210-01**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.02	0.10	mg/L	1.000	ND	102	80-120	1	20	
Matrix Spike Dup (B821158-MSD6)		**Source: 18J0210-02**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.01	0.10	mg/L	1.000	ND	101	80-120	5	20	
Matrix Spike Dup (B821158-MSD7)		**Source: 18J0210-04**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.02	0.10	mg/L	1.000	ND	102	80-120	3	20	
Matrix Spike Dup (B821158-MSD8)		**Source: 18J0210-09**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	1.06	0.10	mg/L	1.000	ND	106	80-120	6	20	
Matrix Spike Dup (B821158-MSD9)		**Source: 8102613-01**		Prepared: 10/16/2018 Analyzed: 10/17/2018						
Phosphorus - total as P	3.85	0.10	mg/L	1.000	2.61	124	80-120	3	20	Q2

LABORATORY RESULTS

Client: Northwater Consulting
Project: Fish Lake **Lab Order:** 18J0210

Notes and Definitions

Q2	Matrix Spike Duplicate failed % Recovery
*	NELAC certified compound.
U	Analyte not detected (i.e. less than RL or MDL).

Chain of Custody Record

Central IL - 1210 Capital Airport Drive - Springfield, IL 62707-8490 - Phone (217) 753-1148 - Facsimile (217) 753-1152
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Central / Southern IL Contact - Phone (217) 414-7762 - Facsimile (217) 753-1152

Prairie Analytical Systems, INCORPORATED www.prairieanalytical.com

Client	Northwater Consultry
Address	960 Clocktower Dr. Suite F
City, State, Zip Code	Springfield IL 62704
Phone / Facsimile	217-725-3181
Project Name / Number	Fish Lake
Project Location	Fish Lake IN
P.O. # or Invoice To	Northwater Consulting
Contact Person	Jeff Boeckler

Reporting: TACO ☐ CCDD ☐ Residential ☐ Industrial / Commercial; CALM ☐ A ☐ B ☐ C ☐ D ☐ E ☐ F; RISC ☐ Residential ☐ Industrial

Sample Description	Sampling Date	Sampling Time	Matrix Code	Preserv Code	No. of Containers	Sample Type Comp	Sample Type Grab	Nitrate	E Coli	Total Phosphorus	Dissolved Organic Carbon	Sampler Comments
IC 10	8/8/18	0945	A	2	4		X	X	X	X	X	
IF	8/8/18	0823	A	2	4		X	X	X	X	X	
2F	8/8/18	0835	A	2	4		X	X	X	X	X	
3F	8/8/18	0845	A	2	4		X	X	X	X	X	
4F	8/8/18	0900	A	2	4		X	X	X	X	X	
5F	8/8/18	0912	A	2	4		X	X	X	X	X	
6F	8/8/18	0935	A	2	4		X	X	X	X	X	
7F	8/8/18	0922	A	2	4		X	X	X	X	X	
8F	8/8/18	0955	A	2	4		X	X	X	X	X	
9F	8/8/18	0905	A	2	4		X	X	X	X	X	

Matrix Code	A - Aqueous	DW - Drinking Water	GW - Ground Water	NA - Non-Aqueous Liquid	S - Solid	O - Oil	X - Other (Specify)
Preservative Code	0 - None	1 - HCl	2 - H2SO4	3 - HNO3	4 - NaOH	5 - 5035 Kit	X - Other (Specify)

Relinquished By	Date	Time	Received By	Date	Time	Method of Shipment
Jeff Boeckler	8/8/18	4:05pm		10-08-18	4:05pm	Hand

Special Instructions:

Turnaround Time: Standard ☐ Rush ☒
Date Required: 5 Day

QC Level: 1 ☐ 2 ☒ 3 ☐ 4 ☐

On wet ice? ☒ Yes ☐ No

Temperature (°C): 1.4

Copies: White - Client / Yellow - PAS, Inc. / Pink - Sampler
PAS COC - Chemical

Revision 4
February 20, 2017

Chain Of Custody Record

Revision 1.2
Effective Date 8/20/2018

Source Molecular Corporation
15280 NW 79th CT Suite 107 Miami Lakes, FL 33016
Tel: (1) 786-220-0379 Fax: (1) 786-513-2733
Email: info@sourcemolecular.com

Company Name	Northwater Consulting
Contact Name(s)	Jeff Boeckler
Send Results To	(email) jeff@northwaterco.com
Phone	217-725-3181
Address	960 Clocktower Dr. Suite F
City/State/Zip	Springfield IL 62704
Billing Info	PO#: [X] Will call with credit card

Analysis Requested (see pg. 2) Mark boxes with "X"

Sample ID	Human qPCR Direct	Human qPCR EPA	Human qPCR Target	Comments (i.e. special requests, # of containers)	Collection Date	Collection Time
1C				Hold Samples Do not run Marker	8/8/18	0945
1F				Hold Samples Do not run Marker	8/8/18	0823
2F				Hold Samples Do not run marker	8/8/18	0835
3F				Hold Samples Do not run marker	8/8/18	0845
4F				Hold Samples Do not run marker	8/8/18	0900
5F				Hold Samples Do not run marker	8/8/18	0917
6F				Hold Samples Do not run marker	8/8/18	0935
7F				Hold Samples Do not run marker	8/8/18	0922
8F				Hold Samples Do not run marker	8/8/18	0955

Completed by Client:
Relinquished By Jeff Boeckler
Signature
Date/Time 8/8/18

To protect confidentiality, confirmation and results will only be sent to email address provided or authorized by contact provided. Signed form indicates agreement with the test limitations on the back of this form and the company's terms of use found here: sourcemolecular.com/about-sourcemolecular/privacy_statement/.

Completed by Source Molecular:
Temperature 10.8C
Thermometer 001
Cooler Number BM026
Received/Filtered Douglas Granajo
Signature
Date/Time 10/8/18 9:47am
DG

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ACCREDITED Certificate 4126.01
ISO 17025 Accredited Testing Laboratory

Human Fecal Quantification ID

Detection and quantification of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Northwater Consulting
Date Received: October 9, 2018
Report Generated: October 30, 2018

ND: Not Detected

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-8J28005	1C	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8J28006	1F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8J24007	2F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8J24008	3F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8J24009	4F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8J24010	5F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8J24011	6F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8J24012	8F	Human Bacteroidetes ID: Dorei	ND	Not Detected
SM-8J24013	1C	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8J24014	1F	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8J24015	2F	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8J24016	3F	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8J24017	4F	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8J24018	5F	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8J24019	6F	Human Bacteroidetes ID: EPA	ND	Not Detected
SM-8J24020	8F	Human Bacteroidetes ID: EPA	ND	Not Detected

Revision 1.3
Effective Date 9/25/18

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ACCREDITED Certificate 4126.01
ISO 17025 Accredited Testing Laboratory

Preliminary Interpretation of Human Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Human gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Northwater Consulting
Date Received: October 9, 2018
Report Generated: October 30, 2018

Sample ID	INTERPRETATION	
	Concentration of Human Fecal Pollution in Sample	Comment
1C	Not Detected	Human fecal biomarker not detected
1F	Not Detected	Human fecal biomarker not detected
2F	Not Detected	Human fecal biomarker not detected
3F	Not Detected	Human fecal biomarker not detected
4F	Not Detected	Human fecal biomarker not detected
5F	Not Detected	Human fecal biomarker not detected
6F	Not Detected	Human fecal biomarker not detected
8F	Not Detected	Human fecal biomarker not detected

The opinions/interpretations identified/expressed in this report are outside the scope of this organization's A2LA Accreditation.

Laboratory Comments

Submitter: Northwater Consulting

Report Generated: October 30, 2018

Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Human Bacteroidetes ID™ Species*: B. dorei*

The **Human Bacteroidetes ID™ Species*: B. dorei*** service targets the species *Bacteroides dorei*. *B. dorei* is an anaerobe that is frequently shed from the gastrointestinal tract and isolated from human feces worldwide. It is a newly discovered species that is widely distributed in the USA.[1,2] The human-associated marker DNA sequence is located on the 16S rRNA gene of *B. dorei*.[3] The marker is the microbial source tracking (MST) marker of choice for detecting human fecal pollution due to its exceptional sensitivity and specificity. Internal validations have been conducted on hundreds of sewage, septage, human and animal host fecal samples collected from throughout the U.S and archived in the Source Molecular fecal bank. The marker has also been evaluated in both inland and coastal waters. A recent, comprehensive, multi-laboratory MST method evaluation study, exploring the performance of current MST methods, concluded the *B. dorei* qPCR assay to be the top performing human-associated assay amongst those tested. The success and consistency of this marker in numerous studies around the world[1,3,4] makes the **Human Bacteroidetes ID™ Species*: B. dorei*** service the primary service for identifying human fecal pollution at Source Molecular.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[5] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The Human Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[3,5,6,7,8] Furthermore, certain strains of *Bacteroidetes* have been found to be associated with humans.[3,6] As such, these bacterial strains can be used as indicators of human fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique *B. dorei* DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the *B. dorei* DNA sequence, then billions of copies of the DNA fragment will be available and detected in real-time. The accumulation of DNA product is plotted as an amplification curve by the qPCR software. The absence of an amplification curve indicates that the *B. dorei* gene biomarker is not detected in the water sample because it is either not present or present at concentrations below the analytical detection limit.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as
Human Bacteroidetes ID™ Species*: B. stercoris*,
Human Bacteroidetes ID™ Species*: B. fragilis,* and
Human Bacteroidetes ID™ Species*: B. thetaiotaomicron*.

[1]Boehm, A., Fuhrman, J., Mrse, R., Grant, S. **Tiered approach for identification of a human fecal pollution source at a recreational beach: case study at Avalon Bay, Catalina Island, California**. Environ Sci Technol. 2003 37**:** 673–680.
[2]Bakir, M., Sakamoto, M., Kitahara, M., Matsumoto, M., Benno, Y. **Bacteroides dorei sp. nov., isolated from human faeces**. Int. J. Syst. Evol. Microbiol. 2006 56: 1639–1641.
[3] Bernhard, A., Field, K. **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Appl. Environ. Microbiol. 2000b 66: 4571-4574.
[4]Ahmed, w., Masters, N., Toze, S. **Consistency in the host specificity and host sensitivity of the Bacteroides HF183 marker for sewage pollution tracking. Lett. Appl. Microbiol. 2012 55: 283-289.**
[5] Scott, T., Rose, J., Jenkins, T., Farrah, S., Lukasik, J. **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. 2002 68: 5796-5803.
[6] Bernhard, A., Field, K. **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Appl. Environ. Microbiol. 2000a 66: 1587-1594.
[7] Fogarty, L., Voytek, M. **A Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species.** Appl. Environ. Microbiol. 2005 71: 5999-6007.
[8] Dick, L., Bernhard, A., Brodeur, T., Santo Domingo, J., *et al*. **Host Distributions of Uncultivated Fecal Bacteroidales Bacteria Reveal Genetic Markers for Fecal Source Identification.** Appl. Environ. Microbiol. 2005 71: 3184-3191.

Human Bacteroidetes ID™: EPA Developed Assay

The **Human Bacteroidetes ID™: EPA Developed Assay** service targets a functional gene biomarker in *Bacteroidales*-like anaerobic bacteria that is present in high concentrations in the human gut. The U.S Environmental Protection Agency (U.S. EPA) was the first to target the biomarker using Polymerase Chain Reaction (PCR) technology in order to detect ground and surface waters impacted by human fecal pollution.[1] Since it's development, the assay has been used succesfully around the U.S to identify fecal pollution originating from human sources, such as sewage and septage wastewaters.

The U.S. EPA Developed assay has been shown to be highly associated with human fecal pollution. It has successfully been validated in multiple nationwide studies using at least 300 individual reference fecal material from 22 different animal species known to commonly contaminate environmental waters.[1,2] A reported 99.2% specificity to human fecal material makes this one of the leading assays to confirm the presence of fecal contamination that is of human origin.[1] The *Bacteroidales*-like bacteria is widely distributed. It was detected in 100% of hundreds of sewage and human reference fecal samples collected from more than 20 human populations, making it highly sensitive. Internal validations have also been conducted on hundreds of wastewater, human and animal host fecal samples archived in the Source Molecular fecal bank.

Fecal anaerobic bacteria are considered for several reasons an interesting alternative to more traditional fecal indicator organisms such as *E. coli* and *Enterococci*.[3] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems.[3] This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*.

The **Human Bacteroidetes ID™: EPA Developed Assay** service is designed around the principle that fecal *Bacteroidales*-like bacteria are found in large quantities in feces of warm-blooded animals.[4,5] Furthermore, certain strains have been shown to be associated with humans.[4,5] As such, these bacterial strains can be used as indicators of human fecal contamination. An advantage of the Human Bacteroidetes ID™ service is that the entire portion of water sampled is filtered to concentrate bacteria. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates. This is an advantage for highly contaminated water systems with potential multiple sources of fecal contamination.

Accuracy of the results is possible because the method amplifies DNA into a large number of small copies of the gene biomarker of interest. This is accomplished with small pieces of DNA called primers that are complementary and specific to the unique B. dorei DNA sequence. Through a heating process called thermal cycling, the double stranded DNA is denatured, hybridized to the complementary primers and amplified to create many copies of the DNA fragment desired. If the primers are successful in finding a site on the DNA fragment that is specific to the B. dorei DNA sequence, then billions of copies of the DNA fragment will be available, detected and quantified.

To strengthen the validity of the results, additional tests targeting other high-ranking, human-associated *Bacteroidetes* species should be performed, such as
Human Bacteroidetes ID™ Species*: B. dorei*,
Human Bacteroidetes ID™ Species*: B. fragilis,* and
Human Bacteroidetes ID™ Species*: B. stercoris*

[1] Shanks, O., Kelty, C., Sivaganesan, M., Varma, M. and Haugland, R. **Quantitative PCR for Genetic Markers of Human Fecal Pollution**. Appl. Environ. Microbiol. 2009 75: 5507-5513.
[2] Layton, B., Cao, Y., Ebentier, D., Hanley, K., Ballesté, E., Brandão, J., *et al*. **Performance of Human Fecal Anaerobe-Associated PCR-Based Assays in a Multi-Laboratory Method Evaluation Study.** Water Research. 2013 In Press.
[3] Scott, T., Rose, J., Jenkins, T., Farrah, S. and Lukasik, J. **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. 2002 68: 5796-5803.
[4] Bernhard, A., Field, K. **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Appl. Environ. Microbiol. 2000a 66: 1587-1594.
[5] Bernhard, A., Field, K. **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Appl. Environ. Microbiol. 2000b 66: 4571-4574.

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ISO 17025 Accredited Testing Laboratory
Certificate 4126.01

Goose Fecal Quantification ID

Detection and quantification of the fecal associated Goose gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Northwater Consulting
Date Received: October 9, 2018
Report Generated: October 30, 2018

ND: Not Detected

SM #	Sample ID	Analysis Requested	Marker Quantified (copies/100 ml)	DNA Analytical Results
SM-8J24021	1C	Goose Bacteroidetes ID: Target 1	ND	Not Detected
SM-8J24022	1F	Goose Bacteroidetes ID: Target 1	ND	Not Detected
SM-8J24023	2F	Goose Bacteroidetes ID: Target 1	ND	Not Detected
SM-8J24024	3F	Goose Bacteroidetes ID: Target 1	ND	Not Detected
SM-8J24025	4F	Goose Bacteroidetes ID: Target 1	ND	Not Detected
SM-8J24026	5F	Goose Bacteroidetes ID: Target 1	ND	Not Detected
SM-8J24027	6F	Goose Bacteroidetes ID: Target 1	ND	Not Detected
SM-8J24028	8F	Goose Bacteroidetes ID: Target 1	ND	Not Detected

Revision 1.3
Effective Date 9/25/18

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ACCREDITED Certificate 4126.01
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Preliminary Interpretation of Goose Fecal "Quantification" ID Results

Detection and quantification of the fecal associated Goose gene biomarker by real-time quantitative Polymerase Chain Reaction (qPCR) DNA analytical technology

Submitter: Northwater Consulting
Date Received: October 9, 2018
Report Generated: October 30, 2018

Sample ID	INTERPRETATION	
	Concentration of Goose Fecal Pollution in Sample	Comment
1C	Not Detected	Goose fecal biomarker not detected
1F	Not Detected	Goose fecal biomarker not detected
2F	Not Detected	Goose fecal biomarker not detected
3F	Not Detected	Goose fecal biomarker not detected
4F	Not Detected	Goose fecal biomarker not detected
5F	Not Detected	Goose fecal biomarker not detected
6F	Not Detected	Goose fecal biomarker not detected
8F	Not Detected	Goose fecal biomarker not detected

The opinions/interpretations identified/expressed in this report are outside the scope of this organization's A2LA Accreditation.

Laboratory Comments

Submitter: Northwater Consulting

Report Generated: October 30, 2018

Non-Detect Results
In sample(s) classified as non-detect, the host-associated fecal gene biomarker(s) was either not detected in test replicates, one replicate was detected at a cycle threshold greater than 35 and the other was not, or one replicate was detected at a cycle threshold less than 35 and the other was not after repeated analysis.

Detected Results
In sample(s) classified as detected, the host-associated fecal gene biomarker(s) was detected in both test replicates suggesting that the host's fecal contamination is present in the sample(s). Copy number measurements reported are relative, not absolute, quantification.

Detected Not Quantified (DNQ) Results
In sample(s) classified as Detected Not Quantified (DNQ), the host-associated fecal biomarker was detected in both test replicates but in quantities below the limit of quantification. This result indicates that fecal indicators associated with the respective host was present in the sample(s) but in low concentrations.

Fecal Reference Samples
The client is encouraged to submit fecal samples from suspected sources in the surrounding area in order to gain a better understanding of the concentration of the host-associated biomarker with the regional population. A more precise interpretation would be available to the client with the submittal of such baseline samples.

Result Interpretations
Quantitative results are reported along with interpretations. Interpretations are given as "non-detect", "low concentration", "moderate concentration", or "high concentration" based on the concentration of the genetic markers found in the sample(s).

The presence of the biomarker does not signify the presence or absence of that form of fecal pollution conclusively. Only repeated sampling will enable you to draw more definitive conclusions as to the contributor(s) of fecal pollution.

Additional Testing
A portion of all samples has been frozen and will be archived for 3 months. The client is encouraged to perform additional tests on the sample(s) for other hosts suspected of contributing to the fecal contamination. A list of available tests can be found at **sourcemolecular.com/tests**

DNA Analytical Method Explanation

Water Samples: Each submitted water sample is filtered through 0.45 micron membrane filter(s). Each filter is placed in a separate, sterile 2ml disposable tube containing a unique mix of beads and lysis buffer. The sample is homogenized for 1min and the DNA extracted using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Devitations to these procedures may occur at the client's request.

Non-Water Samples: Each non-water sample submitted by the client is processed as per internal laboratory extraction procedures. An extracted DNA sample is proceed directly to PCR analysis. Details available upon request.

Amplifications to detect the target gene biomarker were run on an Applied Biosystems StepOnePlus real-time thermal cycler (Applied Biosystems, Foster City, CA) in a final reaction volume of 20ul sample extract, forward primer, reverse primer, probe and an optimized buffer. All assays are run in duplicate. Quantification is achieved by extrapolating target gene copy numbers from a standard curve generated from serial dilutions of known gene copy numbers.

For quality control purposes, a positive control and a negative control, were run alongside the sample(s) to ensure a properly functioning reaction and reveal any false negatives or false positives.

Theory Explanation of Canada Goose Bacteroidetes "Quantification" ID™

The phylum *Bacteroidetes* is composed of three large groups of bacteria with the best-known category being *Bacteroidaceae*. This family of gram-negative bacteria is found primarily in the intestinal tracts and mucous membranes of warm-blooded animals and is sometimes considered pathogenic.

Comprising *Bacteroidaceae* are the genus *Bacteroides* and *Prevotella*. The latter genus was originally classified within the former (i.e. *Bacteroides*), but since the 1990's it has been classified in a separate genus because of new chemical and biochemical findings. *Bacteroides* and *Prevotella* are gram-negative, anaerobic, rod-shaped bacteria that inhabitant of the oral, respiratory, intestinal, and urogenital cavities of humans, animals, and insects. They are sometimes pathogenic.

Fecal *Bacteroidetes* are considered for several reasons an interesting alternative to more traditional indicator organisms such as *E. coli* and *Enterococci*.[1] Since they are strict anaerobes, they are indicative of recent fecal contamination when found in water systems. This is a particularly strong reference point when trying to determine recent outbreaks in fecal pollution. They are also more abundant in feces of warm-blooded animals than *E. coli* and *Enterococci*. Furthermore, these latter two organisms are facultative anaerobes and as such they can be problematic for monitoring purposes since it has been shown that they are able to proliferate in soil, sand and sediments.

The Canada Goose Bacteroidetes ID™ service is designed around the principle that fecal *Bacteroidetes* are found in large quantities in feces of warm-blooded animals.[2,3,4,5,6] Furthermore, certain categories of *Bacteroidetes* have been shown to be predominately detected in Canada geese.[7] Within these *Bacteroidetes*, certain strains of the *Bacteroides* and *Prevotella* genus have been found in Canada geese.[7] As such, these bacterial strains can be used as indicators of Canada geese fecal contamination.

One of the advantages of the Canada Goose Bacteroidetes ID™ service is that the entire water is sampled and filtered for fecal *Bacteroidetes*. As such, this method avoids the randomness effect of culturing and selecting bacterial isolates off a petri dish. This is a particular advantage for highly contaminated water systems with potential multiple sources of fecal contamination.
Accuracy of the results is possible because the method uses PCR DNA technology. PCR allows quantities of DNA to be amplified into large number of small copies of DNA sequences. This is accomplished with small pieces of DNA called primers that are complementary and specific to the genomes to be detected.

Through a heating process called thermal cycling, the double stranded DNA is denatured and inserted with complementary primers to create exact copies of the DNA fragment desired. This process is repeated rapidly many times ensuring an exponential progression in the number of copied DNA. If the primers are successful in finding a site on the DNA fragment that is specific to the genome to be studied, then billions of copies of the DNA fragment will be available for detection in real-time.

References

[1] Scott, Troy M., Rose, Joan B., Jenkins, Tracie M., Farrah, Samuel R., Lukasik, Jerzy **Microbial Source Tracking: Current Methodology and Future Directions.** Appl. Environ. Microbiol. (2002) 68: 5796-5803.

[2] Bernhard, A.E., and K.G. Field (2000a). **Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes.** Applied and Environmental Microbiology, 66: 1,587-1,594.

[3] Bernhard, A.E., and K.G. Field (2000b). **A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA.** Applied and Environmental Microbiology, 66: 4,571-4,574.

[4] Kreader, C.A. (1995). **Design and evaluation of Bacteroides DNA probes for the specific detection of human fecal pollution.** Applied and Environmental Microbiology, 61: 1,171-1,179.

[5] Fogarty, Lisa R., Voytek, Mary **A.Comparison of Bacteroides-Prevotella 16S rRNA Genetic Markers for Fecal Samples from Different Animal Species** Appl. Environ. Microbiol. 2005 71: 5999-6007.

[6] Dick, Linda K., Bernhard, Anne E., Brodeur, Timothy J., Santo Domingo, Jorge W., Simpson, Joyce M., Walters, Sarah P., Field, Katharine G. **Host distributions of uncultivated fecal Bacteroidales bacteria reveal genetic markers for fecal source identification** Appl. Environ. Microbiol. 2005 71: 3184-3191.

[7] Fremaux, B., Boa, T., Yost, C. K. **Quantitative Real-Time PCR Asays for Sensitive Detection of Canada Goose-Specific Fecal Pollution in Water Sources.** Appl. Environ. Microbiol. 2010 76: 4886-4889.