Screening Method for Detecting Nonpoint Source (NPS) Pollutants from Septic Systems Glen Lake, 2019 - 2021

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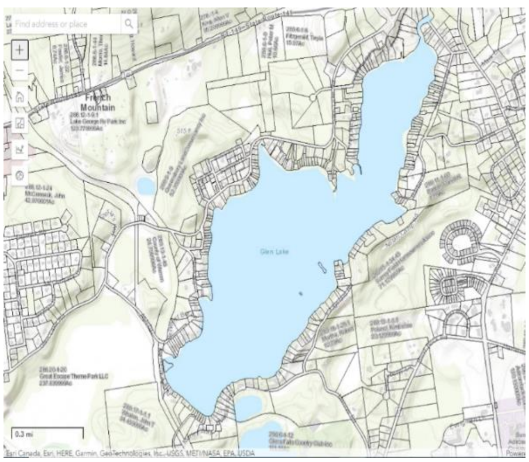
Introduction

Glen Lake is a small lake located in the Southern Adirondack Region of New York that is situated in the Upper Hudson River watershed. The 320-acre lake has 5.1 miles of shoreline, which is highly populated with numerous seasonal and year-round residential properties, as shown on the map of Glen Lake, below[1].



Since 2017, the Glen Lake Protective Association (GLPA) has partnered with SUNY Adirondack and the Warren County Soil and Water Conservation District (WCSWCD) to monitor Glen Lake for chemical and biological indicators associated with septic system runoff. According to the U.S. Environmental Protection Agency (EPA), monitoring small lakes for these indicators serves several purposes, including assessing local water quality problems, developing watershed plans to address current and prevent future problems, and educating the public about lake ecosystems[2].

The goal of this project was to develop a monitoring process to evaluate whether residential/commercial septic systems may be contributing nonpoint source (NPS) pollutants to Glen Lake, which is an established water quality concern. The effects of nonpoint source pollutants in small lakes is variable and may not always be fully assessed; however, these pollutants may have harmful effects on drinking water supplies, recreation, fisheries and wildlife[3]. On residential lakes such as Glen Lake, septic systems are often the primary source of nonpoint source (NPS) pollutants, which is an environmental concern.

Malfunctioning septic systems can leak significant amounts of chemical and biological pollutants into the ground, which are then picked up and carried by runoff from rainfall or snowmelt moving over and through the ground. Even when functioning properly, septic systems can discharge wastewater with pollutant concentrations exceeding established water quality standards. This problem grows when the system lacks proper design or maintenance. The EPA has estimated that half of all existing on-site wastewater systems are more than 30 years old, and at least 20% of systems are malfunctioning to some degree[4].

Failing septic systems may contribute to declining water quality and soil chemistry, and may pose health hazards by potentially introducing viruses, fecal contamination, and nutrients that could lead to growth of invasive water plants, algal blooms and other ecological imbalances. Because septic systems are the predominate wastewater treatment systems in non-urban areas, tracing septic pollution provides data that helps to guide the process of improving water quality[5].

Contamination from septic systems is one of the most difficult sources of nonpoint source (NPS) pollution to quantify, in part because locating a specific malfunctioning septic system within a watershed is very challenging. Fluorescent dyes such as rhodamine have been used to determine whether a septic system is contaminating surface or groundwaters; however, the usefulness of this approach is limited by several factors, including decay of the signal by sunlight, adsorption of the dye to surfaces, background florescence from natural biological sources, high detection limits, and the need to access private residences to perform the test.

Other methods that have been used to detect septic system leaks include detection of elevated concentrations of chemical agents associated with human activity in surface water, such as nitrate, phosphate, chloride, caffeine, and sucralose, and detection of biological indicators of septic contamination, including fecal bacteria or bacterial metabolites. Source-tracking methods that look for genetic markers of fecal bacteria have also been used successfully, however this type of testing requires specific laboratory equipment and trained personnel, and is therefore expensive to perform[4].

Previously, we demonstrated that concurrent detection of fecal bacteria (specifically *Escherichia coli*) and hypochlorite ion ("free" chlorine found in bleach and cleaning products) in near-shore lake samples, using commercially available test kits and procedures, is a cost-effective way to identify areas where septic system NPS pollutants are entering the lake[6]. A primary objective of this project was to optimize the process of sample collection and testing for these two indicators, which will enable trained citizen volunteers to monitor their lakes for septic system-derived pollutants.

Method

Water samples were collected by boat from 24 sampling locations one day per month between May and October. Each sampling location was identified with visual and GPS coordinates using the Google Maps app on an iPhone. The decision to collect samples at sites distributed around the lake allowed us to conduct an unbiased and comprehensive survey with regard to contaminants that might be associated with septic systems. Samples were taken as close to the shore as possible, typically between 2 – 6 feet from land. Additional samples were obtained from sites in the lake's graminoid fen and inlet areas. Other parameters, such as air and water temperature, pH, and weather conditions were recorded as well.

At each site, a "grab" sample was obtained by submerging a sterile 250 mL Nalgene bottle approximately 10 – 20 cm under the surface of the water. All samples were stored in a cooler with ice and transferred to the Microbiology Research Laboratory at SUNY Adirondack, where they were processed within 2 hours of sample collection. Testing was completed in the laboratory according to manufacturer's instruction.

To determine if fecal bacteria were present in the water, we used the IDEXX Colilert kit, which is an EPA approved testing system that can detect both total coliform bacteria and one specific fecal coliform, *Escherichia coli* in a single test[7].

IDEXX Colilert is a qualitative, culture-based test kit containing chromogenic and fluorogenic substrates that specifically react with bacterial enzymes to produce a color change and fluorescence when viewed under long wavelength UV light. As performed, this test indicates only the presence/absence of *E. coli* in water samples. Briefly, 100 mL of lake water was added to a sample bottle, and a packet of the sample reagent was added to the bottle. The bottles were inverted to mix the contents, and then incubated for 24 hours at 35°C, according to manufacturer instructions. After incubation observation of a yellow color indicates the presence of coliform bacteria, while yellow color and fluorescence, which is detected using a black fluorescent light, indicates that *E. coli* is present in the water sample.

In 2021, we evaluated the Roth Bioscience R-CARD, which is a quantitative rapid test for the detection and enumeration of *Escherichia coli*. The R-CARD is user-friendly and requires no specialized laboratory equipment other than an incubator. Briefly, 1 mL of each water sample tested was pipetted on the center of the card, and slowly covered by a plastic film which causes the sample to spread evenly across the card. Inoculated R-CARDs were then incubated at 35°C for 15 – 20 hours. After incubation, blue/green colonies (referred to as colony forming units, or cfu) were counted, which is a direct indication of the number of *E. coli* cells present in the water when the sample was collected. Because this was a new procedure and the concentration of EC in the lake samples was unknown, positive and negative controls, along with serial dilutions of lake samples were prepared and plated on R-CARDS.

The samples were also tested for free chlorine (hypochlorite ion) using a Hach DPD Free Chlorine test kit and Hach DR/800 colorimeter, according to manufacturer's instruction. This test is performed by mixing one packet of the DPD Free Chlorine Reagent with 10 mL of lake water. The DPD reagent reacts with free chlorine and forms a pink color, which is detected using the Hach colorimeter. This assay detects between 0.02 – 2.0 mg/L of chlorine, and any concentration above 0.02 mg/L was considered positive for free chlorine.

Detection of both *E. coli* and hypochlorite in a water sample was considered a positive indicator of septic system leakage into the lake. Although it is not possible to identify a specific septic system from which the pollutants may have originated, this approach can be used to highlight areas where problems may exist.

Results

During the development phase of the project in 2016, we had the opportunity to investigate a sewage spill that resulted in closure of a public beach at the south end of Lake George. A total of ten sites were tested for *E. coli* and hypochlorite. Both indicators were detected only at the beach sampling site, while other testing sites adjacent to the beach were negative for one or both indicators (data not shown).

Over a five-year period, from August of 2017 through October of 2021, water samples taken from Glen Lake were tested for *E. coli* and hypochlorite a total of 25 times. In 2017, all sites were tested once, in August. In 2018, only half of the sites were tested each month, due to funding and logistical considerations. In 2019, 2020, and 2021, all 24 sampling sites were tested, for a total of 18 testing dates. Testing results for these three years is summarized in Table 1, below:

Site #	2019						2020						2021							
	16-May	10-Jun	8-Jul	12-Aug	10-Sep	8-Oct	13-May	10-Jun	6-Jul	18-Aug	14-Sep	12-Oct	10-May	8-Jun	6-Jul	10-Aug	14-Sep	12-Oct	3 Yr Total	% Pos
1	0	0	1	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	5	27.8
2	1	0	0	0	1	0	0	0	1	0	1	0	1	1	1	1	1	0	9	50.0
3	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	0	6	33.3
4	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0	1	0	6	33.3
5	0	0	1	0	1	0	0	0	0	1	1	1	0	1	0	0	0	0	6	33.3
6	0	0	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	3	16.7
7	0	0	1	0	1	1	0	1	0	1	1	0	0	1	1	0	0	0	8	44.4
8	0	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	13	72.2
9	0	0	1	0	1	0	0	1	1	1	1	1	1	1	0	0	0	0	9	50.0
10	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1	1	0	0	5	27.8
11	0	1	1	0	1	0	0	1	0	1	1	1	0	0	1	1	1	1	11	61.1
12	0	0	0	0	0	0	0	1	1	1	1	0	0	0	1	1	0	1	7	38.9
13	0	0	1	0	1	1	0	1	0	1	0	0	0	1	1	0	0	1	8	44.4
14	0	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	5	27.8
15	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	4	22.2
16	0	0	1	0	1	0	1	1	0	0	0	1	0	0	0	0	0	1	6	33.3
17	0	0	1	1	0	1	0	1	0	0	0	1	0	0	1	0	1	1	8	44.4
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
19	1	0	0	1	0	1	1	0	0	0	1	1	0	0	1	0	1	0	8	44.4
20	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	4	22.2
21	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	3	16.7
22	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	5	27.8
23	0	1	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	6	33.3
24	0	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	4	22.2

Table 1: Summary of NPS tracking on Glen Lake from May 2019 – August 2021. Sites that were negative for one or both indicators are reported as “0.” Sites that were positive for both EC and HOCl are reported as “1.” Sites where the rate of positive tests ≥ 50% are highlighted in orange. Site

On Glen Lake, all of the shoreline sampling sites tested positive for both indicators at some point during the study period, with individual sites showing a minimum of 3 positive tests and a maximum of 13. The center of the lake, the deepest location and the most distant from any shore, was used as a negative control. Samples taken from this location were negative across all 18 sampling dates between May 2019 and October 2021. In total, this site was tested 25 times over the 5-year project span, yielding a positive result for both NPS indicators only once, in October of 2018.

For this and in previous studies, any site that showed the presence of both indicators ≥ 50% of the sampling dates was considered significant. During the three-year study period, 20 of the 24 sites were positive for both indicators sporadically, but did not exceed the 50% threshold. Four of the sampling sites were positive for both indicators ≥ 50% of the time.

The general location of these four sites (A, B, C, and D) are highlighted on the Glen Lake map (Figure 1).

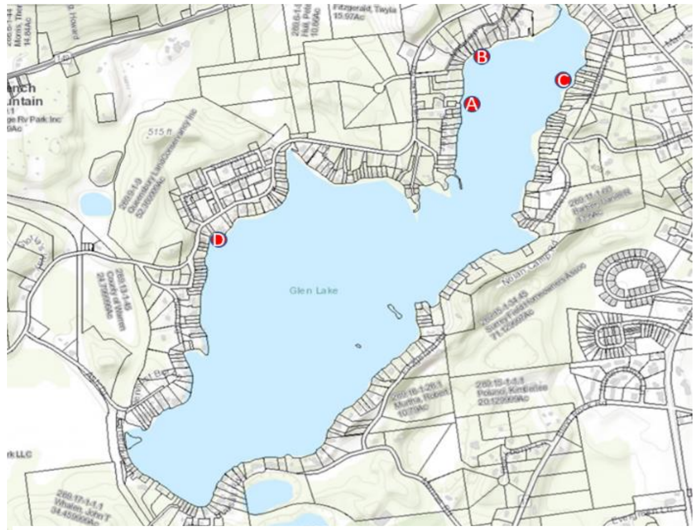


Figure 1: Map of Glen Lake showing the sites positive for both indicators ≥ 50% of the sampling dates.

We also evaluated the Roth Bioscience R-CARD test kit for enumerating *E. coli* present in lake samples, and compared the results with those obtained using IDEXX Colilert. The R-CARD provides a quantitative result indicating how many bacteria are present in the water, where the Colilert test only indicates whether *E. coli* was present or absence in the sample.

Sites where *E. coli* was indicated by a positive Colilert test correlated well with results obtained using the R-CARD, as shown in Table 2. *E. coli* cell counts ranged from 1 cfu/mL to over 50 cfu/mL. Sites where only a single *E. coli* was detected by R-CARD gave both positive and negative results with the Colilert. However, >2 *E. coli* cfu/mL correlated with a positive Colilert test.

Site	Colilert	Roth cfu/ml
1	0	1
2	0	0
3	0	1
4	0	1
5	0	0
6	1	9
7	0	9
8	1	17
9	0	12
10	1	5
11	1	10
12	1	14
13	1	51
14	1	16
15	1	15
16	1	16
17	1	20
18	0	1
19	1	1
20	1	26
21	1	55
22	1	2
23	1	2
24	1	9
Total pos	16	17

Table 2: Comparison of Colilert and R-CARD test for detection of *E. coli*

There were several advantages to using the R-CARD over Colilert. The cost per test was significantly lower than the Colilert test. Although there are advantages to performing the test in a laboratory facility in terms of the availability of microbiological laboratory equipment and disposal of chemical and biological hazardous waste, the R-CARD significantly reduces the need for specialized equipment or procedures, and can be safely disposed of in compliance with regulatory guidelines. Interpretation of the Colilert test is based on the subjective determination of color/fluorescence changes compared to a visual standard, while the R-CARD test is interpreted by counting colonies of *E. coli* present on the card, which provides an objective and quantitative result.

Discussion

Glen Lake participates in the Citizen's Statewide Lake Assessment Program (CSLAP), which is a program of lake monitoring to help understand lake conditions and inform lake management plans. CSLAP volunteers collect water quality data to evaluate chemical nutrient (nitrogen and phosphorus) enrichment, aquatic weed and algae growth, and the recreational quality of the lake. This permits monitoring of lake conditions over time, which reveals existing problems, and detects existing and emerging threats[8].

In the 2020 Report, CSLAP describes Glen Lake as mesoligotrophic (moderately unproductive) based on observed variables including moderate water clarity, moderate algae populations, and low phosphorous and nitrogen (nutrient) levels. Some of the phosphorus and nitrogen detected in the lake is soluble, which indicates a potential for increased plant/algae and microbial growth[9]. In this study, we monitored Glen Lake for two additional indicators, fecal bacteria (specifically *Escherichia coli*) and hypochlorite ion, which are both known as NPS pollutants associated with human activity, and in particular, septic systems.

Of these two metrics, free chlorine (hypochlorite ion) is a more robust indicator of human-associated activity. Hypochlorite is found in bleach and other cleaning compounds, and there are no natural sources of this compound in surface or groundwater. Hypochlorite ion is stable in lake water only for short periods after being introduced by some human-related source. Although detection of either *E. coli* or hypochlorite alone may not implicate a failing septic system with absolute certainty, when these metrics are used together, a more powerful predictive tool emerges.

In this study, we consistently found both indicators present ≥ 50% at two sites, labelled "A" and "B" on the Glen Lake map (Figure 1). Site A was positive for both indicators 72.2% of the testing dates, and Site B 61.1% of the time across a three-year period. This consistent correlation between free chlorine and *E. coli* at these two sites was considered a good indication that septic system leakage was occurring in that area of the lake, although it is not possible to identify a specific septic system as the source of the pollutants.

Two other sites, labelled "C" and "D" on the map, showed the presence of both indicators 50% of the sampling dates. For sites C and D, detection of both indicators was not consistent across years. Site C was positive for both indicators 2 out of 6 testing dates in 2019. This rate increased to 5 out of 6 dates in 2020, and returned to 2 out of 6 dates in 2021. Site D showed an increase in the frequency of positive tests in 2021.

There are several variables that may have contributed to these fluctuations. In 2020 and 2021, Covid19-related shutdowns of worksites, schools, and travel resulted in a higher rate of residential occupancy on Glen Lake than in previous years (Paul McPhillips, personal communication). Site "D" is the only public access point on Glen Lake, which is situated next to a popular lakeside restaurant. Lake residents again noted a substantial increase in the use of the boat launch area during 2021, which ultimately led to the installation of portable toilets to accommodate the increase in the number of people who were accessing Glen Lake from the boat launch.

Sites A, B, and C are all located at the northeast end of the lake, where water depths are 10 feet or less, providing the potential for higher water temperatures that could enhance reproduction of naturally occurring coliform bacteria in the lake. All three of these locations are associated with shallower water (less than 10 feet of depth), and correlate with an observed increase in growth of invasive aquatic plants close to the shore[10]. Collectively these data indicate that anthropogenic factors influence the growth of invasive plants and other organisms in this area, and suggests that management strategies should be considered to improve to preserve water quality.

A logical next step in the monitoring process would be to identify specific properties where septic system leakage may be occurring. This could be accomplished by using a modified approach to standard rhodamine dye testing, in which a large quantity of bleach is flushed in a toilet, and with the Hach test kit and colorimeter monitor the lakefront for 24 – 48 hours for hypochlorite. In our previous study, we were able to narrow the range of possible discharge to an area of approximately 6 feet of shoreline[6].

Evaluation of three years of NPS tracking data on Glen Lake supports the validity of using *Escherichia coli* and hypochlorite ion as reliable indicators of areas where septic system-derived pollutants may be entering the lake. The use of commercially available test kits simplifies the testing process. This method is cost effective and supplements the more comprehensive tests for water quality used in CSLAP and other monitoring programs. These data also provide direction and opportunity for follow-up testing to potentially identify failing septic systems so that updates or changes may be made.

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