

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay



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Executive Summary

The City of Plattsburgh has a municipal beach within the Cumberland Bay of Lake Champlain as well as other public access points. The beach and the lake are a principal economic and recreational driver for the City and the region. Similar to other beaches in New York, Vermont, and Quebec, the Plattsburgh City Beach has recently experienced uncharacteristically high levels of fecal indicating bacteria (FIB) that have resulted in beach closures. Intensive monitoring and investigation in 2015 and 2016 identified potential sources of FIB within Cumberland Bay and contributing watersheds.

This investigation attempted to link previous spatial and temporal patterns of elevated FIB (fecal coliform, *E. coli*, and/or enterococcus) to specific host sources using Microbial Source Tracking (MST) methodologies. Samples were collected during 18 sampling events between May and September 2017, from eight locations, and encompassed dry and wet weather conditions, as well as post-combined sewer overflow (CSO) events. All samples were analysed for Total Coliform and Fecal Coliform enumeration by Method SM 9223 Quantitray, *E. coli* enumeration by Method SM 9223B Colilert, Enterococci enumeration by Enterolert, and membrane filtration/archiving.

A total of 52 filtered/archived samples were selected for host-specific bacterial DNA (bacteroidetes) quantification using quantitative polymerase chain reaction (qPCR) methods, including 43 samples exhibiting *E. coli* and/or enterococcus results above NYS-DOH standards, 7 dry weather samples to assess baseline conditions unaffected by runoff contributions, and one field blank and field duplicate (for QAQC analysis). Goose and gull host bacteroidetes were chosen for enumeration due to their prevalence along the Cumberland Bay shoreline of Lake Champlain. Human, canine, and bovine host bacteroidetes were chosen for enumeration due to the potential impact (from both upstream and urban sources) on the Saranac River and Scotion Creek.

Elevations in FIB concentrations did correlate to wet weather conditions. *E. coli* and enterococcus values were higher at all sample locations after wet-weather events than dry weather events, and were at their greatest detections after CSO events. No correlation could be found between the quantified host-specific bacteroidetes DNA results and quantified *E. coli* results or enterococcus results. However, quantitative qPCR analysis of samples with elevated FIB concentrations did reveal significantly different host sources of bacteroidetes DNA based on weather conditions.

During dry weather (base flow) conditions, the highest detected host source of bacterial DNA was gull at the Plattsburgh City Beach, and bovine at the public access beach. Gulls and bovine were the highest detected host sources of bacterial DNA at the public access beach after wet weather conditions (without combined sewer overflows). Wet weather conditions that generated CSOs produced a mixture of host specific DNA bacteria in Cumberland Bay that included gulls and human as the highest sources, with canine to a lesser extent.

Should beach closures persist in the future the City of Plattsburgh may want to request proposals for host-specific mitigation strategies targeting avian and canine sources along the Cumberland Bay shoreline and at key locations throughout contributing watersheds, and human sources within Cumberland Bay.

Table of Contents

| | Page |
|--|-----------|
| Executive Summary | 3 |
| 1 Project Introduction..... | 5 |
| 2 Tasks Completed | 5 |
| 3 Methodology | 15 |
| 4 Quality Assurance Tasks Completed | 16 |
| 5 Deliverables Completed | 19 |
| 6 Conclusions | 24 |
| 7 References | 27 |
| 8 Appendices | 27 |

1 Project Introduction

The City of Plattsburgh has a municipal beach within the Cumberland Bay of Lake Champlain as well as other public access points. The beach and the lake are a principal economic and recreational driver for the City and the region. Similar to other beaches in New York, Vermont, and Quebec, the Plattsburgh City Beach has recently experienced uncharacteristically high levels of fecal indicating bacteria (FIB) that have resulted in beach closures. As a region that relies on water based tourism and recreation, identifying sources of FIB pollution within contributing watersheds and implementing FIB pollution mitigation and/or prevention measures is a necessity.

In 2015, closures of public bathing beaches in the region due to high levels of FIB spurred an investigation of more than 30 sites around Cumberland Bay, and within the contributing watersheds of the Saranac River and Scotion Creek. The study was narrowed to eight locations in 2016 by correlating the spatial and temporal aspects of elevated FIB (fecal coliform, E. coli, and enterococcus) observed in Cumberland Bay to precipitation patterns, and identified the necessity of wet-weather and post-wet weather monitoring in future data collection. Preliminary DNA analyses in 2016 indicated that geese and gulls may be a significant contributor of FIB at the beach locations.

The goal of this project in 2017 was to document if wet weather conditions would correlate to elevated FIB concentrations in Cumberland Bay, and to confirm the suspected source(s) of the bacteria using Microbial Source Tracking (MST). A specialized laboratory was subcontracted to isolate the DNA of indicator bacteria (bacteroidetes) in filtered water samples through quantitative polymerase chain reaction (qPCR) methods. The signatures of the isolated DNA were attributed to specific hosts identified as possible sources (human, cattle, canine, goose and gull) and were quantified. Goose and gull were chosen due to their prevalence along the Cumberland Bay shoreline of Lake Champlain. Canine (from residential sources) and bovine (agricultural sources) were chosen due to their potential to impact the Saranac River and Scotion Creek watersheds that discharge to Cumberland Bay. The DNA marker for human specific bacteroidetes was chosen to assess the potential impact of Combined Sewer Overflows (CSOs) located throughout the City of Plattsburgh, that have been known to surcharge to the Saranac River during high volume precipitation events, and sewage treatment plants located along the Saranac River and Scotion Creek. This report documents the field and laboratory methodologies, analytical results and findings of 18 sampling events conducted between May and September, 2017.

2 Tasks Completed

Field Tasks

A Quality Assurance Project Plan (QAPP) was approved on June 16th, 2017, and was distributed to City of Plattsburgh - Water Pollution Control Plant staff that would be conducting field sampling. Figure 1, below shows the sample locations (designated "RW" for Raw Water), and the locations of the combined sewers (CSOs) within the City of Plattsburgh, NY. Latitude and longitude coordinates for each location are included in the Appendix Section of this report.

Weather conditions, site conditions and photographs were collected at each sample location and for all sample events. All site photographs have been provided digitally to the Lake Champlain Basin Program Project Manager. Photographs 1 through 11, below, depict typical site conditions at each location.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

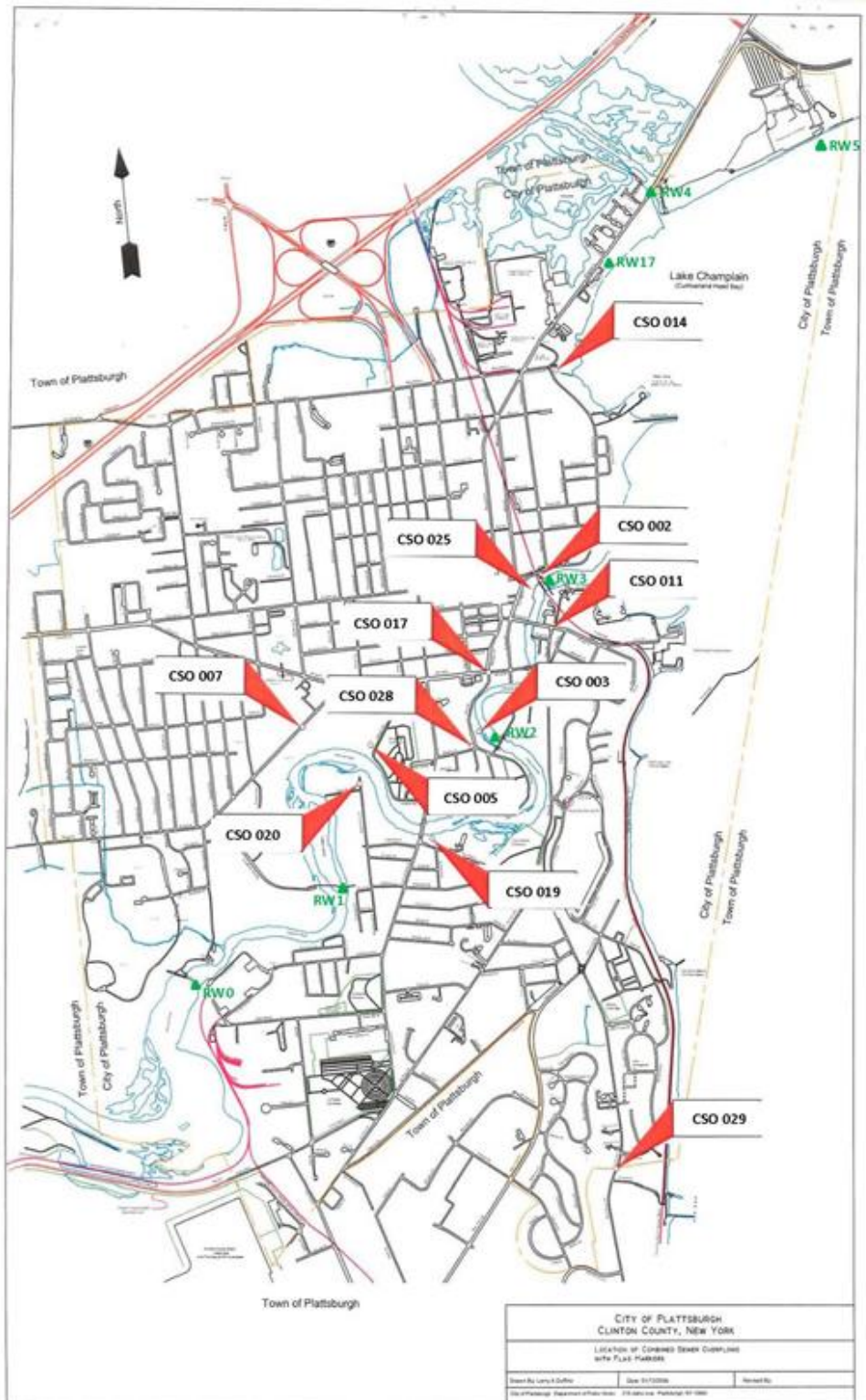


Figure 1. Location Map of Combined Sewer Overflow Outfalls (CSO) and Project Specific Sampling Locations (RW).

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay



Photograph 1. Sample location RW0 – Saranac River upgradient of CSO locations, looking downstream.



Photograph 2. Sample location RW0 – Saranac River upgradient of CSO locations, looking upstream.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay



Photograph 3. Sample location RW1 – Saranac River upgradient of CSO locations, looking upstream.



Photograph 4. Sample location RW2 - Saranac River downgradient of CSO locations #020, #007 and #019, looking upstream.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay



Photograph 5. Sample location RW2 - Saranac River downgradient of CSO locations #020, #007 and #019, looking downstream (dredging/remediation project).



Photograph 6. Sample location RW3 – Saranac River downgradient of all CSO locations, looking upstream.



Photograph 7. Sample location RW3 – Saranac River and example of CSO #002 during discharge.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay



Photograph 8. Sample location RW4 – Scotion Creek, looking downgradient at the Creek's mouth at Lake Champlain.



Photograph 9. Sample location RW5 – Plattsburgh municipal beach bathing area.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay



Photograph 10. Sample location RW6 – outfall (from adjacent parking lot) onto Plattsburgh municipal beach during discharge.



Photograph 11. Sample location RW17 – public access beach behind a McDonald's restaurant on Route 9, Plattsburgh, NY.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

Sample collection and site documentation by Water Pollution Control Plant staff commenced on May 30th, 2017, and concluded on September 6th, 2017. Samples were collected one or more times per week and included 4 dry weather events, 10 wet weather events that did not trigger Combined Sewer Overflows (CSOs), and 4 wet weather event that did trigger CSOs. An "event" includes one or more day(s) following rainfall. Dry weather events followed 72-hours without rainfall. Samples were collected for all lab analyses unless specified in Table 1. Table 1 documents the dates, weather conditions, and locations sampled for each event.

Table 1. Description of 2017 Field Sampling Events

| Sample Date(s) | Event Description and Sample Details | Locations Sampled |
|---------------------|--|---|
| 5/30 5/31 6/1 | <u>Event Type:</u> Rain, No CSOs <u>Rainfall:</u> 0.38" (5/29), 0.16" (5/30), 0.07" (5/31) <u>Sample Time:</u> 24, 48, and 72-hours post rainfall <u>CSOs:</u> N/A | RW0,1,2,3,4,5,17, WPCP Effluent (No membrane filtration on Effluent) |
| 6/7 6/8 | <u>Event Type:</u> Rain, No CSOs <u>Rainfall:</u> 0.08" (6/5), 0.73" (6/6) <u>Sample Time:</u> 24 and 48-hours post rainfall <u>CSOs:</u> N/A | RW0,1,2,3,4,5,17, WPCP Effluent (No membrane filtration on Effluent) |
| 6/13 | <u>Event Type:</u> Dry Weather <u>Rainfall:</u> N/A <u>Sample Time:</u> N/A <u>CSOs:</u> N/A | RW0,1,2,3,4,5,17, WPCP Effluent, Field Duplicate (RW5), Field Blank (No membrane filtration on Effluent) |
| 6/20 | <u>Event Type:</u> Rain, No CSOs <u>Rainfall:</u> 0.03" (6/18), 0.22" (6/19) <u>Sample Time:</u> 24-hours post rainfall <u>CSOs:</u> N/A | RW5, WPCP Effluent (No membrane filtration on Effluent) |
| 6/23 | <u>Event Type:</u> Rain, No CSOs <u>Rainfall:</u> 0.03" (6/20), 0.83" (6/23) <u>Sample Time:</u> First Flush (during rain event) <u>CSOs:</u> N/A | RW0,1,2,3,4,5,6,17, WPCP Effluent (No membrane filtration on Effluent) |
| 6/23 | <u>Event Type:</u> Rain with CSO Surcharges <u>Rainfall:</u> See Previous <u>Sample Time:</u> During CSO <u>CSOs:</u> #002 | RW3 and WPCP Effluent (Membrane Filtrations Only) |
| 6/28 | <u>Event Type:</u> Rain, No CSOs <u>Rainfall:</u> 1.2" (6/27) <u>Sample Time:</u> 24-hours post rainfall <u>CSOs:</u> N/A | RW0,1,2,3,4,5,17, WPCP Effluent (No membrane filtration on Effluent) |
| 6/30 7/1 7/2 | <u>Event Type:</u> Rain with CSO Surcharges <u>Rainfall:</u> 1.2" (6/27), 0.04" (6/28), 1.83" (6/29), 0.21" (6/30), 0.19" (7/1) <u>Sample Time:</u> 12, 36, and 60-hours post CSOs <u>CSOs:</u> #002, #007, #003, #019, #020 (6/29) | RW0,1,2,3,4,5,6,17, WPCP Effluent (No membrane filtration on Effluent) RW6 (6/30 only) |
| 7/5 | <u>Event Type:</u> Dry Weather <u>Rainfall:</u> N/A <u>Sample Time:</u> N/A <u>CSOs:</u> N/A | RW5, Field Duplicate, Field Blank |
| 7/11 | <u>Event Type:</u> Rain with CSO Surcharges <u>Rainfall:</u> 0.58" (7/8) <u>Sample Time:</u> 60-hours post CSOs <u>CSOs:</u> #002, #020 (7/8) | RW0,1,2,3,4,5,17, WPCP Effluent (No membrane filtration on Effluent) |

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

Table 1. (Continued)

| | | |
|----------------------|--|---|
| 7/18 | Event Type: Rain, No CSOs Rainfall: 0.34" (7/17) Sample Time: 24-hours post rainfall CSOs: N/A | RW0,1,2,3,4,5,17, WPCP Effluent |
| 7/21 7/22 7/23 | Event Type: Rain with CSO Surcharges Rainfall: 0.49" (7/20) Sample Time: 24, 48, and 72-hours post CSOs CSOs: #002, Indirect Manhole 1070 | RW0,1,2,3,4,5,17, WPCP Effluent RW5 only (7/22, 7/23) |
| 7/27 | Event Type: Rain, No CSOs Rainfall: 0.32" (7/25) Sample Time: 48-hours post rainfall CSOs: N/A | RW0,1,2,3,4,5,17, WPCP Effluent |
| 8/2 | Event Type: Dry Weather Rainfall: N/A Sample Time: N/A CSOs: N/A | RW0,1,2,3,4,5,17, WPCP Effluent |
| 8/8 | Event Type: Rain, No CSOs Rainfall: 0.48" (8/7) Sample Time: 24-hours post rainfall CSOs: N/A | RW0,1,2,3,4,5,17, WPCP Effluent, Field Duplicate (RW5), Field Blank |
| 8/16 | Event Type: Rain, No CSOs Rainfall: 0.06" (8/15) Sample Time: 12-hours post rainfall CSOs: N/A | RW0,1,2,3,4,5,17, WPCP Effluent |
| 8/23 8/24 8/25 | Event Type: Rain with CSO Surcharges Rainfall: 0.45" (8/22) Sample Time: 17, 41, and 65-hours post CSOs CSOs: #002, #003, #020 (8/22) | RW0,1,2,3,4,5,17, WPCP Effluent RW5 only (8/25) |
| 8/30 | Event Type: Dry Weather Rainfall: N/A Sample Time: N/A CSOs: N/A | RW 0,1,2,3,4,5,17, WPCP Effluent |
| 9/6 | Event Type: Rain, No CSOs Rainfall: 1.27" (9/3), 0.53" (9/5) Sample Time: 24-hours post rainfall CSOs: N/A | RW 0,1,2,3,4,5,17, WPCP Effluent |

Laboratory Tasks

For all field samples collected, Endyne Inc.'s Plattsburgh, New York, office performed Total Coliform and Fecal Coliform enumeration by Method SM 9223 Quantitray, E. coli enumeration by Method SM 9223B Colilert, Enterococci enumeration by Enterolert, and membrane filtration/archiving by as required by Helix Biolab. The laboratory reports generated by Endyne, Inc. can be found in the Appendix Section of this report.

Due to concern from elevated bacteria levels on two sampling events during the 2nd Quarter, archived membrane filtration samples were sent to Helix Biolab, Warren, Michigan, for analysis of host specific (human, canine, goose, gull and bovine) bacteroidetes bacterial DNA markers via qPCR Methods. Upon completion of the field sampling on September 6th, 2017, and after evaluation of the data from Endyne, Inc., thirty-eight additional samples were sent to Helix Biolab during the 3rd Quarter (on September 25th, 2017).

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

All samples were analysed for human, canine, goose, gull and bovine Bacteroidetes DNA markers. The laboratory reports generated by Helix Biolab can be found in the Appendix Section of this report. The following table summarizes the date, location and selection criteria for samples selected for DNA analysis:

Table 2. Archived Samples Submitted to Helix Biolab for DNA Analyses

| Date | Location of Samples Submitted | Selection Criteria |
|------|--|--|
| 6/23 | RW0, RW1, RW2, RW3, RW4, RW6, RW17 | Elevated E. coli and/or Enterococcus at sample locations. |
| 6/30 | RW0, RW1, RW2, RW3, RW4, RW17 | Elevated E. coli and/or Enterococcus at sample locations. |
| 7/1 | RW0, RW1, RW2, RW3, RW4, RW17 | Elevated E. coli and Enterococcus at sample locations. |
| 7/2 | RW2, RW3, RW5, RW17 | Assessment of baseline conditions 60-hours after a large storm event. |
| 7/5 | RW5, RW5D (Field Duplicate), Field Blank | Quality Assurance/Quality Control |
| 7/18 | RW1 | E. coli and Enterococcus at sample location. |
| 7/21 | RW0, RW1, RW2, RW3, RW4, RW5 | Elevated Enterococcus at sample locations (with exception of RW3 & RW5). |
| 7/27 | RW17 | Elevated E. coli at sample location. |
| 8/23 | RW0, RW1, RW2, RW3, RW5, RW17 | Elevated E. coli and/or Enterococcus at sample locations. |
| 8/24 | RW0, RW1, RW2, RW3 | Elevated Enterococcus at sample locations. |
| 8/30 | RW0, RW1, RW2, RW3, RW4, RW5, RW17 | Assessment of baseline conditions (dry weather event). |

3 Methodology

Ancillary Data Collection Methods

Weather data from the Plattsburgh International Airport weather station was obtained from the NOAA - National Weather Service's website (weather.gov). Ancillary field data was documented using a standardized data sheet. At all sample locations, times, field observations (wildlife, algae, boaters, bathers, etc.), analytical parameters (water temperature, turbidity, dissolved oxygen, pH), and photographs were collected. The field acquired parameters (water temperature, dissolved oxygen and pH) were collected by using a grab bucket and an Oakton PD 300 Waterproof Hand-held multi-probe meter. The bucket and probes were rinsed with distilled water between each location. Reusable sample bottles dedicated to each site location were used to collect a sample for turbidity (measured at the WPCP laboratory), and were rinsed with distilled water after each use. All field parameters and weather data are included in the Appendix Section of this report.

Sample Collection and Analytical Methods

Two factory sealed pre-sterilized sample Idexx bottles (120 mL) were used for E. coli and Enterococcus analysis. One new sample bottle (1L) collected filtration/archiving samples. Samples bottles were labeled with sharpie marker or pre-printed waterproof labels. The labels included the Client ID, location, date, preservation, and analyses. Sample containers were attached to a dedicated sampling rod at the Saranac River and Scotion Creek locations. Samples were collected by hand at the beach locations. Sample equipment and clothing (boots or waders) was rinsed and inspected between locations, and new disposable latex gloves were used at each location.

Samples were collected according to the Sample Collection Protocol established as part of the Project QAPP. Any variations to the field conditions and/or sample collection procedures were noted on the Field Data Sheet. The staff member that collected the field data entered results from the Field Data Sheet to an Excel spreadsheet along with his/her initials. An independent staff member verified the data entry and supplied his/her initials.

Samples collected in the field were placed immediately in a cooler with ice until received by Endyne Laboratory, Inc., Plattsburgh, NY, accompanied by a Chain of Custody form. Endyne Laboratory, Inc. conducted all bacteriology analyses and filtered/archived all samples according to protocol established by Helix Biolab, Warren, MI.

Selected (filtered) samples were collected from Endyne Laboratory, Inc., and shipped to Helix Biolab via Fedex Ground (Priority Overnight) preserved with a two pound brick of dry ice and their accompanying Chain of Custody form. Helix Biolab conducted all qPCR analyses for host specific DNA marker quantification.

4 Quality Assurance Tasks Completed

Equipment Calibration

Field analytical equipment used for this project included an Oakton PD 300 Waterproof Hand-held multi-probe meter (water temperature, dissolved oxygen and pH measurements), and a Hach 2100N Turbidimeter (turbidity). Calibration procedures for the equipment were followed according to manufacturer instructions. To maintain field precision and accuracy the instruments were calibrated to known standards prior to every date of sample collection.

Sample Preservation and Custody Review

All samples were handled and transported using the sample containers and preservation established via the Project QAPP, delivered within the appropriate hold time, and accompanied by a Chain of Custody form.

Quality Control Requirements

The Project QA Officer assessed the quality of the data received from the sub-contracted laboratories and collected in the field. The reviews were performed within two weeks of field data collection and receipt of laboratory reports. All reviewed data met the established QAQC objectives for fecal indicating bacteria.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

Precision was assessed through the use of field duplicates/replicates collected at location RW5 once per month. Precision was assessed by percent relative standard deviation (%RSD). Due to the abundance of environmental stressors that can have an effect on the fate of fecal indicating bacteria in the natural environment, the objective RSD was determined to be within 100%.

Sensitivity was assessed through the use of field blanks. Distilled water blanks were handled and transported in the same manner as locational samples. Field blank results were below the parameter specific Method Detection Limits (MDLs) or Reporting Limits (RLs). Table 3 summarizes the results of field duplicate and field blank analyses.

Table 3. RW5 Field Blank and Field Duplicate Comparison of Fecal Indicating Bacteria by Relative Standard Deviation (RSD).*

| | | Date | TC MPN/100mL | FC MPN/100mL | EC MPN/100mL | ENT MPN/100mL |
|--------------|-----------------|-----------|-----------------|-----------------|-----------------|------------------|
| Sample ID | RW5 | 6/13/2017 | 161 | 8.6 | <10 | 1.0 |
| Duplicate ID | RW5D | 6/13/2017 | 213 | 3.1 | <10 | 1 |
| Field Blank | Field Duplicate | 6/13/2017 | <1 | <1 | <1 | <1 |
| | | ST DEV | 36.77 | 3.89 | | |
| | | Mean | 187.00 | 5.85 | | |
| | | RSD | 19.66% | 66.48% | 0.00% | 0.00% |
| | | | | | | |
| | | Date | TC MPN/100mL | FC MPN/100mL | EC MPN/100mL | ENT MPN/100mL |
| Sample ID | RW5 | 7/5/2017 | 1203 | 21.6 | 21.6 | 8.4 |
| Duplicate ID | RW5D | 7/5/2017 | 921 | 19.7 | 20.1 | 7.4 |
| Field Blank | Field Duplicate | 7/5/2017 | <1 | <1 | <1 | <1 |
| | | ST DEV | 199.40 | 1.34 | 1.06 | 0.71 |
| | | Mean | 1062.00 | 20.65 | 20.85 | 7.90 |
| | | RSD | 18.78% | 6.51% | 5.09% | 8.95% |
| | | | | | | |
| | | Date | TC MPN/100mL | FC MPN/100mL | EC MPN/100mL | ENT MPN/100mL |
| Sample ID | RW5 | 8/8/2017 | 1780 | 211 | 132 | 30.0 |
| Duplicate ID | RW5D | 8/8/2017 | 1150 | 75 | 63 | 10 |
| Field Blank | Field Duplicate | 8/8/2017 | <1 | <1 | <1 | <1 |
| | | ST DEV | 445.48 | 96.17 | 48.79 | 14.14 |
| | | Mean | 1465.00 | 143.00 | 97.50 | 20.00 |
| | | RSD | 30.41% | 67.25% | 50.04% | 70.71% |

*TC=Total Coliform, FC=Fecal Coliform, EC=E. coli, ENT=Enterococcus.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

Field duplicate excursions were noted for the DNA marker quantification results from Helix Biolab and are summarized in Table 4. The Field Blank did not have any DNA markers detected from any of the selected hosts. Thus eliminating field sampling and transportation, or filtration/archiving by Endyne Laboratory, Inc. as possible sources of failure to meet the RSD objective. Re-analysis of samples was conducted by Helix Biolab with similar results, eliminating laboratory sources of failure.

The field duplicate samples collected as part of this project were all collected from the municipal beach. It is therefore likely that environmental conditions (such as wind and wave action, sediment mixing, etc.) may be the cause of the high RSD values for the DNA data, therefore the qPCR data was deemed acceptable by the Project QA officer. In addition, despite the numerical deficiency of the data the qualitative results are similar between the RW5 sample and the duplicate (seagulls being the significantly largest contributor of microbial DNA in both samples, and canines/geese being the significantly lowest contributors).

Table 4. RW5 Field Blank and Field Duplicate Comparison of DNA Marker Identification by Host Contributor.*

| | | | Human | Canine | Geese | Gull | Bovine |
|--------------|-----------------|----------|------------------------|------------------------|------------------------|------------------------|------------------------|
| | | Date | <u>Fold Proportion</u> | <u>Fold Proportion</u> | <u>Fold Proportion</u> | <u>Fold Proportion</u> | <u>Fold Proportion</u> |
| Sample ID | RW5 | 7/5/2017 | 16158.44 | 0 | 1 | 17198.6 | 2759.13 |
| Duplicate ID | RW5D | 7/5/2017 | 1520.15 | 1 | 34.54 | 10960.3 | 8422.31 |
| Field Blank | Field Duplicate | 7/5/2017 | 0 | 0 | 0 | 0 | 0 |
| | | ST DEV | 10350.83 | 0.71 | 23.72 | 4411.12 | 4004.47 |
| | | Mean | 8839.30 | 0.50 | 17.77 | 14079.43 | 5590.72 |
| | | RSD | 117.10% | 141.42% | 133.46% | 31.33% | 71.63% |

*Proportional amounts of host source specific DNA markers are expressed as a fold difference between the host source specific DNA marker detected in greater quantity versus the host source specific DNA marker detected in lesser quantity. For example, if host source specific DNA marker "A" has a 2 fold greater proportional fold difference than host source specific DNA marker "B", this means that the quantity of host source specific DNA marker "A" detected was 2 times greater than the quantity of host source specific DNA marker "B" detected at that collection site.

Invasive Species

Invasive species spread was prevented by using new sterilized collection bottles and latex gloves at each sampling location. In addition, samples were collected from upstream to downstream. Sample equipment and clothing (boots/waders) were rinsed and inspected between sampling events.

5 Deliverables Completed

The Project Quality Assurance Project Plan was developed and approved on June 16, 2017. Quarterly reports were submitted and approved on July 10th, 2017, and October 10th, 2017.

Challenges encountered during field sampling were related to the timing of precipitation events. Most rainfall occurred in the evening hours or on weekends when the sub-contracted laboratory was closed and/or sampling conditions were unsafe (after dusk). However, the laboratory did make an exception from 6/30/2017 to 7/2/2017 and from 7/21/2017 to 7/23/2017, due to the multiple combined sewer overflows to the Saranac River during the two events. No other problems were encountered during this study.

Summary tables of Total Coliform, Fecal Coliform, E. coli and Enterococcus results for each sample location are provided in the Appendix Section of this report. There were no detections of Fecal Coliform above the New York State Department of Health freshwater bathing water standard (1,000 fecal coliform bacteria per 100 mL) for any of the samples collected as part of this study. E. coli and enterococcus were detected in samples above the NYS-DOH freshwater bathing standard (235 E. coli per 100 mL, and 61 enterococci per 100 mL) at locations throughout the Saranac River, Scotion Creek, and the two beach locations during some of the wet weather events. Enterococcus was the only parameter that was detected above the NYS-DOH bathing standard during a dry weather sampling event (6/13/2017) at the Saranac River locations RW1, RW2, and RW3. Tables 5 and 6, below, summarize the FIB results.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

Table 5. Summary of E. coli Results by Location

| Weather Event Type* | Date | RW0 EC | RW1 EC | RW2 EC | RW3 EC | RW4 EC | RW5 EC | RW17 EC | RW6 EC | WPCP EFFLUENT |
|---------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---------------|
| | | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL |
| 2 | 5/30/2017 | 36.8 | 47.3 | 162 | 83.6 | 20 | 28.2 | 108 | | 9.8 |
| | 5/31/2017 | 41 | 41 | 52 | 31 | 20 | 10 | 31 | | 5.2 |
| | 6/1/2017 | 41 | 20 | 86 | 10 | 10 | 30 | 63 | | 7.5 |
| 2 | 6/7/2017 | 51 | 75 | 74 | 96 | 31 | < 10 | < 10 | | 7.5 |
| | 6/8/2017 | 10 | 41 | 41 | 41 | 63 | 31 | 97 | | 6.2 |
| 1 | 6/13/2017 | 31 | 41 | 75 | 20 | 20 | < 10 | < 10 | | 5.2 |
| 2 | 6/20/2017 | | | | | | 13.0 | | | 13.4 |
| 2 | 6/23/2017 | 74 | 521 | 576 | 171 | 108 | 10 | 285 | 75 | 411 |
| 2 | 6/28/2017 | 40.4 | 48.8 | 46.4 | 66.3 | 41 | 41 | 63 | | 30.7 |
| 3 | 6/30/2017 | 373 | 496 | 683 | 836 | 3441 | 63 | 203 | 10 | 594 |
| | 7/1/2017 | 594 | 631 | 857 | 578 | 404 | 426 | 299 | | |
| | 7/2/2017 | 211 | 173 | 187 | 228 | 52 | 108 | 146 | | |
| 1 | 7/5/2017 | | | | | | 21.6 | | | 6.2 |
| 3 | 7/11/2017 | 21.3 | 35 | 29.8 | 25.9 | 5.2 | 51 | 158 | | 15.8 |
| 2 | 7/18/2017 | 20 | 298 | 92 | 74 | 63 | 110 | 175 | | 8.5 |
| 3 | 7/21/2017 | 20 | 63 | 20 | 10 | 86 | 86 | < 10 | < 10 | |
| | 7/22/2017 | | | | | | 20 | | | |
| | 7/23/2017 | | | | | | < 10 | | | |
| 2 | 7/27/2017 | 52 | 52 | 10 | 41 | 120 | 30 | 259 | | 16.9 |
| 1 | 8/2/2017 | 85 | 10 | 41 | 30 | 10 | 226 | 52 | | 3.1 |
| 2 | 8/8/2017 | < 10 | 41 | 63 | 52 | 10 | 132 | 148 | | 7.3 |
| 2 | 8/16/2017 | 41 | 30 | 10 | 63 | 20 | 31 | 109 | | 6.3 |
| 3 | 8/23/2017 | 74 | 73 | 98 | 75 | 41 | 990 | 377 | | 14.8 |
| | 8/24/2017 | 41 | 161 | 63 | 63 | 51 | 10 | 41 | | 7.4 |
| | 8/25/2017 | | | | | | 30 | | | |
| 1 | 8/30/2017 | 75 | 62 | 31 | 10 | 20 | < 10 | 10 | | 9.7 |
| 2 | 9/6/2017 | 63 | 73 | 52 | 31 | < 10 | 20 | 108 | | 3.1 |

* 1=Dry Weather Sampling, 2=Wet Weather Sampling, 3=Wet Weather Sampling After Combined Sewer Overflow(s)

Note: Yellow Highlight means the result exceeded standard for Bathing Water Criteria for Fresh Water.

Note: NYS DOH Water Quality Standard: 235 E. coli per 100 mL for freshwater

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

Table 6. Summary of Enterococcus Results by Location

| Weather Event Type* | Date | RW0 ENT | RW1 ENT | RW2 ENT | RW3 ENT | RW4 ENT | RW5 ENT | RW17 ENT | RW6 ENT | WPCP EFFLUENT |
|---------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|---------------|
| | | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL | MPN/100mL |
| 2 | 5/30/2017 | 47.5 | 90.9 | 580.0 | 214.0 | 10.0 | 8.5 | 30.0 | | |
| | 5/31/2017 | 18.0 | 17.0 | 16.0 | 12.0 | 29.0 | 7.4 | 13.0 | | 8.6 |
| | 6/1/2017 | 30.1 | 33.7 | 41.1 | 26.3 | 22.3 | 4.0 | 15.5 | | |
| 2 | 6/7/2017 | 72.3 | 82.9 | 55.4 | 77.6 | 40.7 | 3.0 | 16.8 | | 4.1 |
| | 6/8/2017 | 16.1 | 19.3 | 18.3 | 21.8 | 19.0 | 17.2 | 11.6 | | |
| 1 | 6/13/2017 | 15.6 | 91.4 | 80.3 | 61.9 | 20.0 | 1.0 | < 1.0 | | |
| 2 | 6/20/2017 | | | | | | 12.2 | | | |
| 2 | 6/23/2017 | 84 | 356 | 1550 | 395 | 120 | 10 | 171 | 529 | 343 |
| 2 | 6/28/2017 | 39.1 | 51.2 | 33.2 | 32.0 | 36.3 | 7.0 | 31.8 | | 11.0 |
| 3 | 6/30/2017 | 517 | 1203 | 1203 | 1203 | > 2420 | 30 | 109 | 95 | 135 |
| | 7/1/2017 | 86 | 199 | 197 | 228 | 544 | 301 | 96 | | |
| | 7/2/2017 | 41 | 52 | 74 | 85 | 10 | 31 | 108 | | |
| 1 | 7/5/2017 | | | | | | 8.4 | | | 3 |
| 3 | 7/11/2017 | 3 | 19.9 | 41.9 | 31.2 | 48.4 | 20 | 20 | | |
| 2 | 7/18/2017 | 50.6 | 304.4 | 60.5 | 51.2 | 62 | < 10 | < 10 | | |
| 3 | 7/21/2017 | 400 | 75 | 75.1 | 55.4 | 108 | 31 | 10 | | 10.8 |
| | 7/22/2017 | | | | | | 9.2 | | | |
| | 7/23/2017 | | | | | | < 10 | | | |
| 2 | 7/27/2017 | 32.5 | 39.8 | 40 | 27.5 | 73 | 10 | 41 | | |
| 1 | 8/2/2017 | 10.5 | 34.2 | 31.7 | 28.0 | < 10 | 52 | < 10 | | 2 |
| 2 | 8/8/2017 | 19 | 30 | 50 | 46 | 20 | 30 | 20 | | |
| 2 | 8/16/2017 | 71.8 | 101.2 | 74.5 | 49.9 | 30 | < 10 | 30 | | 20 |
| 3 | 8/23/2017 | 123.5 | 146.2 | 102.6 | 79.9 | 20 | 20 | 10 | | 1 |
| | 8/24/2017 | 107.3 | 113.3 | 70.8 | 78.7 | 20 | < 10 | 20 | | |
| | 8/25/2017 | | | | | | 1.0 | | | |
| 1 | 8/30/2017 | 16.5 | 22.3 | 8.5 | 4.1 | < 10 | < 10 | < 10 | | 1 |
| 2 | 9/6/2017 | 235 | 240 | 222 | 225 | 20 | < 10 | 41 | | 14.5 |

* 1=Dry Weather Sampling, 2=Wet Weather Sampling, 3=Wet Weather Sampling After Combined Sewer Overflow(s)

Note: Yellow Highlight means the result exceeded standard for Bathing Water Criteria for Fresh Water.

NYS DOH Water Quality Standard: 61 enterococci per 100 mL freshwater

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

A total of 52 samples were selected for DNA marker quantification, including 43 samples exhibiting E. coli and/or enterococcus results above NY-DOH standards, 7 dry weather samples to assess baseline conditions unaffected by runoff contributions, and one field blank and field duplicate for QAQC analysis (discussed above). The samples chosen for DNA analysis are summarized in Table 7.

All samples were analysed for DNA bacteroidetes markers that are host specific. Goose and gull hosts were chosen due to their prevalence along the Cumberland Bay shoreline of Lake Champlain. Human, canine, and bovine were chosen due to their potential to impact the Saranac River and Scotion Creek watersheds that discharge to Cumberland Bay.

Summary tables of DNA marker quantification results for each sample location are provided in the Appendix Section of this report. Table 7, below summarizes the results of the DNA analyses by weather condition.

Table 7. Samples Selected for DNA Analysis

| Sample Date | Weather Event Type* | HRS ¹ | RW0 | RW1 | RW2 | RW3 | RW4 | RW5 | RW17 |
|--|---------------------|------------------|------------|------------|------------|------------|------------|-----------|-----------|
| | | | ECENT DNA | ECENT DNA | ECENT DNA | ECENT DNA | ECENT DNA | ECENT DNA | ECENT DNA |
| 6/23/2017 | 2 | FF | X Gull | X X Gull | X X Gull | X Gull | X Gull | | X X Gull |
| 6/30/2017 | 3 | 12 | X X Canine | X X Canine | X X Canine | X X Canine | X X Bovine | | X Gull |
| 7/1/2017 | | 36 | X X Human | X X Canine | X X Bovine | X X Canine | X X Bovine | X X Gull | X X Gull |
| 7/2/2017 | | 60 | | | X Canine | X Bovine | | Human | X Human |
| 7/5/2017 | 1 | | | | | | | Gull | |
| 7/18/2017 | 2 | 24 | | X X Gull | | | | | |
| 7/21/2017 | 3 | 24 | X Canine | X Canine | X Canine | Human | X Goose | Gull | |
| 7/27/2017 | 2 | 48 | | | | | | | X Bovine |
| 8/23/2017 | 3 | 17 | X Gull | X Bovine | X Bovine | X Bovine | | X Human | X Canine |
| 8/24/2017 | | 41 | X Human | X Bovine | X Bovine | X Canine | | | |
| 8/30/2017 | 1 | | Canine | Canine | Human | Canine | Human | Gull | Bovine |
| X=Detection of EC (E. coli) or ENT (Enterococcus) exceeded the NYS DOH Water Quality Standard for freshwater bathing areas | | | | | | | | | |
| * 1=Dry Weather Sampling, 2=Wet Weather Sampling, 3=Wet Weather Sampling After Combined Sewer Overflow(s) | | | | | | | | | |
| ¹ Time in Hours Between Rain Event and Sample Collection, FF=First Flush (Sample Collected During Rain Event) | | | | | | | | | |

Dry weather conditions were assessed on 7/5/2017 and 8/30/2017. The highest detected host in samples from the Saranac River was canine (with the exception of RW2, where the highest signature was human). The highest host specific marker in Scotion Creek was human. In Cumberland Bay, RW5 (Plattsburgh Municipal Beach) had gull as the highest DNA marker. The result of the field duplicate at RW5 was also gull. Bovine was detected as the highest DNA marker at the public access beach (RW17). Refer to Table 8, below.

Wet weather conditions were assessed over three sampling events (a first flush capture on 6/23/2017, 24-hours post storm capture on 7/18/2017, and 48-hours post storm capture on 7/27/2017). Gull was detected as the highest DNA marker from all Saranac River locations and at the Scotion Creek location for all three events. The public access beach in Cumberland Bay (RW17) had gull as the highest DNA host in one sample, and bovine as the highest DNA signature in one sample. Samples from the Plattsburgh Municipal beach were not submitted for DNA analysis for these events because the FIB results were all below freshwater bathing standards. Refer to Table 8, below.


Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

Wet weather conditions generating combined sewer overflows in the Saranac River were assessed over three different events (6/30/2017-7/2/2017, 7/21/2017, and 8/23/2017-8/24/2017). Samples for these events were collected between 12 and 60 hours post sewer overflow(s). Five (5) samples each were submitted from RW0 and RW1 (locations upstream of CSOs). RW0 had dog, human, and gull as the highest host-specific DNA markers. RW1 either had canine or bovine as the highest marker. Six (6) samples each were submitted from RW2 and RW3 (locations downstream of CSOs) and had either canine or bovine as the highest DNA markers (with the exception of one RW3 sample on 7/21/2017 that had human DNA markers as the highest signature). Refer to Table 8, below.

To assess the impact of CSOs to Cumberland Bay, four samples were selected from each of the beach locations, RW5 and RW17. The highest DNA markers at RW5 (municipal beach) were gull for two sample dates, and human for one sample date. RW17 (public access beach) had gull as the highest DNA marker on 2 of the sample dates, human for one sample date, and canine for one sample date. Refer to Table 8, below.

Due to high enterococcus and/or E. coli results, archived samples were sent from Scotion Creek on three different dates, two of which resulted in bovine as the highest DNA marker and one had goose as the highest. Refer to Table 8, below.

Table 8. Summary of Highest Detected Host Source(s) by Location and Weather Condition

| Location Description | Sample Location | Dry Weather Conditions | Wet Weather Conditions -NO CSOs | Wet Weather Conditions After CSOs |
|---|-----------------|------------------------|---------------------------------|-----------------------------------|
| Upstream - Saranac River  | RW0 | Canine | Gull | Canine, Human , Gull |
| | RW1 | Canine | Gull | Canine , Bovine |
| | RW2 | Human | Gull | Canine, Bovine |
| Downstream - Saranac River | RW3 | Canine | Gull | Canine, Bovine, Human |
| | | | | |
| Scotion Creek | RW4 | Human | Gull | Bovine, Goose |
| | | | | |
| Plattsburgh Municipal Beach | RW5 | Gull | No Samples | Gull , Human |
| | | | | |
| Public Access Beach - McDonald's | RW17 | Bovine | Gull, Bovine | Gull, Human , Canine |

In addition to the above sampling data, one sample was selected from RW6, a storm sewer outfall to the public beach from an adjacent parking lot. Sanitary lines are also in the vicinity of the parking lot; therefore the sample was selected to confirm the presence or absence of sanitary sewer influence. The result confirmed gull as the highest DNA marker for the sample.

6 Conclusions

The goal of this project in 2017 was to document if wet weather conditions would correlate to elevated FIB concentrations in the Cumberland Bay of Lake Champlain, and to investigate suspected source(s) of the bacteria using Microbial Source Tracking (MST). Bacteroidetes DNA markers were chosen based on the most likely rural and urban inputs to surface water within the Saranac River watershed. This study did not attempt to identify sources of contamination from river or beach sediments. Samples were successfully collected over 15 consecutive weeks, from eight locations, and encompassed dry and wet weather conditions, as well as post-combined sewer overflow (CSO) events.

Analyses of fecal indicating bacteria (*E. coli* and enterococcus) were conducted on all samples. Elevations in FIB concentrations did correlate to wet weather conditions. *E. coli* and enterococcus values were higher at all sample locations after wet-weather events than dry weather events, and were at their greatest concentrations after CSO events. DNA analysis indicated the highest detected host source of bacteria post CSO-events was canine (Saranac River), ruminant (Scomotion Creek), or gull (beach locations suggesting that the majority of bacterial contamination from wet weather may not be primarily from CSOs). The Saranac River and Scomotion Creek locations had a greater number of samples exceeding NYS-DOH bathing water standards after wet weather events than the beach locations in Cumberland Bay. This could be attributed to mixing and/or dilution of the source waters after entering Lake Champlain.

No correlation could be found between the quantified host-specific bacteroidetes DNA marker results and quantified *E. coli* results or enterococcus results. However, quantitative qPCR analysis of samples with elevated FIB concentrations did reveal significantly different host sources of bacteroidetes DNA based on weather conditions.

Dry Weather Results

During dry weather (base flow) conditions, the highest detected host source of bacterial DNA was gull at the Plattsburgh City Beach, and bovine at the public access beach. Results from the Saranac River and Scomotion Creek locations did not correlate to the beach samples (canines and humans were the highest DNA hosts detected) suggesting that they are not the primary source of bacteria to Cumberland Bay during base flow conditions. The notable numbers of seagulls during field sampling activities during the summer months may be the primary source of gull DNA at the public beach, and could be the focus of future mitigation efforts along the shoreline.

Wet Weather Results

Gulls and bovine were the highest detected host sources of bacterial DNA at the public access beach after wet weather conditions (without combined sewer overflows). All locations along the Saranac River and Scomotion Creek had gull as the highest host-source. This suggests that runoff from these streams contributes to the fecal bacteria pollution to Cumberland Bay, and could be the focus of future mitigation efforts.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

Wet Weather with CSO(s) Results

All locations along the Saranac River had canine as the dominant host-source (with bovine as the second highest host-source) after wet weather conditions that generated combined sewer overflows (CSOs) . Bovine was the highest host-source in samples from Scotion Creek.

Wet weather conditions that generated CSOs produced a mixture of host specific DNA bacteria in Cumberland Bay (beach locations) that included gulls and human as the highest sources, with canine to a lesser extent. Field notes for the dates when the highest host specific markers were from human bacteria indicated that the bathing area of the Plattsburgh City Beach was occupied by 40-60+ people during the sample collection times (60-hours on 7/2, and 17-hours post rainfall on 8/23). It could not be determined from the data if the human sources of bacterial DNA were from the beachgoers, boaters, or the CSOs, and could warrant further investigation, should the need arise.

The experimental design of this study was driven by the assumption that Combined Sewer Overflow events are dominated by human fecal pollution that would impact water quality to Cumberland Bay. Future efforts to evaluate the potential for CSOs to impact the water quality within Cumberland Bay would be aided by discharge measurements at specific CSO locations and DNA analysis of direct capture of CSO effluent during discharge. The City of Plattsburgh is in the process of obtaining and installing flow measurement devices at the CSO outfall locations.

This study has confirmed that the higher the intensity and/or duration of wet weather events, the greater fecal contamination contribution to Cumberland Bay, however, MST efforts to link specific sources of fecal contamination to CSO activity were inconclusive. On the contrary, non-point sources of runoff (canines, bovine and gulls) associated with storm water and wet weather discharges appear to have a greater impact on fecal contamination to Cumberland Bay than CSO discharges. In addition, data confirms upstream sources of fecal contamination as evidenced by results obtained from sites RW-0 and RW-1, both upstream of the combined sewer network.

Using Microbial Source Tracking to Identify and Prevent Fecal Pollution to Cumberland Bay

Source Mitigation

The sampling conducted at two beaches within the Cumberland Bay of Lake Champlain under a variety of weather conditions indicated the majority of bacterial DNA is from gulls, followed by humans, bovine and canine. Should beach closures persist in the future the City of Plattsburgh may want to request proposals for host-specific mitigation strategies including but not limited to:

- Avian Sources along shoreline and at key locations throughout contributing watersheds:
 - Landscaping deterrents
 - Sound deterrents
 - Habitat management
 - No feeding policies/signage
 - Public awareness
- Human Sources within Cumberland Bay
 - Public education and awareness campaign at marinas and popular docking sites
- Canine Sources along shoreline at at key locaitons throughout contributing watersheds
 - Increased pet waste stations
 - Awareness program and signage at beach locations

Possible future work to support the implementation of Opportunities for Action might include follow up surveys and/or FIB testing to confirm the level of success of any future mitigation efforts.

7 References

Hach Company, Model 2100N, Laboratory Turbidimeter, Instruction Manual, 1997.

Instruction Manual, PD 300, Waterproof Hand-held pH/Dissolved Oxygen/Temperature Meter, Rev3 09/06.

8 Appendices

Appended Documents:

- Appendix A – Latitude and Longitude Coordinates of Sample Locations
- Appendix B – Laboratory Reports, Endyne Laboratory, Inc.
- Appendix C – Laboratory Reports, Helix Biolab
- Appendix D – Weather Data - Field Sampling and NOAA Supporting Documents
- Appendix E – Ancillary Field Data
- Appendix F - Summary Data Tables of Fecal Indicating Bacteria
- Appendix G – Summary Data Tables of Bacteroidetes DNA Marker Quantification

Photos and Electronic Data:

All site photographs and electronic data sets have been supplied to the Lake Champlain Basin Program Project Manager

