# Tracking Bacterial Contamination in the Lamprey River Watershed

## Final Report

to the Lamprey Rivers Advisory Committee

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## INTRODUCTION AND BACKGROUND

The main goal of this project is to continue monitoring at key sites in the Lamprey River Watershed (LRW) and expand at sites in areas of concern, especially the Moonlight Brook watershed, to provide essential data for assessing water quality, public health risks and sources of any contamination. Addressing this overarching goal will serve to:

1.) Expand the baseline of information on bacterial pollution to assess water quality status, trends, and contamination sources in the Lamprey River and the Moonlight Brook watersheds.
2.) Target rainfall events to determine if these events trigger elevated bacterial concentrations and/or different pollution sources.
3.) Compile data from ongoing and past bacterial monitoring efforts in the Great Bay watershed.
4.) Assess the potential for eliminating or mitigating pollution sources identified by this study.
5.) Extend findings to interested groups through meetings and published reports.

This Final Report is a summary of all project findings, as well as an updated summary of data from other earlier and ongoing projects related to microbial contamination of the watershed. The report relates particularly to a Goal of the 2013 Lamprey River Management Plan (https://www.lampreyriver.org/about-us-2013-management-plan-draft) under "Enough Clean Water": *Ensure that the Lamprey rivers meet or exceed standards for "fishable and swimmable" water for the health and enjoyment of all species.* The specific focus of this study was assessment of water for swimmable and other recreational uses, using study-generated and other data in comparison to State bacterial indicator standards (NHDES 2019a; 2020a) to enable identifying sites and areas that are clean or of public health concern. The report also sought to identify data trends to track progress or detect new or emerging problems with water quality.

Providing a baseline of information related to bacterial pollution in the Lamprey River and the Moonlight Brook watersheds is important because there are little to no data related to fecal contamination of recreational surface waters other than designated beaches available from the State of New Hampshire in recent years, based on what is presented in their reports related to river water quality (NHDES 2019b; 2020b&c, 2021). These reports include little discussion of this indicator beyond 'designated' beaches and the shellfish program. There is a searchable category for Beaches with posted fecal bacterial data on the NHDES OneStop database (https://www4.des.state.nh.us/DESOnestop/BasicSearch.aspx), while this report provides a convenient way to access bacterial data for other recreational surface-water uses.

**The Intended Audience** and beneficiaries of this work include: 1.) The LRAC and local volunteers and citizens by providing information about the water quality and potential public health risks for recreating in the Lamprey River watershed and surrounding estuary; 2.) Local and state resource, public health and public works personnel who can use the data to focus resources and effort on problem areas where water pollution may pose a threat or restricts use. 3.) Monitoring program managers who can augment their programs with similar efforts. We intend to present the study findings at several meetings in 2024. The PI will discuss findings at the annual Great Bay Estuarine Research Reserve Research Symposium in Stratham, NH in

January 2024 and some of the data will be used by students to present research posters at the UNH Undergraduate Research Conference in April 2024. The data will also be part of an ongoing evaluation and summarization of findings from several dozen recent (2018 to present) microbial source tracking projects conducted by the Jones lab at UNH in areas ranging from Martha's Vineyard, MA to Trenton Harbor, ME.

**The Evaluation Process** for this project includes data analysis and interpretation, using comparisons of data to State water quality standards to enable clear explanation of the potential significance of the findings. We will track who gets involved and their interests, and how many State, Federal and local agencies are provided with the Final Report. It will be important to also track what management actions are undertaken because of this work once it is made available. The elimination of identified pollution sources can be a direct benefit that can also be tracked.

## METHODS

Sample collection by land for analysis of bacterial pollutants occurred at 8 sites where surface water recreation occurs (Tab. 1; Fig. 1). Site 1* is near a site listed as NHEPLRDO16 and was sampled in the tidal portion at low tide. Site 2 is in the dam impoundment area (NHRIV600030709-13) of lower Piscassic River. Site 3 corresponds to the NHDES water quality monitoring program site 07T-LMP and is downstream from 08-LMP. Site 4 is located between NHDES sites 11-LMP and 11A-LMP. Site 5 is in section NHRIV600030703-15 behind the Epping Town Hall; Site 6 is in section NHRIV600030703-07-02 at Carroll Beach behind the Raymond Elementary School. Site PB-MS at the Pecker Bridge on Main Street in Raymond NH corresponds to the NHDES site 20-LMP in section NHRIV600030703-07-01, and Site LfRd, is just downstream of Langford Road, at NHDES site 21-LMP in section NHRIV600030703-05.

| Study Site | Assessment ID number/site ID | Unit Name | Type** of Recreational use | Last sample | Last exceed | Classification Category† |
|---|---|---|---|---|---|---|
| Site 1* | NHRIV600030709-13 | Moonlight Brook | Primary Contact | 2008 | 2000 | 3-ND |
| MBO | | upstream of Moonlight Bk mouth | Secondary Contact | 2008 | 1996 | 3-ND |
| Site 2 | NHIMP600030708-03 | Piscassic Park Boat Launch | Primary Contact | 2007 | 2005 | 3-ND |
| PRBL | near 01-PIS | (Lamprey R. impoundment) | Secondary Contact | 2007 | N/A | 3-ND |
| Site 3 | NHIMP600030709-02 | Wiswall Dam | Primary Contact | 2008 | N/A | 3-ND |
| WD | 08-LMP | just above the dam | Secondary Contact | 2008 | N/A | 3-ND |
| Site 4 | NHRIV600030709-01 | Upstream of Wadleigh Falls | Primary Contact | 2007 | 1999 | 4A-P |
| WF | 11-LMP | Lee public canoe boat launch | Secondary Contact | 2007 | N/A | 3-ND |
| Site 5 | NHRIV600030703-15 | Behind Epping Town Hall | Primary Contact | 2018 | 2018 | 4A-P |
| ETH | 13A-LMP | (Middle Lamprey River) | Secondary Contact | 2018 | 2002 | 2-G |
| Site 6 | NHRIV600030703-07-02 | Carroll Lake Beach | Primary Contact | 2006 | 2006 | 4A-P |
| RES | BCHCLBRAY | Behind Raymond Elem. Sch. | Secondary Contact | 2006 | N/A | 3-ND |
| PB-MS | NHRIV600030703-07-01 | Pecker Bridge Main Street | 20-LMP | 1999 | N/A | 3-ND |
| | 20-LMP | downstream of Carroll Lake | | 1999 | N/A | 3-ND |
| LfRd | NHRIV600030703-05 | Langford Road | 21-LMP | 1999 | 1990 | 3-ND |
| | 21-LMP | Lamprey River crossing | | 1999 | N/A | 3-ND |

*All sites in the Moonlight Brook watershed fall under this same Assessment Unit

†4A-P: Does not meet water quality standards; the impairment is more severe and causes poor water quality;

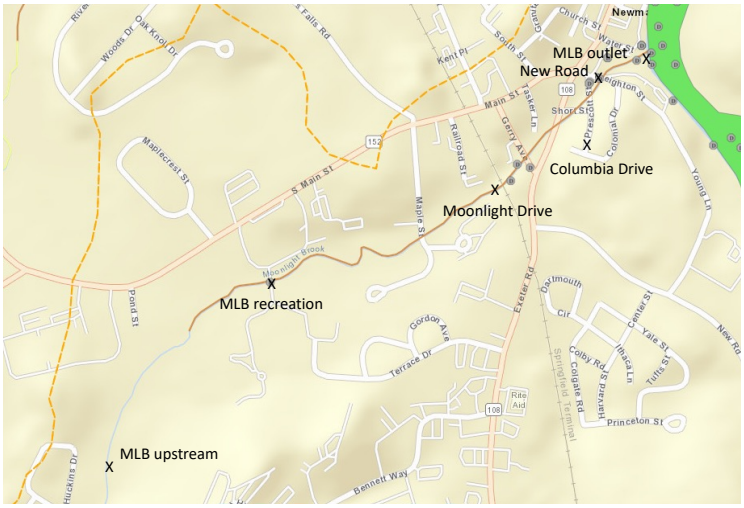
2-G: Meets water quality standards by a relatively large margin; 3-ND: No current data. Insuficient information to make assessment decision.

***Table 1.*** 2020 NHDES Water Quality Assessment categories in the Lower Lamprey River (HUC 12: 010600030709) and the Middle Lamprey River (HUC 12: 010600030703) for the 8 main study sites.

***Figure 1***. Locations of project study sites during 2023. This figure and Figure 2 were developed using the NHDES Surface Water Quality Assessment Viewer:
https://nhdes.maps.arcgis.com/apps/webappviewer/index.html?id=d1ba9c5ec85646538e032580e23174f


To enable more detailed exploration of sources of fecal contamination in Newmarket, we established 6 routine sampling sites in the Moonlight Brook watershed including its one other tributary sub-watershed (Fig. 2). Sample sites included Site MBO the outlet of Moonlight Brook to the tidal portion of the Lamprey River and the same location as Site 1*, then Site MLD upstream next to Moonlight Drive just to the west of the railroad crossing, Site MLBRec recreation next to the recreational area behind the high school, and at Site MLU near the most upstream section of the brook. Two other sites in a tributary in downtown Newmarket included Site NR next to New Road, and Site CD next to Columbia Drive in the Sleepy Hollow trailer park, where it's probable that little surface water recreation occurs. These sites were chosen instead to help determine the location and types of fecal pollution that contributes to what is discharged in the tidal portion of the Lamprey River, where boating is popular. All sites in the Moonlight Brook watershed are classified the same as Site 1* (Table 2) by NHDES.

***Figure 2***. Locations of project study sites in the Moonlight Brook watershed during 2023.



All samples were collected and stored on ice until being transported to the Jackson Estuarine Laboratory (JEL) for analysis within 4 hours of sampling. This sampling occurred approximately once per month during 2023 on April 19, May 11, May 28, June 15, July 17, August 14, September 7, October 24, and November 16. The samples were analyzed to determine concentrations of bacterial indicators of fecal pollution that are used by the State of NH for classifying and managing coastal waters: Enterococci (coastal water recreation), fecal coliforms (shellfish harvesting), and *Escherichia coli* (*E. coli;* freshwater recreation) using standard methods accepted by state agencies for these purposes. Although the fecal coliform test relates to shellfishing which is not the goal of this study, the laboratory test we use provides data for both fecal coliforms and *E. coli,* so we do report data for both here, as it also is useful for understanding contamination sources for downstream areas where shellfishing is allowed. Analyses included negative and positive controls for each sampling day.

Water samples were filtered to capture bacterial cells and their DNA. Samples deemed polluted (above State standards) were further analyzed by established procedures in our lab (Rothenheber and Jones 2018) to identify the presence/absence and to some extent relative quantification of sources of fecal contamination in the sample using PCR (polymerase chain reaction-presence/absence) and qPCR (semi-quantitative) methods. This procedure is called microbial source tracking (MST). The potential source species we have targeted include human, dog, bird, gull, Canada goose, cow, horse, ruminants and mammals for the presence/absence PCR assays and mammal, human and bird for the semi-quantitative qPCR assays.

Water quality measurements were also made using datasondes with sensors for water temperature, salinity, pH, depth, dissolved oxygen, turbidity, and chlorophyll *a*. Data for daily rainfall amounts (inches) were from the UNH Weather statistics online database.

Data analysis involved basic comparisons of fecal indicator concentrations to those used as State water quality standards (Tab. 2; NHDES 2020a) to determine the frequency and location of areas that exceed the standards. Given the array of different standards for different types of uses and water quality classification, we used the Class A freshwater and tidal water standards for comparisons. This is based on the recognition that recreational activities in the watershed often include both boating and swimming, so though the watershed has no designated beaches for which the standards are most strict, we needed to inform potential risks for both activities.

| | **THRESHOLD RISK LEVEL- Primary Contact Recreation** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Class A fresh | | Class B fresh | | Designated beaches | | Tidal | |
| INDICATOR | SSMI* | GM | SSMI | GM | SSMI | GM | SSMI | GM |
| | # cfu or MPN/100 ml | | | | | | | |
| *E. coli* for freshwater recreational uses | **153** | **47** | **406** | **126** | **88** | **47** | N/A | N/A |
| Enterococci for marine water recreational uses | N/A | N/A | N/A | N/A | **104** | **35** | **104** | **35** |
| | | | | | | | | |
| | **THRESHOLD RISK LEVEL- Secondary Contact Recreation** | | | | | | | |
| | Class A fresh | | Class B fresh | | Designated beaches | | Tidal | |
| INDICATOR | SSMI* | GM | SSMI | GM | SSMI | GM | SSMI | GM |
| | # cfu or MPN/100 ml | | | | | | | |
| *E. coli* for freshwater recreational uses | **153** | **235** | **406** | **630** | N/A | N/A | N/A | N/A |
| Enterococci for marine water recreational uses | N/A | N/A | N/A | N/A | N/A | N/A | **520** | **175** |

*SSMI = 'single sample maximum indicator'; GM = geometric mean, or the average of 3 samples within 60 days.

***Table 2.*** State of New Hampshire standard fecal indicator bacteria concentrations for different surface water uses. See citation (State of New Hampshire) in **References** for the source of this information.

The microbial source tracking data were analyzed to determine occurrence and frequency of detection for the different sources at the different sites, noting any temporal trends. The concentrations (copy number per 100 ml) of the human source genetic marker in the qPCR assay are also compared to a threshold (2400 CN/100 ml) above which researchers at EPA and elsewhere have found to exceed acceptable likelihood of human illnesses (Boehm et al. 2013).

**The awarded funds** were used to support time required by Dr. Jones to oversee the project, analyze data, and write the Final Report. Four undergraduate students from UNH and Dr. Jones' Lab Supervisor were also partially supported for their involvement in sampling events and lab analyses. They also helped with data compilation and analysis and providing information for the final report. The project also required purchasing supplies for the water sampling, bacterial analyses, and the pollution source detection analyses, and transportation to sampling sites.

# RESULTS & DISCUSSION

## Review and Summary of Existing Data

There are Draft 2020 NHDES Watershed Report Cards for an approximate 34 square mile area representing the Lower and Middle portions of the Lamprey River (NHDES 2020c). These areas are given Hydrologic Unit Codes (HUC12) of HUC 12: 010600030709 (Lower) and 010600030703 (Middle). Within these areas there are 34 and 63 different Assessment Units, respectively, each also given unique numerical Assessment IDs. In the Lower Lamprey River there were 2 estuarine, 6 impoundment, 1 lake and 25 river Assessment Units. Most (30 of 34) of these Assessment Units have assessment codes for swimming (Primary contact) or boating (Secondary contact) of "3-ND", which is "No current data, insufficient information to make an assessment decision". The assessment codes for the study sites of assessment units closest to the study sites are all '3-ND' (last sample = 2008; Tab. 1), except for Site 2 where there are adequate enterococci data to classify primary contact (swimming) as poor water quality that does not meet water quality standards (4A-P). The secondary contact (boating) classification is '2-G', meaning that the water quality meets standards by a relatively large margin (Tab. 2). One site at Packers Falls also had a 2-G assessment code based on 2017 data for primary and secondary contact uses.

In the Middle Lamprey River portion there were 8 impoundment, 8 lake and 47 river Assessment Units. Most (53 of 63) of these Assessment Units have assessment codes for swimming (Primary contact) or boating (Secondary contact) of "3-ND", which is "No current data, insufficient information to make an assessment decision". The assessment codes for the study sites of assessment units closest to the study sites are all '3-ND' except for Sites 5 and 6 where there are adequate *E. coli* data to classify primary contact (swimming) as poor water quality that does not meet water quality standards (4A-P). The secondary contact (boating) classification is '2-G', meaning that the water quality meets standards by a relatively large margin, for Site 5 and '3-ND' for Site 6 (Tab. 1).

## Lamprey River Watershed

All intended sample collections occurred on 8 dates from April through November 2023. 2023 was a wet summer featuring 14 intensive (>1 in./24 h) rainfall events in the Lamprey River watershed, especially (8 events >1"/24 h) during June through August. The June and July sample dates occurred after >1" rainfall in. the previous 2 days (Tab. 3). The bacterial indicator levels changed with the different monthly sample events, with higher levels of fecal coliforms and *E. coli* especially in June-August, less so in September and October, while all sites had very low levels in May and November and except for 2 sites in April. (Tab. 3).

| Date | Site # | Site name | Fecal coliforms | *E. coli* | Enterococci | Rainfall-daily sample day | prior day | 2 d prior |
|---|---|---|---|---|---|---|---|---|
| | | | CFU/100 ml | CFU/100 ml | CFU/100 ml | "/24 h | "/24 h | "/24 h |
| 4/19/23 | 1 | MB | 360 | 360 | <20 | 0 | 0.02 | 0.21 |
| | 2 | PRBL | 240 | 230 | 65 | | | |
| | 3 | WD | 25 | 25 | <5 | | | |
| | 4 | WF | 20 | 20 | <5 | | | |
| | 5 | ETH | 15 | 15 | 5 | | | |
| | 6 | RES | <5 | <5 | <5 | | | |
| 5/11/23 | 1 | MB | <5 | <5 | 5 | 0 | 0 | 0 |
| | 2 | PRBL | <5 | <5 | 15 | | | |
| | 3 | WD | <5 | <5 | <5 | | | |
| | 4 | WF | 30 | 30 | <5 | | | |
| | 5 | ETH | <5 | <5 | <5 | | | |
| | 6 | RES | <5 | <5 | 5 | | | |
| 6/15/23 | 1 | MB | 2360 | 2360 | <40 | 0 | 0.42 | 0.67 |
| | 2 | PRBL | 64 | 64 | 24 | | | |
| | 3 | WD | 248 | 248 | 24 | | | |
| | 4 | WF | 300 | 300 | 32 | | | |
| | 5 | ETH | 224 | 224 | 44 | | | |
| | 6 | RES | 100 | 100 | <40 | | | |
| 7/17/23 | 1 | MB | 520 | 520 | 360 | 0 | 1.15 | 0 |
| | 2 | PRBL | 400 | 400 | 140 | | | |
| | 3 | WD | 430 | 430 | 610 | | | |
| | 4 | WF | 400 | 400 | 290 | | | |
| | 5 | ETH | 660 | 660 | 300 | | | |
| | 6 | RES | 2660 | 2540 | 770 | | | |
| 8/14/23 | 1 | MB | 500 | 260 | 140 | 0 | 0.28 | 0 |
| | 2 | PRBL | 84 | 80 | 60 | | | |
| | 3 | WD | 124 | 112 | 20 | | | |
| | 4 | WF | 188 | 160 | 88 | | | |
| | 5 | ETH | 96 | 60 | 84 | | | |
| | 6 | RES | 140 | 80 | 12 | | | |
| 9/7/23 | 1 | MB | 500 | 500 | 120 | 0 | 0 | 0 |
| | 2 | PRBL | 20 | 20 | <4 | | | |
| | 3 | WD | 96 | 96 | <4 | | | |
| | 4 | WF | 60 | 48 | 4 | | | |
| | 5 | ETH | 168 | 144 | 24 | | | |
| | 6 | RES | 900 | 880 | 710 | | | |
| 10/24/23 | 1 | MB | 70 | 60 | 80 | 0 | 0 | 0.02 |
| | 2 | PRBL | 320 | 320 | 230 | | | 1.36 |
| | 3 | WD | 40 | 40 | 10 | | | prev day |
| | 4 | WF | 70 | 60 | 40 | | | |
| | 5 | ETH | 130 | 130 | 90 | | | |
| | 6 | RES | 190 | 190 | 150 | | | |
| | 7 | PB-MS | 130 | 120 | 140 | | | |
| | 8 | LfRd | 50 | 50 | 50 | | | |
| 11/16/23 | 1 | MB | <10 | <10 | 5 | 0 | 0 | 0 |
| | 2 | PRBL | 10 | 10 | <10 | | | |
| | 3 | WD | 30 | 20 | 10 | | | |
| | 4 | WF | <10 | <10 | <10 | | | |
| | 5 | ETH | 30 | 30 | 30 | | | |
| | 6 | RES | <10 | <10 | <10 | | | |
| | 7 | PB-MS | <10 | <10 | 10 | | | |
| | | | concentration exceeded State standard | | | | | |

***Table 3***. Fecal indicator bacteria concentrations in water samples collected in the Lamprey River watershed. Site 1: Moonlight Brook-mouth at Lamprey River; Site 2: Piscassic River Boat Launch; Site 3: above Wiswall Dam; Site 4: Wadleigh Falls canoe access. Site 5: behind Epping Town Hall. Site 6: Carroll Lake beach behind Raymond Elementary School. Site 7: Pecker Bridge on Main Street in Raymond. Site 8: below bridge on Langford Road in Raymond.

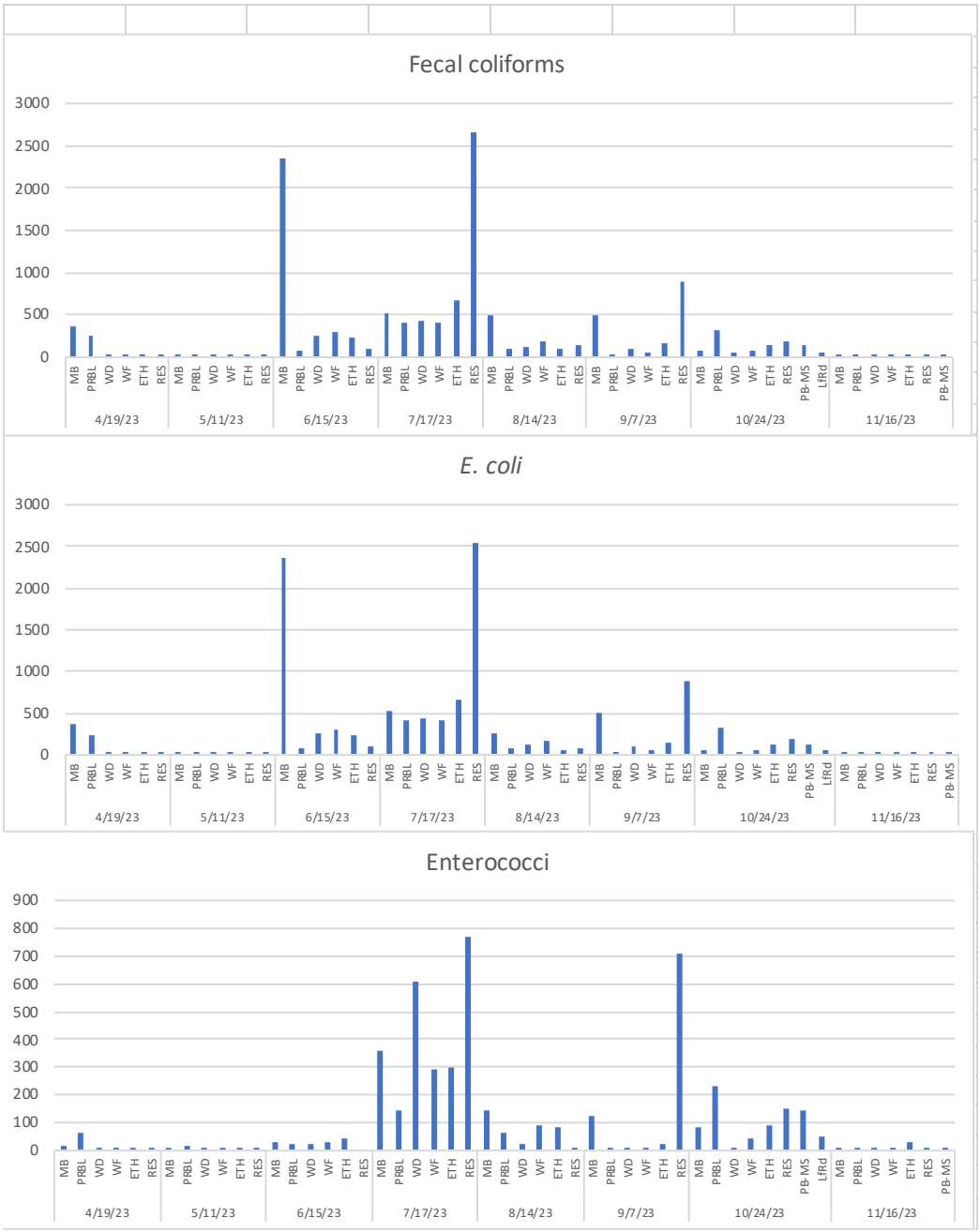
The three bacterial fecal indicators exceeded State water quality standards at varying rates (Tables 3&4). Enterococci levels only exceeded standard (104 enterococci/100 ml) on 1-3 of the 8 sample events at all 6 main sites for a total of 11 out of 48 events, in contrast to fecal coliforms that exceeded standard (14 FC/100 ml) in 33 out of 48 samples. *E. coli* levels, which are most pertinent to this study as they relate to freshwater recreation, exceeded the single sample standard (153 *E.coli*/100 ml) on 2-5 of the 8 sample events the 8 sites for a total of 20 events. This included 11 exceedences out of the 12 samples during June and July, and at 2-3 sites in April, August, and September (Tab. 3). The bacterial indicators were detected at a high frequency, with non-detection occurring in only 4 (enterococci) samples during June to October, although non-detection for all 3 indicators occurred in April, May and November (Tab. 3).

| **2023** | State standard exceedance | | | | Non-detection | |
|---|---|---|---|---|---|---|
| Site | fecal coliform | *E. coli* | Enterococci | fecal coliform | *E. coli* | Enterococci |
| | >14/100 ml | >158/100ml | >104/100 ml | <5 cfu/100ml | <5 cfu/100ml | <5 cfu/100ml |
| 1 | 6 | 5 | 3 | 1 | 1 | 2 |
| 3 | 6 | 2 | 1 | 1 | 1 | 3 |
| 4 | 5 | 3 | 1 | 1 | 1 | 3 |
| 5 | 5 | 3 | 1 | 1 | 1 | 1 |
| 6 | 5 | 4 | 3 | 3 | 3 | 3 |
| Totals | **33** | **20** | **11** | **8** | **8** | **14** |
| % samples | **69%** | **42%** | **23%** | **17%** | **17%** | **29%** |

***Table 4***. Frequency of exceedance of State water quality standards and non-detection of bacterial indicators at the 6 study sites.

In the 2021 and 2022 studies, indicator bacteria were detected at much higher levels at Site 1 compared to all other sites. In 2023, this continued to be the case except Site 6 exceeded State indicator standard concentrations almost as frequently and had higher concentrations on two dates compared to Site 1 (Fig. 3; Tables 3&4), despite this site having somewhat lower contamination levels than Sites 2-5 in 2022. This change induced expansion of water testing to include two more sites in the Carroll Lake section of the Lamprey River in Raymond. The comparison of the 3 fecal contamination indicators in Fig. 3 shows different seasonal dynamics for enterococci compared to the other two indicators. Fecal coliforms and *E. coli* concentrations were elevated in June and July, and somewhat in August-October. Enterococci were only slightly elevated in June, were most consistently elevated in July and to a lesser degree in August and October, with one site, RES-Site 6, showing a high concentration in September. These results illustrate the known differences between different bacterial indicators of fecal contamination that underly different management applications.

***Figure 3***. Concentrations of the 3 fecal coliforms, *E. coli*, and enterococci for all 8 sample dates at each of the 8 sampling sites.




The average concentrations for the fecal indicator bacteria show trends across sites for the full study (Fig. 4A). Fecal coliforms and *E. coli* concentrations were again higher at Site 1 compared to the other 5 sites for the full study time, while the relatively lower enterococci concentrations were highest at Sites 1 and 6 and lower at Sites 2-5, again reflecting a change this year for Site 6. As in 2022, the impact of rainfall and associated runoff was again a focus of this study, but this year instead of a drought the weather was quite rainy which allowed for capturing enough wet weather events to determine potential impacts. In June and July when there was the most rain,

the much higher indicator concentrations (Fig. 4B; see difference in y-axis scales for Figs. 4 A&B) showed similar spatial patterns as for the full study, with Site 1 still having the highest fecal coliform and *E. coli* concentrations; the lowest concentrations were at Site 2, similar concentrations at Sites 3-5, and slightly higher concentrations at Site 6 (Fig. 4B). Average enterococci concentrations varied over a narrower range, but the highest average concentration was at Site 6 instead of at Site 1. Beyond the impacts of rainfall driven runoff as a source of contamination, it is not yet clear if something else may be causing higher levels of contamination at Site 6/RES this year, especially given the relatively high concentrations of all 3 indicators on September 7 compared to other sites (Fig. 3).

***Figure 4A***. Geometric average concentrations (cfu/100 ml) of fecal indicator bacteria at the main 6 sample sites for April to November 2023.

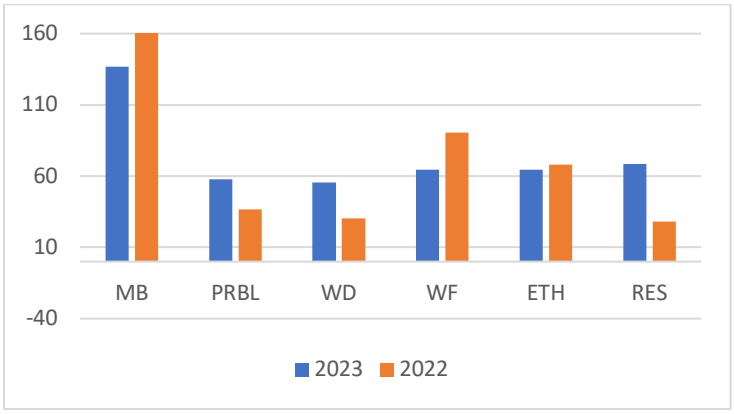
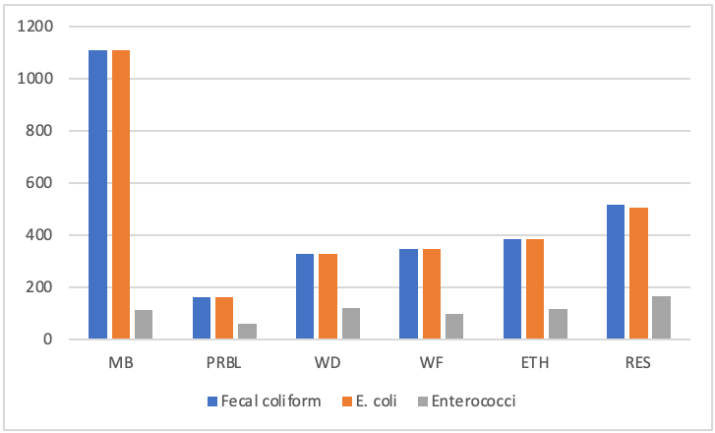
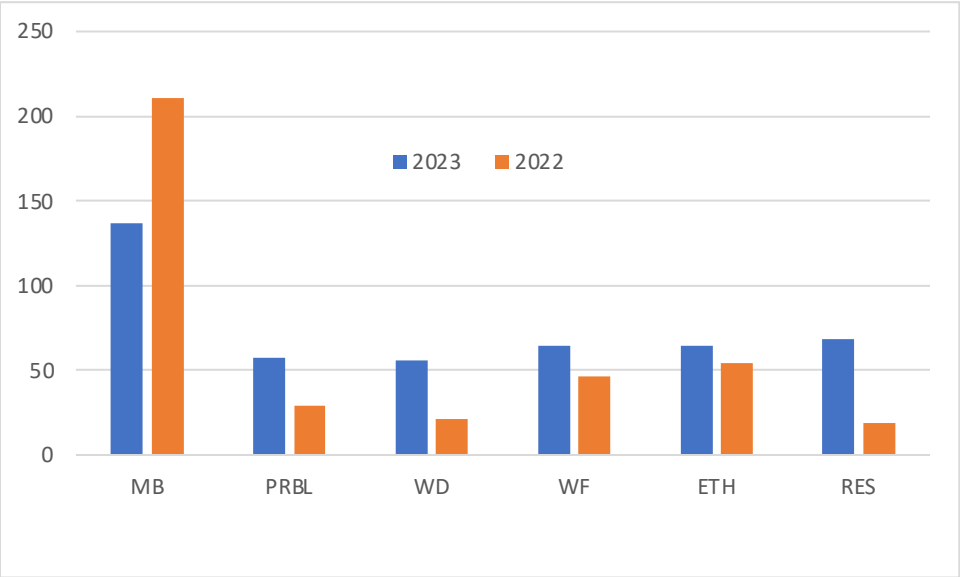



***Figure 4B***. Geometric average concentrations (cfu/100 ml) of fecal indicator bacteria at the main 6 sample sites for June and July 2023.




The average *E. coli* concentrations for all 6 main sites were compared for 2022 and 2023 (Fig. 5). Although Site 1 remained the site with the highest *E. coli* concentrations, the average in 2023 was lower than for 2022, while the *E. coli* concentrations at the other 5 sites were higher in 2023, as expected due to the increased frequency of rainfall/runoff conditions in 2023. It is not clear what caused lower *E. coli* levels at Site 1 in 2023.

***Figure 5***. Geometric average concentrations (cfu/100 ml) of *E. coli* in 2022 and 2023 at the main 6 sample sites for all 8 sample dates.




The bacterial indicator levels at the tidal site at the Newmarket waterfront determined by UNH-JEL for the GBNERR/Piscataqua Regional Estuaries Partnership (PREP) monitoring program, that were included in the 2021 and 2022 LRAC studies (Jones 2022, 2023) are again useful to be compared to levels elsewhere in the upstream Lamprey River watershed determined as part of this 2023 study. In 2023, the levels of all 3 bacterial indicators exceeded State thresholds in April and June, fecal coliforms exceeded the State threshold in every month, and *E. coli* also exceeded the State threshold in July through September. Overall, levels of each indicator showed similar general patterns as this study where the enterococci and *E. coli* levels exceeded standards less frequently than the fecal coliforms for 2021-23 (Tab. 5), except that in November and December 2022, the levels of all three indicators were much higher than for other times, probably due to recent heavy rains and runoff.

| Collection Date | Fecal coliform cfu/100 ml | *E. coli* cfu/100 ml | Enterococci cfu/100 ml |
|---|---|---|---|
| 4/13/21 | 62 | 60 | 10 |
| 5/11/21 | 40 | 32 | 28 |
| 6/8/21 | 84 | 64 | 12 |
| 7/15/21 | 84 | 80 | <4 |
| 8/10/21 | 140 | 32 | 28 |
| 9/20/21 | 76 | 76 | 72 |
| 10/12/21 | 32 | 28 | 8 |
| 11/8/21 | 36 | 32 | 8 |
| 5/17/22 | <4 | <4 | 12 |
| 6/21/22 | 29 | 25 | 8 |
| 7/18/22 | 40 | 36 | 16 |
| 8/15/22 | 20 | 12 | 24 |
| 9/19/22 | n/d | n/d | n/d |
| 10/17/22 | 84 | 80 | 20 |
| 11/15/22 | 3240 | 3100 | 150 |
| 12/2/22 | 1200 | 1100 | 960 |
| 4/10/23 | 8440 | 8200 | 1440 |
| 5/8/23 | 120 | 106 | 6 |
| 6/6/23 | 3280 | 3200 | 440 |
| 7/19/23 | 373 | 310 | 30 |
| 8/7/23 | 220 | 220 | 50 |
| 9/18/23 | 208 | 204 | 12 |
| 10/16/23 | 30 | 30 | <3 |
| 11/6/23 | 50 | 50 | 30 |
| 12/6/23 | 40 | 40 | 100 |

***Table 5.*** Fecal indicator bacteria concentrations in water samples collected at Site GBRLR (Site 2). Yellow highlighted data are levels that exceed water quality standards.

There was evidence of animal (mammal) contamination at all 8 sites for all but 2 of the 51 samples analyzed (Tab. 6). Dog contamination was present at all sample sites in all months except August and November. Bird contamination was also detected in all samples from July through November and to some extent in May through June. Cow contamination was present at some sites each month except in July and November, while ruminants, Canada geese, horses and gulls were detected in diminishingly fewer samples.

| | | | | | | | | | | | qPCR: copies/100 ml | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Sample date | Mammal | Human | Dog | Ruminant | Cow | Bird | Gull | Canada goose | Horse | Mammal | Human | Bird |
| MB | 4/19/23 | + | - | + | - | + | - | - | - | + | 72,974 | - | - |
| PRBL | | + | - | + | - | + | - | - | - | + | 812,709 | - | - |
| WD | | + | - | + | - | + | - | - | - | + | 33,555 | - | - |
| WF | | - | - | + | + | + | + | - | - | - | 54,315 | - | 2,900 |
| ETH | | + | - | + | - | - | + | - | - | - | 115,026 | - | 6,359 |
| RES | | - | - | + | - | - | - | - | - | + | 49,366 | - | - |
| PB-MS | | | | | | | | | | | | | |
| LfRd | | | | | | | | | | | | | |
| MB | 5/11/23 | + | + | + | + | + | + | - | - | - | 1,438,276 | 2,664 | 4,014 |
| PRBL | | + | - | + | - | + | + | - | + | + | 4,035,839 | - | 4,834 |
| WD | | + | - | + | - | + | + | - | - | - | 1,368,538 | - | 12,995 |
| WF | | + | - | + | - | + | + | - | - | - | 1,996,357 | - | 8,452 |
| ETH | | + | - | + | - | + | + | - | - | - | 1,149,199 | - | 10,259 |
| RES | | + | - | + | - | + | - | | - | + | 80,386 | - | - |
| PB-MS | | | | | | | | | | | | | |
| LfRd | | | | | | | | | | | | | |
| MB | 6/15/23 | + | + | + | - | + | + | - | - | + | 18,118,495 | 3,169,542 | 10,311 |
| PRBL | | + | + | + | - | + | - | - | + | + | 2,467,551 | <167 | - |
| WD | | + | - | + | - | + | + | - | - | - | 7,954,666 | - | 3,735 |
| WF | | + | + | + | - | + | + | - | - | + | 3,660,094 | <167 | 2,690 |
| ETH | | + | - | + | - | + | + | - | - | + | 4,672,970 | - | 5,186 |
| RES | | + | - | + | - | + | - | - | - | + | 1,260,457 | - | - |
| PB-MS | | | | | | | | | | | | | |
| LfRd | | | | | | | | | | | | | |
| MB | 7/17/23 | + | + | + | - | - | + | - | - | - | 6,190,589 | 984 | 6,522 |
| PRBL | | + | + | + | - | - | + | - | - | - | 15,477,627 | 92,242 | 7,006 |
| WD | | + | - | + | - | - | + | - | - | - | 19,557,835 | - | 3,959 |
| WF | | + | - | + | - | - | + | - | - | - | 48,500,856 | - | 3,072 |
| ETH | | + | - | + | - | - | + | - | - | - | 2,321,529 | - | 4,467 |
| RES | | + | + | + | - | - | + | - | - | - | 19,699,999 | <167 | - |
| PB-MS | | | | | | | | | | | | | |
| LfRd | | | | | | | | | | | | | |
| MB | 8/14/23 | + | + | + | + | + | + | - | + | - | 26,596,680 | 7,110,544 | 288 |
| PRBL | | + | - | - | - | + | + | - | + | - | 49,451,183 | - | - |
| WD | | + | - | - | - | - | + | - | + | - | 36,793,133 | - | <167 |
| WF | | + | - | + | - | + | + | - | + | - | 63,504,375 | - | 284 |
| ETH | | + | - | - | - | - | + | - | - | - | 19,587,653 | - | 2,684 |
| RES | | + | - | - | - | + | + | - | + | - | 32,267,117 | - | 2,586 |
| PB-MS | | | | | | | | | | | | | |
| LfRd | | | | | | | | | | | | | |
| MB | 9/7/23 | + | - | + | + | + | + | + | - | - | 8,665,941 | - | 52,713 |
| PRBL | | + | - | + | + | + | + | - | + | - | 3,599,131 | - | 11,494 |
| WD | | + | - | + | - | + | + | - | + | - | 11,014,407 | - | 2,059 |
| WF | | + | - | + | + | + | + | - | + | - | 26,055,538 | - | 1,610 |
| ETH | | + | - | + | - | + | + | + | - | - | 14,211,021 | - | 40,328 |
| RES | | + | - | + | - | + | + | + | + | - | 7,658,007 | - | 9,334 |
| PB-MS | | | | | | | | | | | | | |
| LfRd | | | | | | | | | | | | | |
| MB | 10/24/23 | + | + | + | + | + | + | + | - | - | 2,017,118 | 82,599 | 3,180 |
| PRBL | | + | - | + | + | - | + | - | - | - | 23,979,659 | - | 413 |
| WD | | + | + | + | - | - | + | + | - | - | 3,166,780 | 160,654 | 26,498 |
| WF | | + | + | + | + | + | + | - | - | - | 3,100,068 | 3,487,083 | 857 |
| ETH | | + | - | + | - | + | + | - | - | - | 1,742,586 | - | 1,201 |
| RES | | + | - | + | + | + | + | + | - | - | 3,692,919 | - | 485 |
| PB-MS | | + | + | + | + | - | + | - | - | + | 4,610,297 | 11,655 | 347 |
| LfRd | | + | + | + | - | - | + | - | - | + | 2,903,187 | 377,203 | <167 |
| MB | 11/16/23 | + | - | - | + | - | + | - | - | - | 9,196,426 | - | 755 |
| PRBL | | + | - | - | - | - | + | - | - | - | 696,309 | - | <167 |
| WD | | + | - | + | - | - | + | - | - | - | 20,375,343 | - | <167 |
| WF | | + | - | - | - | - | + | - | - | - | 9,920,518 | - | 237 |
| ETH | | + | - | - | - | - | + | - | - | - | 241,595 | - | 778 |
| RES | | + | - | + | + | - | + | - | - | - | 5,817,992 | - | 417 |
| PB-MS | | + | - | + | + | - | + | - | - | - | 5,757,934 | - | <167 |
| LfRd | | | | | | | | | | | | | |

***Table 6***. Detection of the presence of different pollution sources by of PCR and qPCR analyses for all samples from May through November 2023. Green highlight denotes detection, yellow highlight denotes level above human contamination risk threshold level.

The presence of human contamination was detected most frequently (5 of 8 samples) at Site 1 during each month except April, September, and November (Tab. 6). Human contamination was detected more at other sites in 2023 compared to previous years, including twice at Sites 2 and 4, and once each at Sites 6, PB-MS and LfRd. The follow-up semi-quantitative assay (qPCR), which has a higher detection limit, indicated the human contamination concentrations at Site 1 were always higher than levels found at other sites and were highly elevated (>10,000 copy number/100 ml) in May, June, October, and November. The quantified level of human contamination at Site 1 was above a public health safety threshold (4,200 copy number/100 ml; Boehm et al. 2013) at Site 1 in samples collected in June, August, and October, at Site 2 in July, and at Sites 3, 4 PB-MS, and LfRd in October. The high frequency of human contamination detection, and at elevated levels, in October at 5 out of 8 sites across the study area was unusual and is of unknown cause. The quantified human contamination levels were below this threshold in the other 5 instances of detection and below the detection level in 3 of the samples.

The quantified level of bird contamination (by qPCR assay) for the 44 samples where the presence of bird contamination was detected reflected relatively elevated levels of contamination, as only 5 samples were below the qPCR assay detection limit and of the 39 samples with detectable levels by qPCR, 29 exceeded 1,000 CN/100 ml, with the highest level at 52,713 CN/100 ml and the lowest level at 288 CN/100 ml (Tab. 6). The presence of Canada geese did not correspond to elevated levels of bird contamination; however, many ducks were observed at Site 1 where bird contamination was above detection levels from May-November.

There was some seasonality for a few source types, although detection of human, dog, bird, and cow contamination was spread across the full study period (Tab. 6). Canada geese contamination was only detected in August and September, and gulls were detected only in September and October. Ruminants, which can include deer, were detected most frequently in September through November, while horse contamination, whatever the actual source may be, was detected in May through June, and in the 2 new Raymond sites in October.

In addition to showing the highest concentrations of all three indicator bacteria, Site MBO also had the most diverse identified types of contamination in each sample, with an average of 5.4 types (out of 9 possible) per sample (Tab. 7). In 2022 this analysis showed much lower diversity of contamination types at other sites, yet in 2023, Sites PRBL, WF, PB-MS, and LfRd also showed relatively high diversity (4.5 to 5 source types) with other sites between 3.5-4.0 types.

| Site # | Samples # | Ave. # of source types detected | Human source detection | Human source >threshold |
|---|---|---|---|---|
| | | **LAMPREY RIVER WATERSHED** | | |
| **MBO** | 8 | **5.4** | **4** | 4 |
| PRBL | 8 | 4.5 | 2 | 1 |
| WD | 8 | 3.9 | 1 | 1 |
| WF | 8 | 4.5 | 2 | 1 |
| ETH | 8 | 3.5 | 0 | 0 |
| RES | 8 | 4.0 | 1 | 0 |
| PB-MS | 2 | 5.0 | 1 | 1 |
| LfRd | 1 | 5.0 | 1 | 1 |

***Table 7***. The frequency of site-specific fecal-borne bacterial contamination sources.

## Moonlight Brook watershed

One significant focus of the 2023 study was to explore how contaminated Moonlight Brook is in relation to the historically elevated levels of bacterial contamination at the sampling site located in the mouth/outlet of Moonlight Brook next to the Newmarket boat launch (Figs. 1&2). New sampling sites were included that were sampled monthly on different days than the sites on the full Lamprey River watershed. The Moonlight Brook watershed sites included initially one then up to 3 more upstream of the downtown railroad crossing, and initially one then 2 more sites in a tributary waterway to the south of the brook outlet that extends into the Sleepy Hollow trailer park.

All intended sample collections, except for MLD in September, occurred on 7 dates in May, June, then August through November 2023 (Tab. 8). 2023 was a wet summer in the Moonlight Brook watershed, especially during June through August. There were no instances, however, where sampling occurred on a date following significant (>1") rainfall within 2 days prior to the sample dates. Even so, akin to what happened at the Lamprey River sites, concentrations of all three bacterial indicators were higher and more often exceeded State risk thresholds during June, August, and September sample dates (Tab. 8).

| Date | Site | Fecal coliforms CFU/100 ml | *E. coli* CFU/100 ml | Enterococci CFU/100 ml | sample day "/24 h | Rainfall-daily prior day "/24 h | 2 d prior "/24 h |
|---|---|---|---|---|---|---|---|
| 5/16/23 | MBO | 580 | 572 | 28 | 0 | 0 | 0 |
| | NR | 72 | 72 | 48 | | | |
| | MLD | 56 | 56 | 48 | | | |
| 5/24/23 | MBO | 100 | <5 | 80 | 0.21 | 0 | 0 |
| | NR | 72 | 68 | 8 | | | |
| | MLD | 40 | 36 | <4 | | | |
| | CD | 36 | 36 | <4 | | | |
| | MB Rec | 48 | 48 | <4 | | | |
| | LR-Tidal dock | 800 | 780 | 20 | | | |
| 6/22/23 | MBO | 160 | 160 | 200 | 0 | 0 | 0.04 |
| | NR | 504 | 504 | 188 | | | |
| | MLD | 240 | 240 | 80 | | | |
| | CD | 188 | 188 | 72 | | | |
| | MB Rec | 380 | 380 | 372 | | | |
| 8/21/23 | MBO | 476 | 420 | 280 | 0 | 0 | 0 |
| | NR | 316 | 244 | 64 | | | |
| | MLD | 240 | 240 | 80 | | | |
| | CD | 400 | 280 | 64 | | | |
| | MB Rec | 364 | 364 | 108 | | | |
| 9/21/23 | MBO | 190 | 190 | 30 | 0 | 0 | 0.56 |
| | NR | 320 | 320 | 120 | | | 2.75 |
| | MLD | NA | NA | NA | | | prev day |
| | CD | 200 | 180 | 50 | | | |
| | MB Rec | 80 | 60 | 170 | | | |
| 10/19/23 | MBO | 30 | 30 | 20 | 0 | 0 | 0 |
| | NR | 20 | 20 | 20 | | | |
| | MLD | 60 | 50 | <10 | | | |
| | CD | 50 | 50 | 20 | | | |
| | MB Rec | <10 | <10 | 10 | | | |
| | MB-U | 40 | 40 | <10 | | | |
| 11/8/23 | MBO | 440 | 440 | 85 | 0 | 0.06 | 0 |
| | NR | 30 | 30 | 145 | | | |
| | MLD | 10 | 10 | 70 | | | |
| | CD | 40 | 40 | 145 | | | |
| | MB Rec | <5 | <5 | 100 | | | |
| | MB-U | 65 | 55 | 35 | | | |

***Table 8.*** Fecal indicator bacteria concentrations in water samples collected in the Moonlight Brook watershed. Site MBO: Moonlight Brook Outlet-mouth at Lamprey River; Site NR: New Road 3; Site MLD: Moonlight Drive upstream of the railroad crossing; Site CD: Columbia Drive, upstream of New Road; Site MBRec: Moonlight Brook behind the Newmarket High School near the recreational facilities; MBU: Moonlight Brook upstream.

The three bacterial fecal indicators exceeded State water quality standards to varying degrees (Tables 8&9). Enterococci levels only exceeded standard (104 enterococci/100 ml) at 3 sites in June, 2 sites in November and 1 site in August, twice at Sites MBO and NR, and once at Site MBRec. In contrast, fecal coliforms exceeded the standard (14 FC/100 ml) in 30 out of the 34 samples. *E. coli* levels, which are most pertinent to this study as they relate to freshwater recreation, exceeded the single sample standard (153 *E.coli*/100 ml) at all sampled sites in June,

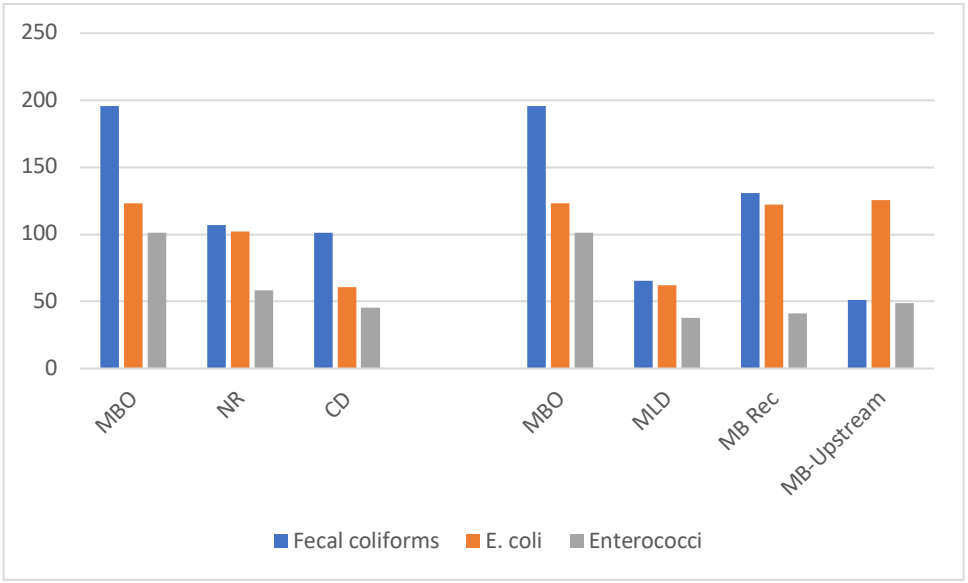
August and September (except Site MBU in September), and only at Site MBO on May 8 and November 8. The bacterial indicators were detected at a high frequency, with non-detection occurring only twice for fecal coliforms, 3 times for *E. coli*, and 4 times for enterococci (Tab. 9).

| | State standard exceedance | | | Non-detection | | |
|---|---|---|---|---|---|---|
| Site | fecal coliform | *E. coli* | Enterococci | fecal coliform | *E. coli* | Enterococci |
| MBO | 7 | 5 | 2 | 0 | 1 | 0 |
| NR | 7 | 3 | 3 | 0 | 0 | 0 |
| MLD | 5 | 2 | 0 | 0 | 0 | 2 |
| CD | 6 | 3 | 1 | 0 | 0 | 1 |
| MBRec | 5 | 2 | 3 | 2 | 2 | 1 |
| MBU | 2 | 0 | 0 | 0 | 0 | 1 |
| Totals | **32** | **15** | **9** | **2** | **3** | **5** |
| % samples | **91%** | **43%** | **26%** | **6%** | **9%** | **14%** |

***Table 9***. Frequency of exceedance of State water quality standards and non-detection of bacterial indicators at the 6 study sites in the Moonlight Brook watershed.

The average concentrations for the fecal indicator bacteria show trends across the 6 sites in Figure 6, which separates the two sub-watersheds and includes MBO in both. Fecal coliform concentrations were substantially higher at Site MBO compared to the other 5 sites. *E. coli* concentrations were highest at Sites MBO, MBRec, and MBU, slightly lower at Site NR, then lowest at Sites CD and MLD. The relatively lower enterococci concentrations were highest at Site MBO and at consistently lower concentrations at all other sites.

***Figure 6***. Geometric average concentrations (cfu/100 ml) of fecal indicator bacteria at the 6 sample sites in the Moonlight Brook watersheds: May-November 2023. Site MBO is at the mouth of both watersheds.



There was evidence of animal (mammal) contamination at all 8 sites for all the 35 samples analyzed (Tab. 10). Bird contamination was present in 33 of the 35 samples analyzed and at all sample sites on May 16 and in August through November. Dog contamination was detected in 23 of the analyzed samples and at one site or more on each sample event. Cow contamination was present in 23 analyzed samples and at most sites each month except in September and October, while horses, ruminants, Canada geese, and gulls were detected in diminishingly fewer samples.

| | | | | | | | | | | | qPCR: copies/100 ml | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Sample date | Mammal | Human | Dog | Ruminant | Cow | Bird | Gull | Canada goose | Horse | Mammal | Human | Bird |
| MBO | 5/16/23 | + | + | + | - | + | + | - | - | + | 1,884,336 | 36,465 | 252 |
| NR | | + | - | + | - | + | + | - | - | + | 1,764,061 | - | 11,294 |
| MLD | | + | + | + | - | + | + | - | - | + | 271,737 | 27,233 | 14,689 |
| CD | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| MBREC | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| MBU | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| LRTD | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| MBO | 5/24/23 | + | + | + | - | + | + | - | - | + | 1,044,230 | 502 | 9,075 |
| NR | | + | - | - | - | + | + | - | - | + | 3,789,115 | - | 13,721 |
| MLD | | + | + | + | - | + | + | - | - | + | 1,389,770 | - | 1,990 |
| CD | | + | + | + | - | + | + | - | - | + | 1,400,652 | - | 13,183 |
| MBREC | | + | + | + | - | + | - | - | - | + | 1,031,844 | 539 | 20,597 |
| MBU | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| LRTD | | + | + | + | - | + | + | - | - | - | 5,278,679 | - | 2,335 |
| MBO | 6/22/23 | + | + | + | - | + | + | + | - | + | 6,645,327 | 253,208 | 10,544 |
| NR | | + | + | + | - | - | + | - | - | + | 1,456,362 | <167 | 5,585 |
| MLD | | + | + | + | - | + | - | - | - | - | 1,256,207 | 83,516 | - |
| CD | | + | + | + | - | + | + | - | - | + | 5,312,388 | 17,793 | 4,345 |
| MBREC | | + | + | + | - | + | + | - | - | - | 2,082,238 | 37,259 | 3,927 |
| MBU | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| LRTD | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| MBO | 8/21/23 | + | + | + | + | + | + | - | - | - | 20,575,263 | 30,502 | 619 |
| NR | | + | - | + | + | + | + | - | - | - | 71,948,283 | - | 1,658 |
| MLD | | + | - | - | - | - | + | - | - | - | 4,641,089 | - | 922 |
| CD | | + | - | + | + | + | + | - | - | + | 116,881,795 | - | 2,106 |
| MBREC | | + | - | - | | + | + | + | - | - | 9,716,235 | - | 3,650 |
| MBU | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| LRTD | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| MBO | 9/21/23 | + | + | - | + | - | + | - | + | + | 88,231,002 | 27,537 | 770 |
| NR | | + | - | - | + | - | + | + | + | + | 47,594,560 | - | 1,004 |
| MLD | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| CD | | + | - | - | + | - | + | - | + | + | 162,198,556 | - | 1,179 |
| MBREC | | + | - | + | - | - | + | - | + | + | 121,042,976 | - | 834 |
| MBU | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| LRTD | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| MBO | 10/19/23 | + | + | + | - | - | + | - | + | - | 2,924,080 | 27,361 | 712 |
| NR | | + | + | + | + | - | + | - | + | - | 8,303,139 | 38,696 | 688 |
| MLD | | + | - | + | - | - | + | - | + | - | 1,559,006 | - | <167 |
| CD | | + | - | - | + | - | + | - | + | - | 24,196,166 | - | 1,552 |
| MBREC | | + | + | + | - | - | + | - | + | - | 888,612 | 38,899 | 513 |
| MBU | | + | + | + | + | - | + | - | + | - | 552,952 | 10,957 | 1,313 |
| LRTD | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |
| MBO | 11/18/23 | + | + | - | + | + | + | - | - | - | 13,088,640 | 786,671 | 585 |
| NR | | + | - | - | + | + | + | - | - | - | 8,518,207 | - | 1,169 |
| MLD | | + | - | + | - | + | + | - | - | - | 766,367 | - | 1,782 |
| CD | | + | - | - | + | - | + | - | - | - | 5,370,792 | - | 1,834 |
| MBREC | | + | - | - | - | + | + | - | - | - | 852,601 | - | 2,014 |
| MBU | | + | - | - | + | + | + | - | - | - | 671,034 | - | 3,725 |
| LRTD | | ND | ND | ND | ND | ND | ND | ND | ND | ND | | | |

***Table 10***. Detection of the presence of different pollution sources by of PCR and qPCR analyses for all samples from May through November 2023. Green highlight denotes detection, yellow highlight denotes level above human contamination risk threshold level.

Human contamination was always (7 of 7 samples) detected at Site MBO (Tab. 10). Human contamination was detected at least once for the other 6 sites in the Moonlight Brook watershed, including the single sample collected below the tidal dam on May 24, 2023, when there was a leaky sewer pipe in the tidal portion of the Lamprey River. Human contamination was detected more than once at Sites NR, MLD, CD, and MBRec.

The follow-up semi-quantitative assay (qPCR), which has a higher detection limit than the presence/absence PCR assay, indicated the human contamination at Site MBO was higher than levels found at other sites for all dates except October 19th (Table 10). The quantified level of human contamination at Site MBO was above a public health safety threshold (4,200 copy number/100 ml; Boehm et al. 2013) in samples collected on all dates except May 24th. The levels of human contamination in the 13 of 16 instances where it was detected at all sites were highly elevated (>10,000 copy number/100 ml). The relatively common detection of human contamination, and at elevated levels, at all sites and thus in both sub-watersheds, is a concern and suggests that at least some of the human contamination may come from sources upstream of the downtown section where both branches of the brook are underground.

The quantified level of bird contamination for the 33 samples where bird contamination was also detected by the non-quantitative PCR assay reflected relatively elevated levels of contamination, as only 1 sample was below the qPCR assay detection limit and of the 32 samples with detectable levels by qPCR, 23 exceeded 1,000 CN/100 ml, with the highest level at 20,597 CN/100 ml and the lowest level at 252 CN/100 ml (Tab. 10). The presence of Canada geese did not correspond to elevated levels of bird contamination; however, many ducks were observed at Site MBO where bird contamination occurred above detection levels from May through November.

There was some seasonality for a few source types, although detection of human and bird contamination was spread across the full study period (Tab. 10). Canada geese contamination was only detected in September and October, gulls were detected only in June to September, and cows were not detected in September and October. Ruminants, which can include deer, were detected most only from August through November, while horse contamination, whatever the actual source may be, was detected in May through September.

In addition to showing the highest concentrations of all three indicator bacteria, Site 1 also had the most diverse identified types of contamination in each sample, with an average of 5.9 types (out of 9 possible) per sample (Tab. 11). All the other 6 sites also showed relatively high diversity (4.3 to 5.0 source types). Again, human contamination was detected at all sites and, for most samples, at elevated levels above the risk threshold.

| Site # | Samples # | Ave. # of source types detected | Human source detection | Human source >threshold |
|---|---|---|---|---|
| **MBO** | 7 | **5.9** | **7** | **6** |
| NR | 7 | 5.0 | 2 | 1 |
| MBD | 6 | 4.3 | 3 | 2 |
| CD | 6 | 5.0 | 2 | 1 |
| MBREC | 6 | 4.5 | 2 | 2 |
| MBU | 2 | 5.0 | 1 | 1 |
| LRTD | 1 | 5.0 | 1 | ND |

***Table 11***. The frequency of site-specific fecal-borne bacterial contamination sources.

Significant Findings, Accomplishments and Next Steps

This study represents an up-to-date and comprehensive summary of the sanitary water quality conditions in the Lower and Middle Lamprey River watershed. This is important as New Hampshire rivers, streams and impoundments are increasingly used by boaters and swimmers, who may be at risk for water-borne illnesses under contaminated conditions.

The detailed review of existing data on microbial pollution in the watershed showed very few of the assessment units had any available or recent data to provide water quality assessments for swimming and boating uses. The findings from this study are useful as a starting point for all watershed users and groups like LRAC to communicate with NHDES and other agencies about where to focus potential monitoring that could provide data to inform protecting people involved in recreational uses from water-borne illnesses. The new data generated by this study represent a continuation of a 3-year synoptic dataset for 6 key sites in the watershed related to recreational uses, and thus serve as a start for continued monitoring and water quality assessments. This report will be provided to the New Hampshire Department of Environmental Services (NHDES) to inform the State Surface Water Quality Assessment process that is required by Sections 305(b) and 303(d) of the Clean Water Act.

The expansion of water quality assessment to the Moonlight Brook sub-watershed provided context for previous detection of consistent and elevated levels of bacterial contamination at Site MBO that are probably in part a result of upstream sources of pollution. There was some evidence of potential pollution sources upstream, like at Site MBRec where bacterial indicators were detected at levels higher than at the downstream Site MLD, however it does appear that there may be some sources of contamination to MBO from downtown portions of the brook. There is an effort to upgrade a section of sewer pipe on New Road that is suspected of having leaks that may be a source of the elevated levels of contamination at Site MBO.

In addition to the expansion of water quality assessment efforts to Moonlight Brook, there arose concerns about early summer elevated bacterial contamination at Site 6/RES in Raymond. In response sampling also was initiated at two sites: Site PB-MS and LfRd, but neither site has yet to provide clarity about what the sources of contamination may be in that section of the Lamprey

River, mainly because contamination levels were somewhat lower later in the study period when the two new sites were added.

The abundant rainfall in 2023 allowed for assessment of the impacts of rainfall and associated runoff on bacterial contamination in the Lamprey River and Moonlight Brook watersheds. During June and July, sample events followed rainy periods and the levels of bacterial indicators were substantially higher than levels detected during drier conditions. Bacterial indicator levels, especially *E. coli*, the freshwater recreation indicator, became elevated to levels that exceeded State water quality thresholds for safe recreational activities at all sites. These results provide further insights into when and where water quality concerns occur throughout the two watersheds, and thus a better understanding of where and how to manage the area to improve water quality.

Microbial source tracking is an invaluable tool for assessing watershed water quality, as it shows what sources are contributing contamination and where resources for eliminating pollution sources should be used. Human sources are the highest priority/of most concern. Previous year results showed Site 1/MBO was a consistent concern due to elevated bacterial indicator concentrations accompanied by consistent detection of elevated levels of human contamination, and the rare detection of human contamination at Sites 2 through 6 was encouraging. The impacts of rainfall/runoff conditions this year showed that human contamination is more widespread and present at almost all sites in both watersheds. The source(s) of the human contamination is not yet apparent, so the towns along the Lamprey River and around the Moonlight Brook will need to conduct further investigations to pinpoint the sources. More in-depth sampling at sites upstream and following rainfall events could help that process.

The next most manageable source is probably dogs. Dog contamination was consistently present at all sites in both 2022 and 2023. Several management approaches are typical for reducing the significance of this source including signage that is located at water access points (all sites in this study) that alerts dog owners to pick up and dispose of dog feces, plus the provision of dog feces collection bags at the signage locations. The NHDES has a Scoop the Poop Campaign webpage that can help: https://www.des.nh.gov/home-and-recreation/your-health-and-environment/pet-health-and-environment.

The LRAC will be able to use the findings to help communicate to recreational users about potential water quality issues and precautions to be taken. These were delineated in a separate 2-page document provided in 2022 that is based on NH Dept. of Health and Human Services/Division of Public Health Services and US CDC fact sheets and information.

Future work could take several directions, the most obvious being a continuation of routine monitoring for bacterial pollution indicators at key sites. One dimension that remains uncaptured is the duration of impacts of rainfall and associated runoff, a condition that is now known to be widely responsible for elevated levels of bacterial pollution in the two study watersheds. Typically, watersheds impacted by runoff-borne contamination require one to several days before elevated levels of contamination are transported out of the system. Three years of data reflecting both dry and wet conditions provides for a solid baseline to compare to future findings that include more rainy condition results. As our regional climate continues to change, rainfall

patterns are expected to become more extreme and may change the dynamics of bacterial contamination levels and types of contamination sources, including birds and animal migration patterns that are influenced by climate change.

This Final Report will be made available to key people involved in the PREP and GBNERR monitoring programs, the Town of Newmarket, as well as water quality managers and the Shellfish Program Manager in NHDES.

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