Microbial Source Tracking in the Lamprey River and Moonlight Brook Watersheds Interim Report to the LRAC September 3, 2024 Steve Jones and Meadow Gregory

NOTE:

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Background and Purpose:

The main goal of this project is to continue and expand monitoring at several key sites in the Lamprey River Watershed to provide essential data for assessing water quality, public health risk, and sources of the contamination. Data have been collected for three years but changing conditions from one year to the next are often reflected in changing water quality. This has been significant when the drought in 2022 was so intense that little rainfall/runoff occurred, while in 2023, abundant rainfall was a significant factor affecting water quality in early summer. In 2024, bacterial tracking will enable the following outcomes:

- 1. Expand the baseline of information on bacterial pollution to assess water quality status, spatial and temporal trends, and contamination sources in the Lamprey River watershed.
- 2. Continuation of targeting rainfall events to determine the extent to which these events trigger elevated bacterial concentrations and/or different pollution sources.
- 3. Compile data from ongoing and other related 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 Agreement advances mutual objectives of the 2013 Lamprey Rivers Management Plan and multiple Great Bay partners.

1. "Ensure that the Lamprey rivers meet or exceed standards for "fishable and swimmable" water for the health and enjoyment of all species. "

This project would provide data that can be compared to state standards and enable identifying problem and clean areas, as well as to track progress or new problems with water quality.

2. "Improve and increase appropriate, non-motorized opportunities for public enjoyment along and in the Lamprey River and its designated tributaries (Little, North, North Branch, Pawtuckaway, and Piscassic rivers).

This project will provide data to enable management and improvement of water quality to create new areas safe for recreation on and beside the water.

3. "Protect and restore the ecological functions and resources of the Lamprey River that are critical to wildlife and humans."

This project will provide the framework to identify and eliminate sources of fecal- borne pollution that are a direct public health threat but also contain other pollutants that can impair ecological function (Memorandum of Agreement, LRAC proposal 2024).

Project tasks and timelines:

The following locations are being sampled once monthly from April through November. Samples will be taken, if possible, to capture impacts of rainfall events of 0.5 inches or more over 24 hours along with dry weather conditions.

Lamprey River Watershed

Site 1-the mouth of Moonlight Brook in Newmarket

Site 2-Piscassic River Boat Launch in Newmarket

Site 3-above Wiswall Dam in Durham

Site 4-Wadleigh Falls canoe access

Site 5-behind Epping Town Hall

Site 6-Carroll Beach behind Raymond Elementary School

Moonlight Brook Watershed

Site 1-the mouth of Moonlight Brook in Newmarket

Site 2-New Road

Site 3-Moonlight Drive

Site 4-Columbia Drive

Site 5-Moonlight Brook near the recreational area

Site 6-Moonlight Brook upstream

| Task | Date |
|--|---------------------|
| Start date | April 1, 2024 |
| Monthly water sample collection | April-November 2024 |
| Water sample analysis: indicator bacterial cultures & MST. | April-November 2024 |
| Data compilation and QA/QC | April-November 2024 |
| Interim report | August 2024 |
| Final report | December 31, 2024 |
| (Memorandum of Agreement, LRAC proposal 2024). | |

Lamprey River Watershed

Sample collection occurred on six dates from April to August 2024. Unlike 2023, 2024 has not been a wet summer, and the collections have taken place only one date with >0.5" of rainfall in the previous two days. The bacterial indicator levels changed with the different monthly sample dates, with higher levels in May and August and the lowest levels in April (Table 1). Levels of all indicator bacteria were much lower at the mouth of Moonlight Brook (Site 1) than in past years.

| | | Site | Fecal | | | | Rainfall-daily | |
|---------|-------|---------|------------|------------|-------------|------------|----------------|-----------|
| Date | Site# | name | coliforms | E. coli | Enterococci | sample day | prior day | 2 d prior |
| | | run MST | CFU/100 ml | CFU/100 ml | CFU/100 ml | "/24 h | "/24 h | "/24 h |
| 4/25/24 | 1 | MB | 40 | 40 | <40 | 0 | 0.1 | 0 |
| | 2 | PRBL | 12 | 12 | <4 | | | |
| | 3 | WD | 8 | 8 | <4 | | | |
| | 4 | WF | 16 | 16 | <4 | | | |
| | 5 | ETH | 8 | 8 | <4 | | | |
| | 6 | RES | 8 | 8 | <4 | | | |
| 5/7/24 | 1 | МВ | 260 | 240 | ? | 0 | 0 | 0.4 |
| | 2 | PRBL | 10 | 10 | ? | | | |
| | 3 | WD | <10 | <10 | ? | | | |
| | 4 | WF | 40 | 40 | ? | | | |
| | 5 | ETH | 80 | 80 | ? | | | |
| | 6 | RES | 6,610 | 6,610 | 300 | | | |
| 5/22/24 | 1 | MB | 500 | 500 | 220 | 0.01 | 0.1 | 0 |
| | 2 | PRBL | 640 | 600 | 460 | | | |
| | 3 | WD | 340 | 320 | 100 | | | |
| | 4 | WF | 90 | 90 | 40 | | | |
| | 5 | ETH | 255 | 255 | 35 | | | |
| | 6 | RES | 665 | 665 | 285 | | | |
| 6/3/24 | 1 | MB | 100 | 100 | 40 | 0 | 0 | 0 |
| | 2 | PRBL | <10 | <10 | <10 | | | |
| | 3 | WD | 10 | 10 | <10 | | | |
| | 4 | WF | 80 | 80 | 20 | | | |
| | 5 | ETH | 80 | 80 | 60 | | | |
| | 6 | RES | 10 | 10 | <10 | | | |
| 7/22/24 | 1 | МВ | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2 | PRBL | 60 | 80 | 0 | | | |
| | 3 | WD | 100 | 120 | 1860 | | | |
| | 4 | WF | 40 | 40 | 20 | | | |
| | 5 | ETH | 240 | 240 | 40 | | | |
| | 6 | RES | 160 | 160 | 0 | | | |
| 8/12/24 | 1 | MB | 380 | 380 | 180 | 0.01 | 0 | 0.51 |
| | 2 | PRBL | 140 | 140 | 80 | | | |
| | 3 | WD | 140 | 140 | <10 | | | |
| | 4 | WF | 100 | 100 | <10 | | | |
| | 5 | ETH | 260 | 260 | 100 | | | |
| | 6 | RES | 540 | 540 | <10 | | | |

Table 1. 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.

The three bacterial fecal indicators exceeded State water quality standards differently (Table 2). Enterococci levels exceeded the standard (104 enterococci/100 ml) on 7 out of 36 (19%) samples, whereas fecal coliforms exceeded the standard (14 FC/100 ml) in 26 out of 36 (72%) samples. *E. coli* levels, which are most relevant to this study as they relate to freshwater recreation, exceeded the standard (153 *E.coli*/100 ml) on 14 of the 36 (39%) samples, most often at Site 6 in Raymond.

| 2024 | State | standard exceed | dance | | Non-detection | |
|-----------|----------------|-----------------|-------------|---|---|---------------------|
| Site | fecal coliform | E.coli | Enterococci | fecal coliform | E.coli | Enterococci |
| | >14/100 ml | >150/100 ml | >104/100 ml | <mdl< td=""><td><mdl< td=""><td><mdl< td=""></mdl<></td></mdl<></td></mdl<> | <mdl< td=""><td><mdl< td=""></mdl<></td></mdl<> | <mdl< td=""></mdl<> |
| 1 | 4 | 2 | 1 | 1 | 1 | 3 |
| 2 | 2 | 1 | 1 | 2 | 2 | 4 |
| 3 | 2 | 1 | 1 | 0 | 0 | 4 |
| 4 | 5 | 0 | 0 | 0 | 0 | 3 |
| 5 | 4 | 2 | 0 | 0 | 0 | 2 |
| 6 | 3 | 3 | 2 | 0 | 0 | 4 |
| Totals | 26 | 14 | 7 | 3 | 3 | 20 |
| % samples | 72% | 39% | 19% | 8% | 8% | 56% |

Table 2. Frequency of exceedance of State water quality standards and non-detection of bacterial indicators at the 6 study sites through August 2024

The DNA extracted from water samples were assayed to identify sources of the fecal-borne bacterial contamination. This microbial source tracking analysis was only conducted on samples where bacterial indicator concentrations exceeded state standards (Table 2) or were relatively elevated (Table 3). The mammal genetic marker was detected in all samples, while 75% of the samples were positive for the bird marker. Other sources were detected at the following levels ruminant (deer, cow, etc.) -63%, dog - 38%, human, cow, gull - 19%, and Canada geese - 13%. The samples where detection is designated \pm represent samples with relatively low levels of the source marker; this is significant for human source detection because 3 of the 4 positive samples were \pm , and for ruminant where 3 of the 10 positive samples were \pm . Some sources like birds were consistently detected, while others were detected more sporadically, probably reflecting changes in animal, human and climatic conditions.

| Site | Sample date | Mammal | Human | Dog | Ruminant | Cow | Bird | Gull | Horse | Canada Goose |
|------|--------------|--------|-------|-----|----------|-----|------|------|-------|--------------|
| MB | 5/7/2024 | + | + | + | - | - | + | - | | |
| PRBL | | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| WD | | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| WF | | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| ETH | | + | - | + | - | + | + | - | - | - |
| RES | | + | - | + | - | - | + | - | - | - |
| MB | 6/3/2024 | + | - | + | + | - | + | | - | |
| PRBL | | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| WD | | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| WF | | + | - | + | + | - | + | - | - | - |
| ETH | | + | - | + | + | - | | - | - | - |
| RES | | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| MB | 7/22/2024 | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| PRBL | | + | - | - | | + | + | + | - | - |
| WD | | + | - | - | | + | + | + | - | - |
| WF | | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| ETH | | + | - | - | + | - | + | - | _ | + |
| RES | | + | - | - | | - | | - | | |
| MB | 8/12/2024 | + | - | - | + | - | +/- | - | - | - |
| PRBL | | + | +/- | - | +/- | - | +/- | - | - | + |
| WD | | + | - | - | +/- | - | + | - | - | - |
| WF | | + | +/- | - | + | - | - | - | - | - |
| ETH | | + | +/- | - | + | - | - | - | - | - |
| RES | | + | - | - | +/- | - | + | +/- | - | - |
| | % positive = | 100% | 19% | 38% | 63% | 19% | 75% | 19% | 0% | 13% |

Table 3. Detection of the presence of different pollution sources by PCR analyses for all samples from May through July 2024. Green highlight indicates detection. ND= not determined.

Moonlight Brook Watershed

Continuing from 2023, an important part of the 2024 study has been to examine how contaminated the Moonlight Brook watershed is as compared to levels of bacterial contamination at the sampling site near the Moonlight Brook outlet next to the Newmarket boat launch. The Moonlight Brook watershed sites were sampled monthly on different days than the full Lamprey River watershed.

| | | Fecal | | | | Rainfall-daily | |
|---------|-------|-----------------|----------------|--------------|------------|----------------|-----------|
| Date | Site | Coliforms | E.coli | Enteroccocci | sample day | prior day | 2 d prior |
| | | CFU/100mL | CFU/100 mL | CFU/100 mL | "/24 h | "/24 h | "/24 h |
| 5/1/24 | MBO | 310 | 310 | 50 | 0.29 | 0.4 | 0 |
| | NR | 100 | 95 | <5 | | | |
| | CD | 165 | 165 | 5 | | | |
| | MBD | 730 | 730 | 30 | | | |
| | MBRec | 905 | 905 | 110 | | | |
| | MBU | 15 | 10 | <5 | | | |
| 5/22/24 | MBO | 500 | 500 | 260 | 0.01 | 0.1 | 0 |
| | NR | 665 | 665 | 305 | | | |
| | CD | 255 | 255 | 45 | | | |
| | MBD | 340 | 320 | 120 | | | |
| | MBRec | 640 | 600 | 500 | | | |
| | MBU | 90 | 90 | 80 | | | |
| 6/5/24 | MBO | 60 | 60 | 140 | 0 | 0 | 0 |
| | NR | 20 | 40 | 680 | | | |
| | CD | 180 | 180 | 100 | | | |
| | MBD | 90 | 90 | 410 | | | |
| | MBRec | 80 | 80 | 20 | | | |
| | MBU | 1830 | 1790 | 4380 | | | |
| 7/1/24 | MBO | 280 | 280 | <10 | 0 | 0 | 0 |
| | NR | 280 | 280 | 120 | | | |
| | CD | 280 | 280 | 240 | | | |
| | MBD | <10 | <10 | <10 | | | |
| | MBRec | 120 | 120 | 160 | | | |
| | MBU | 80 | 80 | <10 | | | |
| 7/31/24 | MBO | 960 | 960 | 350 | 0 | 0 | 0 |
| | NR | 1280 | 1280 | 200 | | | |
| | CD | 4480 | 4480 | 220 | | | |
| | MBD | 1120 | 1120 | 640 | | | |
| | MBRec | 2200 | 2200 | 280 | | | |
| | MBU | 3080 | 3080 | 880 | | | |
| | | concentration e | exceeded State | standard | | | |

Table 4. Fecal indicator bacteria concentrations in water samples collected in the Moonlight Brook watershed through July 2024 Site MBO: Moonlight Brook Outlet-mouth at Lamprey River; Site NR: New Road 3; Site MBD: 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; Site MBU: Moonlight Brook upstream.

The three bacterial indicators exceeded State water quality standards to a varying extent. Through July 2024, fecal coliform levels exceeded the state standard (14 cfu/100 ml) in all but one of the samples. *E. coli* levels, vital in determining the safety of freshwater recreation, exceeded the state standard (153 *E.coli*/100 ml) in 21 of 30 samples. Enterococci exceeded the State standard (104 cfu/100 ml) in 20 of 30 samples. All the bacterial indicators exceeded the State standards to the greatest extent in late July, although exceedance was consistently present since the beginning of the 2024 sampling events in early May.

The three bacterial fecal indicators exceeded State water quality standards at levels higher than in the 6 Lamprey River sample sites (Table 5). Enterococci levels exceeded the standard (104 enterococci/100 ml) on 20 out of 30 (67%) samples, whereas fecal coliforms exceeded the standard (14 FC/100 ml) in 29 out of 30 (97%) samples. *E. coli* levels, which are most relevant

to this study as they relate to freshwater recreation, exceeded the standard (153 *E.coli*/100 ml) on 21 of the 30 (70%) samples, most often at Site CD on Columbia Drive.

| 2024 | State | standard exceed | dance | | Non-detection | |
|-----------|----------------|-----------------|-------------|---|---|---------------------|
| Site | fecal coliform | E.coli | Enterococci | fecal coliform | E.coli | Enterococci |
| | >14/100 ml | >150/100 ml | >104/100 ml | <mdl< td=""><td><mdl< td=""><td><mdl< td=""></mdl<></td></mdl<></td></mdl<> | <mdl< td=""><td><mdl< td=""></mdl<></td></mdl<> | <mdl< td=""></mdl<> |
| MBO | 5 | 4 | 3 | 0 | 0 | 1 |
| NR | 5 | 3 | 4 | 0 | 0 | 1 |
| CD | 5 | 5 | 3 | 0 | 0 | 0 |
| MBD | 4 | 3 | 3 | 1 | 1 | 1 |
| MBRec | 5 | 3 | 4 | 0 | 0 | 0 |
| MBU | 5 | 3 | 3 | 0 | 0 | 2 |
| Totals | 29 | 21 | 20 | 1 | 1 | 5 |
| % samples | 96% | 62% | 58% | 3% | 3% | 17% |

Table 5. Frequency of exceedance of State water quality standards and non-detection of bacterial indicators at the 6 study sites through August 2024.

Microbial source tracking analysis was conducted on all collected water samples (Table 6). The mammal genetic marker was detected in all samples, while 86% of the samples were positive for the bird marker. Other sources were detected at the following levels: dog - 46%, ruminant (deer, cow, etc.) -43%, human- 29%, cow - 7%, and horse– 4%. Some sources like birds were consistently detected, while others were detected more sporadically, probably reflecting changes in animal, human and climatic conditions. Detection of the human marker in the Moonlight Brook watershed was higher than the level of detection in the Lamprey River sites.

| Site | Sample date | Mammal | Human | Dog | Ruminant | Cow | Bird | Gull | Horse | Canada Goose |
|-------|-------------|--------|-------|-----|----------|-----|------|------|-------|--------------|
| MBO | 5/1/2024 | + | + | + | + | - | + | - | - | - |
| NR | | + | - | + | - | - | + | - | - | - |
| CD | | + | - | + | _ | - | + | - | - | - |
| MBD | | + | - | + | _ | - | + | - | - | - |
| MBrec | | + | - | + | - | - | + | - | - | - |
| MBO | 5/22/2024 | + | + | + | - | - | + | - | - | - |
| NR | | + | - | + | + | | + | - | - | - |
| CD | | + | - | - | + | - | + | - | - | - |
| MBD | | + | - | - | - | - | - | - | - | - |
| MBrec | | + | - | - | - | - | + | - | - | - |
| MBO | 6/5/2024 | + | - | - | + | - | + | | - | - |
| NR | | + | + | + | + | - | + | | - | - |
| CD | | + | + | + | + | - | + | | - | - |
| MBD | | + | + | + | + | - | + | | - | - |
| MBrec | | + | + | + | - | - | + | | - | - |
| MBU | | + | | - | + | - | + | | - | - |
| MBO | 7/1/2024 | + | - | - | - | - | + | - | - | - |
| NR | | + | - | + | + | - | + | - | - | - |
| CD | | + | + | - | + | - | + | - | - | - |
| MBD | | + | - | - | - | - | | - | - | - |
| MBrec | | + | + | - | | - | + | - | - | - |
| MBU | | + | - | - | | - | - | - | - | - |
| MBO | 7/31/2024 | + | - | - | + | - | + | - | - | - |
| NR | | + | - | + | - | + | + | - | + | - |
| CD | | + | - | - | | - | + | - | - | - |
| MBD | | + | - | - | + | + | + | - | - | - |
| MBrec | | + | - | - | - | - | + | - | - | - |
| MBU | | + | - | - | | - | | - | - | - |
| | % positive | 100% | 29% | 46% | 43% | 7% | 86% | 0% | 4% | 0% |

Table 6. Detection of the presence of different pollution sources by PCR analyses for all samples from May through July 2024. Green highlight indicates detection.

Next Steps

This study represents an interim report on the bacterial contamination in the Lamprey River watershed from April-August 2024. This research is vital as it helps to determine the safety of freshwater recreation, detect potentially manageable sources of microbial contamination, and be used to convey information about water conditions to the community. The paucity of sampling events following intensive rainfall in 2024 provides an opportunity to assess the impacts of drier conditions on bacterial contamination and allows for comparison to 2023, when the rainy conditions significantly elevated bacterial indicator levels in the summer months. As in 2023, there were consistent positive results for dog thus far in 2024. Management strategies like signage to notify dog owners and providing dog bags can help to reduce contamination. The UNH-Jones lab will continue to use microbial source tracking to monitor the Lamprey River and Moonlight Brook watersheds through November 2024. Efforts will also be focused on identifying trends in bacterial indicator levels as well as pollution sources.