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.) Continue 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 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; 2024a) 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; 2022; 2024 b&c). 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 continue to present study findings at relevant regional meetings, and some of the data will be used by students to present research posters at the UNH Undergraduate Research Conference in

April 2025. The data are also 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

Water samples collection from the shorelines of the Lamprey River from Newmarket to Raymond, and the Moonlight Brook watershed in Newmarket for analysis of bacterial pollutants. Sampling in the Lamprey River watershed occurred at 6 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.

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	direct drain to E*01-01 area	upstream of Moonlight Bk mouth	Secondary Contact	2008	1996	3-ND/5-P
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	3-ND
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	3-ND
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

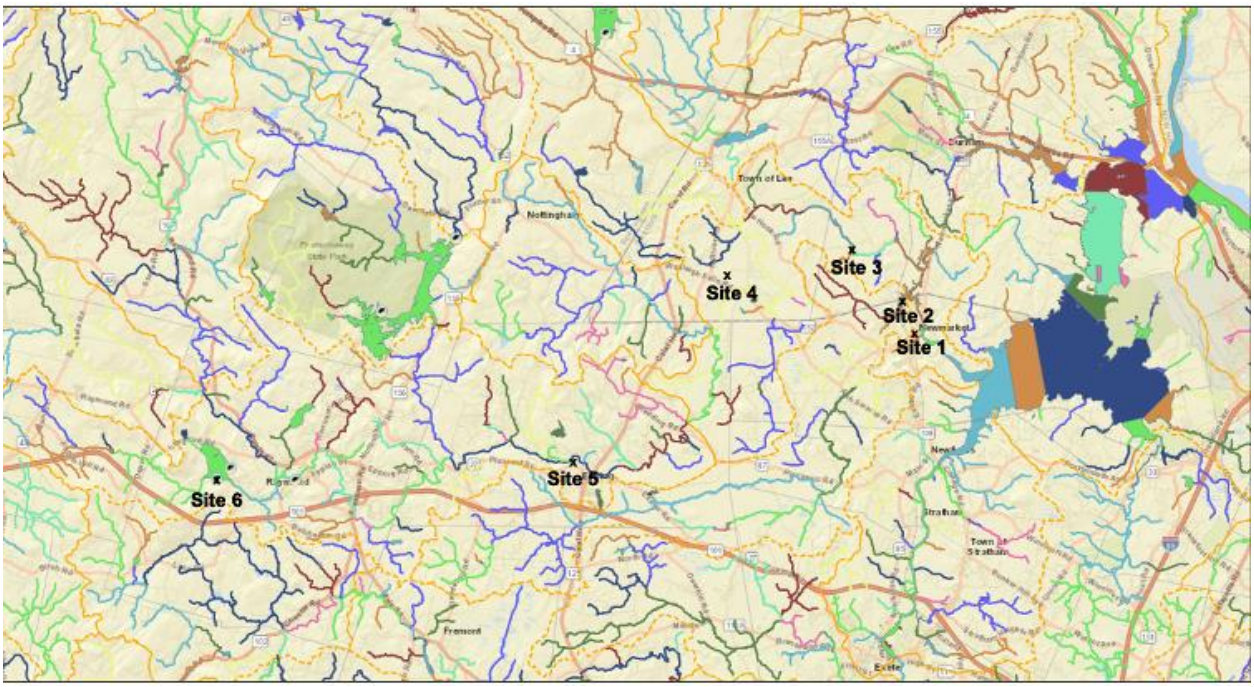
*All sites in the Moonlight Brook watershed fall under this same Assessment Unit; E*01-01 area is classifed as P-5 'not supporting-severe'.

†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 relatively large margin; 3-ND: No current data/insufficient information to make assessment decision.

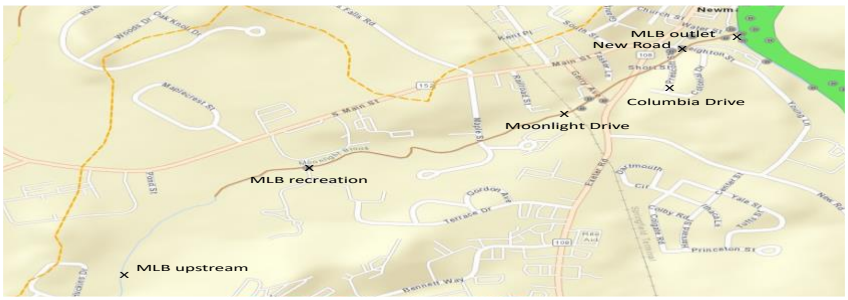
Table 1. 2024 NHDES Water Quality Assessment categories in the Lower Lamprey River (HUC 12: 010600030709) and the Middle Lamprey River (HUC 12: 010600030703) for the 6 main study sites.

Figure 1. Locations of project study sites during 2024 (NHDES 2024d). This figure and Figure 2 were developed using the NHDES Surface Water Quality Assessment Viewer:
https://experience.arcgis.com/experience/23aca0560af74cfa9f0d39f4125ce479#data_s=id%3A83f83eddd9b8476580896fe20c0e4c5b-4abcbca5f2ae49d784eea43ebcbbb593%3A3379



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 contribute to what is discharged into 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 2024.



All samples were collected and stored on ice while being transported to the Jackson Estuarine Laboratory (JEL) for analysis within 4 hours of sampling. The sampling in the Lamprey River watershed occurred approximately once per month during 2023 on May 1, June 5, July 1, July 31, August 12, September 19, October 17, and November 14. The sampling in the Moonlight Brook watershed occurred approximately once per month during 2023 on May 7, May 22, June 3, July 22, September 24, October 17, and November 21. 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 (elevated relative to 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) are from the UNH Weather daily statistics online database.

Data analysis involved basic comparisons of fecal indicator concentrations to those used as State water quality standards (Tab. 2; NHDES 20204a) 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 need 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. 2015).

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 2024 NHDES Watershed Report Cards for an approximate 34 square mile area representing the Lower and Middle portions of the Lamprey River (NHDES 2024b). 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 24 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 translates to: "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 1, which is at the mouth of Moonlight Brook where fresh and tidal water from the Lamprey River mix. The tidal portion of the Lamprey River in that area is classified as 5-P, while the classification of the whole Moonlight Brook is 3-ND. The secondary contact (boating) classification is '2-G', meaning that the water quality meets standards by a relatively large margin (Tab. 2).

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 '3-ND' for Sites 5 & 6 (Tab. 1).

Lamprey River Watershed

All intended sample collections occurred on 9 dates from April through November 2024. 2024 was a dry summer featuring only 2 intensive (>1 in./24 h) rainfall events (1 each in June and September) in the Lamprey River watershed, with numerous periods where no rain fell for more than a week. The wettest conditions for sample dates were in May and August, where sampling occurred after 0.4" rainfall inches fell 2 days prior to the sample date (Tab. 3). The bacterial indicator levels changed with the different monthly sample events but were probably only minimally affected by previous rainfall/runoff conditions.

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.

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
4/25/24	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	MB	260	240	<20	0	0	0.4
	2	PRBL	10	10	<10			
	3	WD	<10	<10	<10			
	4	WF	40	40	<10			
	5	ETH	80	80	<10			
	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	MB	<20	<20	<20	0	0	0
	2	PRBL	60	80	<20			
	3	WD	100	120	1860			
	4	WF	40	40	20			
	5	ETH	240	240	40			
	6	RES	160	160	<20			
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			
9/19/24	1	MB	1250	1240	1760	0.04	0	0
	2	PRBL	<20	30	670			
	3	WD	<10	<10	160			
	4	WF	90	90	870			
	5	ETH	570	570	520			
	6	RES	60	50	500			
10/17/24	1	MB	10	10	<10	0	0	0
	2	PRBL	10	10	<10			
	3	WD	<10	<10	<10			
	4	WF	50	50	<10			
	5	ETH	60	50	<10			
	6	RES	10	10	<10			
11/14/24	1	MB	155	155	<5	0	0	0
	2	PRBL	35	15	<5			
	3	WD	40	40	<5			
	4	WF	65	55	<5			
	5	ETH	15	15	<5			
	6	RES	<5	<5	<5			
			concentration exceeded State standard					

The three bacterial fecal indicators exceeded State water quality standards at varying rates (Tables 3 & 4). Enterococci levels exceeded the State standard (104 enterococci/100 ml) on up to 4 of the 9 sample events at 6 sites for a total of 16 of 72 events, in contrast to fecal coliforms that exceeded the 14 FC/100 ml standard in 38 of 72 samples. *E. coli* levels, which are most pertinent to this study as they relate to freshwater recreation, exceeded the State standard (153 *E. coli*/100 ml) on up to 5 of the 9 sample events at the 6 sites for a total of 24 events. The May and August samples exceeded the State standard at 5 out of 12 site samples while concentrations exceeded the State standard at only 1 to 2 sites in the other 7 sample dates (Tab. 3 & 4). Fecal coliforms and *E. coli* were detected at high frequencies (8-10%), enterococci were not detected in 44% of samples, and non-detection occurred in all months except May (Tab. 3 & 4).

2024	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	7	5	3	1	1	5
2	5	2	3	2	1	6
3	4	2	4	3	3	6
4	9	0	1	0	0	5
5	8	4	1	0	0	4
6	5	4	4	1	1	6
Totals	**38**	**17**	**16**	**7**	**6**	**32**
% samples	**53%**	**24%**	**22%**	**10%**	**8%**	**44%**

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

In the 2021 to 2023 studies, indicator bacteria were detected at much higher levels at Site 1 (MB) compared to all other sites (Jones 2023). In 2024, Site 1 continued to be contaminated at a relatively high frequency but at generally lower levels than in past years, while Site 6 had the highest fecal coliform and *E.* coli single sample concentration (6,600/100 ml) and Site 3 (WD) had the highest enterococci concentration (1,800/100 ml) (Fig. 3; Tables 3 & 4). A 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, which are detected using the same assay, were elevated in May, July and August, with the highest concentrations (6,610/100 ml) at Site 6 (RES) on May 5th. Enterococci were elevated at all sites in September, and at most sites in May and August, with the highest concentration (1,860/100 ml) at Site 3 (WD) on July 22nd. These results illustrate the known differences between different bacterial indicators of fecal contamination that underly different management applications.

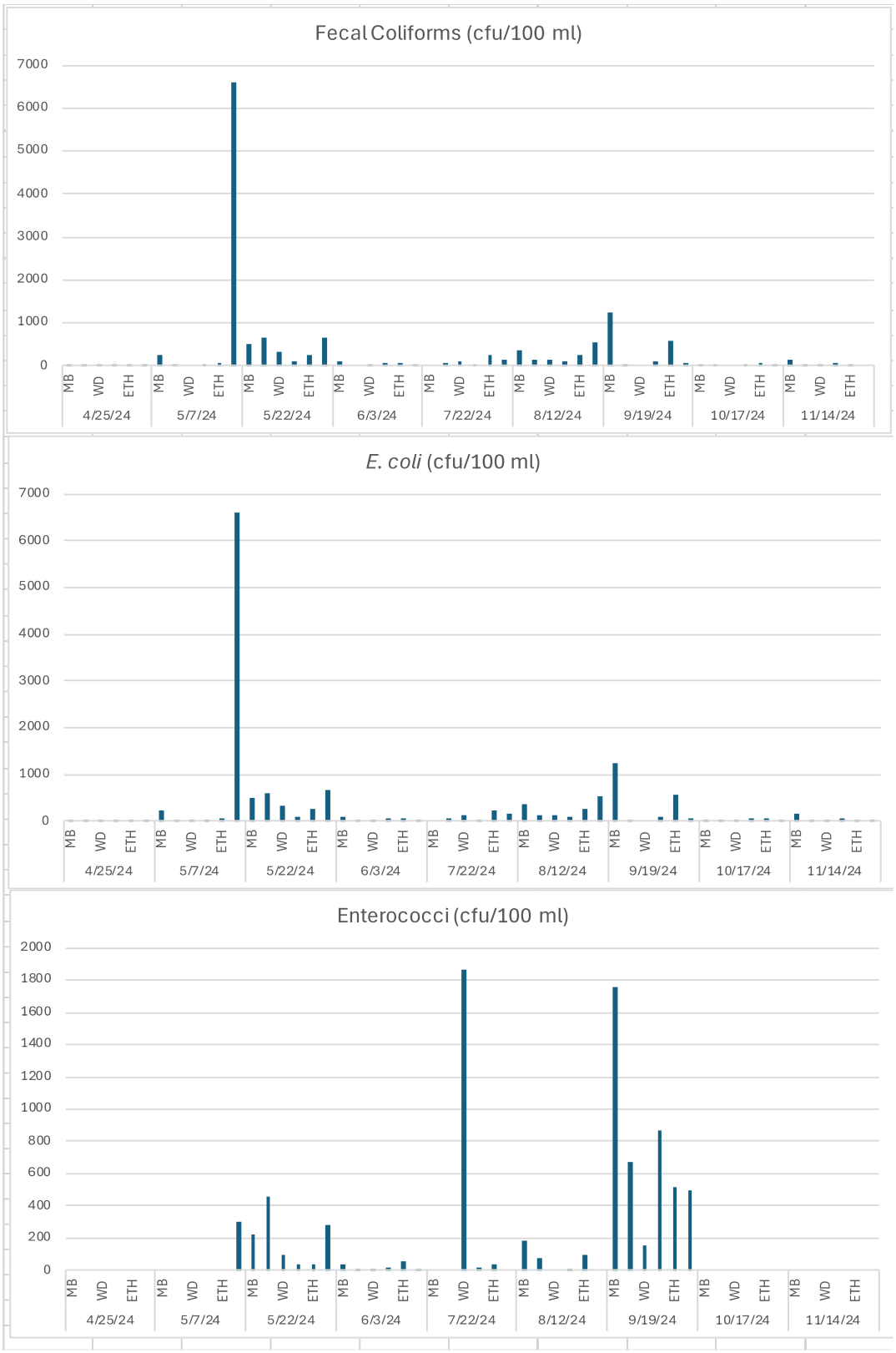
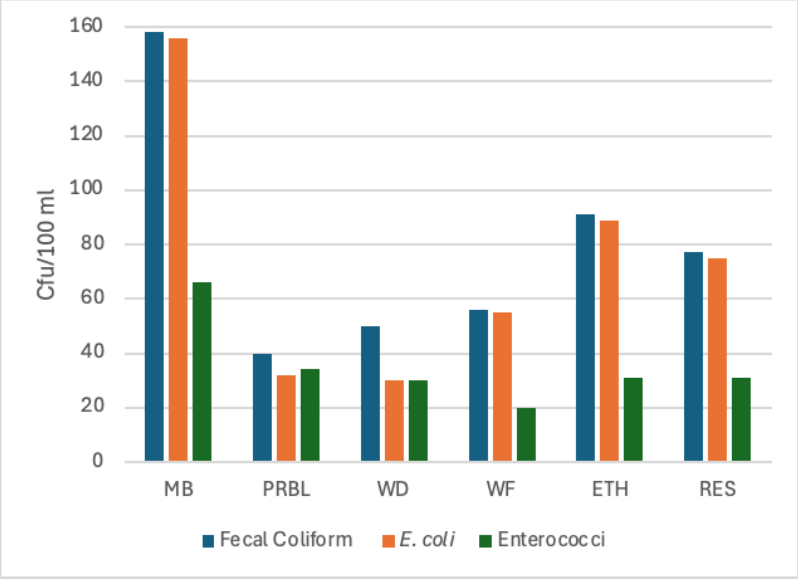


Figure 3. Concentrations of the 3 fecal coliforms, *E. coli*, and enterococci for all 9 sample dates at each of the 6 sampling sites.

As in past years (Jones 2022; 2023), the geometric mean concentrations for the fecal indicator bacteria for the full study show differences between sites (Fig. 4). Fecal coliforms and *E. coli* average concentrations were once again highest at Site 1 and elevated at Sites 5 & 6, while the relatively lower enterococci concentrations were slightly higher at Site 1 and uniformly lower at the other sites. As in 2022 and 2023, the impact of rainfall and associated runoff was again a focus of this 2024 study, but drought conditions dominated the weather which did not allow for capturing wet weather events to determine potential impacts.

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



The bacterial indicator levels at a tidal site at the Newmarket waterfront just upstream from Site 1 (MB) determined by UNH-JEL for the GBNERR/Piscataqua Regional Estuaries Partnership (PREP) monitoring program for the previous two years (2022 & 2023) were useful for comparisons to the 2024 results for the upstream watershed. In 2024, the levels of all 3 bacterial indicators exceeded State thresholds only in August and fecal coliforms exceeded the State threshold in all but 2 months. Overall, levels of each indicator were lower and exceeded State standards less frequently compared in 2024 to the previous two years, especially 2023 when rainfall/runoff occurred more frequently and there was a sewage leak from a broken pipe under the upper tidal river.

Collection Date	Fecal coliform cfu/100 ml	*E. coli* cfu/100 ml	Enterococci cfu/100 ml
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
4/15/24	<10	<10	<10
5/13/24	40	40	8
6/11/24	60	60	100
7/9/24	40	40	40
8/5/24	230	230	120
9/9/24	17	13	43
11/5/24	28	28	2
12/9/24	8	8	<4

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 6 sites for all the 30 samples analyzed (Tab. 6). Bird contamination was the most prevalent contamination source as it was detected in all but 3 samples. Ruminant contamination was present at some sites each month except for May, while Canada geese were detected only after June and especially in November. Contamination from dogs, cows and gulls were detected in 6 to 8 of the samples, but no contamination from horses was detected.

						PCR (presence/absence)						qPCR (copy #/100 ml)		
Site	**Date**	**Vol**	Mammal	Human	Dog	Ruminant	Cow	Bird	Gull	Horse	Canada goose	Mammal	Human	Bird
MB	5/7/24	300	+	+	+	-	-	+	-	-	-	1.83E+07	2.75E+02	3.25E+03
ETH	5/7/24	300	+	-	+	-	+	+	-	-	-	7.63E+06		3.71E+03
RES	5/7/24	300	+	-	+	-	-	+	-	-	-	8.56E+06		1.47E+04
MB	6/3/24	300	+	-	+	+	-	+	+	-	-	1.32E+07		7.58E+03
WF	6/3/24	300	+	-	+	+	-	+	+	-	-	6.11E+07		6.71E+03
ETH	6/3/24	300	+	-	+	+	-	+	-	-	-	2.23E+07		9.64E+03
PRBL	7/22/24	300	+	-	-	-	+	+	+	-	-	8.06E+06		2.72E+03
WD	7/22/24	300	+	-	-	-	+	+	+	-	-	1.07E+06		5.40E+03
ETH	7/22/24	300	+	-	-	+	-	+	+	-	-	4.85E+06		<167
RES	7/22/24	300	+	-	-	+	-	+	-	-	+	3.08E+06		2.23E+03
MB	8/12/24	300	+	-	-	+	-	+	-	-	-	4.86E+06		<167
PRBL	8/12/24	300	+	-	-	-	-	+	-	-	+	4.00E+06		1.98E+03
WD	8/12/24	300	+	-	-	-	-	+	-	-	-	3.18E+06		1.90E+03
WF	8/12/24	300	+	+	-	+	-	-	-	-	-	6.84E+07	<167	-
ETH	8/12/24	300	+	-	-	+	-	-	-	-	-	5.32E+07		-
RES	8/12/24	300	+	-	-	-	-	+	+	-	-	3.60E+07		<167
MB	9/19/24	300	+	+	-	-	-	+	-	-	-	1.23E+08	9.68E+02	5.26E+03
PRBL	9/19/24	300	+	-	-	-	-	+	-	-	+	1.62E+07		<167
WD	9/19/24	300	+	-	-	-	-	+	-	-	+	2.41E+07		3.00E+03
WF	9/19/24	300	+	-	-	+	-	+	-	-	-	1.02E+08		3.86E+03
ETH	9/19/24	300	+	-	-	+	-	+	-	-	-	1.51E+08		8.73E+03
RES	9/19/24	300	+	-	-	-	-	+	-	-	-	2.41E+07		2.61E+03
WD	10/17/24	300	+	-	-	-	-	-	-	-	-	2.32E+06		-
WF	10/17/24	300	+	-	+	+	-	+	-	-	-	5.20E+07		2.28E+03
ETH	10/17/24	300	+	-	-	+	-	+	-	-	-	1.16E+07		3.83E+03
MB	11/14/24	300	+	+	-	+	-	+	+	-	+	1.21E+07	3.39E+03	5.45E+03
PRBL	11/14/24	300	+	-	-	+	+	+	+	-	+	2.56E+07		9.68E+02
WD	11/14/24	300	+	-	-	+	+	+	-	-	+	1.83E+07		2.36E+03
WF	11/14/24	300	+	-	-	+	-	+	-	-	+	1.18E+07		2.05E+03
ETH	11/14/24	300	+	-	-	+	+	+	-	-	+	1.23E+07		4.57E+03
		# positive =	30	4	7	17	6	27	8	0	9			

Table 6. Detection of the presence of different pollution sources by of PCR and qPCR analyses for all samples from May through November 2024. Green highlight denotes detection, yellow highlight denotes level above human contamination risk threshold level.

Human contamination was detected only in 4 samples, 3 of them from Site 1 (MB) (Tab. 6), unlike in 2023 when it was detected at 4 of the 6 study sites. The follow-up semi-quantitative assay (qPCR), which has a higher detection limit, indicated the human contamination concentrations at Site 1 were higher (968 to 3,390 CN/100 ml) than the below detection level found at the other site (Site 4-WF). The concentrations were all below a public health safety threshold (4,200 copy number/100 ml; Boehm et al. 2015).

The quantified level of bird contamination (by qPCR assay) for the 27 samples where it was detected reflected relatively elevated levels of contamination (Tab. 6). Only 4 samples were below the qPCR assay detection limit and of the 23 samples with detectable levels by qPCR, 22 exceeded 1,000 CN/100 ml. The highest contamination level was14,700 CN/100 ml and the lowest level at 968 CN/100 ml. The presence of Canada goose and gull contamination did not correspond to more elevated levels of bird contamination.

There was some seasonality for a few source types, although detection of human, bird, ruminant, gull and cow contamination was spread across the full study period (Tab. 6). Canada geese

contamination was detected mainly in September and November, and dog contamination was detected mainly in May and June.

In addition to showing the highest concentrations of all three indicator bacteria, Site 1 (MB) also had a slightly higher diversity for identified types of contamination, with an average of 3.6 types (out of 8 possible, excluding mammal) per sample (Tab. 7). In 2023 this analysis showed higher diversity of contamination types at all sites, more detection of human contamination, and some that were above the human contamination threshold (Jones 2023).

Site	Samples #	Ave. # of source types detected	Human source detection	Human source >threshold
		LAMPREY RIVER WATERSHED		
MB	5	**3.6**	**3**	0
PRBL	4	3.0	0	0
WD	5	2.0	0	0
WF	5	2.8	1	0
ETH	7	2.7	0	0
RES	4	2.0	0	0

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

Moonlight Brook watershed

A significant focus of the 2024 study was to continue exploration of how contaminated Moonlight Brook is in relation to the historically elevated levels of bacterial contamination at the sampling site (Site 1-MB/MBO) located in the mouth/outlet of Moonlight Brook next to the Newmarket boat launch (Figs. 1 & 2). The Moonlight Brook watershed sites were sampled monthly on different days than the sites on the full Lamprey River watershed. The Moonlight Brook watershed sites included 3 upstream of the downtown railroad crossing, and 2 more sites in a small tributary waterway to the south of the brook outlet that crosses New Road and that extends into the Sleepy Hollow trailer park.

All intended sample collections occurred on 7 dates in May through November 2024 (Tab. 8). Like the Lamprey River watershed, 2024 was dry in the Moonlight Brook watershed. There were no instances where sampling occurred on a date following significant (>1") rainfall within 2 days prior to the sample dates. Concentrations of all three bacterial indicators were higher and more often exceeded State risk thresholds during late July, and at lower concentrations in early July and September (Tab. 8).

		Fecal			Rainfall-daily		
Date	Site	coliforms	*E. coli*	Enterococci	sample day	prior day	2 d prior
		CFU/100 ml	CFU/100 ml	CFU/100 ml	"/24 h	"/24 h	"/24 h
5/1/24	MB outlet	310	310	50	0.29	0.4	0
	New Road	100	95	<5			
	CD	165	165	5			
	Moonlight Dr	730	730	30			
	MB Rec	905	905	110			
	MB-U	15	10	<5			
6/5/24	MB outlet	60	60	140	0.69	0	0
	New Rd	20	40	680			
	Moonlight Dr	180	180	100			
	CD	90	90	410			
	MB Rec	80	80	20			
	MB-U	1830	1790	4380			
7/1/24	MB outlet	40	40	1700	0	0.56	0.05
	New Rd	240	240	780			
	CD	80	80	1840			
	Moonlight Dr	60	60	420			
	MB Rec	1200	1200	1240			
	MB-U	560	560	240			
7/31/24	MB outlet	960	960	350	0.66	0.01	0.14
	New Rd	1280	1280	200			
	CD	4480	4480	220			
	Moonlight Dr	1120	1120	640			
	MB Rec	2200	2200	280			
	MB-U	3080	3080	880			
9/24/24	MB outlet	960	960	1400	0	0	0
	New Rd	1640	1580	2020			
	CD	360	360	920			
	Moonlight Dr	140	120	560			
	MB Rec	160	160	760			
	MB-U	800	800	4480			
	MBO-pipe	320	280	1680			
10/17/24	MB outlet	280	280	20	0	0	0
	New Rd	140	140	<10			
	CD	160	160	<10			
	Moonlight Dr	240	220	<10			
	MB Rec	100	100	<10			
	MB-U	840	840	<10			
11/21/24	MB outlet	530	520	210	0.28	0	0
	New Rd	580	540	90			
	CD	230	230	110			
	Moonlight Dr	70	70	70			
	MB Rec	30	30	30			
	MB-U	80	80	20			

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.

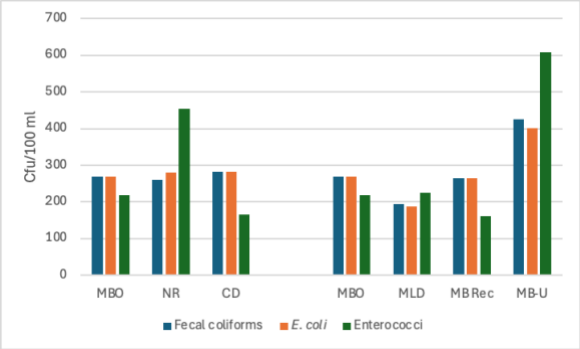
The three bacterial fecal indicators exceeded State water quality standards to varying degrees (Tables 8 & 9). Fecal coliforms exceeded the standard (14 FC/100 ml) in all the 42 samples. *E. coli* levels, which are most pertinent to this study as they relate to freshwater recreation, exceeded the single sample standard (153 *E. coli*/100 ml) in 60% of the samples including all 6 sites in July. Enterococci levels exceeded the State standard (104 enterococci/100 ml) in 50% of sample and at all sites in July through September. The bacterial indicators were detected at a high frequency, with fecal coliforms always detected, and non-detection occurring only 3 times for *E. coli* and 7 times for enterococci (Tab. 9).

	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
MBO	7	5	5	0	1	0
NR	7	4	3	0	0	2
MLD	7	2	0	0	0	1
CD	7	5	5	0	0	1
MBRec	7	4	4	0	2	1
MBU	7	5	4	0	0	2
Totals	**42**	**25**	**21**	**0**	**3**	**7**
% samples	**100%**	**60%**	**50%**	**0%**	**7%**	**17%**

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 geometric mean 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 and *E. coli* concentrations were relatively equal across all sites except Site MBU, where both were substantially higher. Enterococci concentrations were highest at Site MBU then at Site NR, with relatively equal levels at the other 4 sites. These results contrast with the 2023 study results where Site MBO clearly had the highest fecal coliform and enterococci concentrations. Overall, indicator levels were higher at all sites in 2024 compared with 2023 and despite being a dry year, and the elevated levels of all 3 indicators at MBU is a new development.

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 based on all the 47 samples analyzed (Tab. 10). Bird contamination was present in 45 of the 47 samples analyzed and at all sample sites except at Site MBU in May and early July. Ruminant contamination was detected in 32 of the analyzed samples and at one site or more on each sample event. Dog and human contamination were present in 16 and 14 of the analyzed samples, respectively, with human contamination at all sites, including a pipe discharge at the MBO site on 9/24/24, and dog contamination each month except October and November. Canada goose, gull and horse contamination was detected in 5, 4 and 2 samples, respectively.

			PCR (presence/absence)									qPCR (copy #/100 ml)		
Site	**Date**	**Vol**	Mammal	Human	Dog	Ruminant	Cow	Bird	Gull	Horse	Canada goose	Mammal	Human	Bird
MBO	5/1/24	300	+	+	+	+	-	+	-	+	-	7.50E+06	1.10E+03	3.73E+03
NR	5/1/24	300	+	-	+	-	-	+	-	-	+	2.04E+07	-	2.03E+03
CD	5/1/24	300	+	-	+	-	-	+	-	-	-	1.53E+07	-	9.88E+03
MBD	5/1/24	300	+	+	+	-	-	+	-	-	-	8.99E+06	5.73E+02	2.51E+03
Mbrec	5/1/24	300	+	-	+	-	-	+	-	-	-	4.02E+06	-	3.41E+03
MBO	5/22/24	300	+	+	+	-	-	+	-	-	-	1.92E+07	3.44E+02	5.65E+03
NR	5/22/24	300	+	-	+	+	-	+	-	-	-	9.89E+06	-	2.54E+03
CD	5/22/24	300	+	-	-	+	-	+	-	-	-	2.84E+07	-	2.48E+03
MBD	5/22/24	300	+	-	-	-	-	-	-	-	-	1.90E+07	-	-
MBrec	5/22/24	300	+	-	-	-	-	+	-	-	-	1.70E+07	-	< 167
MBO	6/5/24	300	+	-	-	+	-	+	-	-	-	1.31E+07	-	< 167
NR	6/5/24	300	+	-	+	+	-	+	-	-	-	4.83E+07	-	< 167
CD	6/5/24	300	+	+	+	+	-	+	-	-	-	1.67E+07	<167	6.44E+03
MBD	6/5/24	300	+	+	+	+	-	+	-	-	-	3.63E+06	-	7.18E+03
MBrec	6/5/24	300	+	-	+	-	-	+	-	-	-	4.05E+06	-	< 267
MBU	6/5/24	300	+	+	-	+	-	+	-	-	-	3.21E+06	1.71E+02	5.68E+03
MBO	7/1/24	300	+	-	-	-	-	+	-	-	-	9.22E+06	-	1.72E+03
NR	7/1/24	300	+	-	+	+	-	+	-	-	-	2.72E+07		3.64E+03
CD	7/1/24	300	+	+	-	+	-	+	-	-	-	5.78E+07	<167	< 167
MBD	7/1/24	300	+	-	-	-	-	+	-	-	-	2.37E+06		3.02E+03
MBrec	7/1/24	300	+	+	-	+	-	+	-	-	-	2.88E+07	3.79E+03	< 167
MBU	7/1/24	300	+	-	-	+	-	-	-	-	-	4.33E+06		-
MBO	7/31/24	300	+	+	-	-	-	+	-	-	-	3.26E+06	<167	1.48E+04
NR	7/31/24	300	+	-	+	-	+	+	-	+	-	9.24E+06		3.12E+03
CD	7/31/24	300	+	-	-	+	-	+	-	-	-	1.73E+08		2.39E+04
MBD	7/31/24	300	+	-	-	+	+	+	-	-	-	6.23E+05		8.91E+03
MBrec	7/31/24	300	+	-	-	-	-	+	-	-	-	1.53E+07		3.32E+03
MBU	7/31/24	300	+	-	-	+	-	+	-	-	-	2.65E+07		2.07E+03
MBO	9/24/24	300	+	+	-	+	-	+	-	-	-	4.11E+07	4.12E+03	2.66E+03
NR	9/24/24	300	+	-	+	+	-	+	-	-	-	6.85E+07		1.09E+03
CD	9/24/24	300	+	-	-	+	-	+	-	-	-	9.90E+07		1.33E+03
MBD	9/24/24	300	+	-	-	+	-	+	-	-	-	3.44E+07		4.26E+03
MBrec	9/24/24	300	+	-	-	+	-	+	-	-	-	5.57E+07		8.10E+02
MBU	9/24/24	300	+	-	-	+	-	+	-	-	-	1.40E+08		1.00E+03
MB PIPE	9/24/24	300	+	+	+	+	-	+	+	-	-	1.42E+08	<167	4.18E+02
MBO	10/17/24	300	+	+	-	+	-	+	-	-	+	1.21E+07	2.69E+03	6.28E+03
NR	10/17/24	300	+	-	-	+	-	+	-	-	-	5.27E+07		2.21E+03
CD	10/17/24	300	+	-	-	+	-	+	-	-	-	2.08E+08		6.67E+02
MBD	10/17/24	300	+	-	-	+	-	+	-	-	+	2.96E+07		1.32E+03
MBrec	10/17/24	300	+	-	-	+	-	+	-	-	+	7.77E+06		3.92E+03
MBU	10/17/24	300	+	-	-	-	-	+	-	-	-	3.27E+07		3.86E+02
MBO	11/21/24	300	+	+	-	+	+	+	+	-	-	4.60E+07	2.65E+04	1.96E+03
MBD	11/21/24	300	+	+	-	+	-	+	-	-	-	1.50E+07	3.14E+02	1.74E+03
MBrec	11/21/24	300	+	-	-	+	-	+	+	-	-	2.05E+07		4.66E+02
MBU	11/21/24	300	+	-	-	+	+	+	-	-	+	8.28E+06		7.94E+02
CD	11/21/24	300	+	-	-	+	+	+	-	-	-	9.70E+07		1.39E+03
NR	11/21/24	300	+	-	-	+	+	+	+	-	-	6.89E+07		1.59E+03
		# positive =	47	14	16	32	6	45	4	2	5			

Table 10. Detection of the presence of different pollution sources by of PCR and qPCR analyses for all samples from May through November 2024. Green highlight denotes detection, yellow highlight denotes level above or nearly equal to a human contamination risk threshold level.

Human contamination was detected in 14 of the 48 total samples and in 6 of 8 samples at Site MBO (Tab. 10). Human contamination was detected at least once for the other 5 sites in the Moonlight Brook watershed except at Site NR, including the single sample collected in a pipe discharge at Site MBO, and more than once at Sites MLD and CD.

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 (Tab. 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. 2015) in 1 sample collected on November 11th, and at just below the threshold on September 24th and at MBrec on July 1st. The levels of human contamination in 4 of 14 analyzed samples were below the detection limit and the concentration in the 1 sample from MBO that exceeded the safety threshold was 26,500 copy number/100 ml). The relatively common detection of human contamination at MBO remains a concern, but the results suggest that at least some of the human contamination may come from sources upstream of the downtown section of the watershed.

The quantified level of bird contamination for the 45 samples where bird contamination was also detected by the non-quantitative PCR assay reflected relatively low levels of contamination, as 6 samples were below the qPCR assay detection limit, 6 had copy numbers below 1,000/100 ml, and only 2 samples detectable levels exceeded 10,000 CN/100 ml, with the highest level at 23,900 CN/100 (Tab. 10). The presence of Canada geese and gulls did not correspond to elevated levels of bird contamination, even during the fall when contamination was detected in 4 of 4 gull and 4 of 5 Canada goose samples.

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 detected in three October samples and single samples in May and November, gulls were detected only in September to November, cows were detected only in July and November, and dogs were detected mainly in May and June. Ruminants, which can include deer, were detected most often during fall months, while horse contamination, whatever the actual source may be, was only detected in single samples at MBO or NR in May and July.

Sites MBO and NR had slightly more diverse identified types of contamination, with an average of 4.1 and 4.0 types (out of 8 possible) , respectively, per sample (Tab. 11). Sites MLD and CD had somewhat lower average number of types (3.5 & 3.4), while Sites MBrec and MBU showed the lowest level of type diversity (2.8 & 2.5 source types). Again, human contamination was detected at 5 of 6 sites at levels below the safety threshold except for 1 sample.

Site #	Samples #	Ave. # of source types detected	Human source detection	Human source >threshold
MBO	8	**4.1**	**6**	**1**
MBO-pipe	1	6.0	1	0
NR	8	4.0	0	0
MLD	8	3.4	3	0
CD	8	3.5	2	0
MBREC	8	2.8	1	0
MBU	6	2.5	1	0

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 4-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.

The continued exploration of water quality assessment to the Moonlight Brook sub-watershed provides 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 MBU where bacterial indicators were detected at levels higher than at the downstream Sites MBRec and MLD. There may also be some sources of contamination to MBO from downtown portions of the brook. There has been 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. The levels of bacterial contamination at MB/MBO in 2024 are overall lower that in previous years, though again it was a dry year.

The concerns about elevated bacterial contamination at Site 6/RES in Raymond during 2023 were still evident in 2024. Levels of fecal coliforms and *E. coli* were higher at Sites 5 and 6 than at other sites except MB during 2024, and Site 6 had the overall highest single sample concentration of these 2 indicators. These results show concerns are present in wet and dry years.

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. In 2024, no samples were collected after substantial rainfall events, yet contamination was still present at somewhat lower overall levels. Thus, water quality concerns are not only tied to rainfall/runoff conditions as indicated by previous comparisons of the 2023 samples with previous year samples.

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. Site 1/MBO continued to be a consistent concern due to elevated bacterial indicator concentrations accompanied by consistent detection of elevated levels of human contamination. The lack of detection of human contamination at all other sites except for one sample at Site 4 was encouraging. The sources of the human contamination are not yet apparent, so the towns along

the Lamprey River and 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, however, in 2024 detection was rare after frequent detection in May. These multi-year results suggest runoff from rainfall events may exacerbate water quality contamination from dogs. 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 Jones (2022) that is based on NH Dept. of Health and Human Services/Division of Public Health Services and US CDC fact sheets and information (NHDES 2019a).

Future work could take several directions, the most obvious being a continuation of routine monitoring for bacterial pollution indicators at key sites. This is especially important as the Town of Newmarket will be conducting 2 new sewage infrastructure projects in 2025. 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. Four years of data reflecting both dry and wet conditions provides for a solid baseline to compare to future findings that hopefully can 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. The differences in rainfall frequency and amounts over the past 2 years exemplifies these points.

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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