Tracking Bacterial Contamination in the Lamprey River Watershed

Finals Report

to the Lamprey Rivers Advisory Committee

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INTRODUCTION and BACKGROUND

The main goal of this project was to initiate monitoring at several key sites in the Lamprey River Watershed (LRW) to provides essential data for assessing water quality, public health risks and sources of the contamination. Addressing this overarching goal served to:

1.) Provide a baseline of information related to bacterial pollution to help assess water quality status and potential contamination sources in freshwater and tidal Lamprey River areas.
2.) Compile data from ongoing and past bacterial monitoring efforts in the Lamprey River watershed to allow for spatial and long-term temporal trend determination for concentrations of three indicators of fecal-borne bacterial pollution.
3.) Extend findings to interested groups through meetings and the final published report.

This Final Report is a summary of all project findings, as well as a 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 that 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 watershed is important because there are little to no data related to fecal contamination of recreational surface waters other that 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. Although 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), there either are no such data or no convenient way to access 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 poses a threat or restricts use. 3.) Monitoring program managers who can consider augmenting their programs with similar efforts.

We intend to present the study findings at the Lamprey River Symposium if it is held in January 2022. The data will also be part of UNH student effort to conduct an ongoing evaluation and summarization of the 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,

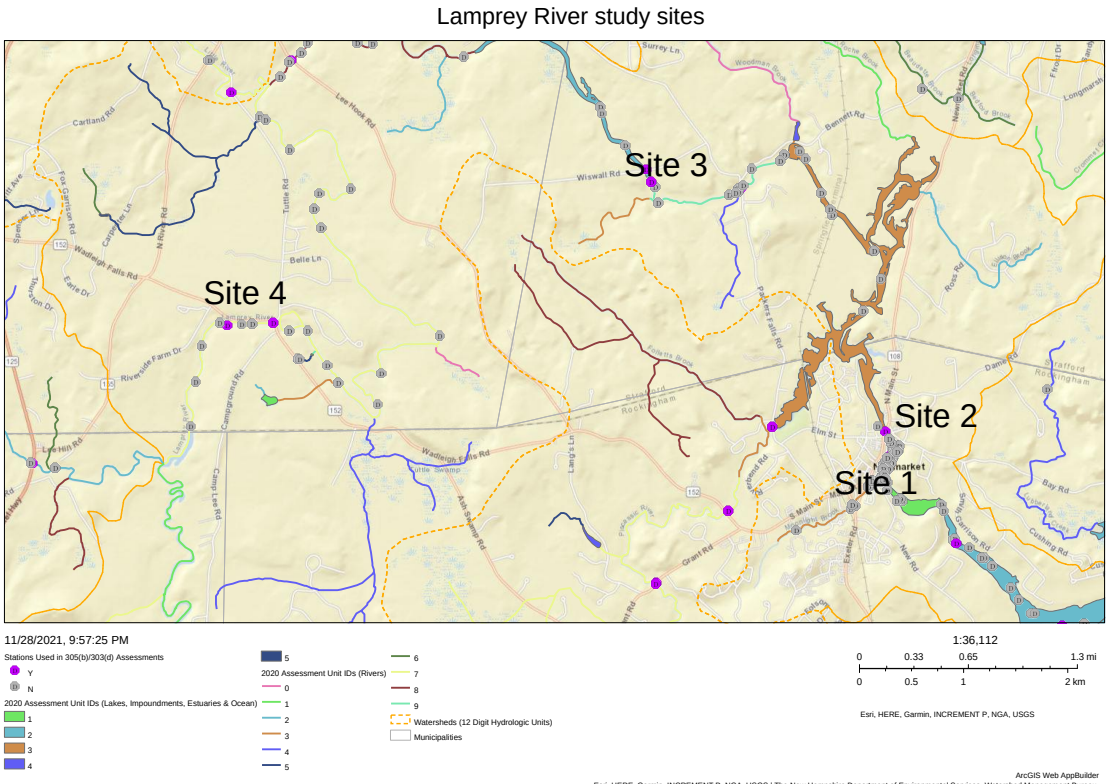
MA to Trenton Harbor, ME. The student(s) will present findings at the UNH Undergraduate Research Conference in April 2022.

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 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 as a result 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 for bacterial pollutants occurred at 4 sites where surface water recreation occurs (Figure 1). The sampling in the tidal water occurred at low tide.
The tidal Site 2 corresponds to the NHDES water quality monitoring program site 05-LMP, and is the same site- GRBLR- as currently monitored by the Great Bay National Estuarine Research Reserve (https://cdmo.baruch.sc.edu/dges/). Site 1 is near a site listed as NHEPLRDO16. 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.

Figure 1. Locations of project study sites. Site 1: upstream of the mouth of Moonlight Brook; Site 2: the tidal portion of the Lamprey River around downtown Newmarket; Site 3: Wiswall Dam. Site 4: upstream of the dam at the Lee public canoe access site near Wadleigh Falls. This figure was developed using the NHDES Surface Water Quality Assessment Viewer: https://nhdes.maps.arcgis.com/apps/webappviewer/index.html?id=d1ba9c5ec85646538e032580e23174f7)



Samples were transported to the UNH/Jackson Estuarine Laboratory (JEL) for analysis. This sampling occurred once per month on April 30, May 28, June 7, July 7 and August 11. 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* (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 test we use provides data for both fecal coliforms and *E. coli* so we do report it 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 from all but the April samples were filtered to capture bacterial cells/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 quantification, of sources of fecal contamination in the sample using PCR (polymerase chain reaction- presence/absence) and qPCR (semi-quantitative) methods. This approach 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 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, and dissolved oxygen. Data for daily rainfall amounts (inches) were also collected from the UNH Weather statistics online database.

Data analysis involved basic comparisons of fecal indicator concentrations to those used as State water quality standards (Table 1; 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 capture the potential 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 1. 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.

For microbial source tracking, the 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 above which researchers at EPA and elsewhere have found to exceed acceptable likelihood of human illnesses.

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 were also partially supported for their involvement in all 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.

RESULTS & DISCUSSION

Review and Summary of Existing Data

There is a Draft 2020 NHDES Watershed Report Card for an approximate 34 square mile area representing the Lower Lamprey River (NHDES 2020c). This area is given a Hydrologic Unit Code (HUC12) of HUC 12: 010600030709. Within this area there are 34 different Assessment Units, each also given unique numerical Assessment IDs, including 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), 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 (Table 2). One other site at Packers Falls also had a 2-G assessment code based on 2017 data for primary and secondary contact uses.

	Assessment Unit		Type* of			Classification
Study Site	ID number	Name	Recreational use	Last sample	Last exceed	Category†
Site 1	NHEST600030709-01-01	Lamprey R. Estuary North	Primary Contact	2017	2016	4A-P
			Secondary Contact	2017	2008	2-G
Site 2	NHRIV600030709-13	Moonlight Brook	Primary Contact	2008	2000	3-ND
			Secondary Contact	2008	1996	3-ND
Site 3	NHIMP600030709-02	Wiswall Dam	Primary Contact	2008	N/A	3-ND
			Secondary Contact	2008	N/A	3-ND
Site 4	NHRIV600030709-01	Upstream of Wadleigh Falls	Primary Contact	no data	no data	3-ND
			Secondary Contact	no data	no data	3-ND
*Primary contact = swimming; Secondary contact = boating.						
†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. Insufficient information to make an assessment decision.					

Table 2. Draft 2020 NHDES Water Quality Assessment categories for primary and secondary contact uses in the Lower Lamprey River (HUC 12: 010600030709) assessment units at or near the 4 study sites.

The bacterial indicator levels at Site 2-the tidal site at the Newmarket waterfront- can be compared to levels determined at the same location by UNH-JEL for the Piscataqua Regional Estuaries Partnership (PREP) - GBNERR monitoring program. There was a long-term decreasing, and thus favorable, trend for enterococci and even more so for *E. coli* levels at this site (Figure 2) over a 27-year period from 1991-2017.

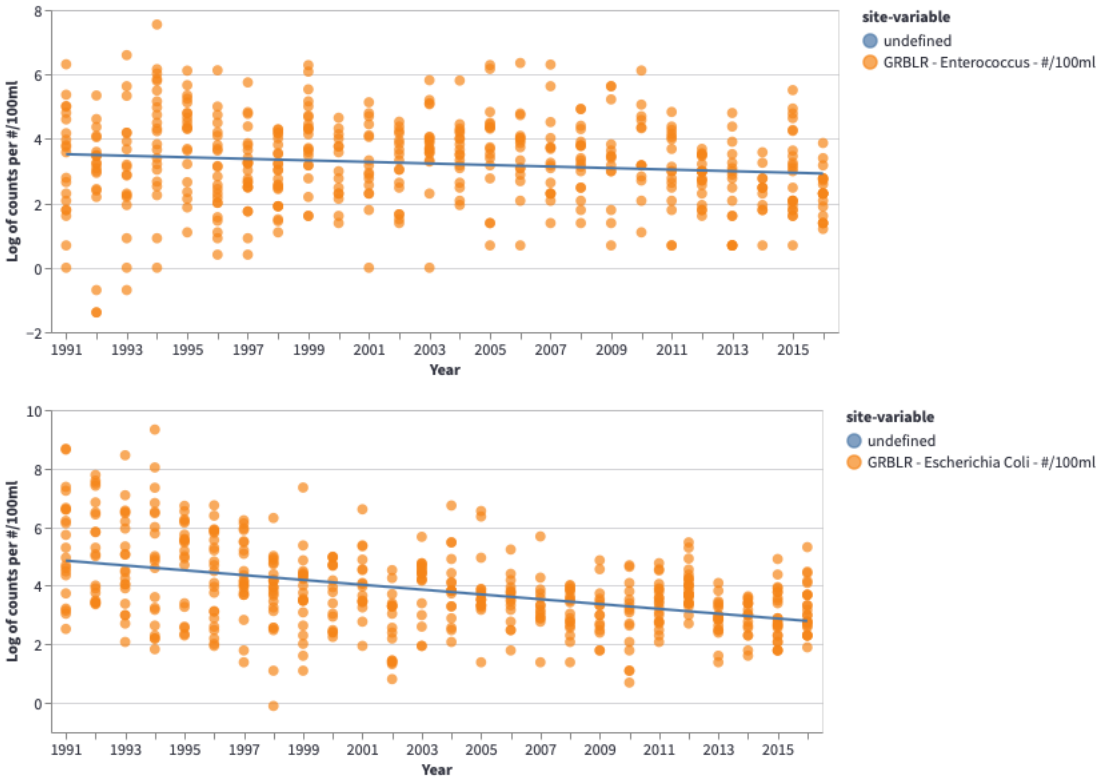


Figure 2. Enterococci and *E. coli* concentrations (cfu/100 ml) at the GBNERR-PREP GRBLR site next to Site 2 from this study: 1991-2017 (**courtesy of PREP**).

2021 Study Supported Sampling and Analyses

All intended sample collections occurred on the 5 dates from April 30 through August 11, 2021. April 30th and May 28th were preceded by some precipitation while there was no precipitation in the two days prior to sampling during June, July and August (Table 3).

The three fecal indicator bacteria were detected at all sites during April and May, but enterococci were not detected at Sites 2, 3 and 4 in June, at no site in July and not at Sites 1 and 2 in August. All water samples contained fecal coliforms at concentrations that exceeded the State of NH standard (14 FC/100 ml), and only samples from Site 1 in May to August, and at Site 4 in August, contained *E. coli* concentrations above the State of NH single sample standard (153 *E.coli*/100 ml; Table 1) for Class A freshwater. The State of NH enterococci single sample standard (104 enterococci/100 ml) was exceeded three times (May, June and August) at Site 1

and once (April) at Site 3. There is no shellfish harvesting allowed in the area so the exceedances of the fecal coliform standard do not raise any direct issue for the Lamprey River watershed, however, the pollution along with the less frequent exceedance of *E. coli* and enterococci standards in study samples suggests that downstream areas may be affected.

		Bacterial Indicator					
				Fecal		Rainfall-daily	
Date	Site	Enterococci	*E. coli*	coliforms	sample day	prior day	2 d prior
		CFU/100 ml	CFU/100 ml	CFU/100 ml	"/24 h	"/24 h	"/24 h
4/30/21	1	32	48	88	0.67	0.15	0.05
	2	20	76	156			
	3	164	132	196			
	4	88	144	156			
5/28/21	1	1320	600	600	0	0.25	0
	2	80	60	90			
	3	5	15	15			
	4	15	90	90			
6/7/21	1	760	440	520	0	0	0
	2	<20	100	140			
	3	<5	30	30			
	4	<5	65	70			
7/7/21	1	<40	640	640	0	0	0
	2	<20	100	180			
	3	<5	120	125			
	4	<5	150	195			
8/11/21	1	200	1200	1680	0	0	0
	2	<20	60	380			
	3	5	25	80			
	4	90	205	205			
		sample 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: Lamprey River-tidal at Newmarket waterfront; Site 3: Lamprey River- above Wiswall Dam; Site 4: Lamprey River- Wadleigh Falls canoe access.

For all dates except the April sample date, when the highest amount of rain fell prior to a sample event, indicator bacteria were detected at much higher levels at Site 1 compared to Sites 2, 3 and 4 (Figures 3 A-C). The average concentration for each of the fecal indicator bacteria was much higher at Site 1 compared to Sites 2, 3 and 4 (Figure 4), with no significant difference between Sites 2, 3 and 4. Concentrations of fecal coliforms and *E. coli* are highly similar because fecal coliforms are a group of bacteria that include *E. coli* while enterococci are a completely different type of bacteria group. There was no apparent impact of other water conditions (temperature, salinity, dissolved oxygen; data not shown) on bacterial levels. All 5 sample events occurred following relatively dry conditions except for the first event on April 30, which was also the earliest and coldest date. The impact of rainfall and associated runoff would have to be the focus of another study to capture enough wet weather events to determine any potential impacts.

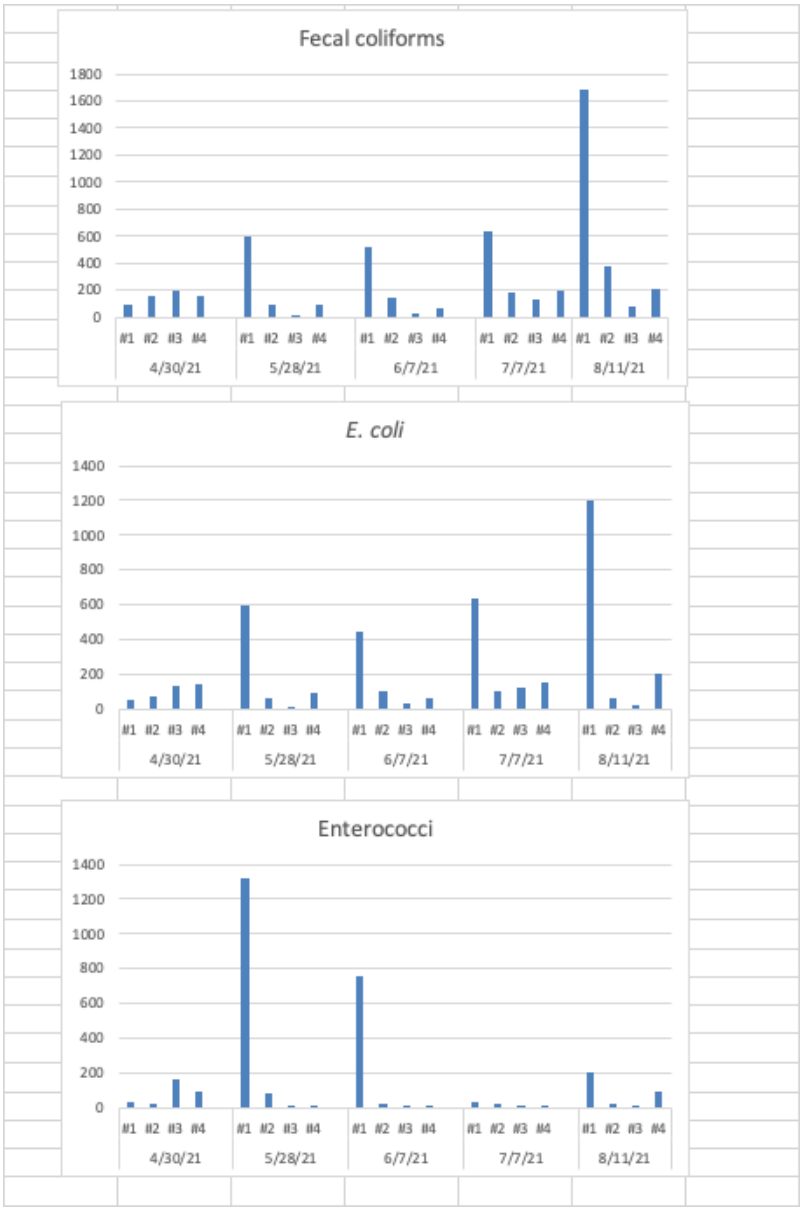


Figure 3. Concentrations of the 3 fecal indicator bacteria (A, B, C) for all 5 sample dates at each of 4 sampling sites (#1-4).

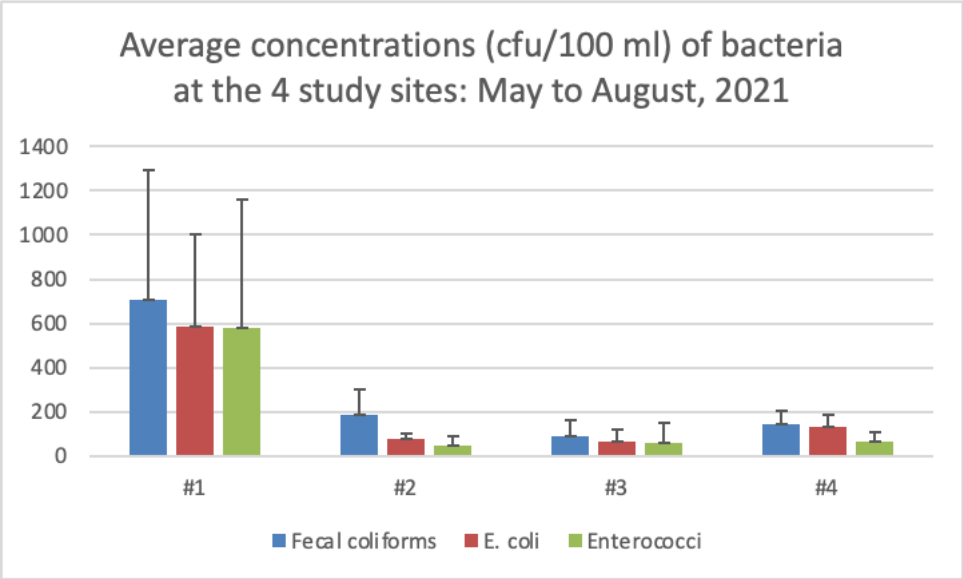


Figure 4. Average concentrations of fecal indicator bacteria at each of the 4 sample sites for 5 months: April to August 2021.

The bacterial indicator levels at Site 2-the tidal site at the Newmarket waterfront- were compared to levels determined at the same location by UNH-JEL for other monitoring program. Levels of each indicator showed similar patterns during the same time as this study in 2021 where the enterococci and *E. coli* levels did not exceed standards while the fecal coliforms exceeded the standard on every date (Table 4). The three years of data show no trends related to exceedences, however, there is a long-term decreasing trend for enterococci and, especially, *E. coli* levels at this site (Figure 2).

Collection Date	Fecal coliform cfu/100 ml	*E. coli* cfu/100 ml	Enterococci cfu/100 ml
4/23/19	60	50	<10
5/21/19	20	13	10
6/18/19	28	26	6
7/9/19	58	56	104
8/6/19	34	32	16
9/17/19	8	8	8
10/14/19	12	12	22
11/19/19	46	40	32
12/4/19	36	30	20
5/11/20	n/d	n/d	<2
6/9/20	32	32	30
7/7/20	6	6	<4
8/24/20	80	56	48
9/21/20	8	6	9
10/19/20	n/d	n/d	n/d
11/2/20	28	28	40
12/7/20	120	120	100
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

Table 4. 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 and bird) contamination at all 4 sites for all 4 sampling events, and dog contamination except for Site 1 in May and August (Table 5). There was evidence of cow/ruminant contamination at all 4 sites on June 7 and at Site 4 in August, and ruminant contamination at Sites 1, 2 and 4 in May. There was evidence of sporadic seagull and Canada goose contamination at several sites. Human contamination detected by PCR was restricted to Sites 1 and 2, however, the follow-up semi-quantitative assay indicated the human contamination at Site 2 was very low (below the qPCR detection limit) and may be a result of contamination from Site 1. The quantified level of human contamination at Site 1 was highest in May and above a public health safety threshold in all months except in July when levels remained above the detection limit.

			PCR Markers: + or -									qPCR Markers: copy number/100 ml		
SampleID	Date	vol. filtered	Mammal	Human	Dog	Ruminant	Cow	Bird	Gull	Canada Goose	Horse	Mammal	Human	Bird
1: Moonlight Brook	5/28/21	300	+	+	-	+	-	+	-	-	-	15,492,094	94,619	797
2: Tidal Dock	5/28/21	300	+	+	+	+	-	+	-	-	-	10,491,098	<167	<1.67E+02
3: Wiswall Dam	5/28/21	300	+	-	+	-	-	+	-	-	-	1,030,801	-	314
4: Wadleigh Falls	5/28/21	300	+	-	+	+	-	+	-	+	-	6,023,544	-	<1.67E+02
1: Moonlight Brook	6/7/21	300	+	+	+	+	+	+	-	+	-	17,700,000	12,400	1,990
2: Tidal Dock	6/7/21	300	+	-	+	+	+	+	-	-	-	9,750,000	<1670	3,040
3: Wiswall Dam	6/7/21	300	+	-	+	+	+	+	+	+	-	7,850,000	-	4,240
4: Wadleigh Falls	6/7/21	300	+	-	+	+	+	+	-	-	-	16,200,000	-	3,170
1: Moonlight Brook	7/7/21	300	+	+	+	-	-	+	-	-	-	15,500,000	1,083	4,955,231
2: Tidal Dock	7/7/21	300	+	+	+	-	-	+	-	-	-	23,900,000	<167	351,679
3: Wiswall Dam	7/7/21	300	+	-	+	-	-	+	-	+	-	14,100,000	-	<1.67E+02
4: Wadleigh Falls	7/7/21	300	+	-	+	-	-	+	-	-	-	19,400,000	-	<1.67E+02
1: Moonlight Brook	8/11/21	300	+	+	-	-	-	+	+	-	-	4,120,000	16,355	1,151
2: Tidal Dock	8/11/21	300	+	+	+	-	-	+	+	-	-	12,100,000	<167	991
3: Wiswall Dam	8/11/21	300	+	-	+	-	-	+	+	-	-	15,900,000	-	255
4: Wadleigh Falls	8/11/21	300	+	-	+	+	+	+	+	-	-	12,900,000	-	2,203
"-" indicates no signal									indicates above research-based risk threshold of 4200 CN/100 ml					

Table 5. Detection of the presence of different pollution sources by of PCR and qPCR analyses for all samples from May through August 2021.

Microbial Source Tracking is useful because it provides information on what is causing detected contamination, and thus allows for focusing resources to mitigate actual sources of pollution. The semi-quantitative qPCR assays are useful to gauge relative amounts of targeted genetic

markers found at different sites on different dates from this and other studies. The mammal and bird qPCR data are useful for comparisons between dates and sites, but do not relate to any risk threshold at present even though that is the focus of some ongoing work in the Jones Lab. The human qPCR data, however, have been related to risk of unacceptable levels of human illness (Boehm et al. 2015). The threshold they determined, 4200 copy number/100 ml for the human marker, was exceeded on 3 of the 4 samples dates at Site 1. This study so far suggests that the consistent fecal and human contamination at Site 1 may require some investigation to mitigate this source area as a potential public health concern.

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 Lamprey River watershed. This is important as the rivers, streams and impoundments are increasingly used by boaters and some swimmers, who may be at risk for water-borne illnesses under contaminated conditions.

The research for this report included a detailed review of existing data on microbial pollution in the watershed. Very few of the assessment units had any available or recent data to provide a water quality assessment for swimming and boating uses. This finding is useful as a starting point for 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 synoptic dataset for 4 key sites in the watershed related to recreational uses, and thus serves as a start for continued monitoring and water quality assessments.

The inclusion of microbial source tracking in this study is an invaluable addition, 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, so the study results showing no discernable detection of human contamination at Sites 3 and 4 is encouraging. Conversely, Site 1 is an obvious concern for the downstream upper tidal Lamprey River area, and the Town of Newmarket.

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 are delineated in a separate 2-page document 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 not captured in this study was the impact of rainfall and associated runoff, a condition that is widely responsible for elevated levels of bacterial pollution. As our regional climate continues to change, rainfall patterns are expected to become more extreme and may change the dynamics of bacterial contamination. There also could be future follow up sampling into some key tributaries- like Moonlight Brook to determine where and how pollution problems arise.

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

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