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 and expand 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 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 watershed.
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 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 only 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 consider augmenting their programs with similar efforts.

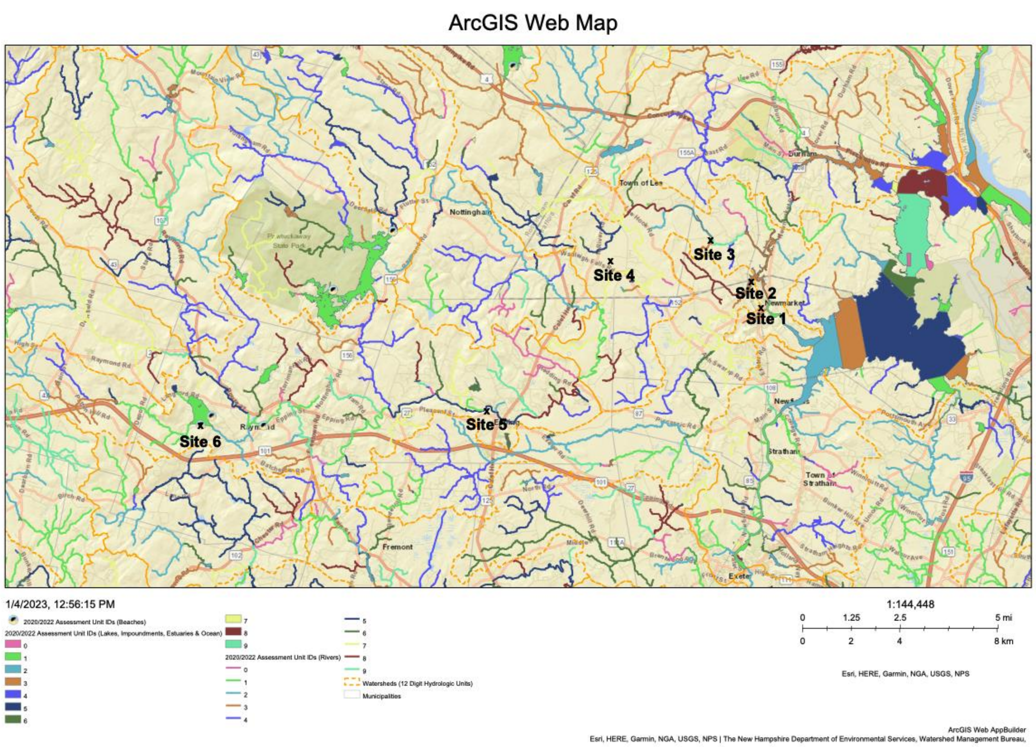
We intend to present the study findings at several meetings in 2023. The PI will present findings at the annual National Shellfisheries Association Conference in Baltimore MD in March 2023 and some of the data will be used by students to present research posters at the UNH Undergraduate Research Conference in April 2023. 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.

<u>METHODS</u>

Sample collection by land for analysis of bacterial pollutants occurred at 6 sites where surface water recreation occurs (Figure 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 Lamprey River Elementary School in Raymond.

Figure 1. Locations of project study sites during 2022.



Site 1: upstream of the mouth of Moonlight Brook; Site 2: the Piscassic Park Boat Launch in Newmarket; Site 3: below the Wiswall Dam. Site 4: upstream of the dam at the Lee public canoe access site near Wadleigh Falls; Site 5: behind the Epping Town Hall; Site 6: at Carroll Beach behind the Lamprey River Elementary School in Raymond. This figure was developed using the NHDES Surface Water Quality Assessment Viewer: https://nhdes.maps.arcgis.com/apps/webappviewer/index.html?id=d1ba9c5ec85646538e032580e23174f

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 on May 22, May 28, June 2 and 22, July 12, August 2, October 24, October 31 and November 28. A delay in accessibility to required analytical supplies during the early summer caused a delay in sampling through September and into October before sampling twice in late October. 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 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 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, chlorophyll *a*, and other parameters. 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 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 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 (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 and one graduate student 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, 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 areas 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), 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.

In the Middle Lamprey River 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 (Table 2).

	Assessment Unit		Type* of			Classification
Study Site	ID number	Name	Recreational use	Last sample	Last exceed	Category†
2021 Site 1	NHEST600030709-01-01	Lamprey R. Estuary North	Primary Contact	2017	2016	4A-P
			Secondary Contact	2017	2008	2-G
Site 1	NHRIV600030709-13	Moonlight Brook	Primary Contact	2008	2000	3-ND
		(upstream of likely source)	Secondary Contact	2008	1996	3-ND
Site 2	NHIMP600030709-03	Piscassic Park Boat Launch	Primary Contact	2007	2005	3-ND
		(Lamprey R. impoundment)	Secondary Contact	2007	N/A	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	2007	1999	4A-P
		canoe boat launch	Secondary Contact	2007	N/A	3-ND
Site 5	NHRIV600030703-15	Behind Epping Town Hall	Primary Contact	2018	2018	4A-P
		(Middle Lamprey River)	Secondary Contact	2018	2002	2-G
Site 6	NHRIV600030703-07-02	Carroll Lake Beach	Primary Contact	2006	2006	4A-P
		Behind Lamprey R. Elem. Sch.	Secondary Contact	2006	N/A	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) and the Middle Lamprey River (HUC 12: 010600030703) assessment units at or near the 6 study sites.

<u>2021 Study Supported Sampling and Analyses</u>

All intended sample collections occurred on 8 dates from May through November 2022. 2022 was a dry summer featuring severe drought conditions in the Lamprey River watershed. Only the November 28 sample event was preceded by any significant (0.87” within a day of sampling) precipitation while there was little to no precipitation in the day prior to sampling during May, June, July, August and October (Table 3). The bacterial indicator levels changed with the different monthly sample events, with higher levels of fecal coliforms and *E. coli* in spring, and did not appear to have elevated levels after the single rainfall event in late November.

		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
5/25/22	1	360	1200	2400	0	0	0
	2	<5	175	190			
	3	<5	145	145			
	4	20	155	155			
	5	20	180	200			
	6	5	65	65			
6/2/22	1	800	880	880	0	0.12	0.31
	2	10	70	70			
	3	<5	60	60			
	4	50	180	180			
	5	<5	230	230			
	6	10	35	35			
6/22/22	1	40	200	240	0	0	0
	2	5	<5	<5			
	3	<5	5	10			
	4	10	30	35			
	5	10	20	20			
	6	<5	20	20			
7/12/22	1	240	200	200	0	0	0.71
	2	15	10	25			
	3	5	20	25			
	4	<5	<5	<5			
	5	35	50	50			
	6	10	25	35			
8/2/22	1	5	5	70	0	0	0
	2	<5	15	30			
	3	<5	<5	<5			
	4	<5	80	135			
	5	10	35	90			
	6	10	15	25			
10/24/22	1	80	280	320	0	0.04	0.12
	2	<5	40	40			
	3	10	165	185			
	4	25	35	40			
	5	<5	50	50			
	6	24	68	72			
10/31/22	1	171	440	480	0	0	0
	2	9	15	15			
	3	17	40	40			
	4	40	40	40			
	5	35	35	40			
	6	13	10	15			
11/28/22	1	480	200	400	0	0.87	0.12
	2	100	165	170			
	3	30	15	15			
	4	65	40	40			
	5	85	25	30			
	6	5	5	5			
			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.

The three bacterial fecal indicators exceeded State water quality standards at varying rates (Table 4). Enterococci levels only exceeded standard ((104 enterococci/100 ml) at Site 1 on 5 of the 8 sample events in contrast to fecal coliforms that exceeded standard (14 FC/100 ml) in 35 out of 40 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 7 of the 8 sample events at Site 1, in 2 of the 8 events at Sites 2, 4 and 5, once at Site 3, and not at all at Site 6. The bacterial indicators were detected more often than not, with non-detection occurring in only 3 samples for both enterococci and fecal coliforms, and in 10 samples for *E. coli* (Table 4).

Site	State standard exceedance			Non-detection		
	Enterococci >104/100 ml	*E. coli* >158/100ml	fecal coliform >14/100 ml	Enterococci <5 cfu/100ml	*E. coli* <5 cfu/100ml	fecal coliform <5 cfu/100ml
1	5	7	8	0	0	0
2	0	2	7	1	2	1
3	0	1	6	1	4	1
4	0	2	7	1	2	1
5	0	2	8	0	2	0
6	0	0	7	0	0	0
Totals	**5**	**14**	**35**	**3**	**10**	**3**

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

For all dates except for August 2nd in the middle of the summer drought, indicator bacteria were detected at much higher levels at Site 1 compared to all other sites (Figures 2 A-C). The average concentration for each of the fecal indicator bacteria was also much higher at Site 1 compared to the other 5 sites (Figure 3A), with somewhat lower concentrations at Site 6 compared to Sites 2-5. 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 6 sample events occurred following relatively dry conditions except for the last event on November 28, which was also the latest and coldest date. Although the impact of rainfall and associated runoff was supposed to be one focus of this study, the drought was long-lasting and intense, so it is necessary to assess this factor in another study to capture enough wet weather events to determine any potential impacts.

In comparison to the 3 sites studied in common with the 2021 study, enterococci levels were higher in 2021 at all 3 sites, *E. coli* levels were higher in 2021 at Sites 3&4, and fecal coliform levels were higher in 2022 at all 3 sites. The site numbers are for 2022, with #1-Moonlight Bk, #3-Wiswall Dam, and #4-Wadliegh Falls.

Figure 2. Concentrations of the 3 fecal indicator bacteria (A, B, C) for all 8 sample dates at each of 6 sampling sites (#1-4).

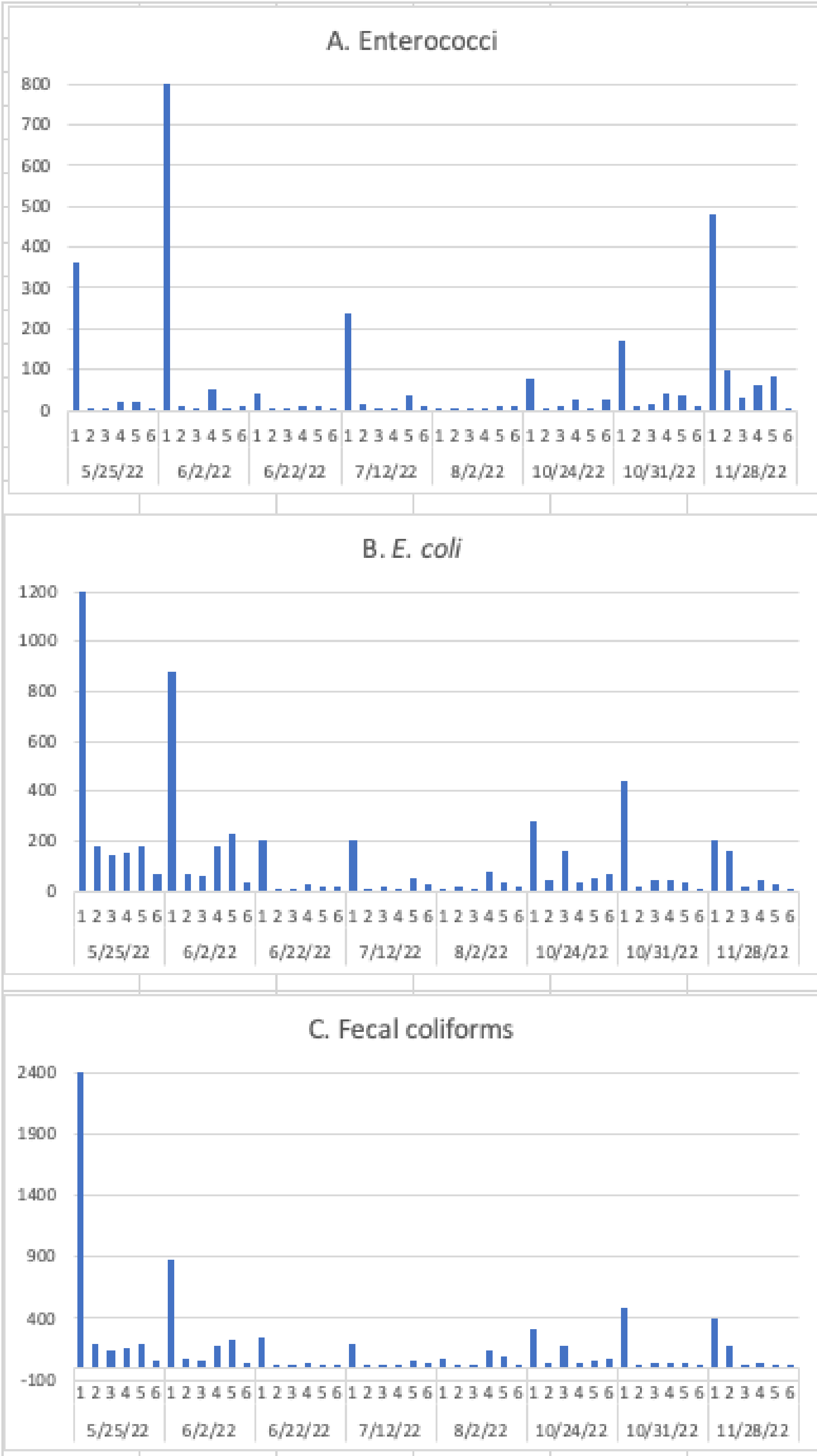
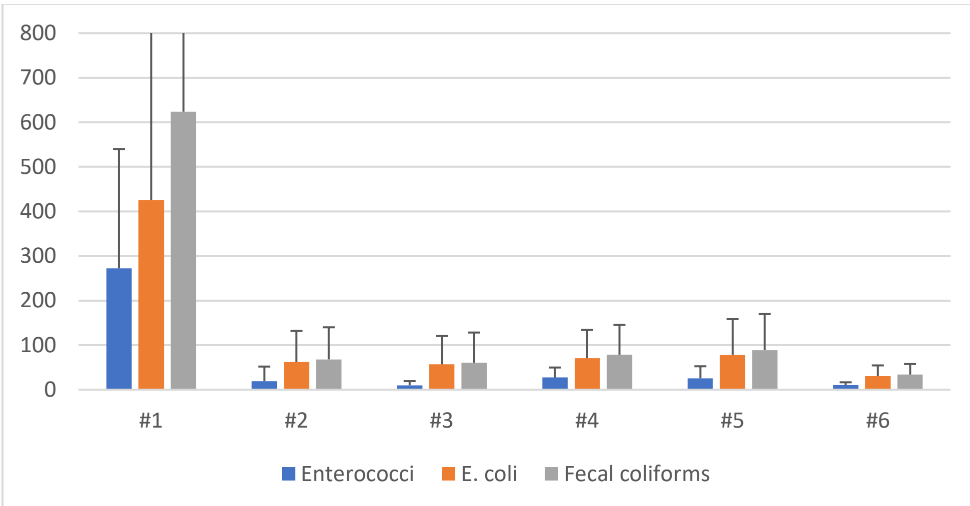
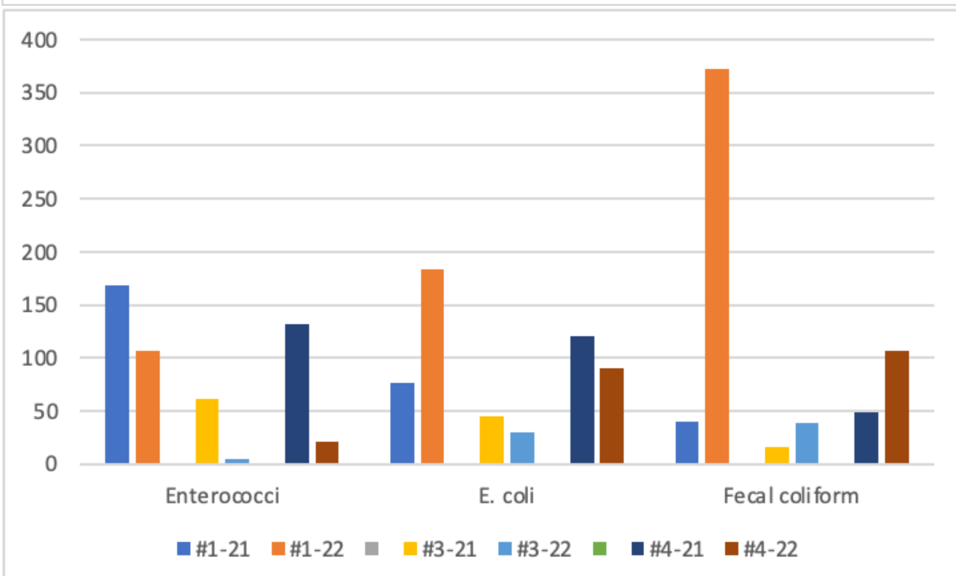


Figure 3A. Geometric average concentrations of fecal indicator bacteria at each of the 6 sample sites for 7 months: May to November 2022.

3B. Geometric average concentrations of indicator bacteria at three common sites in 2021 and 2022.





The bacterial indicator levels at the tidal site at the Newmarket waterfront determined by UNH-JEL for the Piscataqua Regional Estuaries Partnership (PREP)- GBNERR monitoring program that were included in the 2021 study (Jones 2022), are again useful to be compared to levels elsewhere in the Lamprey River watershed determined as part of this 2022 study. 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 through all four years (Table 5), only in November and December the levels of all three indicators were much higher than for other times due to heavy rains and runoff. These data help to address the issue of whether rainfall and runoff cause increased levels of bacterial contamination in the watershed. There is also a long-term decreasing trend for enterococci and for *E. coli* levels at this site (Figure 4) over a 30-year period from 1991-2020. The decreasing trend is a good sign indicating bacterial levels are decreasing over time.

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
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

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.

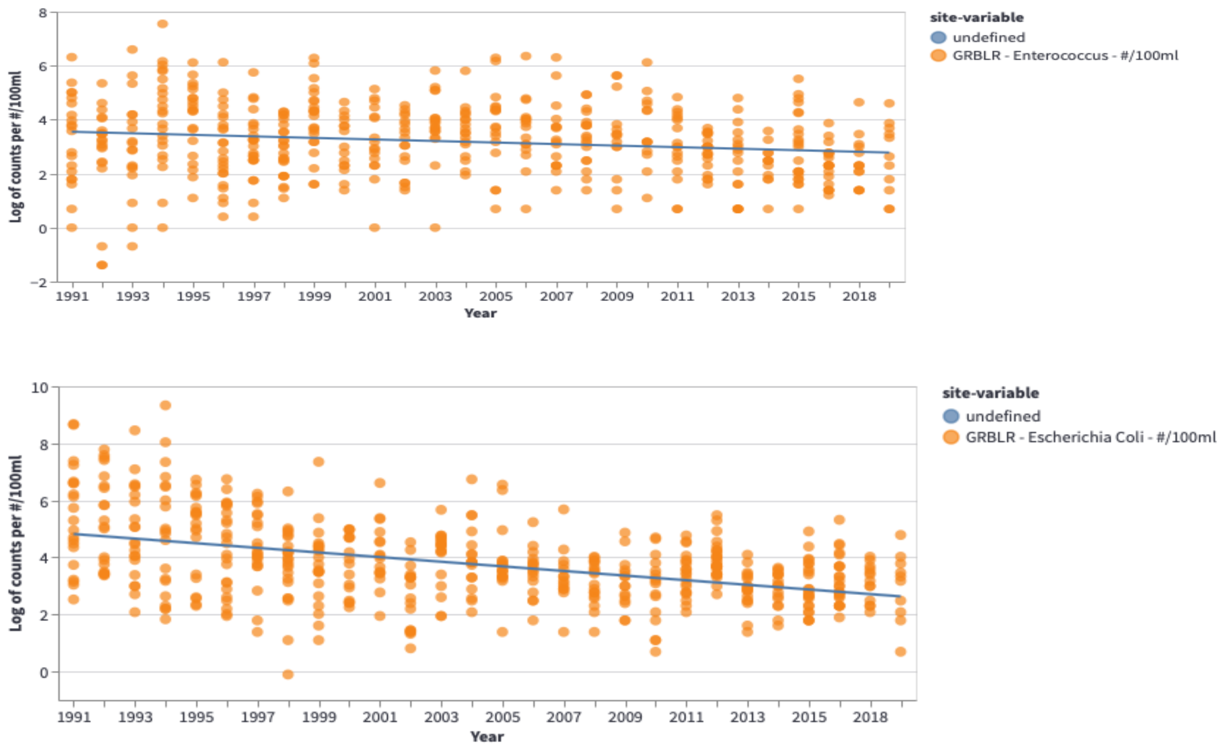


Figure 4. Enterococci and *E. coli* concentrations (cfu/100 ml) at the GBNERR-PREP GRBLR site next to Site 2 from the 2021 study (Jones 2022): 1991-2020 (data and figures **courtesy of PREP**).

There was evidence of animal (mammal) contamination at all 6 sites for all 48 sampling events, (Table 5). Bird contamination was present in all samples on June 2nd and in all 5 samples from July to November. Dog contamination was also detected in all samples from July 12 to October 31, while cow contamination was present at some sites each month, and ruminants, Canada geese and horses were detected in diminishingly fewer samples.

Human contamination detected by PCR was most frequently (7 of 8 samples) detected at Site 1 and only once at each of the other 5 sites. The follow-up semi-quantitative assay (qPCR), which has a higher detection limit, indicated the human contamination at Site 1 was 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) in all samples except on July 12 and August 2. The quantified level of human contamination at the other sites only exceeded the 4,200 CN/100 ml threshold at Wiswall Dam on June 2 and were otherwise below this threshold level. The quantified level of bird contamination for the 35 samples where bird contamination was detected by the non-quantitative PCR assay reflected relatively low levels of contamination, as 20 out of the 35 samples were below the qPCR assay detection limit(s) and of the 15 samples with detectable levels by qPCR, few exceeded 1,000 CN/100 ml, with the highest level at 7,769 CN/100 ml and the lowest level at 176 CN/100 ml. The presence of Canada geese did not correspond to elevated levels of bird contamination; however, many ducks were observed at Site 1 when the level of bird contamination was 7,769 CN/100 ml on November 28.

			PCR Markers									qPCR Markers		
SampleID	Date	Volume	Mammal	Human	Dog	Ruminant	Cow	Bird	Gull	Canada Goose	Horse	Mammal	Human	Bird
			"-" indicates no signal									Copy number/100 ml		
Moonlight Brook	5/25/22	300	+	+	-	-	+	+	-	-	-	7,622,974	5,133	957
PRBL	"	"	+	-	+	-	-	-	-	-	-	1,921,981	-	-
Wiswall Dam	"	"	+	-	-	-	+	-	-	-	-	521,997	-	-
Wadleigh Falls	"	"	+	+	-	-	-	-	-	-	-	2,950,381	-	-
ETH	"	"	+	-	-	-	-	-	-	-	-	652,166	-	-
RES	"	"	+	-	-	-	-	-	-	-	-	1,446,587	-	-
Moonlight Brook	6/2/22	300	+	+	-	-	+	+	-	-	-	18,854,924	502,893	939
PRBL	"	"	+	-	-	-	+	+	-	-	-	2,553,547	-	1,143
Wiswall Dam	"	"	+	-	-	-	+	+	-	-	-	1,958,885	67,752	-
Wadleigh Falls	"	"	+	-	+	-	-	+	-	-	-	8,465,609	-	-
ETH	"	"	+	-	-	-	+	+	-	-	-	1,791,263	-	1,835
RES	"	"	+	-	+	-	-	+	-	-	-	2,258,825	-	-
Moonlight Brook	6/22/22	300	+	+	-	-	-	-	-	-	-	2,538,555	8,928	-
PRBL	"	"	+	-	-	-	-	-	-	-	-	5,838,358	-	-
Wiswall Dam	"	"	+	-	-	-	-	-	-	-	-	4,328,575	-	-
Wadleigh Falls	"	"	+	-	-	+	+	-	-	-	-	6,384,004	-	-
ETH	"	"	+	-	-	-	-	+	-	-	-	4,401,921	-	-
RES	"	"	+	-	-	+	+	+	-	-	-	4,017,966	-	1,691
Moonlight Brook	7/12/22	300	+	-	+	-	+	+	-	-	-	13,604,935	-	2,480
PRBL	"	"	+	-	+	-	-	+	-	-	-	50,909,210	-	<1,670
Wiswall Dam	"	"	+	-	+	-	+	+	-	-	-	38,995,451	-	<1,670
Wadleigh Falls	"	"	+	-	+	-	+	+	-	-	-	4,629,993	-	<1,670
ETH	"	"	+	+	+	-	-	+	-	-	-	33,144,772	<1,670	2,238
RES	"	"	+	-	+	-	+	+	-	-	-	6,944,213	-	2,442
Moonlight Brook	8/2/22	300	+	+	+	+	-	+	+	-	-	9,361,333	702	2,812
PRBL	"	"	+	-	+	-	-	+	-	+	-	24,581,222	-	<1,670
Wiswall Dam	"	"	+	-	+	+	+	+	-	+	-	10,372,627	-	<1,670
Wadleigh Falls	"	"	+	-	+	+	+	+	-	-	-	22,639,411	-	<1,670
ETH	"	"	+	-	+	-	-	+	-	-	-	27,994,847	-	1,903
RES	"	"	+	-	+	-	-	+	-	-	-	5,812,952	-	<1,670
Moonlight Brook	10/24/22	300	+	+	+	+	+	+	+	+	-	17,609,623	8,151	<167
PRBL	"	"	+	-	+	-	+	+	+	-	-	90,327,870	-	<167
Wiswall Dam	"	"	+	+	+	-	-	+	-	+	-	6,220,454	509	<167
Wadleigh Falls	"	"	+	-	+	-	-	+	-	-	-	6,997,505	-	1,315
ETH	"	"	+	-	+	-	-	+	-	+	-	6,213,109	-	<167
RES	"	"	+	-	+	-	-	+	-	-	-	5,938,820	-	<167
Moonlight Brook	10/31/22	300	+	+	+	+	+	+	+	-	-	38,942,850	85,055	<167
PRBL	"	"	+	-	+	-	-	+	-	-	-	62,628,227	-	<167
Wiswall Dam	"	"	+	-	+	+	+	+	-	-	-	37,241,729	-	<167
Wadleigh Falls	"	"	+	-	+	-	-	+	-	-	-	11,649,539	-	<167
ETH	"	"	+	-	+	-	-	+	-	-	-	13,041,626	-	<167
RES	"	"	+	-	+	+	+	+	-	-	-	20,673,187	-	226
Moonlight Brook	11/28/22	300	+	+	+	+	+	+	+	-	-	14,841,156	55,024	7,769
PRBL	"	"	+	+	-	+	-	+	-	-	-	26,829,920	622	<167
Wiswall Dam	"	"	+	-	-	+	-	+	-	-	-	18,282,405	-	168
Wadleigh Falls	"	"	+	-	+	-	-	+	-	-	+	28,397,553	-	<167
ETH	"	"	+	-	+	-	-	+	-	-	-	17,326,563	-	<167
RES	"	"	+	+	+	+	+	+	-	-	-	5,078,347	452	176

Table 5. Detection of the presence of different pollution sources by of PCR and qPCR analyses for all samples from May through August 2021. Yellow highlight denotes detection, red highlight denotes level above human contamination risk threshold level.

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 5.3 types (out of 9 possible) per sample including 8 different identified sources on 10/24. The sites with the next most diverse contamination types were Sites 3 and 6, followed in decreasing order by Sites 4, 2 and 5. The number of different types of identified contamination sources also increased through the study period, with an average of 17 types identified in each sample for all 6 sites from May 25 to July 12, compared to an average of 27 for August 2 to November 28.

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 6 of the 8 samples dates at Site 1 and once at Site 3 (Wiswall Falls). This study so far suggests that the consistent fecal and human contamination at Site 1 could be managed with 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 and Middle 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 6 key sites in the watershed related to recreational uses, and thus serves as a start for continued monitoring and water quality assessments. The data will be provided to the New Hampshire Department of Environmental Services (NHDES) to be part of and inform the 2024 Surface Water Quality Assessment process that is currently required by Sections 305(b) and 303(d) of the Clean Water Act.

The inclusion of microbial source tracking 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 only rare detection of human contamination at Sites 2 through 6 is encouraging. Conversely, Site 1 is an obvious concern for the upper tidal Lamprey River area and the Town of Newmarket. The source of the human contamination is not apparent in the area above ground near the final discharge into the tidal portion of the Lamprey River, so the Town of Newmarket will need to conduct further investigations to pinpoint the source. Further upstream sampling could help that process. The next most manageable source is probably dogs. Dog contamination was consistently present at all sites after early June. 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) and that alerts dog owners to pick up 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 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 that remains uncaptured is the impact of rainfall and associated runoff, a condition that is widely responsible for elevated levels of bacterial pollution. There was some evidence from this study that rainfall events may trigger greater concerns, including the fact that human contamination was detectable at 3 of the 6 sites on November 28 including an elevated level (85,055 CN/100 ml) at Site 1, which also had the highest level of bird contamination (7,769 CN/100 ml) detected in this study on that date. November 28 is later in fall and thus reflects different conditions than in the summer, so more samples on days with elevated rainfall will be necessary to sort out different factors that can affect bacterial contamination concentrations and source types. Two years of data under relatively dry conditions provides for a solid baseline to compare findings under future rainy conditions. 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; birds and animal migration patterns are influenced by climate change. 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, as well as water quality managers and the Shellfish Program Manager in NHDES.

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