

PEMAQUID RIVER

2019 RIVER MONITORING REPORT



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PREPARED FOR



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Little Falls Brook above Carl Bailey Road. Photo: FBE

INTRODUCTION

The lower Pemaquid River and its estuary are located in the Town of Bristol, Maine (Figure 1). The river and estuary are listed as impaired on the Maine Department of Environmental Protection (Maine DEP) 303(d) list due to elevated fecal indicators and the proximity of the local marina.¹ The northern portion of the Pemaquid River estuary is classified as conditionally approved for shellfish harvesting and the southern portion of the estuary is listed as prohibited. As shellfishing is an important resource for the surrounding community, the closures have led the Bristol Shellfish Committee and the Town of Bristol to track potential pollution sources in the surrounding waterways and landscape.

In 2017, Maine DEP began sampling the freshwater tributaries of the Pemaquid River to identify possible sources of fecal contamination in the watershed contributing to the impairment in the estuary. As a continuation of this effort, in 2018 FB Environmental Associates (FBE) worked with the Town of Bristol, the Shellfish Committee, and the Pemaquid Water Association (PWA) to sample twelve sites in the Pemaquid River watershed to improve our understanding of fecal contamination in the freshwater tributaries that could be contributing to fecal contamination in the estuary. In 2018, sampling was conducted across one storm event in June with a cumulative precipitation of 1.86 inches over 24 hours. Samples were collected before the storm (6/27), during the storm (6/28) and the day after the storm (6/29). Using 2017 and 2018 data, FBE identified several higher priority “hotspots” for tracking the source(s) of fecal contamination in the watershed. In 2019, FBE worked with the Town of Bristol to identify specific locations to perform bracket sampling in an attempt to further isolate potential sources of fecal contamination contributing to these hotspots. Bracket sampling is used to locate the sources of bacteria entering a tributary by sampling upgradient and downgradient of potential sources to “bracket” (or isolate) pollutant source locations. Four tributaries that enter the Pemaquid River and its estuary were identified for sampling locations (Figure 1):

- Two tidewater tributaries located on the east side of the estuary, School House Cove and Coombs Cove, were sampled for fecal indicator bacteria and co-indicators at four and two locations each, respectively.
- One freshwater tributary, Redonnett Mill Road tributary, entering the Pemaquid River approximately five miles above its outlet into the estuary, was sampled at five locations for fecal indicator bacteria and co-indicators.
- One tidewater tributary located on the west side of the estuary, Little Falls Brook, was sampled at three locations for molecular microbial source tracking.

Fecal contamination is one of the most difficult pollutants to remediate. There are a few reasons for this:

- (1) It is a nonpoint source pollutant, meaning that it can come from many different locations on the landscape.
- (2) Human health concerns are caused by potentially harmful pathogens such as viruses and bacteria, that are present within fecal matter. However, it would be too expensive to track and monitor each harmful virus and bacteria individually. Because of this, we use indicator organisms (such as *Escherichia coli* or *E. coli*, Enterococci, and Fecal Coliform). These indicator organisms are chosen based on similarities to pathogens in behavior and transport in the environment.
- (3) Synchronicity in behavior between FIB and the pathogens-of-concern for public health risk (e.g., salmonella, campylobacter, rotavirus, giardia, norovirus, hepatitis, etc.) may break down under certain environmental conditions. Therefore, caution must be used when interpreting FIB data in the context of risk management decisions.
- (4) Fecal contamination tracking is an evolving science, with new technologies consistently making their way to the market. We do our best to use the tools at our disposal while recognizing their limitations.

¹ https://www.maine.gov/dep/water/monitoring/305b/2016/28-Feb-2018_2016-ME-IntegratedRptLIST.pdf

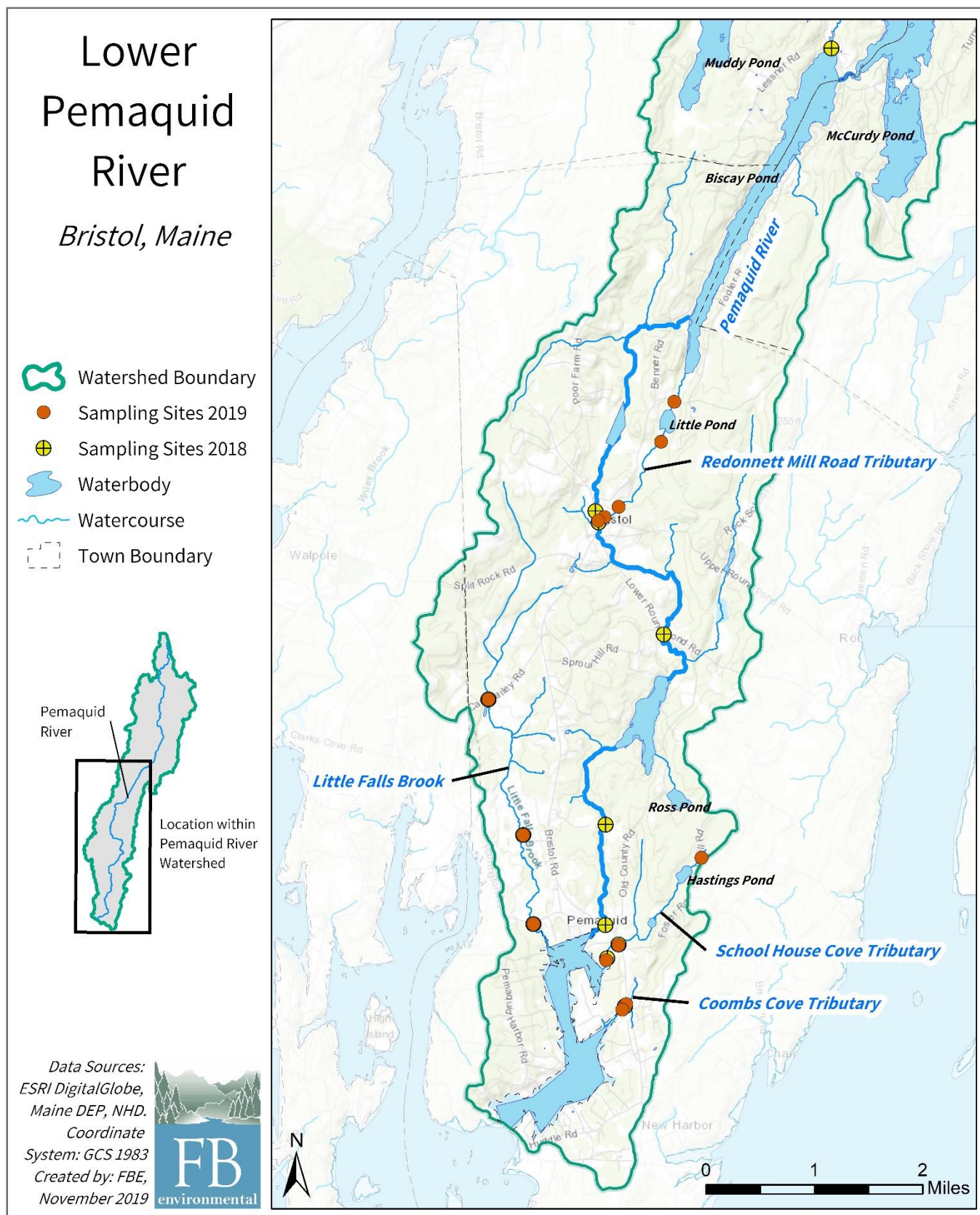


Figure 1. The lower Pemaquid River and estuary, located in Bristol, Maine. Orange points indicate sites sampled in 2019. Sampling locations from 2018 are shown as yellow points with a crosshatch.

METHODOLOGY

BRACKET SAMPLING

FBE completed surface water sampling on 7/15/2019 and 7/16/2019 to further track sources of fecal indicator bacteria entering the Pemaquid River and estuary. Bracket sampling is a technique that assists in pinpointing sources of bacteria entering a tributary, done by sampling upgradient and downgradient of potential sources to isolate pollutant source locations. Based on “hotspots” for fecal contamination highlighted in 2018 sampling, FBE completed bracket sampling on the two tidewater tributaries on the east side of the estuary; four sites on School House Cove tributary and three sites on Coombs Cove tributary, as well as five sites at the Redonnett Mill Road tributary

Samples were collected during dry weather conditions (<0.1” rain within 24 hours) in an attempt to isolate sources not caused by runoff. Surface water samples were collected and analyzed as follows:

- Field parameters included dissolved oxygen, temperature, salinity, and specific conductance and were recorded using a YSI 85 field meter.
- *Escherichia coli* bacteria samples and nitrate/nitrite samples were analyzed at Maine Environmental Laboratory (MEL) in Yarmouth, Maine.
- Ortho-phosphate samples were filtered in the field using a 0.45-micron filter and analyzed at MEL.
- Ammonia was determined using HACH ammonia test strips.
- Optical brighteners were measured using a handheld Aquaflor fluorometer, based on methods described in SOP 3.4.1.4 Measuring Optic Brighteners in Ambient Water Samples Using a Fluorometer, by Erick Burres, dated March 2011.



Sampling at School House Cove tributary outlet. Photo: FBE

The State of Maine uses *Escherichia coli* (*E. coli*) as the primary indicator bacteria for fecal source tracking, however, several other parameters have been successfully used as “co-indicators” to fecal indicator bacteria; the combination of these parameters can help determine whether the contamination source is likely from humans. Nutrients (nitrate and phosphate) can indicate human sewage if in extremely high concentrations. Ammonia is used in illicit discharge detection screenings and high concentrations can indicate sewage contamination, however not all discharges have high concentrations, so it is best used in combination with other parameters. Orthophosphates provide an estimation of the amount of phosphorus available for algae and plant growth, and as they are often the main constituent in fertilizers used agriculturally and residentially, they can indicate human presence. Optical brighteners are commonly used for wastewater detection. Optical brighteners are not naturally occurring and are typically added to laundry soaps, detergents, cleaning agents, and toilet papers to aid in the brightening of fabrics and/or surfaces. Testing positive for optical brighteners in groundwater, definitively indicates the presence of greywater from leach fields.

MICROBIAL SOURCE TRACKING

Elevated fecal indicator bacteria and nutrients (discussed above) do not alone constitute a contamination because fecal indicator bacteria can be sourced from wildlife or proliferate on certain substrates within the environment. Microbial source tracking (MST) is a scientific technique that uses mitochondrial (mtDNA) host-specific markers to determine the source animal of mtDNA found in water samples. Available biomarkers include human, bovine, canine, avian, Canada Goose, and beaver. FBE collected an initial three samples for mtDNA analysis from Little Falls Brook on 7/16/2019 to analyze for a human marker, Canada goose marker, and beaver marker. Little Falls Brook tributary had elevated levels of fecal indicator bacteria in 2018. Host gene markers were selected based on site characteristics; beaver dams and presence of geese have been noted on Little Falls Brook in previous years.

Additionally, FBE use the analysis from bracket sampling on School House Cove tributary, Coombs Cove tributary, and Redonnett Mill Road tributary to determine highest priority sites for follow-up mtDNA analysis. Samples were preemptively collected at the three tributaries on 7/15/2019-7/16/2019, filtered, and frozen to await analysis. Five sites from bracket sampling were chosen for mtDNA analysis based on elevated *E. coli* levels; three on the Redonnett Mills Road tributary, one on the School House Cove tributary, and one on the Coombs Cove tributary. All were analyzed for the human marker, and the Redonnett Mills Road tributary sites was analyzed for the Canada Goose Marker as well. **A preliminary biomarker for beaver was used on the Little Falls Brook tributary because of the presence of extensive beaver dams. This biomarker is under development and therefore should be interpreted with care.**

The mtDNA analysis was performed at the UNH Microbial Source Identification Laboratory using molecular biomarkers to assess water quality. All samples were filtered and frozen upon being received at the lab. Analysis results for the host-specific markers were reported as present or absent.² If absent, the target biomarker was not detected or detected in quantities below the limit of detection and considered absent. Additional sampling, for example during variable flow in dry conditions and during a storm, may provide more data to draw definitive conclusions about the animal source contributor(s) of fecal pollution in surface water.³ If positive, the source of a present sample is detected within the standard microbial source guidelines.

APPLICABLE CRITERIA

Table 1, below, outlines applicable state criteria for the lower Pemaquid River watershed. The main stem of the lower Pemaquid River and its tributaries – including the Redonnett Mills Road Tributary – are categorized as Class A waters. The tributaries to the tidal estuary – including Little Falls Brook, School House Cove Tributary, and Coombs Cove Tributary – are categorized as Class B waters. State criteria evaluate only *E. coli*; co-indicator parameters are used for investigation of pollution sources that could be causing elevated *E. coli* bacteria in downstream waterbodies.

Note that the units used in this analysis are most probable number (MPN) per 100 mL in contrast to the state criteria in Table 1 that is listed in colony forming units (CFU) per 100 mL. MPN is a statistical estimate of the colonies present using the IDEXX colilert method. CFU represents an actual count of colonies using culture-based membrane filtration methods. The US Environmental Protection Agency has approved both methods for enumeration and they are generally considered relatively interchangeable.

² Each water sample was filtered through a micron membrane filter from 250-500 mL of surface water. Each filter was processed further using a conventional DNA extraction method. Samples were run on an Applied Biosystems 7500 real-time thermal cycler (Foster City, CA) in a final reaction volume including the sample DNA extract and assay reagents. For quality control purposes, a positive control consisting of genomic DNA and a negative control consisting of PCR-grade water were run alongside the samples to ensure an efficient reaction and to reveal any false negatives or false positives. A Bacteroidetes marker was used from a library based on a 16S rRNA gene sequence. The accumulation of PCR product is detected in an amplification plot with replicates. If the fecal indicator is absent in the sample, this accumulation is not detected, and the sample is considered absent. If accumulation of PCR product is detected, the sample is reported as present.

³ Note: The method used to produce these results is part of a research program. Bucci JP, Shattuck M, Carey R, Aytur S, McDowell WH. 2017. A case study characterizing animal fecal sources in surface water using a mitochondrial DNA marker. Environ Monitoring Assess. 189: 406. Layton A, McKay L, Williams D, Garrett V, Gentry R, Sayler G. 2006. Development of Bacteroides 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. Appl Environ Microbiol. 72:4214-4224.

Table 1. Applicable state and federal thresholds for all parameters applicable to the Lower Pemaquid River.

PARAMETER	TYPE	CLASSIFICATION	THRESHOLD JUSTIFICATION	THRESHOLD	APPLICABLE TO
E. coli *	Freshwater	Class A	Maine DEP	236 CFU/100mL (in more than 10% of samples in any 90-day interval); 64 CFU/100mL (geometric mean over 90-day interval)	Pemaquid River and its freshwater tributaries
		Class B	Maine DEP	236 CFU/100mL (in more than 10% of samples in any 90-day interval); 64 CFU/100mL (geometric mean over 90-day interval)	Freshwater tributaries to Pemaquid River estuary
Dissolved Oxygen	Freshwater	Class A/B	Maine DEP	7 ppm and 75% saturation**	All fresh waters
	Estuarine	Class SB	Maine DEP	85% saturation	All estuarine waters
Temperature	Freshwater	None	Maine DEP	Recommended <24°C for cold water fish survival	All fresh waters
Specific Conductance	Freshwater	None	Maine DEP	Recommended <854 µS/cm	All fresh waters
Ammonia	Freshwater	None	Center for Watershed Protection and US EPA “IDDE Guidance Manual”; A concentration of 1 mg/L Ammonia is generally assumed raw waste and 0.3 mg/L is an indicator of a potential problem and worth exploring other parameters.	0.3 mg/L	All fresh waters
		None	US EPA Ecoregion VIII Ambient Water Quality Criteria; threshold set higher than reference condition (0.07 mg/L).	0.10 mg/L	All fresh waters
Nitrate/Nitrite-N	Freshwater	None	US EPA Ecoregion VIII Ambient Water Quality Criteria; threshold set at reference criteria for total phosphorus concentration.	.012 mg/L	All fresh waters
Orthophosphate	Freshwater				

* E. coli criteria only applicable seasonally (between May 15th and October 31st)

**Except for Oct 1 – May 14 during spawning and egg incubation. 7-day mean dissolved oxygen not less than 9.5 ppm and 1-day minimum not less than 8.0 ppm in identified fish spawning areas.

QUALITY CONTROL

A field duplicate was taken at one site during the sampling; site SHC-1 (Table 2). Field duplicates for bacteria are notoriously challenging because despite rapid sample collection, each sample represents a slightly different parcel of water that could be carrying FIB particulates. The field duplicate relative percent difference was deemed acceptable and does not indicate sampling error.

Table 2. QA/QC duplicate result for sampling in the lower Pemaquid River watershed on 7/15/2019.

SITE NAME	ORTHOPHOSPHATE (mg/L)	E. COLI (MPN/100 mL)	NITRATE/ NITRITE-N (mg/L)	ORTHOPHOSPHATE (PRESENT/ABSENT)
SHC-1	0.007	6	<0.05	Absent
DUPE	0.006	12	<0.05	Absent
Relative Percent Difference (%)	15	67	NA	NA

RESULTS

Three of the original 16 sampling sites were omitted due to lack of access or a dry stream bed. Standard metrics of water quality – including temperature, dissolved oxygen, specific conductance, and flow level, were measured at all sites to establish a baseline of parameters. Baseline levels can be used to help discern future effects on water quality and to inform source tracking. Salinity was measured for all samples to ensure samples were taken in freshwater (above head of tide) because research shows *E. coli* can break down in saltwater. Redonnett Mill Road Tributary sites 1, 2, and 3 and School House Cove site 1 experienced low dissolved oxygen, likely due to low flow conditions (Table 2).

Two of the ten sites (sites 4 and 5 on the Redonnett Mill Road tributary) sampled for *E. coli* exceeded the Maine DEP *E. coli* instantaneous threshold for Class A and B waters of 236 MPN per 100 mL (Figure 2, Table 2 and 3). These sites also exceeded the Nitrate/Nitrite-N threshold set by US EPA Ambient Water Quality Criteria Recommendations.

Three of the eight sites sampled for microbial source tracking DNA results had hits for source markers. On Little Falls Brook, sites one and three had positive hits for Canada Goose presence, and site one has a positive hit for Beaver presence (Figure 2, Table 2 and 3).

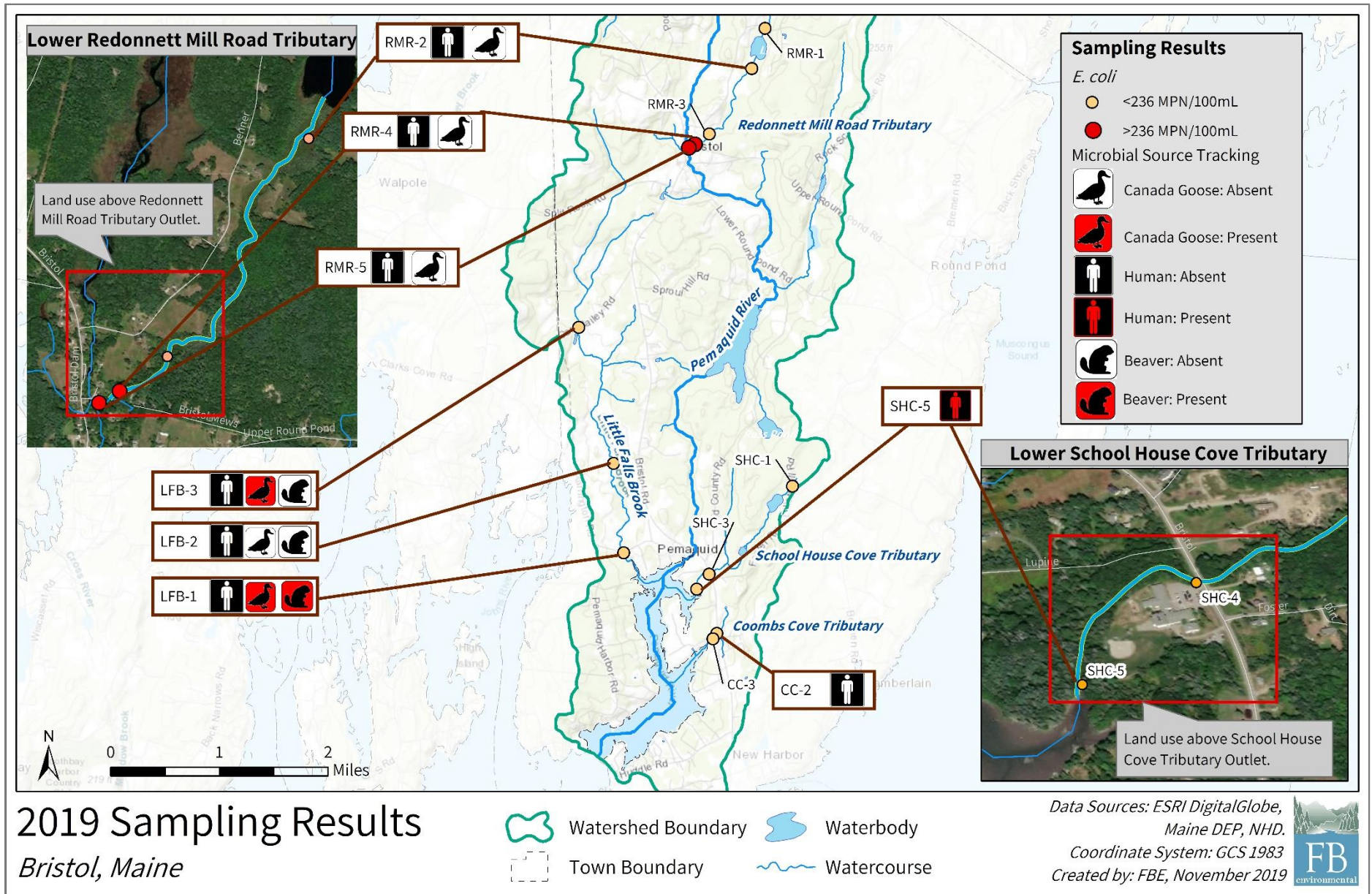


Figure 2. Results from 2019 sampling event in 7/15/19-7/16/19.

Table 3. Field parameter results for the ten sites sampled for baseline water quality conditions. Results highlighted in red indicate an exceedance of State water quality standards.

SITE NAME	DATE COLLECTED	TRIBUTARY	FIELD PARAMETERS					
			TEMPERATURE (°C)	DISSOLVED OXYGEN (PPM)	DISSOLVED OXYGEN (%)	SALINITY (PPM)	SPECIFIC CONDUCTANCE (µS/cm)	AMMONIA (PPM)
		Threshold	<24°C	7 ppm	75%	-	<854 µS/cm	0.3 mg/L
CC-2	7/15/19	Coombs Cove	22.2	7.1	88.37	0.0	98.3	0
CC-3	7/16/19	Coombs Cove	19.6	8.81	96.3	0.0	91.1	0
RMR-1	7/15/19	Redonnett Mill Road	23.0	2.56	29.6	0.0	39.7	0.12
RMR-2	7/15/19	Redonnett Mill Road	19.4	1.05	11.7	0.0	48.9	0
RMR-3	7/15/19	Redonnett Mill Road	19.3	1.82	20.1	0.0	47.5	0.125
RMR-4	7/15/19	Redonnett Mill Road	17.1	8.67	90.1	0.0	55.6	0
RMR-5	7/15/19	Redonnett Mill Road	17.7	9.05	95.1	0.0	55.8	0
SHC-1	7/15/19	School House Cove	17.3	6.07	63.3	0.0	51.8	0.125
SHC-4	7/15/19	School House Cove	21.5	8.72	99.0	0.0	40.0	0.125
SHC-5	7/15/19	School House Cove	21.3	9.20	104.0	0.0	42.6	0

CC-1 was omitted due to dry stream bed.

SCH-2 and SHC-3 were omitted due to lack of access.

Table 4. Results from laboratory analysis and microbial source tracking analysis. Results highlighted in red indicate an exceedance of State/Federal water quality standards or recommendations or a “presence” of mtDNA target.

SITE NAME	LABORATORY ANALYSIS				MICROBIAL SOURCE TRACKING		
	OPTICAL BRIGHTENERS (PRESENT/ ABSENT)	ORTHOPHOSPHATE (mg/L)	E. COLI (MPN/100 mL)	NITRATE/ NITRITE-N (mg/L)	HUMAN TARGET (DNA RESULT)	CANADA GOOSE TARGET (DNA RESULT)	BEAVER TARGET ⁴ (DNA RESULT)
	Present	0.12 mg/L	236 MPN/100 mL	0.10 mg/L	Present	Present	Present
CC-2	Absent	0.013	34	<0.05	Absent	-	-
CC-3	*	0.013	32	0.053	-	-	-
RMR-1	Absent	0.004	84	<0.05	-	-	-
RMR-2	Absent	0.004	36	<0.05	Absent	Absent	-
RMR-3	Absent	0.018	57	<0.05	-	-	-
RMR-4	Absent	0.019	326	0.12	Absent	Absent	-
RMR-5	Absent	0.017	488	0.13	Absent	Absent	-
SHC-1	Absent	0.007	6	<0.05	-	-	-
SHC-4	Absent	0.013	52	0.061	-	-	-
SHC-5	Absent	0.014	31	<0.05	Present	-	-
LFB-1	-	-	-	-	Absent	Present	Present
LFB-2	-	-	-	-	Absent	Absent	Absent
LFB-3	-	-	-	-	Absent	Present	Absent
<i>*Error in sample processing</i>							

⁴ The mtDNA marker for beaver is in preliminary testing and therefore, results are preliminary.

RECOMMENDATIONS

Because bacteria is highly variable across time and space and available indicators for tracking fecal contamination sources have understood limitations, it is important to note that results stated here are preliminary. If possible, further testing at all sites in both wet and dry weather conditions would improve our understanding of fecal contamination in the watershed. However, given our understanding of the expense involved in microbial source tracking, we have prioritized our recommendations based on results from 2017 – present, below.

Little Falls Brook (Sites LFB-1, LFB- 2, and LFB-3): Sampling and analysis in 2017 and 2018 by Maine DEP, FBE, and the Town of Bristol has indicated Little Falls Brook appears to contribute FIB to the estuary. Microbial Source Tracking in 2019 indicated that contamination is most likely wildlife due to positive host marker presence for beaver and Canada Goose. Site LFB-1 (above outlet to estuary) had a positive hit for beaver presence and a hit for Canada Goose. Site LFB-3 (above outlet to Estuary) had a positive hit for Canada Goose. Analysis for presence of the human marker was negative at all three sites. Previous sampling has indicated that FIB results have been highest during storm events. High FIB during the storm could be the result of the mobilization of fecal waste settled in and behind beaver dams. *To conserve and prioritize available funds, we do not recommend further testing on Little Falls Brook because sources of fecal contamination from wildlife are much more difficult to control. Increasing vegetated buffers around streams can minimize access to waterbodies by waterfowl.*

School House Cove Tributary (Site SHC-5): Previous sampling at tidewater tributaries has indicated possible groundwater contamination due to elevated bacteria prior to storm events. Microbial source tracking at the outlet of School House Cove Tributary for human presence was positive, indicating possible groundwater contamination from human sewage. Land use in the surrounding area includes the Bristol Consolidated School and residential properties. Microbial source tracking in Coombs Cove Tributary for human presence did not result in any positive hits, suggesting that FIB contamination on the east side of the tributary is from School House Cove.

We recommend a septic system inventory to isolate and correct any malfunctioning septic or sewer systems that may be contributing to FIB contamination. A septic system risk assessment would facilitate management of septic systems by ranking systems according to their risk of contamination if the system fails. This assessment would utilize ERSI® ArcGIS desktop analysis to identify the risk from the following categories:

1. *Infrastructure Risk Factors*
 - a. *Design suitability, installation quality, maintenance history, age of system*
2. *Soil and Environmental Factors*
 - a. *Soils (filtering capacity, flooding potential, ponding, depth to bedrock, slope, depth to saturated zone, seepage, restricted permeability), wetlands, surface water exposure, and flooding occurrence.*

An understanding of septic system risks in the areas with high fecal contamination will serve as a tool to prioritize investigation and correction of any malfunctioning septic systems.

Redonnett Mill Road Tributary (Sites RMR-4 and RMR-5): Previous sampling has indicated high *E. coli* levels during dry and wet weather at the outlet of the Redonnett Mill Road Tributary into the main stem of the Pemaquid River. Sampling in 2019 showed *E. coli* exceedances at sites RMR-4 and RMR-5, the two sites above the outlet, but not at sites further up the tributary, indicating possible contamination sources from the surrounding land. Land use in the surrounding area includes agriculture and homes with private septic systems (Figure 2). Microbial source tracking at sites RMR-2, RMR-4, and RMR-5 for human presence and Canada Goose presence were negative, indicating that FIB contamination from the Redonnett Mill Road Tributary is most likely a result of agriculture, not wildlife or human sources.

We recommend following up in the area with the agricultural landowner to install best management practices to prevent contamination from agricultural land uses in the tributary.

- *Engage the farm owner and create a collaborative effort to protect the stream and greater watershed.*

- *Install a buffer for storm runoff by planting native plants along the stream, particularly at the southwest corner of the property.*
- *Relocate animal pens further away from the stream.*
- *Consider further microbial source tracking for host-specific markers such as ducks and pigs to pinpoint the dominate source. Sample suspected sites along a transect during a period of low flow and soon after a storm event (such as a spring flush).*

We also recommend including this area in a septic system inventory. A negative result for human presence from microbial source tracking does not eliminate the possibility of a malfunctioning septic system as a cause of fecal contamination.