FALL 2021 PEMAQUID RIVER HOTSPOT SAMPLING | MEMORANDUM

TO: Christopher Hall, Ph.D., Town Administrator Rachel Bizarro, Town Clerk, Town of Bristol
FROM: Maggie Kelly, FB Environmental Associates (FBE)
SUBJECT: Fall 2021 Pemaquid River Hotspot Sampling
DATE: November 29, 2021
CC: Forrest Bell, FB Environmental Associates

INTRODUCTION & SUMMARY OF RESULTS

FB Environmental Associates (FBE) has been working with the Town of Bristol and Bristol Shellfish Committee since 2018 to investigate potential pollution sources in the Pemaquid River and Estuary. 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. 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.

In October 2021, FBE completed hotspot sampling above the Pemaquid River Falls for bacteria (Enterococci) and mitochondrial DNA (mtDNA) analysis (chicken fecal marker) based on concerns of pollution from a farm located along the river. Results indicated that bacteria levels were elevated at one location on the Pemaquid River (site PF-2) and the mtDNA chicken marker was detected at low concentrations at two locations on the River (sites PF-2 and PF-3) (Map 1). **This indicates that chicken fecal waste is present in the river under wet weather conditions at LOW concentration levels. This sampling event represents a single point in time and does not confirm pollution from the poultry farm however, it does warrant follow-up monitoring and site assessment.** As such, FBE contacted the landowner of the farm who is interested in a site assessment of the property to ensure there is no stormwater runoff carrying nonpoint source pollution into the Pemaquid River and to discuss best management practices, if stormwater runoff is noted. FBE will conduct a site visit at the farm to identify best management practices in early December that may be completed under future grant funding. The landowner has also already applied for grant funding to complete a Nutrient Management Plan in 2022 through the Natural Resources Conservation Service.

Given that the Pemaquid River and estuary has had elevated levels of bacteria under different conditions (wet and dry weather) and in multiple locations, and this sampling indicates chicken fecal bacteria is present at low levels, this is likely not the sole contributor of bacteria to the river. We recommend the Town of Bristol continue investigating potential pollutant sources upstream of the Pemaquid River Falls.

METHODOLOGY

FBE collected water quality samples at four sites upstream of Pemaquid River Falls on October 26, 2021 after 0.25 inches of precipitation in the prior 24 hours (PF-C, PF-1, PF-2, and PF-3). Site PF-C was located upstream of the farm and served as the control site. Sites PF-1 and PF-2 were located adjacent to the farm and PF-3 was just downstream of the farm. Sampling was completed during wet weather conditions to understand the potential impact of runoff from the adjacent land uses. Wet weather often mobilizes fecal sources on the landscape and transports them via overland flow to nearby rivers and streams. Samples were analyzed for Enterococci at Maine Environmental Laboratory and for mitochondrial DNA (mtDNA) analysis for chicken fecal targets by Dr. John Bucci at the University of New Hampshire. Field parameters were also collected to serve as standard metrics of water quality to help with data interpretation, including dissolved oxygen, temperature, salinity, and specific conductance.

Using Enterococci as a Fecal Indicator Bacteria

Fecal indicator bacteria (such as *Escherichia coli* or *E. coli*, Enterococci, and Fecal Coliform) are used to track a wide variety of potentially harmful pathogens such as viruses and bacteria found in fecal waste that would otherwise be too expensive to monitor comprehensively. High in-stream fecal indicator bacteria levels during wet weather events can point to fecal sources on the landscape via stormwater runoff. Enterococci is the most appropriate indicator bacteria for fecal source tracking in estuarine waters (*E. coli* does not survive in saltwater). Head of tide on the Pemaquid River is at the Route 130 bridge over the Pemaquid River approximately 800 feet upstream of Pemaquid Falls, indicating that there is tidal influence at the sites used in this study. Sampling was completed at low tide to best avoid sampling water coming in from the estuary.

Using Microbial Source Tracking

Elevated fecal indicator bacteria alone do not constitute a contamination from domestic waste 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. The mtDNA analysis was performed at the UNH Microbial Source Identification Laboratory by John Bucci, Ph.D., using molecular biomarkers to assess water quality (refer to Appendix A for full methodology). The chicken fecal marker was selected based on the surrounding land use. The chicken marker used by Dr. Bucci is for chickens specifically and does not include ducks or waterfowl.

RESULTS

Results are displayed in Table 1 and Map 1. Site PF-2 (located between the Route 130 bridge and Pemaquid Falls) was the only site that had enterococci bacteria levels elevated above Maine's EPA-approved instantaneous (one sample) threshold of 104 MPN/100 mL. Sites PF-2 and PF-3 (PF-3 is located at Pemaquid Falls) were present for mtDNA chicken marker at low concentrations. **This indicates that chicken fecal waste may be contributing bacteria to the river adjacent to and downstream of the property under wet weather conditions at LOW concentration levels. Low concentrations indicate that this source may be contributing bacteria to the Pemaquid River, but it is unlikely that it is the sole source of elevated bacteria levels in the Pemaquid River and estuary. Fecal bacteria tracking has many limitations and should be cautiously interpreted – i.e., these sampling results represent one moment in time and do not indicate that there is ongoing, severe pollution from this property.** Field parameters were within the expected range for all sites. During low tide conditions, sites PF-C, PF-1, and PF-2 had minimal tidal influence, while PF-3 was tidally influenced.

Table 1. Field parameter and laboratory results for the sample sites from 10/26/21. Bold, italicized ***red*** text indicate results exceeding state criteria. Sites PF-C, PF-1, and PF-2 had minimal tidal influence. Site PF-3 (furthest downstream) was tidally influenced.

Site Name	Salinity (ppt)	Dissolved Oxygen (%)	Temp (°C)	Specific Conductivity (µS/cm)	Enterococci (MPN/100 mL)	mtDNA Chicken Marker
Threshold	*<0.5 indicates freshwater*	*75% (freshwater) 85% (tidal)*	*<24 °C*	*<854 (freshwater) Not applicable to tidal waters*	*104 MPN/100mL*	*Present*
PF-C	0.03	77.5	10.6	64.2	20	Absent
PF-1	0.03	86.2	10.4	64.4	30	Absent
PF-2	0.03	93.0	10.6	66.2	***132***	***Present (Low)***
PF-3	0.34	97.3	10.6	680.0*	52	***Present (Low)***

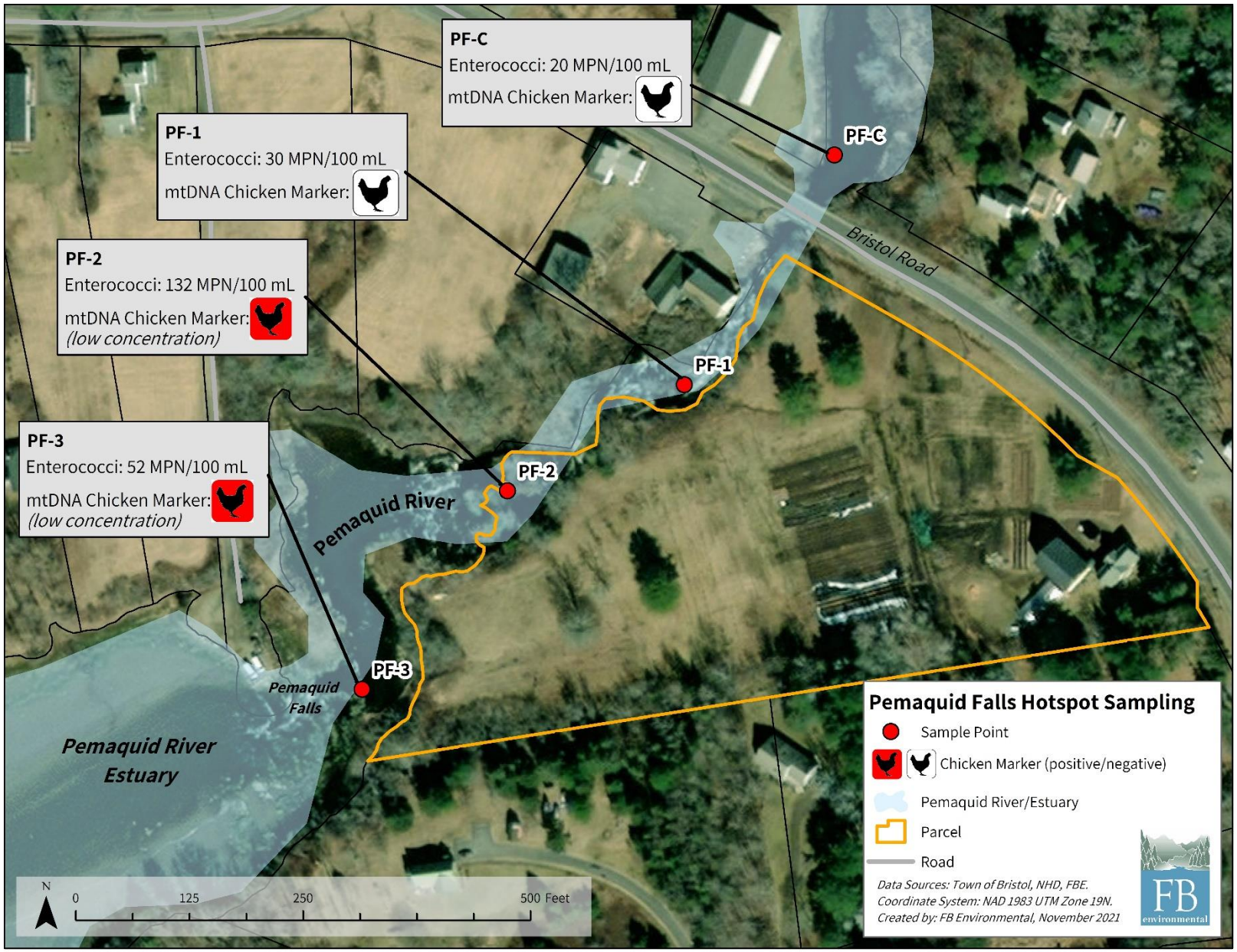
**elevated conductance at this site is because of tidal influence and does not represent contamination.*

DISCUSSION & RECOMMENDATIONS

FBE spoke with the adjacent farm's landowner and the landowner has already taken action to reduce the risk of pollution entering surface waters from the farm. In September 2021, the landowner applied for funds to complete a nutrient management plan with the Natural Resource Concentration Service in 2022. A nutrient management plan assists agricultural landowners with efficiently using nutrient resources for their agricultural operation while minimizing the transport of nutrients to nearby surface waters. NRCS also performed a site walk in September 2021 and is developing planting plans for Best Management Practices (BMPs) that will assist with stormwater control. FBE will meet with the landowner in early December to identify locations for buffer plantings or other best management practices along the length of the property adjacent to the river, if needed to reduce polluted runoff from reaching the Pemaquid River.

FBE recommends the following action items:

- If identified as a need, include best management practice implementation efforts at the farm in the 2022 Maine Shellfish Resiliency Fund grant application.
- Repeat monitoring at these four sites under both dry and wet weather conditions.
- Continue investigating potential pollutant sources upstream of the Pemaquid River Falls. Given that the Pemaquid River has had historically elevated levels of bacteria under different conditions (wet and dry weather) at multiple locations both below and upstream of the falls, and this sampling indicates chicken fecal bacteria is present at low levels, this is likely not the sole contributor of bacteria to the river.



Map 1. Results from Pemaquid Falls hotspot sampling in October 2021. Enterococci bacteria levels were elevated at site PF-2, and low concentration levels of the chicken mtDNA marker were detected at sites PF-2 and PF-3.

CITATIONS

Monitoring, Notifications, and Illness. 2021. Maine Department of Environmental Protection. https://www.maine.gov/dep/water/beaches/monitoring.html

Standards for classification of estuarine and marine waters (§465-B). 2017. Maine Legislature. http://www.mainelegislature.org/legis/statutes/38/title38sec465-B.html

USEPA. 2001. Ambient Water Quality Criteria Recommendations: Information Supporting the Development of State and Tribal Nutrient Criteria: Rivers and Streams in Nutrient Ecoregion VIII. United States Environmental Protection Agency, Office of Water 4304, EPA 822-B-01-015. Retrieved online from http://www2.epa.gov/sites/production/files/documents/rivers8.pdf.

Weather History for KMEBRIST7. 2021. https://www.wunderground.com/dashboard/pws/KMEBRIST7/table/2021-10-26/2021-10-26/daily

APPENDIX A: MICROBIAL SOURCE TRACKING METHODOLOGY

The mtDNA analysis was performed at the UNH Microbial Source Identification Laboratory using molecular biomarkers to assess water quality. Analysis results for the host-specific markers were reported as present or absent. 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. If present, the source of a present sample is detected within the standard microbial source guidelines.[1] To confirm a fecal source, additional testing may be recommended.[2]

Samples identified as present for the chicken marker also had the concentration level estimated to assist with analysis of the results. Detection of the target fecal DNA biomarker (i.e., Bacteroides, mtDNA)[3] by quantitative Polymerase Chain Reaction (qPCR) is based on the concentration (copies by reaction volume) of replicates using a standard curve method. Unknowns are measured for presence/absence based on a series of standards of known quantity. Results designated as present are a clear DNA signal above the limit of detection. Low concentration is defined as the level above the limit of detection (≥ 1 copy/25uL reaction) threshold.

Each water sample was filtered through a 0.45-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 a real-time qPCR thermal cycler (BioRad™ Hercules, CA) including the sample DNA extract and Taq polymerase reagents. For quality control purposes, a positive control consisting of known 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 positives. A Bacteroidetes marker was used from a library based on a 16S rRNA gene sequence. The accumulation of PCR product is detected in the amplification phase. If the fecal marker is not amplified in the sample, replication is not detected and the sample is considered absent. If accumulation of PCR product is detected, the sample is reported as present.

The method used to produce these results is part of a research program. MSI technology is in the optimization phase and in the process of being standardized with an international certification program. For example, a process goal is to translate concentration levels (i.e., low, medium, high) to be interpreted per 100mL of sample water.[4] For most accurate interpretation of source detection, it is recommended that this microbial source tool be used in combination with additional water quality parameters such as fecal coliform bacteria concentration, temperature, turbidity, and flow.

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[1] Bustin, SA. et al. 2009. MIQE Guidelines. Clin.Chem. 55: 611-622. Harwood V. et al. 2014. Federation of European Microbiology Societies. Microbiol Rev. 38: 1-40. 2

[2] 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

Balleste E, et al. 2020. Implementation and integration of microbial source tracking in a river watershed plan. Science of the Total Environment. 736. http://doi.org/10.1016/j.scitotenv.2020.139573.

[3] Kobayashi A, et al. 2013. Chicken and duck associated Bacteroides-Prevotella genetic markers for detecting fecal contamination in environmental water. Environmental Biotechnology. 97: 7427-7437.

[4] Zhuang FF, Li H, Zhou XY, et al. 2017. Quantitative detection of fecal contamination with domestic poultry feces in environments in China. AMB Expr 7: 80. https://doi.org/10.1186/s13568-017-0379-0.