

OARS ASSABET IN MAYNARD BACTERIA STUDY—2023

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The following is a summary of OARS’ monitoring and source-tracking results for *E. coli* bacteria in the Assabet River Maynard special study. This study was funded by a grant from the Greater Lowell Community Foundation. We also thank our team of dedicated volunteers for helping collect the river water samples.

This study was prompted by frequent elevated *E. coli* bacteria levels in our 2019–2022 sampling in the Assabet River at the Route 27 bridge in Maynard. *E. coli* bacteria come from the digestive systems of warm-blooded animals, and a high concentration of the bacteria is an indicator of fecal water contamination. The Sudbury, Assabet and Concord rivers have a Class B water quality standard, meaning that they should be safe for fishing and swimming. OARS is working to have the three rivers meet this goal by tracking down sources of bacteria contamination so they can be eliminated.

During the summer of 2023, for this study, our volunteers collected bi-weekly samples for bacteria analysis at 6 sites along the Assabet River and Mill Pond in Maynard. Our focus was to identify the source of bacterial pollution that we had discovered at the Route 27 bridge. We conducted three different source tracking tests: bacteria monitoring, DNA analysis, and detergent testing. Below are our 2023 results.

Bacteria Monitoring

OARS’ bacteria monitoring program (launched in 2019) highlighted frequent elevated *E. coli* bacteria levels at the Assabet River sampling site in Maynard upstream of the Route 27 bridge (site # ABT-077). During the four years between 2019 and 2022, 35% of bacteria samples at this site (out of 54 total samples) exceeded the EPA’s Beach Action Value (BAV) swimming threshold of 235 CFU-MPN per 100 ml¹. One sample on 8/3/20 even exceeded the MassDEP boating threshold of 1260 CFU-MPN per 100 ml. An analysis of the separate wet and dry weather² sample data shows no statistical difference between dry and wet weather results (Figure 1). When wet weather bacteria counts are not significantly different than dry weather counts, it tends to mean that the source of the bacteria pollution is sewer-related because it is not significantly affected by precipitation or stormwater runoff.

In the summer of 2023, OARS volunteers collected samples at five additional locations upstream of the ABT-077 site in an attempt to pinpoint the source of the bacterial pollution (see Appendix A for a map of the sites). The results of this sampling are shown in the graph in Figure 2. Contrary to the earlier results, bacteria levels did follow precipitation in 2023, with

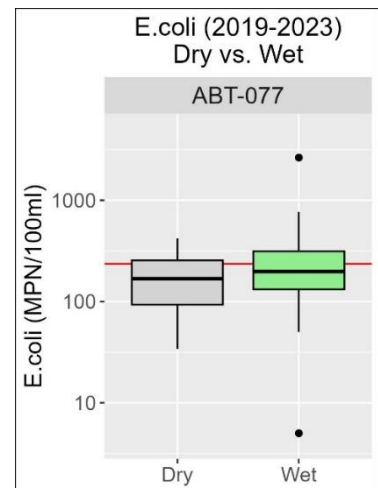


Figure 1: Maynard ABT-077 bacteria dry vs. wet all years. Red line represents the BAV threshold of 235 MPN/100ml.

¹ Culturable bacteria can be enumerated in either CFU/100 ml (Colony Forming Units) or MPN/100 ml (Most Probable Number) depending on the method used for analysis. The two units of measure are statistically interchangeable for bacteria monitoring purposes in surface waters. OARS has used both during this time period.

² Wet weather is defined as 48-hour precipitation exceeding 0.1 inches.

precipitation-related high counts on both July 17 and September 11. There were some very large rainfall events which made 2023 an unusual year. The Bedford airport recorded more precipitation between June 1st and August 31st than any year in the past 25+ years³. The graph also shows that up until the June 26 sampling event there was a relatively consistent spread in bacteria counts between upstream and downstream sites. Precipitation was moderate until June 26 but increased significantly afterward as shown in the cumulative precipitation graph in Figure 3. The spread in bacteria counts is easier to evaluate in Figure 4, which shows that the pre-July results for the upstream site (ABT-089) and the Mill Pond sluiceway (MLP-003) were significantly lower than all the downstream sites. This implies that the source of dry-weather bacteria contamination is located somewhere between ABT-089 (Rt. 117) and ABT-084 (Town Hall). However, from July 17 onwards, there was no significant spread between any of the sites, implying that wet-weather bacteria contamination is coming from upstream of the Ben Smith dam. Wet-weather sources are probably from stormwater runoff or flooding of land and could be from animal or human waste⁴.

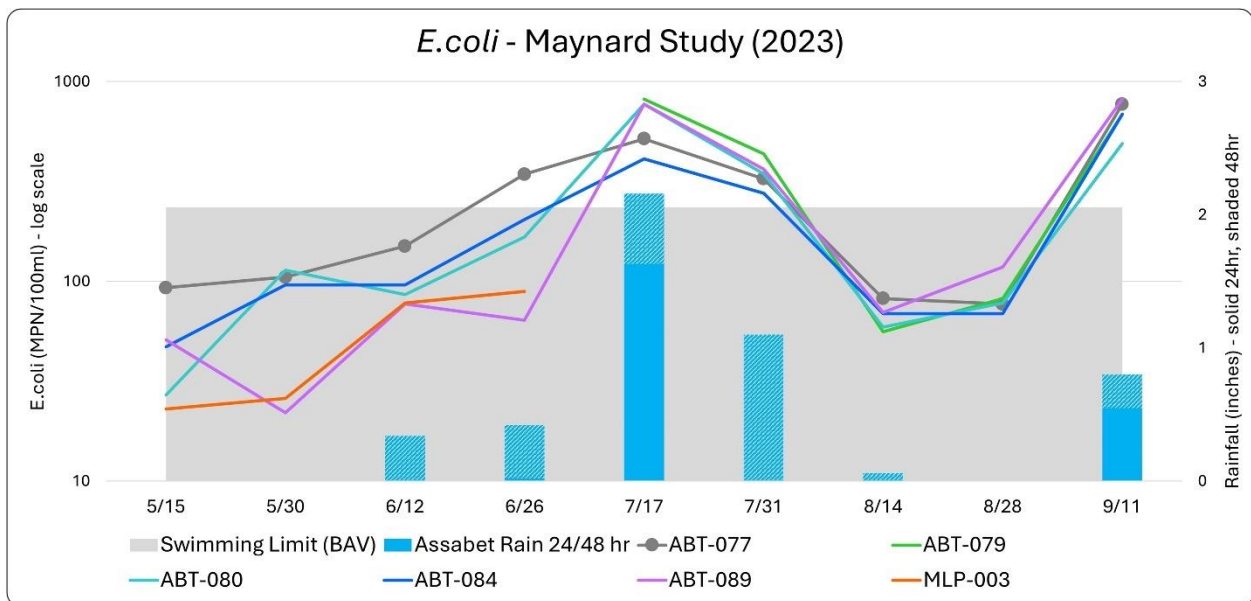


Figure 2: Bacteria and precipitation data for Assabet Maynard sites. The downstream ABT-077 site is drawn with gray dots for reference. All the upstream sites are depicted with lines only.

³ Bedford Hanscom Field, MA. Data sourced from The National Weather Service, a division of NOAA, <https://www.weather.gov/wrh/climate?wfo=box>. Period of record 1999-2023.

⁴ Ahmed, Warish, et.al., 2019, "A review on microbial contaminants in stormwater runoff and outfalls: Potential health risks and mitigation strategies", Science of the Total Environment 692 (2019) 1304–1321.

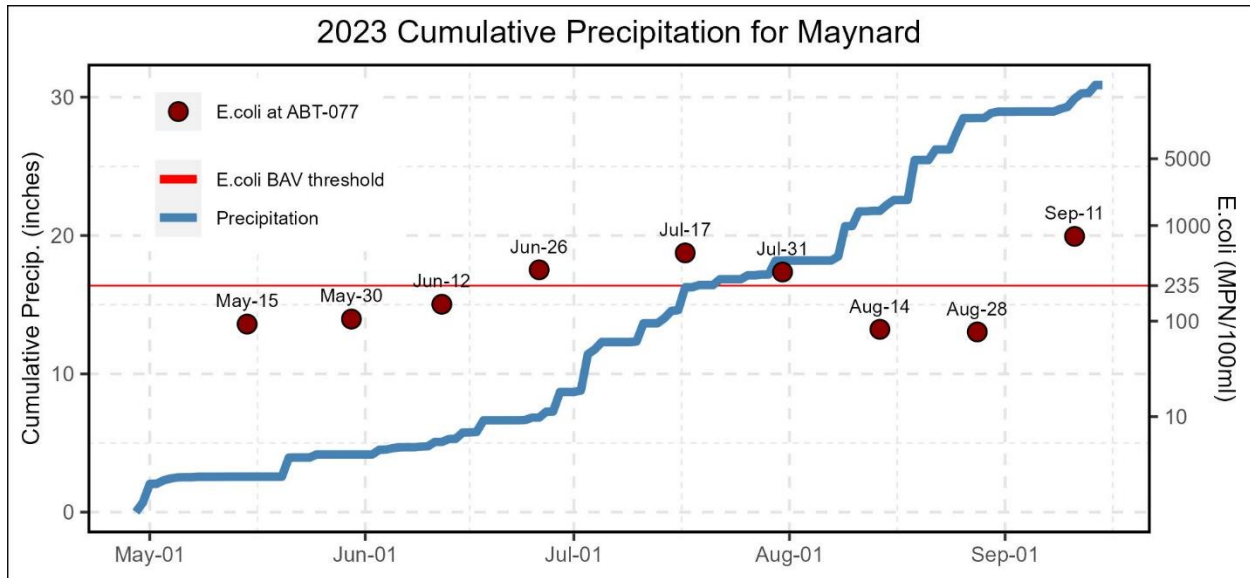


Figure 3: Cumulative precipitation in the Maynard area 4/29/23–9/15/23 (source CoCoRaHS.org).

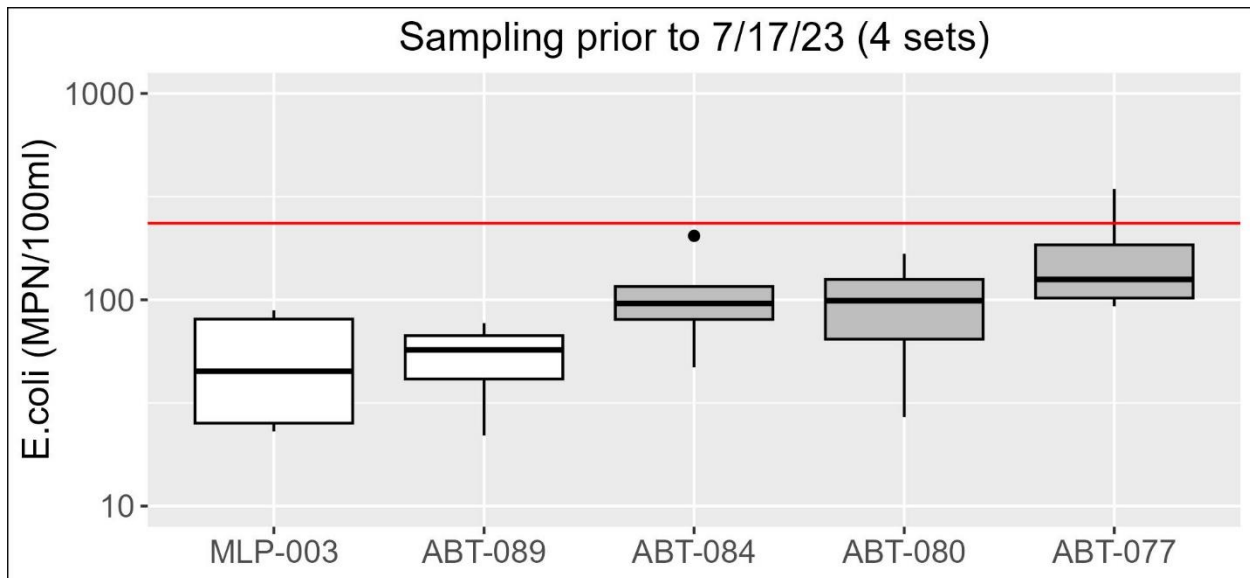


Figure 4: Boxplot analysis of E. coli results prior to major precipitation, prior to 7/17/23. The upstream sites (white shading) had significantly less contamination than the downstream sites (gray shading).

DNA Analysis

DNA analysis can pinpoint which animal species are responsible for contamination. OARS had one water sample analyzed for DNA on 9/11/23. The sample was collected from the ABT-077 site. The results did confirm human contamination. However, since it was a wet-weather sample, it was not a good analysis of dry weather conditions. Due to the rainy summer, there were no opportunities for low precipitation or low flow sampling after mid-June in 2023. We are considering collecting another sample in 2024, if there are low-flow conditions.

The DNA sample was analyzed by Jonah Ventures (Boulder, CO), who used qPCR technology to measure the number of DNA replicates found in the water sample for four different species: human, beaver,

goose, and dog. The test results returned DNA copy counts of 165 for human, 386 for beaver, 5 for goose, and 0 for dog (Table 1). These results confirmed that humans were a source of DNA in the sample⁵. The results also show high amounts of beaver DNA in the sample. This sample corresponded to the 9/11/23 sampling event shown in Figure 2, when all sites returned very high bacteria levels, meaning that during wet weather the bacteria source was upstream of the Rt. 117 bridge (also upstream of the Ben Smith dam). There are known beaver populations around the Ben Smith impoundment, and these results imply that the upstream beaver populations could be a major source of bacterial contamination during wet weather. However, this is probably not the case during dry weather, when bacteria levels at the upstream Rt. 117 site tended to be lower than downstream. Additional testing would be useful. Neither goose nor dog DNA was present in any significant amount.

Table 1: DNA results, analyzed by Jonah Ventures. Results are averages based on three replicates. All replicates had similar detection levels.

	ABT-077 9/11/23 7:30 AM	
Beaver DNA by qPCR	386	Copies/100 ml
Human DNA by qPCR	165	Copies/100 ml
Canadian Goose DNA by qPCR	5	Copies/100 ml
Dog DNA by qPCR	0	Copies/100 ml
<i>E. coli</i> Bacteria by Collilert-18	770	MPN/100 ml
Notes	High flow, wet weather, 0.8" of rain in previous 48 hours	

Detergent Monitoring

Detergent in stormwater indicates a cross-connection with sewer pipes. With the help of one of our intrepid volunteers, OARS conducted a survey of all the outfall pipes draining into the Assabet River between ABT-077 (furthest downstream site) and ABT-089 (1.2 miles upstream at Rt. 117). We walked up the river on 11/16/23 and tested for detergents in each pipe that had flowing water, using a Chemets K-9400 test kit. There were many pipes along the river that did not have any flow at the time of the survey, which probably makes it safe to exclude them as dry-weather sanitary sewer sources. Of the pipes that were tested, most did not show any noteworthy results, but one pipe at mile 8.15 did show significantly elevated levels (Figure 5). The mile 8.15 pipe is located next to the Florida Rd. bridge on the north side of the river (see Figure 6 for a picture of the pipe). Three other discharges had slightly elevated results, though not conclusive in relation to the sensitivity of the test: two pipes at miles 8.05 and 8.12, and a stream at mile 8.10.

This detergent survey clearly identified one pipe that needs to be addressed. The pipe at Florida Rd. should be easy to map and resolve. The other pipes would be worth further evaluation by the Maynard Department of Public Works. The detergent survey also highlighted two other interesting findings. First, the outfalls under the Mill & Main buildings did not show any indications of detergent contamination, nor did the bacteria results point to any increased contamination in that area. Second, while the bacteria results pointed to a source of bacteria between Rt. 117 and the Maynard Town Hall, our survey did not find any above-water pipes with flowing water in that section. Finding a pollution

⁵ The copy count of 165 for human DNA is significant in comparison to OARS' 2022 DNA test results of 271 copies from River Meadow Brook in Lowell, where OARS has demonstrated significant bacterial pollution and a dominance of human sources in a dense urban environment. See "OARS River Meadow Brook Bacteria Monitoring Results—2022", Feb. 7, 2022.

source in this section will require further infrastructure information. Maps show a stream entering from the west side of the river 200 ft. downstream of the Rt. 117 bridge. This stream was not tested in our survey and should be checked.

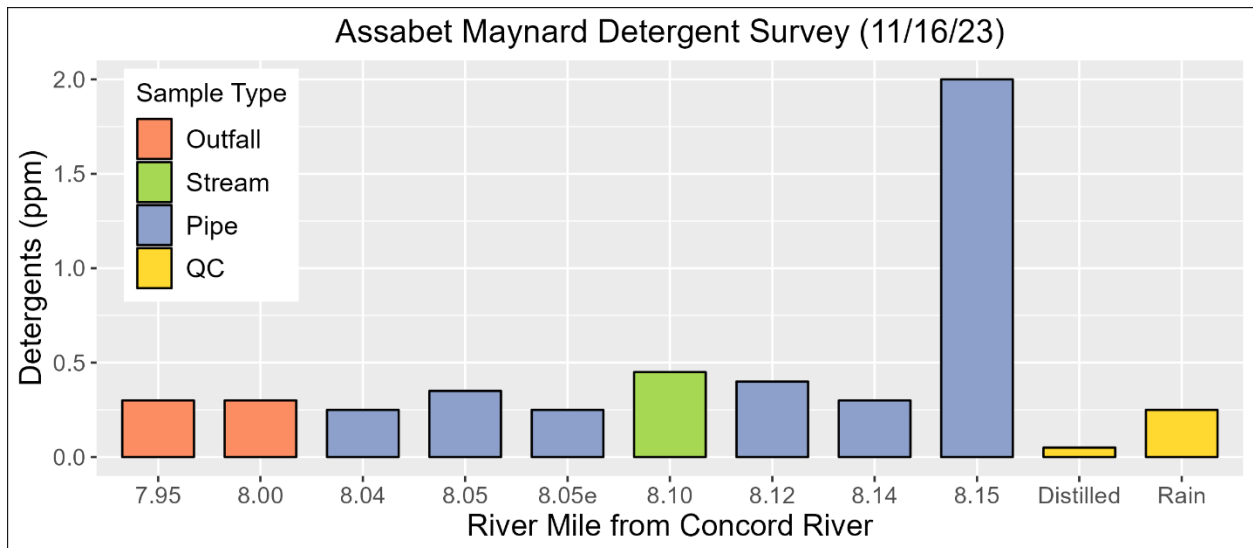


Figure 5: Assabet Maynard detergent survey 11/16/23. Samples listed by river mile of outfall pipe. Mileage is measured from the USGS gauge upstream of Rt. 27, which is mile 7.70. Each bar represents one sample. The two sites with type “Outfall” are the outfalls from Mill Pond that flow under the Mill & Main building.



Figure 6: Inflows with high detergent levels (mile 8.15 on left next to Florida Rd. bridge; mile 8.10 on right 200 ft. downstream of Florida Rd. bridge).

Conclusion

OARS’ monitoring over the last five years has shown a persistent level of dry-weather bacterial pollution in the Assabet River in Maynard. Of 37 dry-weather samples over this period, 32% of them exceeded the EPA BAV swimming threshold. Our research this year has provided more information about the source of the pollution. Focused bacteria monitoring highlighted the section of river between the Rt. 117 bridge and the Maynard Town Hall as a probable source of dry-weather bacteria pollution and provided evidence that wet-weather pollution is heavily influenced by beaver populations and is coming from upstream of the Ben Smith Dam. The DNA analysis of water samples at our downstream site provided evidence that human sources do constitute a significant portion of bacterial contamination and confirmed the influence of beavers in wet-weather contamination. The detergent testing identified one

pipe that we suggest the Maynard Department of Public Works should follow up on urgently and three other inflows that deserve some additional research. A significant finding of the study is that the bacteria and detergent results both imply that, contrary to assumptions about old buildings and infrastructure, the Mill & Main buildings and the Mill Pond are probably not sources of bacterial pollution. Our study was somewhat compromised by the unusually wet weather in 2023, but the study did help to highlight several areas of opportunity for closer research and to rule out some sources of question.



Figure 7: Volunteer J. Clarke checking a storm sewer pipe for discharge.

Appendix A: Map of River Meadow Brook Study Area



Appendix B: OARS site list with coordinates

Site #	DESCRIPTION	TOWN	WATERBODY	LATITUDE	LONGITUDE
ABT-077	Route 27/62 bridge (near USGS gauge)	Maynard	Assabet River	42.43206352	-71.4497411
ABT-079	Walnut St Bridge	Maynard	Assabet River	42.43085948	-71.4534336
ABT-080	Main St & Walnut St	Maynard	Assabet River	42.43179564	-71.4551611
ABT-084	Maynard Town Hall	Maynard	Assabet River	42.43129419	-71.4618084
ABT-089	Rt. 117 Bridge	Maynard	Assabet River	42.42835791	-71.4685656
MLP-003	Sudbury St at Mill Pond	Maynard	Mill Pond	42.42870125	-71.4594878

Appendix C: Bacteria Data

2023 *E. coli* lab results MPN/100ml

Site #	Description	River	5/15	5/30	6/12	6/26	7/17	7/31	8/14	8/28	9/11
ABT-077	USGS gauge, Maynard	Assabet	93	105	150	344	517	326	82	77	770
ABT-079	Walnut St Bridge, Maynard	Assabet					816	435	56	82	687
ABT-080	Main St & Walnut, Maynard	Assabet	27	114	86	167	770	345	59	78	489
ABT-084	Maynard Town Hall	Assabet	47	96	96	204	411	276	69	69	687
ABT-089	Rt. 117, Maynard	Assabet	51	22	77	64	770	365	70	118	816
MLP-003	Sudbury St, Maynard	Mill Pond	23	26	78	89					