

## SUDBURY RIVER ASHLAND BACTERIA STUDY—2024

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The following is a summary of OARS' monitoring and source-tracking results for *E. coli* bacteria in the Sudbury River Ashland 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 2019-2023 sampling in the Sudbury River at the Chestnut Street access point in Ashland. *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 bacterial contamination so they can be eliminated.

During the summer of 2024, with the help of volunteers, OARS collected many local water samples along the Sudbury River in Ashland and carried out a suite of tests. Our focus was to identify the source of the bacterial pollution that we had discovered at the Chestnut Street access. We conducted three different source tracking tests: bacteria monitoring, DNA analysis, and detergent testing. We found one major source of pollution and several other potential sources. Below are our results.

### Bacteria Monitoring

OARS' bacteria monitoring program (launched in 2019) highlighted frequent elevated *E. coli* bacteria levels at the Sudbury River sampling site along Chestnut Street in Ashland between the Union Street bridge and Cold Stream Brook (site # SUD-236). During the six years between 2019 and 2024, 35% of bacteria samples at this site (out of 71 total samples) exceeded the EPA's Beach Action Value (BAV) swimming threshold of 235 CFU-MPN per 100 ml<sup>1</sup>. Three samples (6/29/20, 8/3/20, and 7/17/23) even exceeded the MassDEP boating threshold of 1260 CFU-MPN per 100 ml. An analysis of the separate wet and dry-weather<sup>2</sup> sample data (Figure 1) shows that median wet-weather results are statistically greater than median dry-weather results, but it also shows that 24% of dry-weather samples exceeded the BAV. High wet-weather bacteria levels can imply contamination from surface and stormwater runoff and could be from

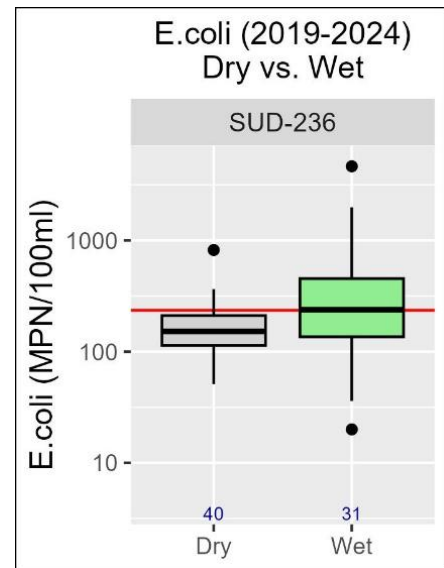


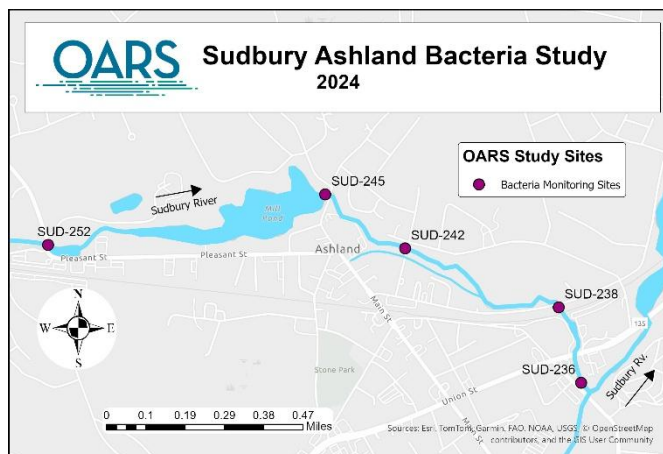
Figure 1: Ashland SUD-236 bacteria dry vs. wet all years. Red line represents the BAV threshold of 235 MPN/100ml.

<sup>1</sup> 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.

<sup>2</sup> Wet weather is defined as 48-hour precipitation exceeding 0.1 inches.

animal or human waste<sup>3</sup>. High dry-weather bacteria levels tend to mean there is a sewer-related source of bacteria pollution. Our sampling data show both concerns for this Ashland site.

In the summer of 2024, OARS volunteers collected samples at four additional locations upstream of the SUD-236 site in an attempt to pinpoint the source of the bacterial pollution (Figure 2, see Appendices A and B for a larger map and list of the sites). The results of this sampling are graphed by date in Appendix C. Other than the clear alignment of the sites in the first three samples and the spike in all values following the May 28 precipitation, it is hard to discern conclusive patterns when viewing the data by date. It is easier to see patterns in the data when the results are organized by site and separated by wet and dry weather (Figure 3). In dry weather, the SUD-238 site (Front Street bridge) produced significantly higher bacteria counts than any of the upstream sites. This highlights a chronic dry-weather pollution source between SUD-242 (Concord St) and SUD-238 (Front St). In wet weather, the SUD-238 pollution source is still evident, though diluted by higher flows, and there is also a clear pollution source upstream of SUD-252 (Cordaville Road). The area upstream of Cordaville Road has low population density and is bordered by wetlands, so it is probable that the upstream wet-weather pollution could be animal sourced. However, the persistence of the pollution upstream of SUD-238 in dry and wet weather indicates a sewer pollution source in the section between Concord Street and Front Street.



*Figure 2: Map of bacteria sampling sites. See Appendix A for a larger map of all testing sites.*

<sup>3</sup> 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.

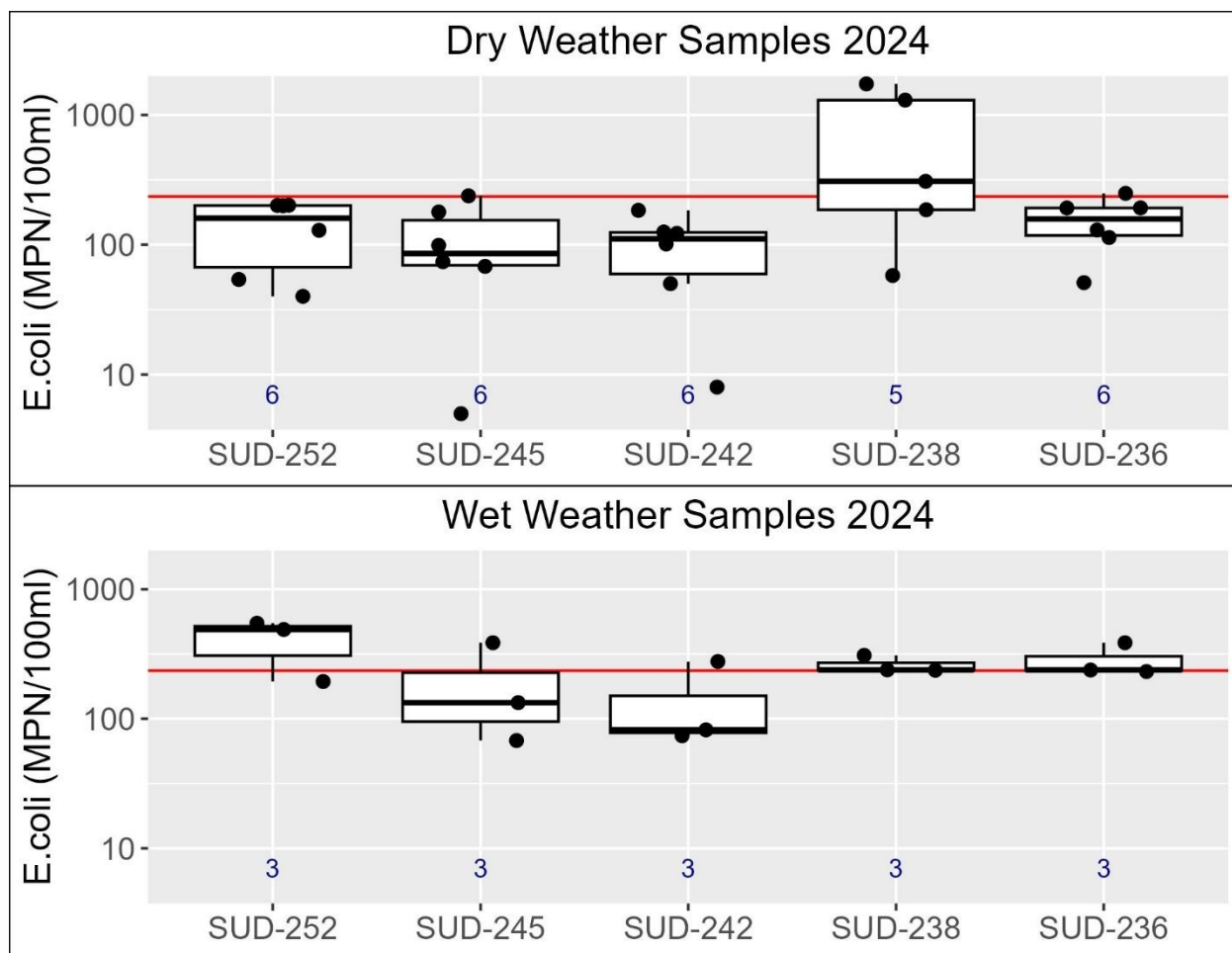


Figure 3: Boxplot analysis of *E. coli* results for dry and wet-weather sampling dates. The dots represent actual samples. Wet weather is defined as >0.1 inches of rain in the previous 48 hours. The sites are listed left to right from upstream to downstream. Dry and wet-weather pollution is evident upstream of the SUD-238 site. The red line represents the BAV threshold of 235 MPN/100ml.

## DNA Analysis

DNA analysis can pinpoint which animal species are responsible for contamination. OARS had two Sudbury River water samples analyzed for DNA. The samples were collected from the SUD-236 site on 6/17/24 and 9/3/24, coinciding with bacteria sampling dates. The samples were analyzed by Jonah Ventures (Boulder, CO), who used qPCR technology to measure the number of DNA replicates found in the water samples for four different species: human, beaver, goose, and dog.

The results of the two samples were quite different (Figure 4). The first sample was collected during wet weather in moderately high flows. This sample showed a large proportion of beaver DNA and also some goose DNA, supporting the hypothesis that upstream wet-weather bacteria counts are probably from wildlife. Both samples had a significant portion of human DNA, and the second sample, which was collected during a long period of dry weather, was almost 100% human DNA. It had no beaver or goose

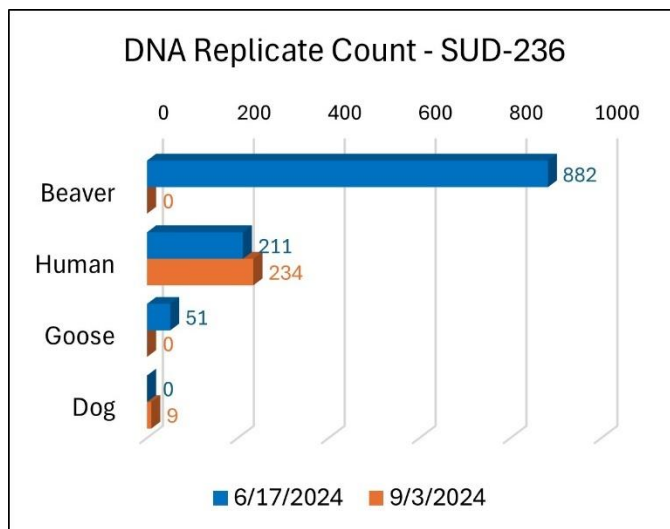
DNA at all. These results confirmed that humans were a significant source of DNA in the samples<sup>4</sup>. The dry-weather results support the suspicion of potential sewage pollution upstream of the dry-weather bacteria hot-spot, SUD-238. Dog DNA was not present in any significant amount in either sample.

## 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 Sudbury River between 0.3 miles upstream of SUD-252 (furthest upstream site) and SUD-236 (furthest downstream site). We canoed and walked

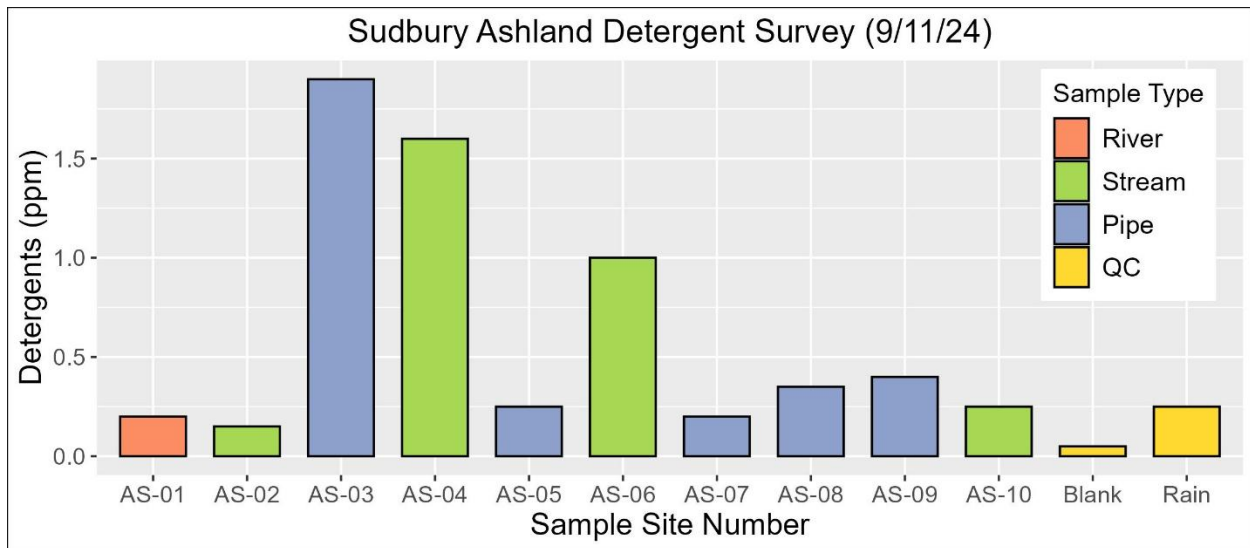
down the river on 9/11/24 and tested for detergents in each pipe that had flowing water, using a Chemets K-9400 test kit. There were many pipes along the river that did not have any flow at the time of the survey, which probably makes it safe to exclude them as dry-weather sanitary sewer sources. We did find several pipes and a side stream with noteworthy results (Figure 5). Pipes AS-03, AS-08, and AS-09 all had elevated results (see Figure 6 for pictures of the pipes). In particular, pipe AS-03 had extremely high detergent levels at 1.9 ppm. This pipe was located on river right (facing downstream) next to the parking lot for 50 Main Street. The side stream that we found also had very high detergent levels at or above 1 ppm. Sites AS-04 and AS-06 were both along this side stream, which runs parallel to the Sudbury River and joins the Sudbury 1100 feet upstream of site SUD-238. This stream originates between 50 Main Street and 98 Main Street at a municipal stormwater outfall (Figure 7).

This detergent survey clearly identified two pipes that need to be addressed. The AS-03 pipe behind the parking lot should be easy to map and resolve. This is most likely a short pipe from the 50 Main Street facility. The other pipe, the outfall pipe feeding the side stream and AS-04/AS-06, is connected to a large network of street sewers for the whole downtown area of Ashland. This will require more work on the part of the Public Works Department, but there are clearly some very strong pollution sources connected to this street sewer. The combination of high detergent levels in the stream and a bacteria hot-spot downstream of the stream's confluence and dry-weather human DNA downstream of the confluence is strong evidence of a sanitary sewer connection feeding this stormwater outfall.



*Figure 4: DNA results, analyzed by Jonah Ventures. Results are averages based on three replicates. The 6/17 sample was a wet-weather sample with moderately high flows. The 9/3 sample was dry-weather with very low flows.*

<sup>4</sup> The copy counts of 211 and 234 for human DNA are 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.



*Figure 5: Sudbury Ashland detergent survey 9/11/24. Samples listed left to right from upstream to downstream. Each bar represents one sample. See the map in the appendix for exact locations of each site. The two stream sites AS-04 and AS-06 are different locations on the same side stream.*



*Figure 6: Pipes with elevated detergent levels. Left to right: AS-03, AS-08, AS-09. AS-03 had very high levels – located river right facing downstream near the middle of the parking lot for 50 Main Street.*



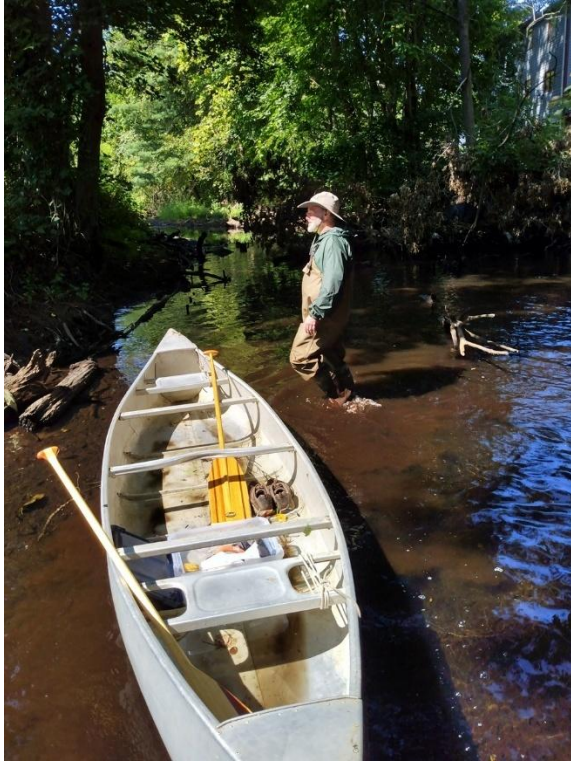


Figure 7: Ashland stormwater system map showing on the left side of the image the municipal outfall (red dot) from Main St. feeding the side stream (blue line) that was measured with high detergent levels. The Sudbury River is just north of the side stream.

## Conclusion

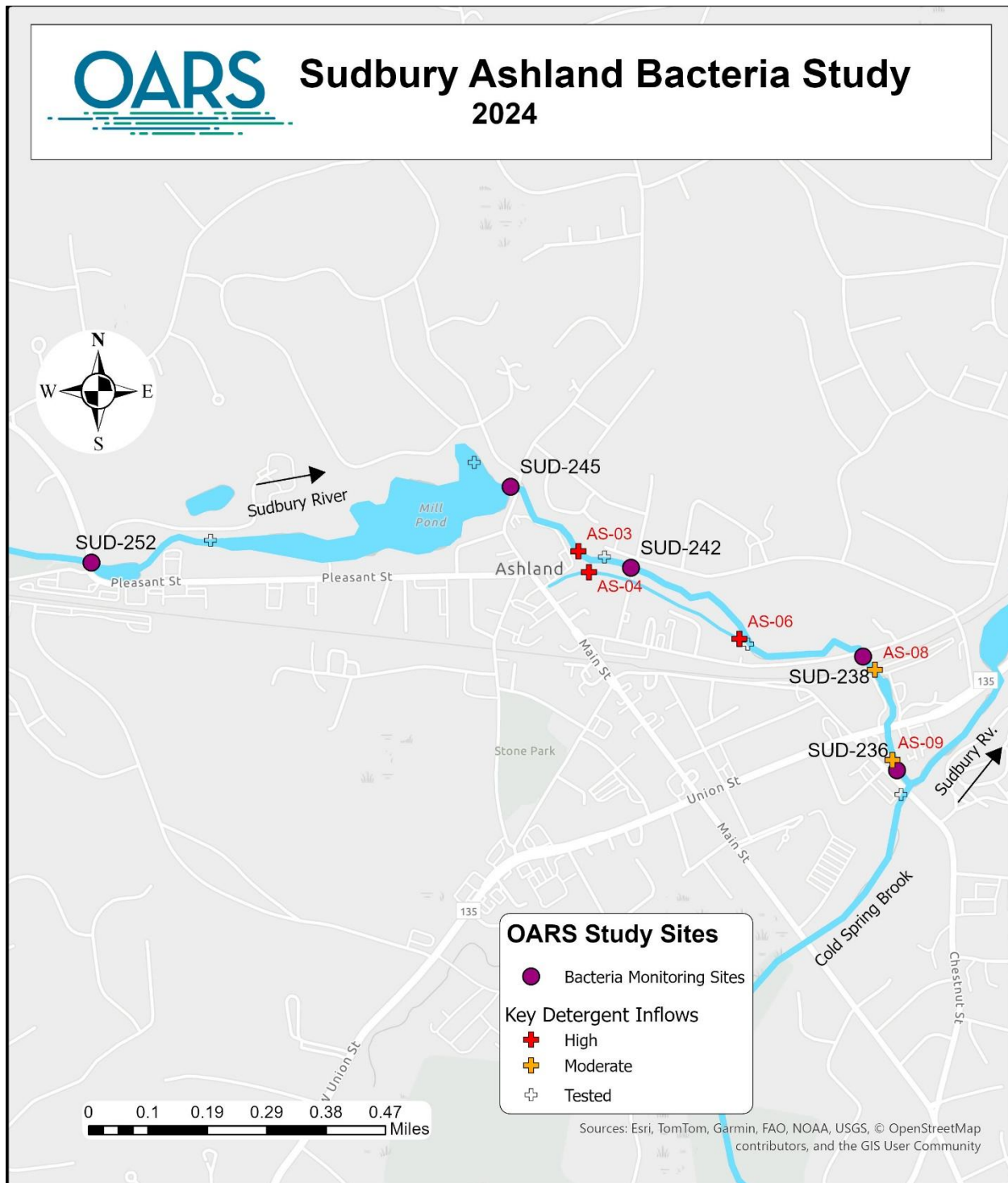
OARS' monitoring over the last six years has shown a persistent level of dry-weather and wet-weather bacterial pollution in the Sudbury River in Ashland. Of 71 samples collected over this period, 35% of them exceeded the EPA BAV swimming threshold. Our research this year has provided some very good leads about the source of the pollution. Focused bacteria monitoring highlighted the section of river upstream of the Front Street bridge as a probable source of dry-weather bacteria pollution and provided evidence that wet-weather pollution is influenced by beaver populations and is coming from upstream of the Cordaville Road bridge. The DNA analysis of water samples at our downstream site provided evidence that human sources constitute almost 100% of the bacterial contamination during dry weather and confirmed the influence of beavers in wet-weather contamination. The detergent testing identified two highly polluted pipes that we suggest the Ashland Department of Public Works should follow up on urgently and two other pipes that deserve some additional research. The combination of these three sets of evidence points to the municipal storm sewer outfall between 50 Main Street and 98 Main Street as the primary source of pollution in this section of the river. This outfall feeds the stream which measured high detergent levels and which joins the Sudbury just upstream of the bacteria monitoring hot spot at the Front Street bridge. And, the dominance of human DNA in the dry-weather DNA analysis confirms that there must be a sanitary sewer connection polluting this outfall. OARS is eager to work

with the town of Ashland to resolve these issues and continue monitoring the recreational safety of the river.



*Figure 8: Volunteer J. Clarke searching for discharge pipes.*

## Appendix A: Map of Study Area





## Appendix B: OARS site list with coordinates

Site #	DESCRIPTION	TOWN	WATERBODY	LATITUDE	LONGITUDE
SUD-236	Chestnut Street access	Ashland	Sudbury River	42.257609	-71.454952
SUD-238	Front Street bridge	Ashland	Sudbury River	42.260228	-71.455945
SUD-242	Concord Street bridge	Ashland	Sudbury River	42.262324	-71.463429
SUD-245	Mill Pond Dam	Ashland	Sudbury River	42.264444	-71.467519
SUD-252	Cordaville Road bridge	Ashland	Sudbury River	42.262492	-71.480271
AS-01	in river	Ashland	Sudbury River	42.262805	-71.476570
AS-02	Mill Pond offshoot	Ashland	Sudbury River	42.264606	-71.468345
AS-03	broken pipe, right side	Ashland	Sudbury River	42.262550	-71.465087
AS-04	parallel stream south side	Ashland	Sudbury River	42.262062	-71.464759
AS-05	pipe under blocks, left side	Ashland	Sudbury River	42.262413	-71.464279
AS-06	outflow of parallel stream	Ashland	Sudbury River	42.260517	-71.460067
AS-07	pipe, right side	Ashland	Sudbury River	42.260391	-71.459815
AS-08	overhanging pipe, right side	Ashland	Sudbury River	42.259799	-71.455841
AS-09	pipe, right side	Ashland	Sudbury River	42.257709	-71.455299
AS-10	Cold Spring Brook	Ashland	Sudbury River	42.256912	-71.455032

## Appendix C: Bacteria Data

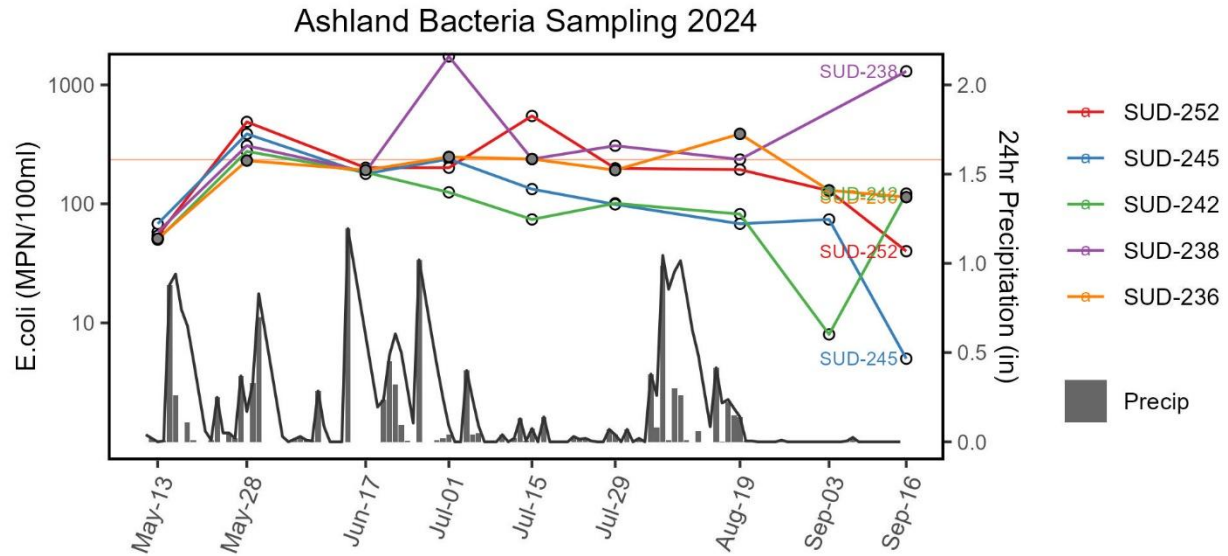


Figure 9: Bacteria and precipitation data for Sudbury Ashland sites. The downstream SUD-236 site is drawn with gray dots for reference. All the upstream sites are depicted with white circles. The red line represents the BAV threshold of 235 MPN/100ml. Daily precipitation is depicted with gray bars. The black line represents effective precipitation assuming the rainwater clears out of the system within 3 days.

### 2024 *E.coli* lab results MPN/100ml

Site #	Description	River	5/13	5/28	6/17	7/1	7/15	7/29	8/19	9/3	9/16
SUD-236	Chestnut Street access	Sudbury	51	231	192	248	238	192	387	130	114
SUD-238	Front Street bridge	Sudbury	58	308	186	1733	238	308	236		1300
SUD-242	Concord Street bridge	Sudbury	50	276	184	125	74	101	82	8	122
SUD-245	Mill Pond Dam	Sudbury	68	387	179	238	133	99	68	74	5
SUD-252	Cordaville Road bridge	Sudbury	54	488	201	201	548	199	194	129	40