



Pharmaceuticals and Personal Care Products as Indicators of Sewage Contamination in Urban Streams

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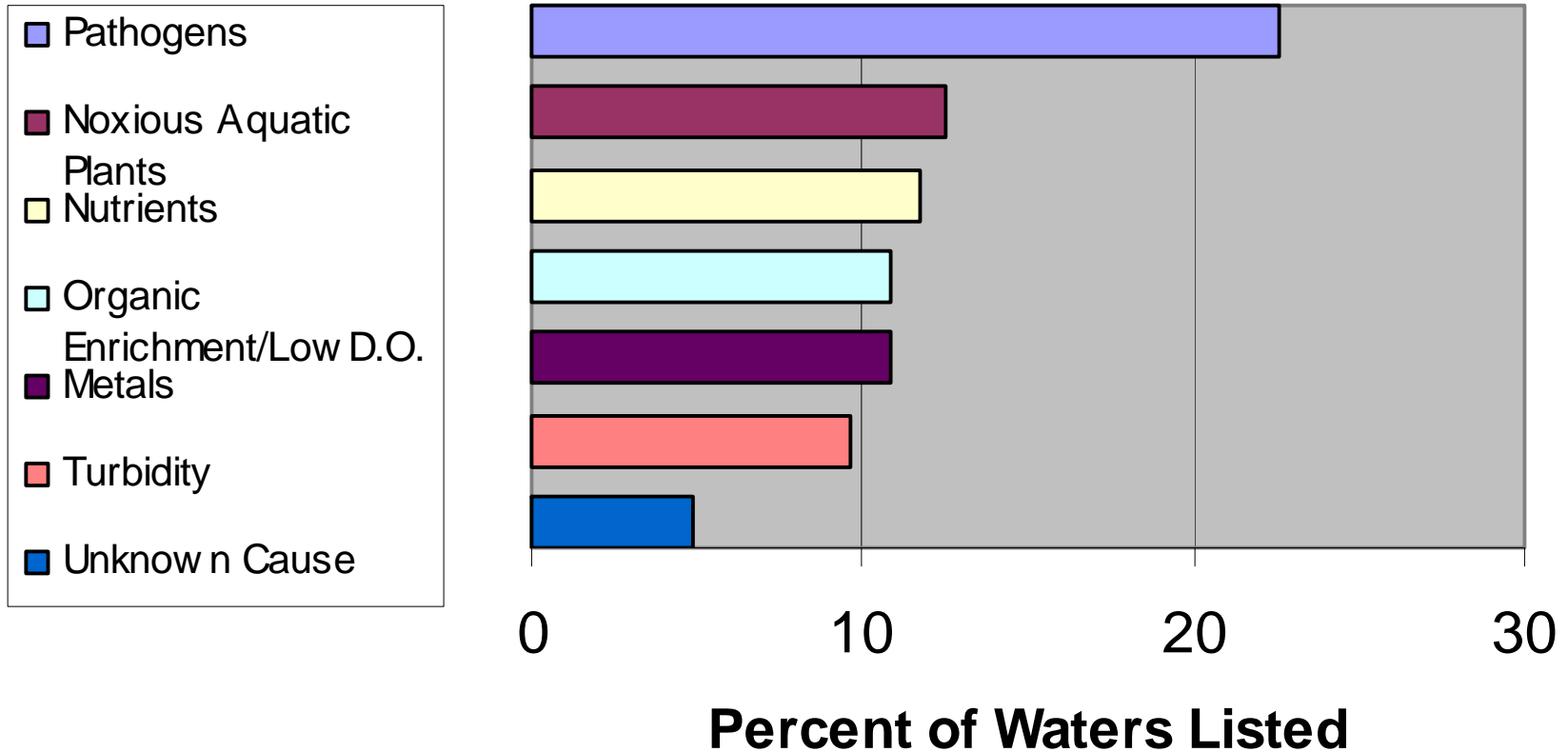
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U.S. Environmental Protection Agency, Office of Research and Development

Problem:

Figure 1
**Top Water Quality Impairments in Massachusetts (MA
 DEP 2002 Integrated List of Waters)**



Need for MST methods:

- Fecal-indicator bacteria are not specific to human sewage.
- Ability to distinguish human from non-human sources can inform potentially costly decisions as to how best to eliminate or manage the problem.
- MST can also help determine whether or not the response actually fixes the problem.
- Methods that attempt to match gene patterns in fecal-indicator bacteria with those in bacteria isolated from known sources (library-dependent methods) have not been successful.

Multiple-lines-of-evidence approach:

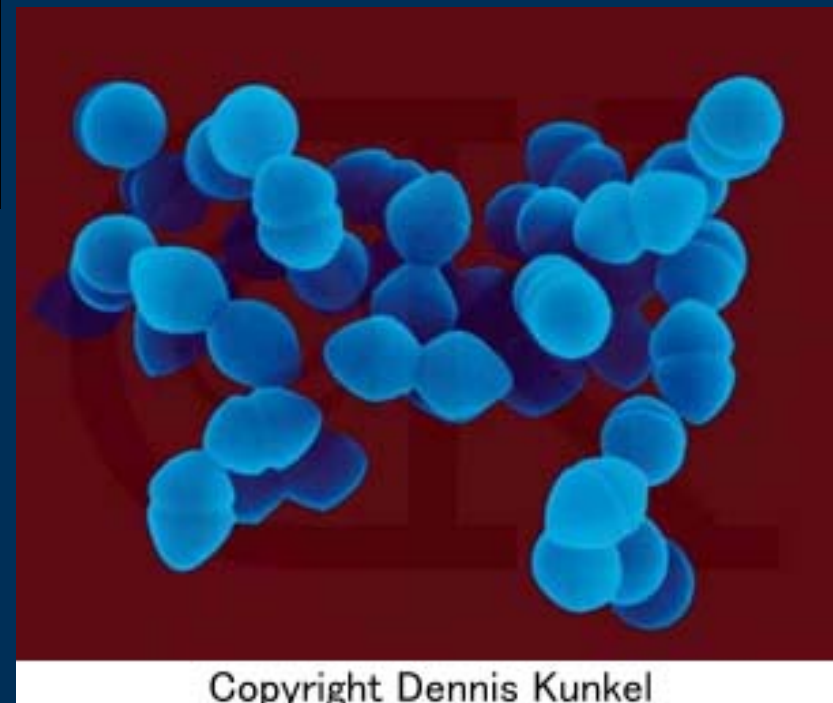
- Library-independent methods take advantage of established bacteria-host associations (human-specific genetic markers) and do not require culturing of bacteria.
- Multiple lines of evidence (including fecal-indicator bacteria counts, PCR assays for human-specific genetic markers, and chemical analysis for fluorescent whitening agents and PPCPs) can help account for false-positive and false-negative results inherent in all microbial source-tracking methods.

Potential library-independent target species:



Bernhard, A.E. et al., 2000, A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides/Prevotella genes encoding 16S rRNA: Applied and Environmental Microbiology, v. 66, no.10, p. 4571-4574.

Scott, T.M. et al., 2005, Potential use of a host-associated molecular marker in *Enterococcus faecium* as a index of human fecal pollution: Environmental Science and Technology, v. 39, no. 1, p. 283-287.



Study objectives

- Develop/refine PCR methods for detecting human-specific genetic markers in *Bacteroidetes* sp. and *Enterococcus faecium*.
- Validate the molecular methods by use of single-blind proficiency testing.
- Use multiple lines of evidence to document sewage contamination in tributary and mainstem sites in the lower Charles River watershed.



Fecal samples collected from known sources in the Lower Charles River watershed



USGS-WES single-blind proficiency test:

Sample id	Source	Date collected	WES determination based on detection or non-detection of <i>Bacteroidetes</i> human-specific marker
1	Human #1	8-23-05	Human
2	Cat	8-23-05	Human
3	Dog #1	8-24-05	Non-human
4	Human #2	8-24-05	Non-human
5	Human #3	8-23-05	Human
6	Dog #2	8-24-05	Non-human
7	Seagull	8-24-05	Non-human
8	Canada goose	8-31-05	Non-human
9	Human #1	8-23-05	Human
10	Blank	8-24-05	Non-human

Potential indicators of human sewage:

Bacterial indicators:

Fecal coliforms
E. coli
 Enterococci

Genetic indicators:

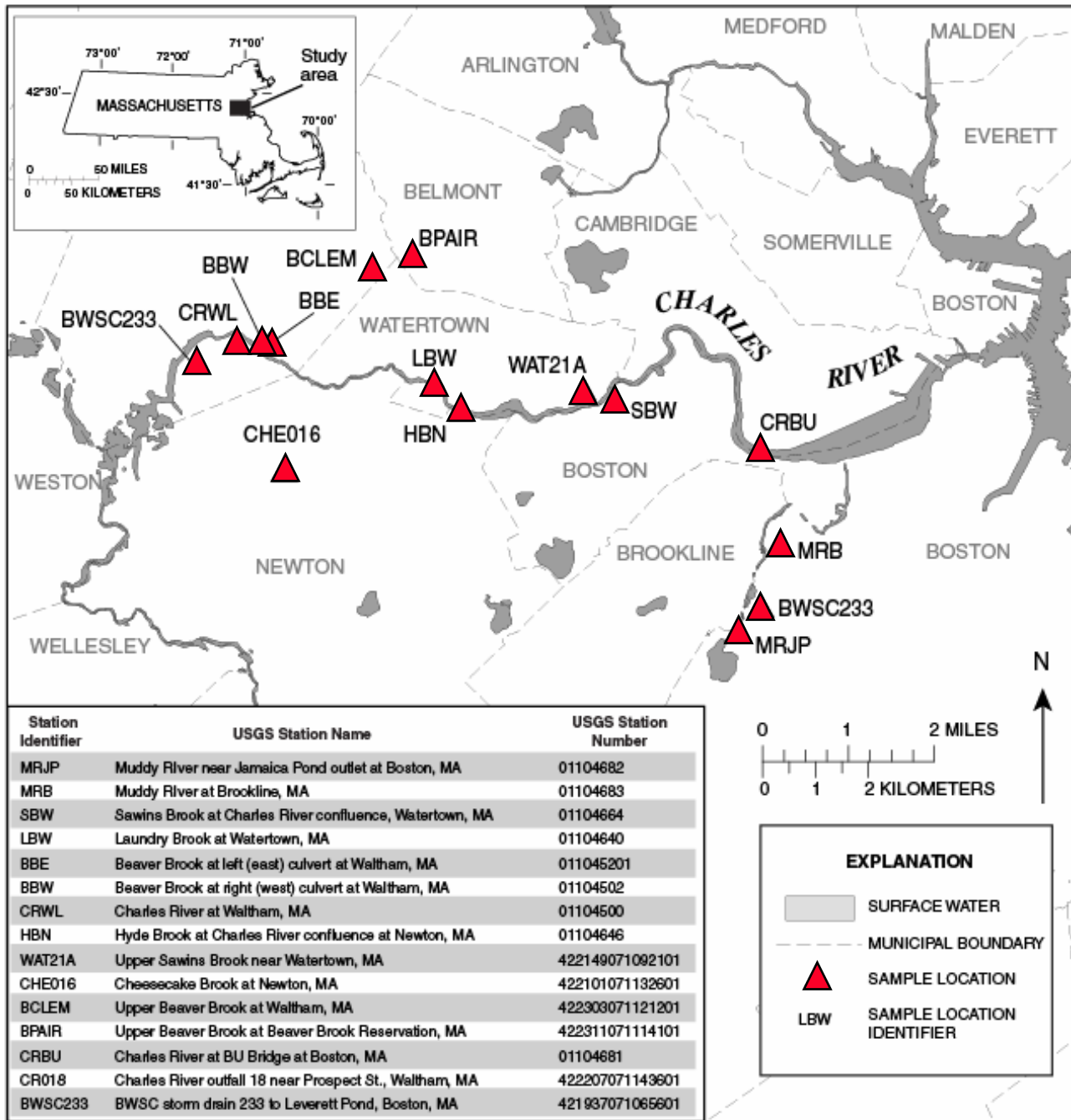
Bacteriodes human-specific genetic marker
Enterococcus faecium esp sewage marker

Whitening agents:

Optical brightener 1 (OB-1)
 Optical brightener 2 (OB-2)
 Fluorescent whitening agent 1 (FWA-1)
 Fluorescent whitening agent 2 (FWA-2)
 Fluorescent whitening agent 4 (FWA-4)

Pharmaceuticals and personal care products:

1,7-dimethylxanthine
 Acetaminophen
 Albuterol
 Caffeine
 Carbamazepine
 Codeine
 Cotinine
 Dehydronifedipine
 Diltiazem
 Diphenhydramine
 Fluoxetine
 Ranitidine
 Sulfamethoxazole
 Thiabendazole
 Trimethoprim
 Warfarin



Dry-weather sampling:



*Control site: Muddy River
below Jamaica Pond*

*Sample processing, Muddy River
at Brookline, Mass.*



Dry-weather sampling:



Hyde Brook at Charles River Confluence

Beaver Brook, East Culvert, at Charles River Confluence



Wet-weather sampling:



*Laundry Brook at Charles River
confluence near Watertown, Mass.*

*Laundry Brook culvert instrumented
with v-notch weir and pressure
transducer for monitoring stage*



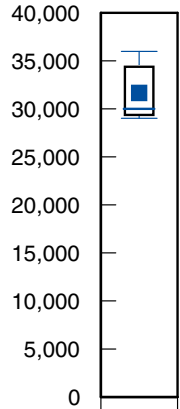
Frequency of detection (%)

<i>Sewage indicator</i>	Dry	Wet
Fecal coliform bacteria	94	100
<i>E. coli</i>	97	100
Enterococci	100	100
<i>Bacteroidetes</i> human-specific genetic marker	52	64
<i>Enterococcus faecium</i> esp sewage marker	26	7
OB-1	60	50
OB-2	0	0
FWA-1	53	57
FWA-2	10	0
FWA-4	0	0

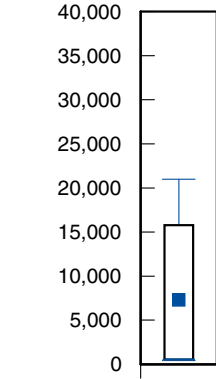
Frequency of detection (%)

<i>Sewage indicator</i>	Dry	Wet
1,7-dimethylxanthene	60	15
Acetaminophen	93	100
Albuterol	0	0
Caffeine	97	100
Carbamazapine	0	0
Codeine	0	0
Cotinine	90	92
Dehydronfedipine	0	0
Diltiazem	0	0
Diphenhydramine	0	0
Fluoxetine	0	0
Ranitidine	0	0
Sulfamethoxazole	0	0
Thiabendazole	0	0
Trimethoprim	0	0
Warfarin	0	0

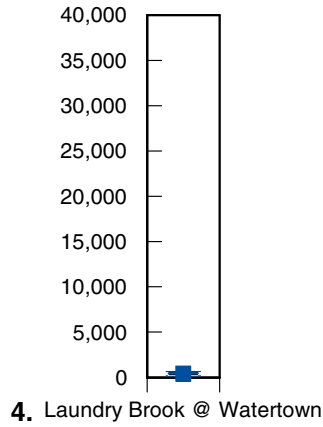
E. coli (CFU/100 mL)



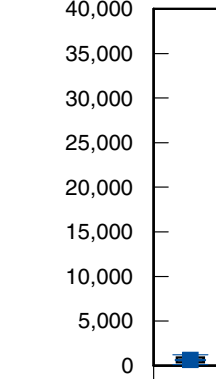
6. Beaver Brook @ west culvert



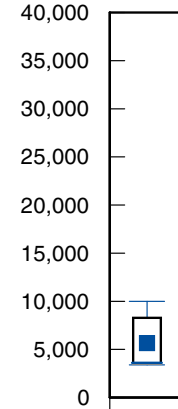
5. Beaver Brook @ east culvert



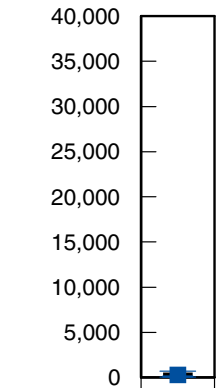
4. Laundry Brook @ Watertown



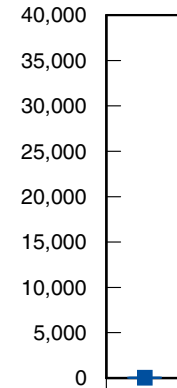
3. Sawins Brook @ Watertown



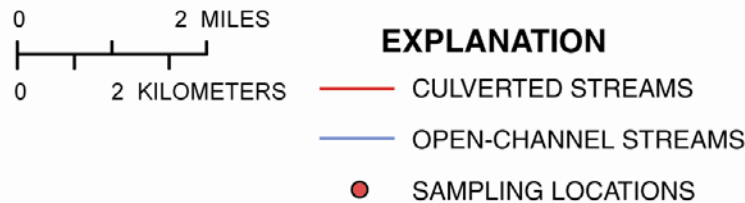
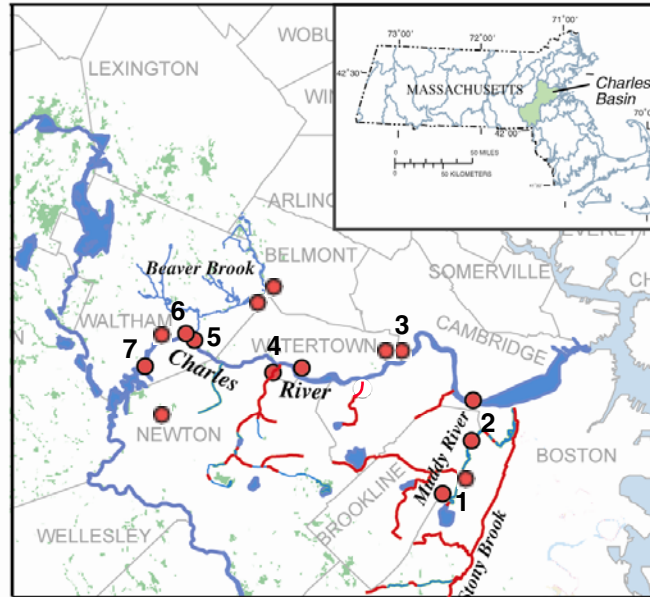
2. Muddy River @ Brookline



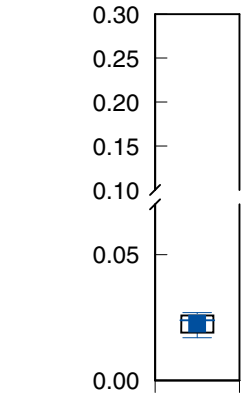
7. Charles River @ Waltham



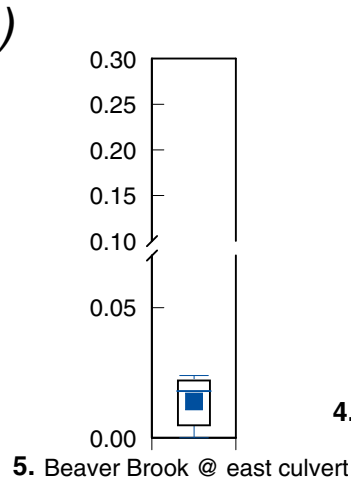
1. Muddy River below Jamaica Pond



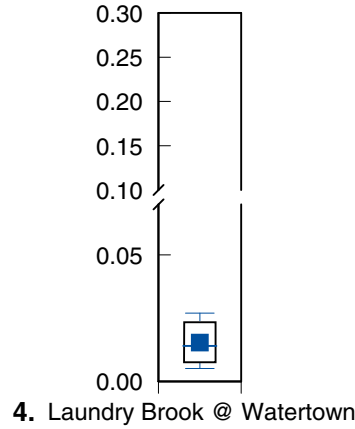
FWA-1 (g/L)



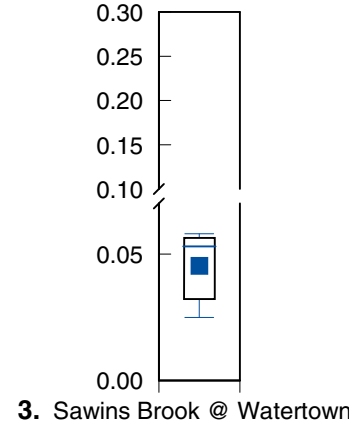
6. Beaver Brook @ west culvert



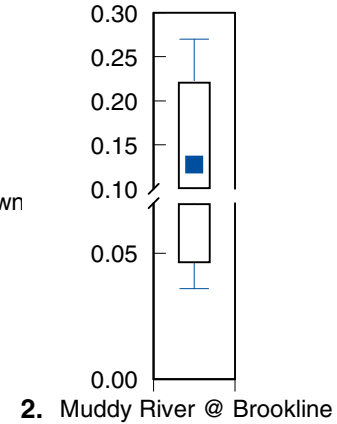
5. Beaver Brook @ east culvert



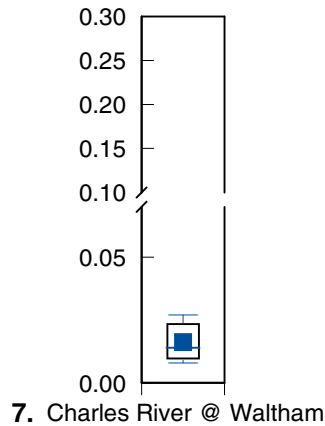
4. Laundry Brook @ Watertown



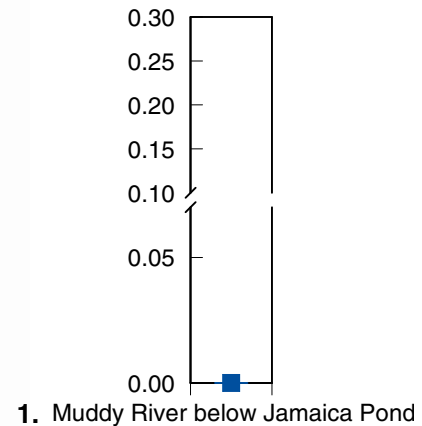
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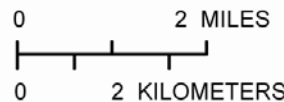
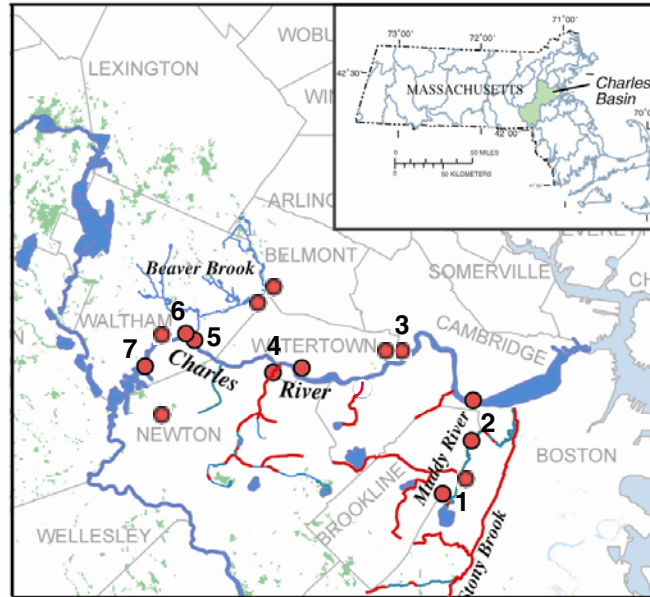
2. Muddy River @ Brookline



7. Charles River @ Waltham



1. Muddy River below Jamaica Pond

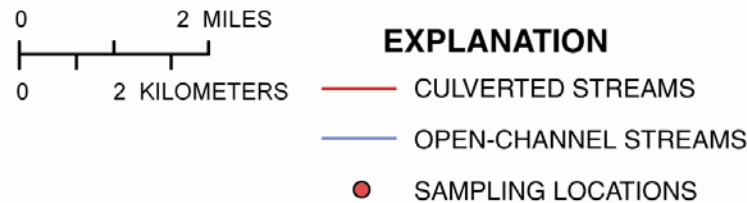
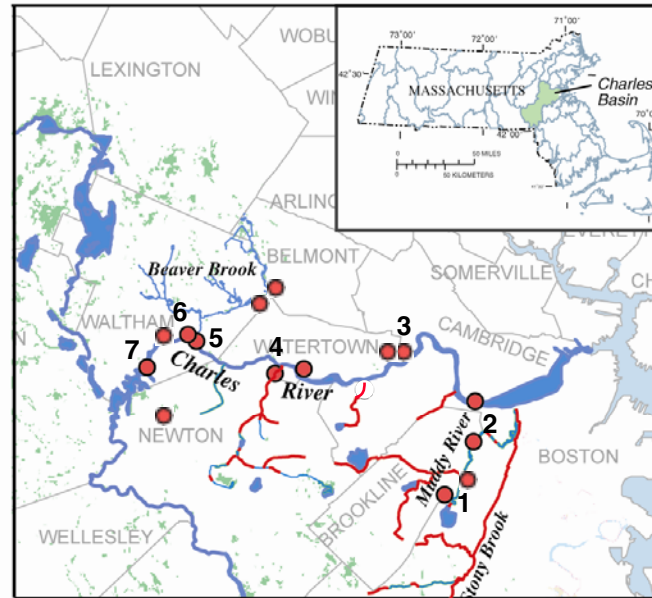
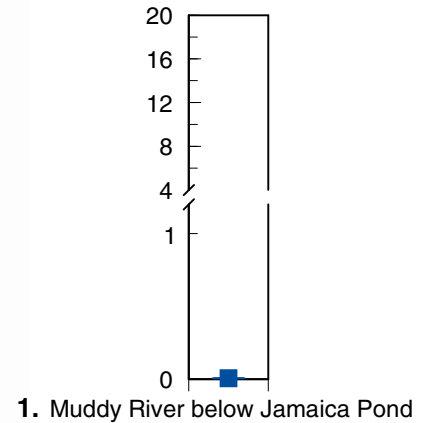
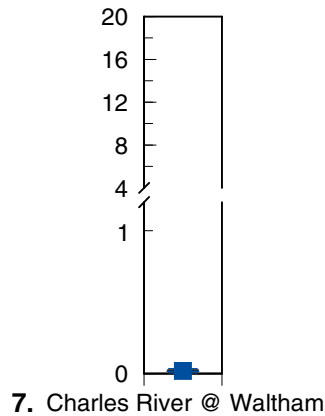
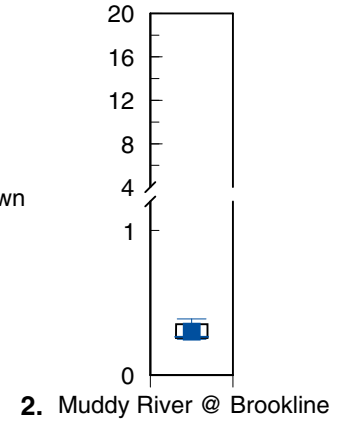
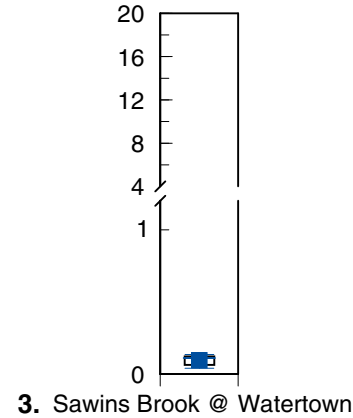
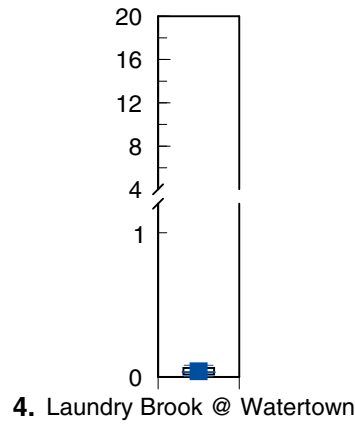
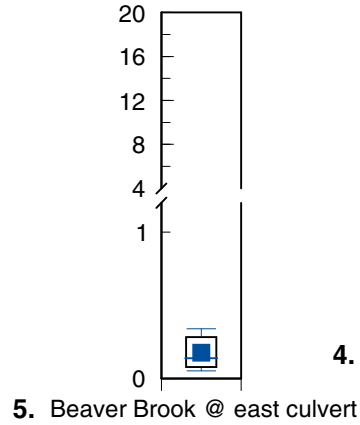
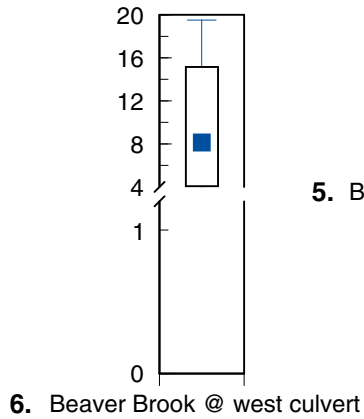


EXPLANATION

- CULVERTED STREAMS
- OPEN-CHANNEL STREAMS
- SAMPLING LOCATIONS



Acetaminophen (g/L)



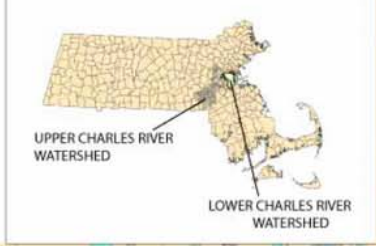
LAUNDRY BROOK

DRAINAGE AREA

12.7 km² (4.9 mi²)

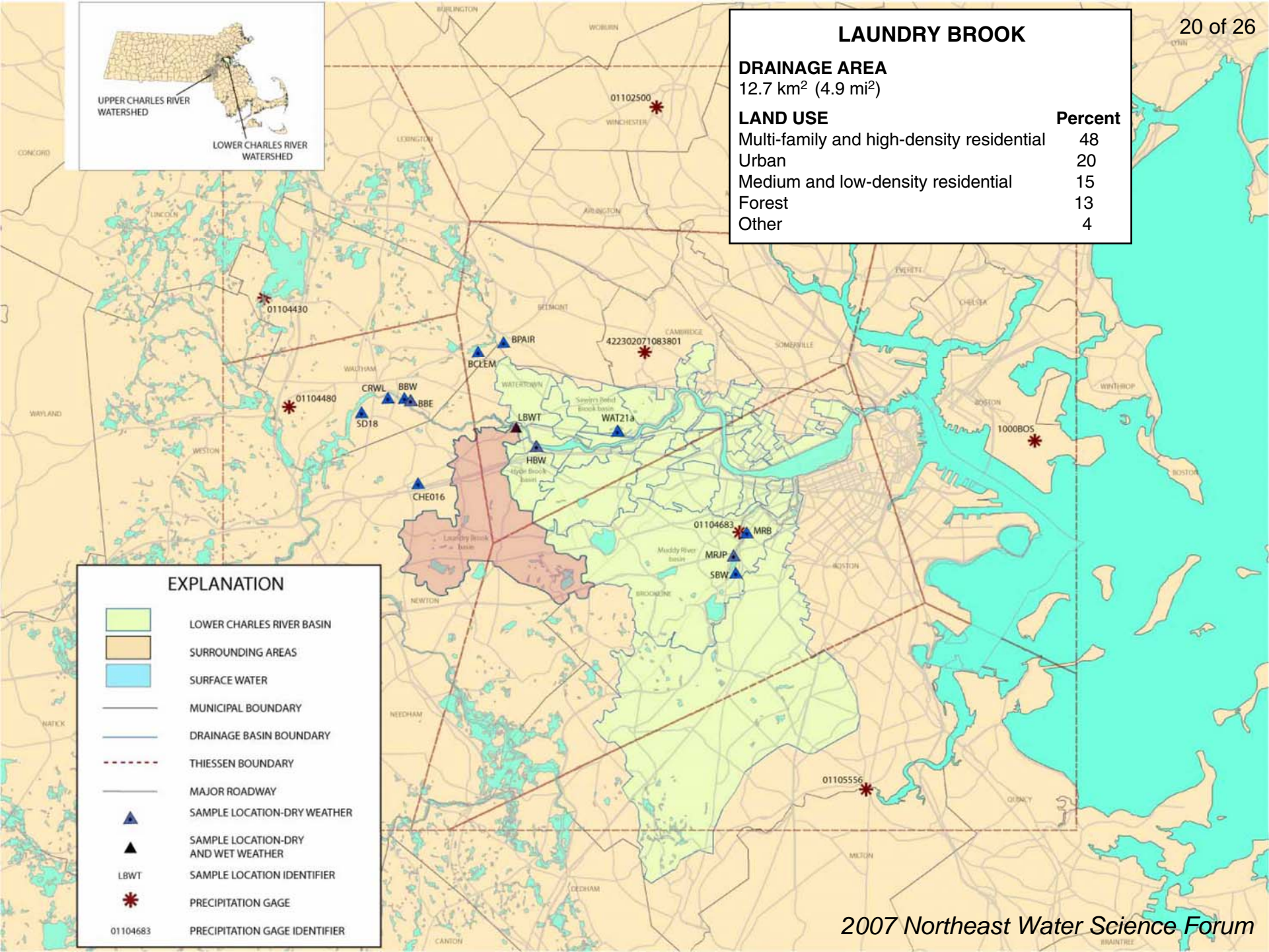
LAND USE

	Percent
Multi-family and high-density residential	48
Urban	20
Medium and low-density residential	15
Forest	13
Other	4

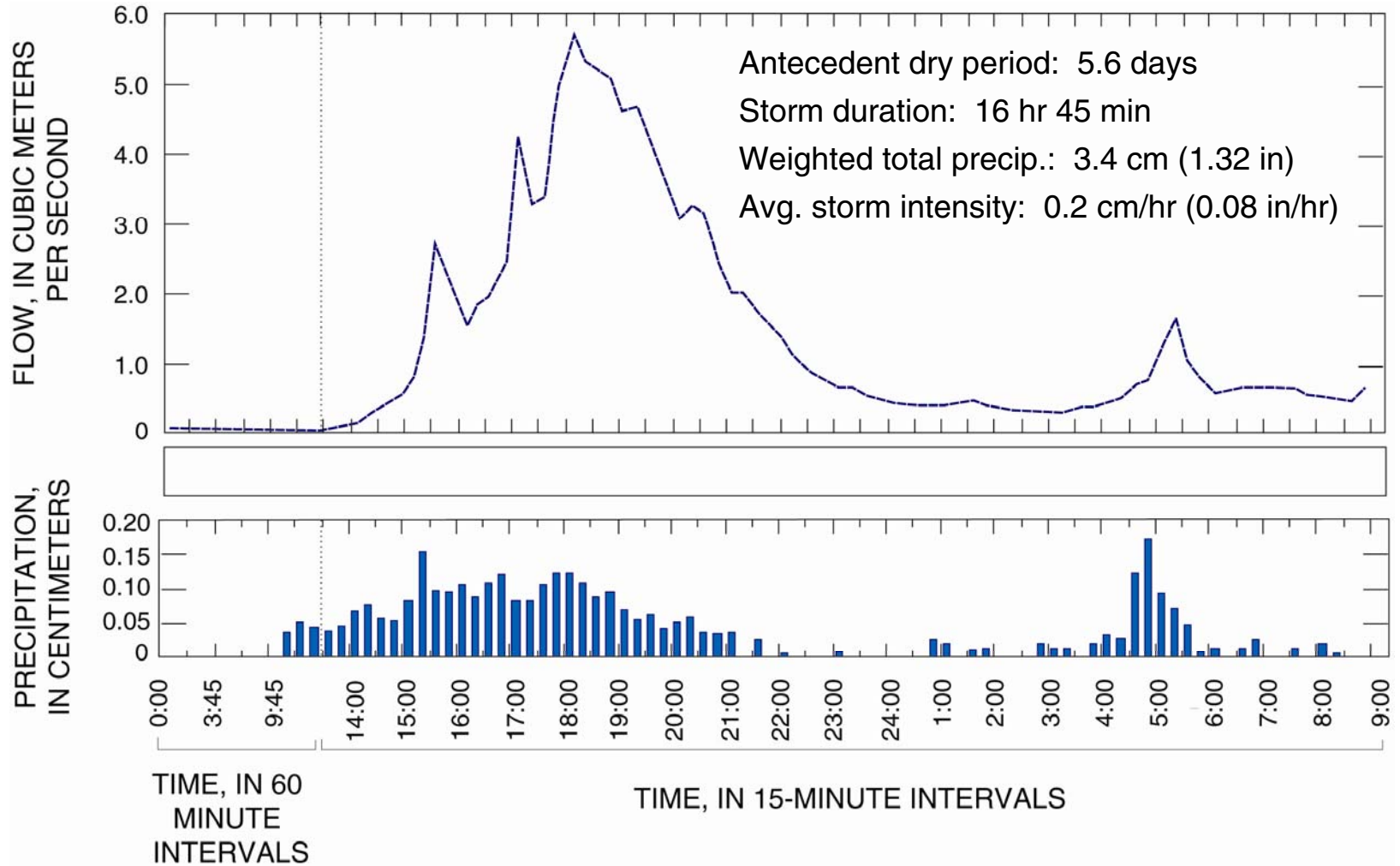


EXPLANATION

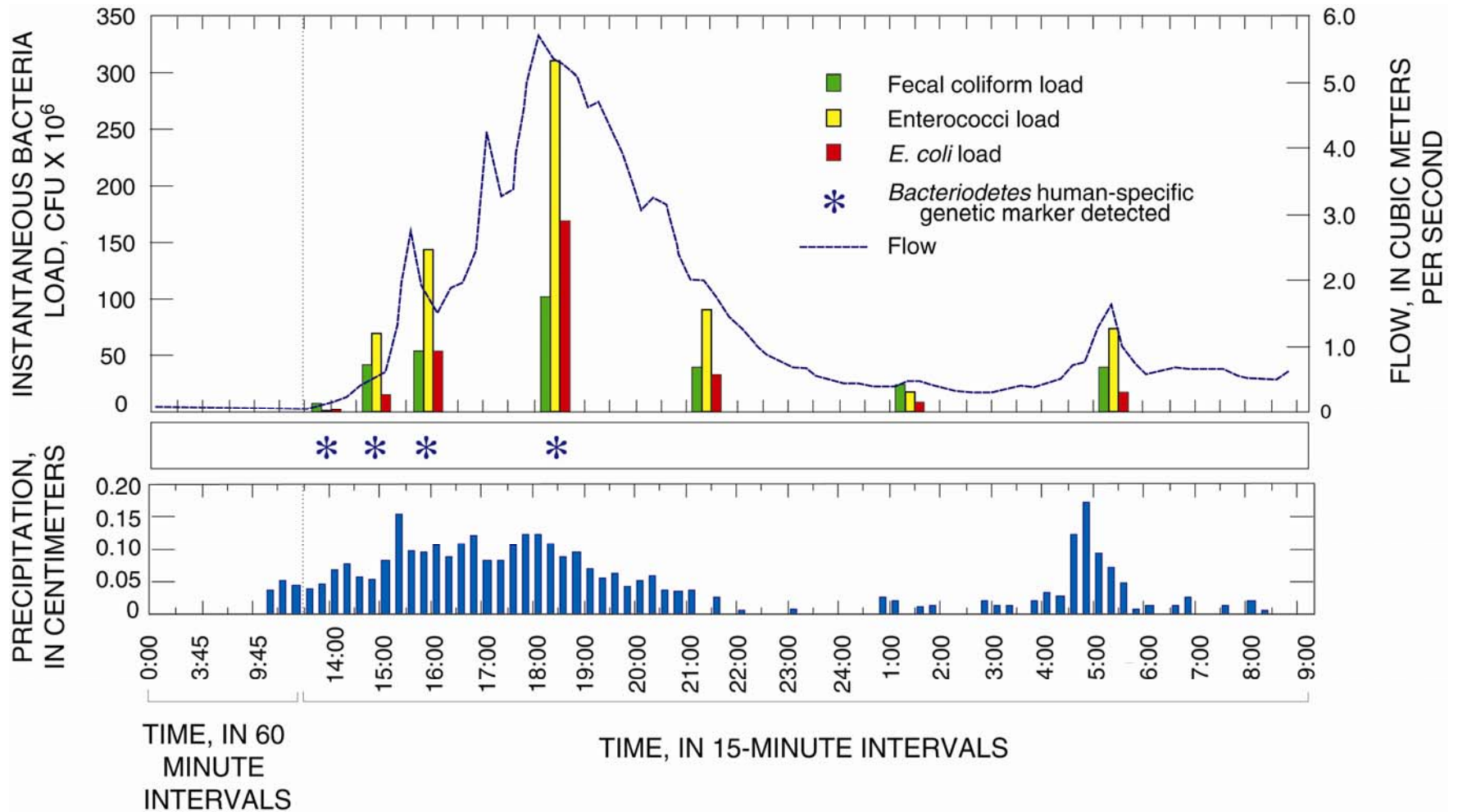
- LOWER CHARLES RIVER BASIN
- SURROUNDING AREAS
- SURFACE WATER
- MUNICIPAL BOUNDARY
- DRAINAGE BASIN BOUNDARY
- THIESSEN BOUNDARY
- MAJOR ROADWAY
- SAMPLE LOCATION-DRY WEATHER
- SAMPLE LOCATION-DRY AND WET WEATHER
- LBWT SAMPLE LOCATION IDENTIFIER
- PRECIPITATION GAGE
- 01104683 PRECIPITATION GAGE IDENTIFIER



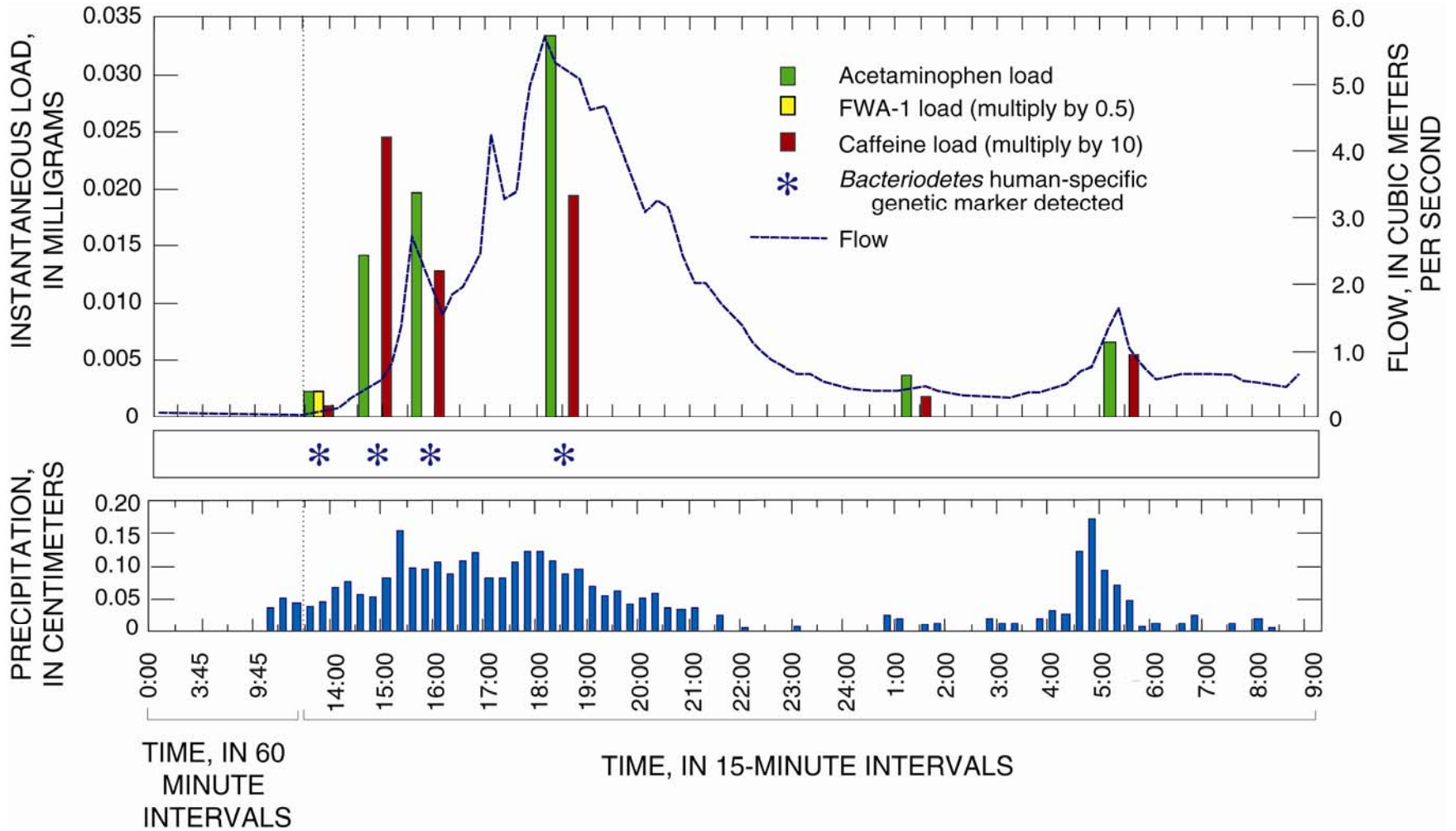
May 9-10, 2006 storm:



Fecal-indicator bacteria:



Pharmaceuticals and personal care products:



Fecal-indicator bacteria and PPCPs are highly correlated during dry-weather conditions...

Rank correlations (Kendal's tau-b) and significance values ($p > \text{tau-b}$) for microbial indicators and selected PPCPs in dry-weather samples

	Fecal coliforms								
Fecal coliforms		<i>E. coli</i>	Enterococci		OB-1	FWA-1	Acetaminophen	Caffeine	Cotinine
<i>E. coli</i>	0.850 ***								
Enterococci	0.657 ***	0.639 ***							
OB-1	0.286	0.415 *	0.415 *						
FWA-1	0.384 *	0.447 **	0.413 **	0.607 ***					
Acetaminophen	0.489 ***	0.521 ***	0.441 **	0.507 **	0.424 **				
Caffeine	0.304 *	0.321 *	0.382 *	0.503 **	0.358 *	0.460 ***			
Cotinine	0.049	0.128	0.230	0.214	0.099	0.119	0.185		
<i>p</i> -xanthene	0.324 *	0.410 *	0.490 ***	0.527 **	0.450 **	0.621 ***	0.387 *	0.161	

*** $p < 0.0001$

** $p < 0.001$

* $p < 0.05$



But not during wet weather:

Rank correlations (Kendal's tau-b) and significance values ($p > \tau\text{-}b$) for microbial indicators and selected PPCPs during the May 9-10, 2006 storm

Fecal coliforms							
Fecal coliforms	<i>E coli</i>			Enterococci			
<i>E coli</i>	0.113						
Enterococci	-0.109	0.539					
OB-1	---	---	---				
FWA-1	0	0	-0.432	---			
Acetaminophen	0.619	0.412	0.238	---	-0.150		
Caffeine	0.524	0.309	0.153	---	-0.150	0.905 *	
Cotinine	0.429	0.412	0.238	---	-0.250	0.810 *	0.905 *

*** $p < 0.0001$

** $p < 0.001$

* $p < 0.05$

Summary:

- Fecal-indicator bacteria were detected at all sampling sites; other, more specific indicators of human sewage were not always detected.
- Some PPCPs (e.g., acetaminophen) were detected at concentrations representing several orders of magnitude, making them highly useful indicators of sewage presence.
- Concentrations of fecal indicator bacteria are correlated with concentrations of whitening agents and PPCPs during dry weather, but not during storms, indicating that storms involve multiple sources (sewage and non-sewage).
- Multiple lines of evidence provide a more robust indication of sewage presence than any single test.