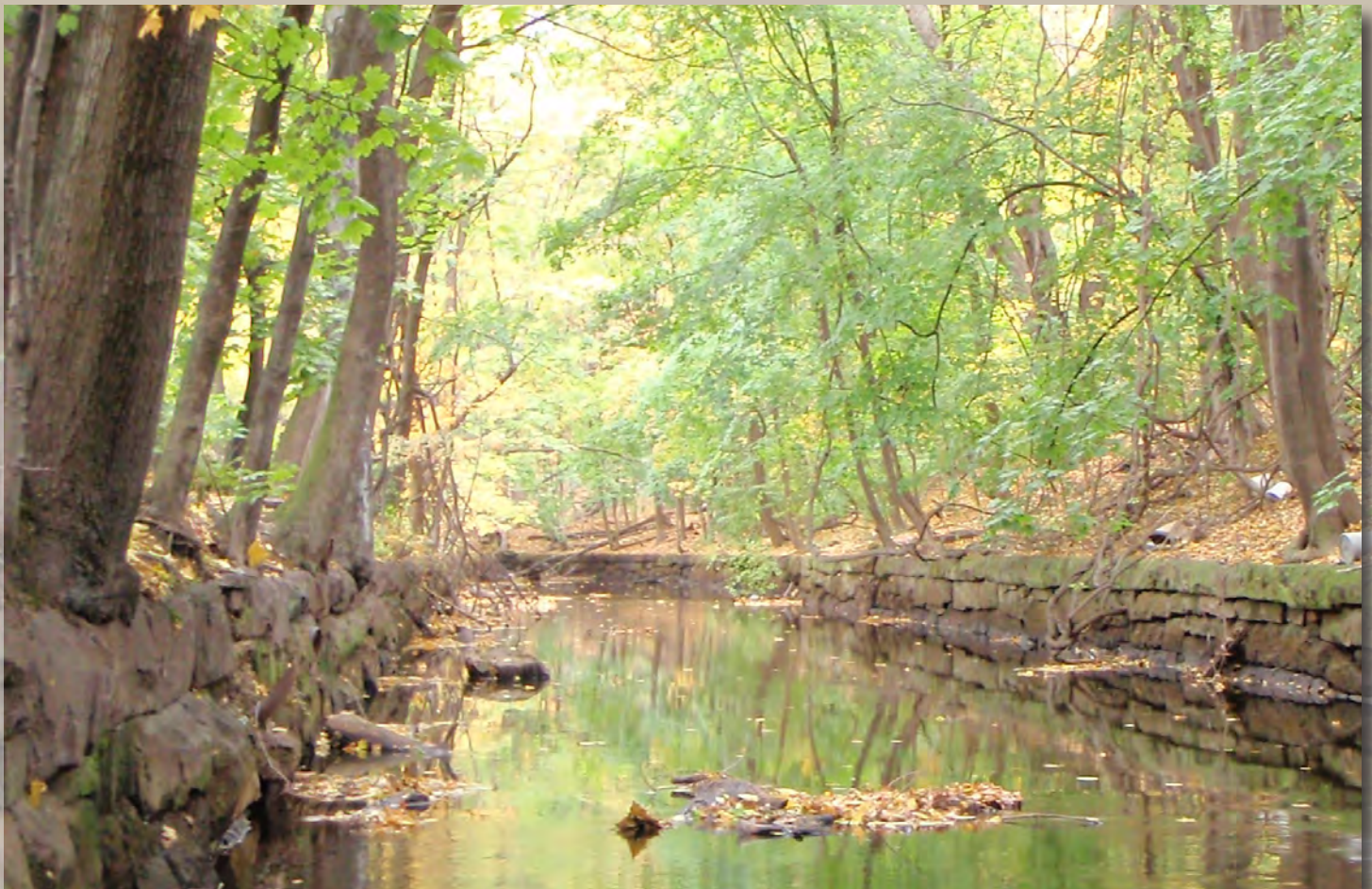Prepared in cooperation with the Pioneer Valley Planning Commission, the U.S. Environmental Protection Agency, and the Massachusetts Department of Environmental Protection

Assessment of the Presence of Sewage in the Mill River Under Low-Flow Conditions, Springfield, Massachusetts, 2010–11

Scientific Investigations Report 2019–5027

U.S. Department of the Interior
U.S. Geological Survey

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DAVID BERNHARDT, Secretary

U.S. Geological Survey
James F. Reilly II, Director

Acknowledgments

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Conversion Factors

U.S. customary units to International System of Units

Multiply	By	To obtain
Length		
inch (in.)	2.54	centimeter (cm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
Area		
square mile (mi^2)	2.590	square kilometer (km^2)
Volume		
gallon (gal)	3.785	liter (L)
Flow rate		
cubic foot per second (ft^3/s)	0.02832	cubic meter per second (m^3/s)
million gallons per year (Mgal/yr)	3,785	cubic meter per year (m^3/yr)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as °F = (1.8 × °C) + 32.

Datum

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Supplemental Information

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (μS/cm at 25 °C).

Concentrations of chemical constituents in water are given in micrograms per liter (μg/L).

Concentrations of bacteria in water are given in colony-forming units per 100 milliliters (CFU/100 mL).

Filter-mesh sizes are given in micrometers (μm).

Time is reported in military time format as hours and minutes.

Abbreviations

CSO	combined sewer overflow
CSS	combined sewer system
E. Coli	*Escherichia coli*
EPA	U.S. Environmental Protection Agency
EWI	equal width increment
FWA	fluorescence whitening agent
MassDEP	Massachusetts Department of Environmental Protection
NWQL	National Water Quality Laboratory
OB	optical brightener
PCR	polymerase chain reaction
QA	quality assurance
QC	quality control
RL	analytical reporting level
TCRTWI	Tri-State Connecticut River Targeted Watershed Initiative
USGS	U.S. Geological Survey
WES	Wall Experiment Station

Assessment of the Presence of Sewage in the Mill River Under Low-Flow Conditions, Springfield, Massachusetts, 2010–11

By Andrew J. Massey,[1] Marcus C. Waldron,[1] R. Jean Tang,[2] and Thomas G. Huntington[1]

Abstract

The U.S. Geological Survey, in cooperation with the Pioneer Valley Planning Commission, the U.S. Environmental Protection Agency, and the Massachusetts Department of Environmental Protection Senator William X. Wall Experiment Station, assessed the presence of 14 commonly used human-health pharmaceutical compounds, fecal indicator bacteria, and other man-made compounds indicative of the presence of human sewage in the lower reach of the Mill River near its confluence with the Connecticut River in Springfield, Massachusetts. The study was part of the Tri-State Connecticut River Targeted Watershed Initiative and involved the collection and analysis of raw river water at three sites along the reach, extending from Watershops Pond to the mouth, over the course of a low-flow period, July through November 2010. Previous studies in the region indicated that nonpoint or undocumented sources of wastewater contributed a variety of organic contaminants and potentially harmful bacteria to rivers under both high- and low-flow conditions. Additional samples, including a raw sewage sample collected near a Mill River combined sewer overflow during a non-overflow period, were collected in March 2011.

The study was designed to determine if city sewage or other domestic sources of wastewater were entering the river within this reach during low-flow conditions. No definitive evidence of sewage was measured in Mill River water samples collected during the study period. Fecal indicator bacteria, including *Escherichia coli* (*E. coli*) and enterococci bacteria, were detected in all Mill River water samples. In the DNA analysis of enterococci cultures from the Mill River, samples generally tested negative for the *Enterococcus faecium* (*esp*) human-specific genetic marker, whereas the raw sewage sample tested positive. Samples also generally tested negative in the human-specific rDNA marker assay for the anaerobic bacterium *Bacteroidetes*. Samples tested negative in 2010 for two *Bacteroidetes* human-specific genetic markers, HF134 and HF183, except samples from near the mouth of the Mill River, which tested positive. Samples collected in March 2011 from all three measurement sites tested positive for both markers. The results of bacterial analyses suggest that the fecal bacteria in summer and fall months are most likely of animal origin rather than human. Despite the urban setting, long history of development, and many potential sources of man-made contamination in the Mill River, none of the 12 water samples collected during the study contained targeted pharmaceutical compounds at concentrations greater than the analytical reporting levels. Other man-made compounds, like fluorescent whitening agents, were measured and detected in samples at low concentrations 4 out of 5 times the samples were collected; however, the other lines of evidence do not support a sewer source but rather other nonpoint sources upstream in the watershed.

The results of this study do not support the hypothesis that aging sewer lines or combined sewer overflow infrastructure leak into the Mill River as tested during the low-flow conditions during sampling for this study. None of the results from Mill River samples offer conclusive evidence of the presence of sewage. Some low-level detections of pharmaceutical compounds, other man-made chemicals, and bacteria suggest an upstream, nonpoint source.

A single raw sewage sample was collected, diluted, and examined for comparison with Mill River water samples and to ensure that the analytical methods could detect typical wastewater constituents. High levels of bacteria were measured, and low levels of three anthropogenic pharmaceutical compounds were detected, confirming the effectiveness of the sub-part-per-million method. The concentration of fluorescent whitening agent-1 in the sewage sample was 90,000 times greater than the median concentration in the Mill River samples.

[1]U.S. Geological Survey.

[2]Massachusetts Department of Environmental Protection.

Introduction

The city of Springfield, Massachusetts, located along the lower Mill River, has a long history of development. The city includes many underground sewers, and residential neighborhoods and suburbs have developed around the headwater reaches. The watercourse is an example of the highly urbanized tributaries typically found in and near cities along the main stems of major rivers in the eastern United States. Previous studies in the region indicated that nonpoint or undocumented sources of wastewater contributed a variety of organic contaminants and potentially harmful bacteria to rivers under both high- and low-flow conditions (Poiger and others, 1996; Breault and others, 2002; Barnes and others, 2008; Massey and Waldron, 2011). Aging public works infrastructure, high-density, onsite septic systems, and various nonpoint sources are among several possible sources of contaminants and bacteria.

The causes and sources of bacteria and contaminant loading from the surrounding landscape to the Connecticut River are not completely understood. One substantial knowledge gap is the extent to which inflows from urbanized tributaries, which may contain flows from relic and illicit sewer connections and various nonpoint sources, contribute to water-quality impairment during low-flow conditions. The Mill River component (this study) of the Tri-State Connecticut River Targeted Watershed Initiative (TCRTWI) was designed to quantify indicators of sewage and fecal bacteria in the most urbanized reach of the river during low-flow hydrologic conditions.

A longitudinal sampling approach was implemented as a first step to identify any in-river water-quality impairment during a low-flow period. Multiple sites were sampled from July through November 2010 to help geographically identify source areas of water-quality impairment. The objective of this study was to determine whether a suite of 14 commonly used human-health pharmaceutical compounds, fecal indicator bacteria, and other man-made materials indicative of human sewage could be detected in a reach of the Mill River under low-flow conditions. Another objective was to determine the source (human or animal) of fecal coliform bacteria found in the Mill River.

This study, a component of the TCRTWI, was designed and carried out by the U.S. Geological Survey (USGS) in cooperation with the Pioneer Valley Planning Commission and the U.S. Environmental Protection Agency (EPA). The TCRTWI is a cooperative project, funded by more than a dozen local organizations, that addresses some of the most important water-quality impairments in the Connecticut River watershed, including combined sewer overflows (CSOs), episodic river-bank erosion, increased threats to sources of public water supplies, and nutrient loading by runoff from agricultural operations and other nonpoint sources. Because of the presence of CSOs, several sections of the Connecticut River do not support recreational use designation (Connecticut Department of Environmental Protection, 2011).

Purpose and Scope

This report documents the Mill River component of the TCRTWI. The report describes the methods of selecting sites, measuring and processing field observations, collecting and analyzing water samples, and applying quality-assurance (QA) and quality-control (QC) procedures. Results are presented for field parameters, pharmaceutical compounds, fecal coliform bacteria and host-specific genetic markers, and fluorescent whitening agents. The results are discussed as evidence in the assessment of the presence of sewage in the Mill River during the low-flow study period. All data generated or analyzed during this study are included in the main text of this publication.

Hydrologic Setting of the Mill River, Springfield, Massachusetts

The Mill River is a tributary to the Connecticut River on the eastern slope of the Pioneer Valley in south-central Massachusetts (fig. 1). The Mill River drains a small (33-square-mile) watershed with diverse land use. Land use in the watershed ranges from mixed rural, agricultural, and forested land in the headwaters to suburban and dense urban development along the lower reaches down to the confluence with the Connecticut River at Springfield, Massachusetts. Watershops Pond, the impoundment formed by the Springfield Armory Watershops mill complex, marks the transition to the heavily developed urban reach of the Mill River along the final mile of the river. The pond is bordered by a narrow, wooded buffer along most of the shoreline; however, dense housing developments, schools, a large hospital, a cemetery, businesses, and industrial and other urban infrastructure are near the river corridor.

The lower Mill River has played an important role in the cultural and hydrologic history of Springfield. Its steep gradient, abundant water power, and proximity to the developing downtown made it an ideal location for mills and other heavy industry such as the Springfield Armory Watershops, which dammed the river at Allen Street. The Watershops were constructed in 1855 as the mill counterpart to the Springfield Armory "Hilltops," the primary campus of the Nation's first national armory, on State Street (fig. 2) (Bauer, 1975).

During the 19th and early 20th centuries it was common for untreated sewage to be discharged directly to the river. Remnants of outfall pipes remain along the man-made hardened walls of the river below Watershops Pond. Many improvements have been made to sewer and water infrastructure in Springfield, but at the time of this study (2010), the sewer system near the lower Mill River was still a combined sewer system (CSS) where up to 7 CSOs discharged approximately 3.2 million gallons per year (Mgal/yr) directly to the lower Mill River during intense rain or snowmelt events (Pioneer Valley Planning Commission, 2005b). The wastewater treatment plant that serves the sewered, urbanized part of the Mill River watershed is outside of the Mill River watershed,
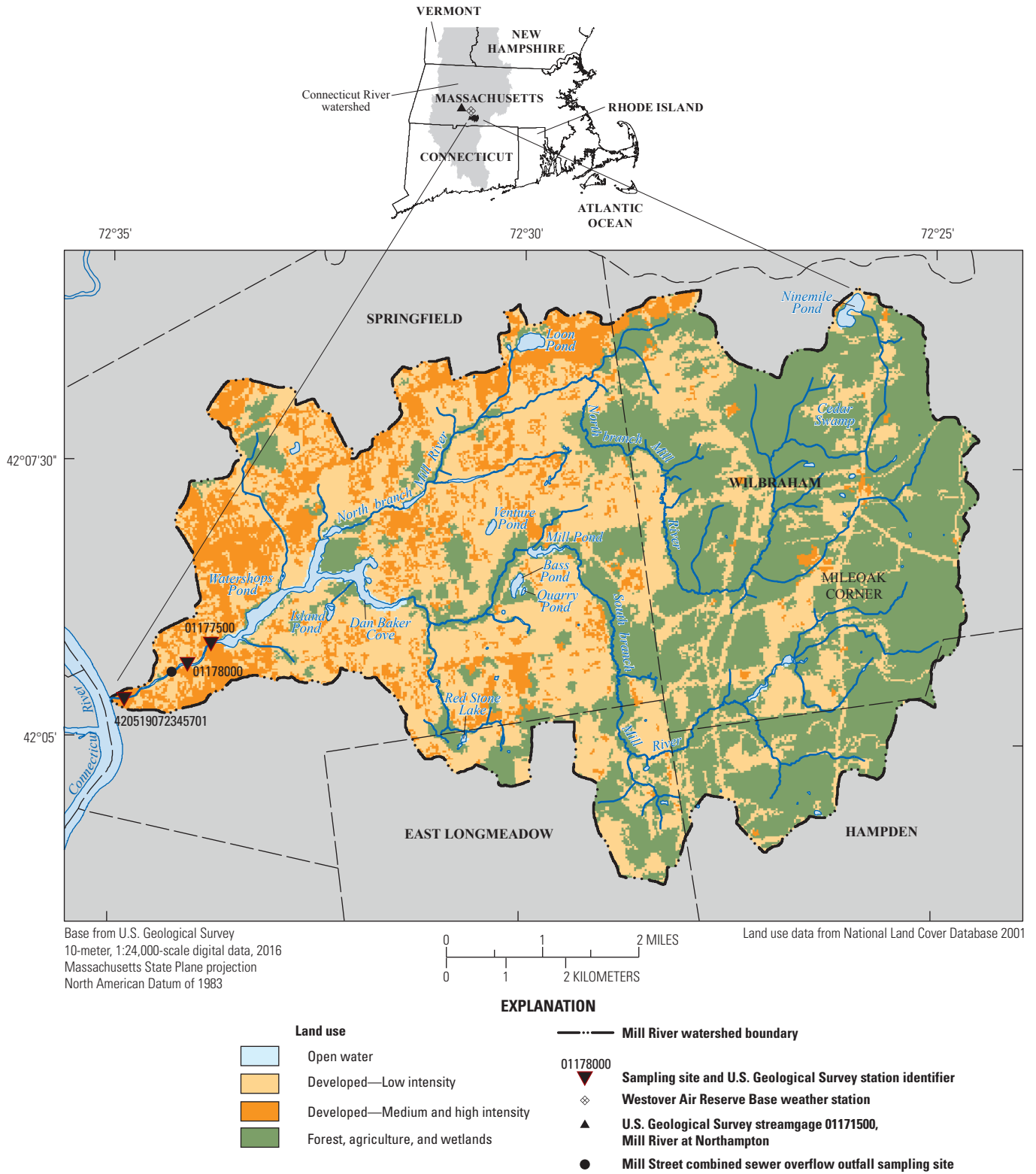
Figure 1. The Mill River watershed in central Massachusetts and the location of the study area, land use, and sample sites on the Mill River, Springfield, Massachusetts.

A

B

C

Figure 2.   The Mill River in Springfield, Massachusetts. *A*, Watershops mill complex. *B*, Mill River channel above Hancock Street. *C*, A relic drain. Photographs by Andrew J. Massey, U.S. Geological Survey.

and its outfall is to the Connecticut River downstream from the mouth of the Mill River.

The Mill River watershed has been developed for many uses since the founding of Springfield in 1636. Historic farms and old mill sites throughout the upper watershed took advantage of fertile soils, varied terrain, and proximity to the Connecticut River. During the 19th century, the lower river was extensively harnessed through several large mill complexes and was channelized for further control and use of its flow. The steep, rocky channel through Springfield's neighborhoods near the Mill River proved ideal and provided abundant power. Many man-made structures remain and continue to convey water through the Watershops area.

Potential Sources of Contamination in the Mill River Watershed

Several point and nonpoint sources potentially contribute wastewater contaminants to the Mill River. At the time of this study, there were several CSOs in the densely populated urban area along the Mill River near the confluence with the Connecticut River (Pioneer Valley Planning Commission, 2005a; U.S. Environmental Protection Agency, 2009). A CSO can contribute more pharmaceutical compounds than the associated wastewater treatment plant discharge, even though the total volume of water released by the CSO is much smaller (Phillips and others, 2012). Although CSOs are designed to

release wastewater under high-flow conditions, during which dilution rates in the receiving stream are high, some contaminants may remain in the receiving stream reach, mixed in with new sediment deposits, and may be remobilized long after the initial CSO release. The fate and persistence of some pharmaceutical compounds in surface waters, groundwater, and aquatic sediments is poorly understood. Some sewage indicator compounds are easily biodegraded or photo-oxidized, but other compounds can persist, particularly in aquatic sediments or along groundwater flow pathways (Fono and others, 2006; Cantwell and others, 2010; Radke and others, 2010; Musolff and others, 2010).

Combined sewer systems (CSSs) collect domestic wastewater for much of the Mill River watershed and convey it to a wastewater treatment plant. However, some CSSs discharge untreated sewage to surface waters during periods of heavy runoff (a CSO event); the overflow occurs when the outdated infrastructure exceeds sanitary-design capacity. CSSs, in which sewage and stormwater flow to a treatment plant through a single pipe, were in use in the area around the lower Mill River during the study. CSOs may convey untreated domestic, commercial, and industrial wastes along with stormwater runoff into receiving waters like the Mill River. Major efforts have been underway since the 1990s to address CSSs by separating stormwater and wastewater systems along the Connecticut River, and future efforts are proposed to address the remaining CSSs (Pioneer Valley Planning Commission, 2005a; Springfield Water and Sewer Commission, 2012). However, from the 1990s through the study period (2010–11), multiple heavy rainfall events per year still resulted in untreated wastewater discharge through CSOs. In 2005, the Pioneer Valley Planning Commission reported a CSO discharge reduction of 3.2 Mgal/yr to the Mill River (Pioneer Valley Planning Commission, 2005b), and in 2012, the Springfield Water and Sewer Commission reported extensive improvements to sewer infrastructure near the Mill River. These infrastructural improvements, mandated

through an administrative consent order issued by the EPA, further reduced Mill River CSO discharge by 98 percent, from an original 1990s-era discharge volume of 61.2 Mgal/yr to a current volume of 1.2 Mgal/yr (Springfield Water and Sewer Commission, 2012). CSOs may contribute smaller amounts of fecal coliform and *Escherichia coli (E. coli)* bacteria to the river system than other nonpoint sources on an annual basis yet, on a daily or weekly basis, be the main source of these and other anthropogenic contaminants to downriver sections of the river.

In more rural areas of the watershed, contaminants can have various nonpoint sources (U.S. Environmental Protection Agency, 2002; Brown and Trombley, 2009). In these rural areas, household wastewater is treated with onsite septic systems. Drainage from these systems, particularly if they are not functioning properly, can contribute contaminants to nearby surface waters (U.S. Environmental Protection Agency, 2014; Del Rosario and others, 2014; Phillips and others, 2015). Many residential areas in the Mill River watershed are on public sanitary sewer lines, through which sewage is transported to a regional treatment plant outside the watershed; however, aging infrastructure can result in leakage from pipes and seals, contributing to contamination of urban streams (Fono and Sedlak, 2005; Phillips and Chalmers, 2009).

Other nonpoint sources of contaminants and bacteria can also contribute to river water-quality impairment. Runoff from livestock-affected areas, domestic animal wastes from residential areas, application of fertilizer, and groundwater discharge containing poorly treated septic effluent may also contribute nutrients and bacteria to the river system (Kolpin and others, 2002; Barnes and others, 2008; Focazio and others, 2008). Water fowl, especially large flocks of migratory species, which typically stop only briefly at resting areas, may become year-round residents at locations with abundant food, rearing habitat, or supplemental feeding from human activity (fig. 3).

Figure 3.   Resident geese at Watershops Pond near Springfield College and Wesson Park, Springfield, Massachusetts. Photograph by Andrew J. Massey, U.S. Geological Survey.

Site Selection and Sample Collection

The Pioneer Valley Planning Commission, Connecticut River Joint Commissions, Franklin Regional Council of Governments, University of Massachusetts Water Resources Research Center, and USGS chose a portion of the Mill River below Watershops Pond (fig. 1) to address concerns about water quality in the Mill and Connecticut Rivers. These concerns centered on the densely populated, urbanized lower reaches of Mill River because of the uncertainty associated with contaminant inputs from nonpoint sources under low-flow conditions. Initially, two sample sites were chosen along the river to allow comparisons of water quality between upriver (near the outlet of Watershops Pond, USGS site number 01177500) and downriver (Hancock Street, USGS site number 01178000) sites (fig. 1). Water samples were collected four times, each with an antecedent dry period of at least 3 days, following a monthly schedule from late July 2010 through early November 2010 (fig. 4). In October, a third sample site (USGS site number 420519072345701) was added at the river mouth, just upriver from the confluence with the Connecticut River. Water samples were collected by using trace-level (parts-per-billion) protocols (Wilde and others, 2004). Samples were collected under low-flow conditions to determine whether wastewater contaminants indicative of sewage were present in the Mill River under these conditions, as have been observed for other rivers in New England.

Samples of Mill River water were collected by using various equipment and procedures depending on the conditions at each site, following guidance from the USGS "National Field Manual for the Collection of Water-Quality Data" (U.S. Geological Survey, variously dated). Where access was available and where water depth was sufficient, a US DH–81 wading sampler was used. Where wading was not possible but water depth was sufficient and access was appropriate, a tethered weighted-bottle sampler equipped with a 1-liter sample bottle of baked amber glass was used. At certain sites, where water depths were too shallow to permit sampling with a US DH–81 or weighted-bottle sampler, grab samples were collected in sterile plastic flasks or 3-liter Teflon bottles. For grab samples, water was collected by multiple grabs from several verticals to approximate equal-width-increment (EWI) procedures (Wilde and others, 2004). This adaptation is required for low-water conditions, where flow velocities are usually inadequate to meet the strict EWI criteria. All samples were immediately put on ice after collection and transported to the Massachusetts Department of Environmental Protection (MassDEP) Senator William X. Wall Experiment Station (WES) laboratory in Chelmsford, Massachusetts, or the USGS laboratory in Northborough, Massachusetts, on the same day that they were collected. Samples for pharmaceutical analysis were shipped chilled overnight to the USGS National Water Quality Laboratory (NWQL) in Lakewood, Colorado.

A grab sample of raw sewage was collected from the Springfield sewer at Mill Street, near the Mill Street CSO (fig. 1), on March 17, 2011. The sample was diluted with deionized water (50:1) prior to analysis. This raw sewage sample was analyzed for the same wastewater contaminants as the samples from the Mill River. Two samples were also
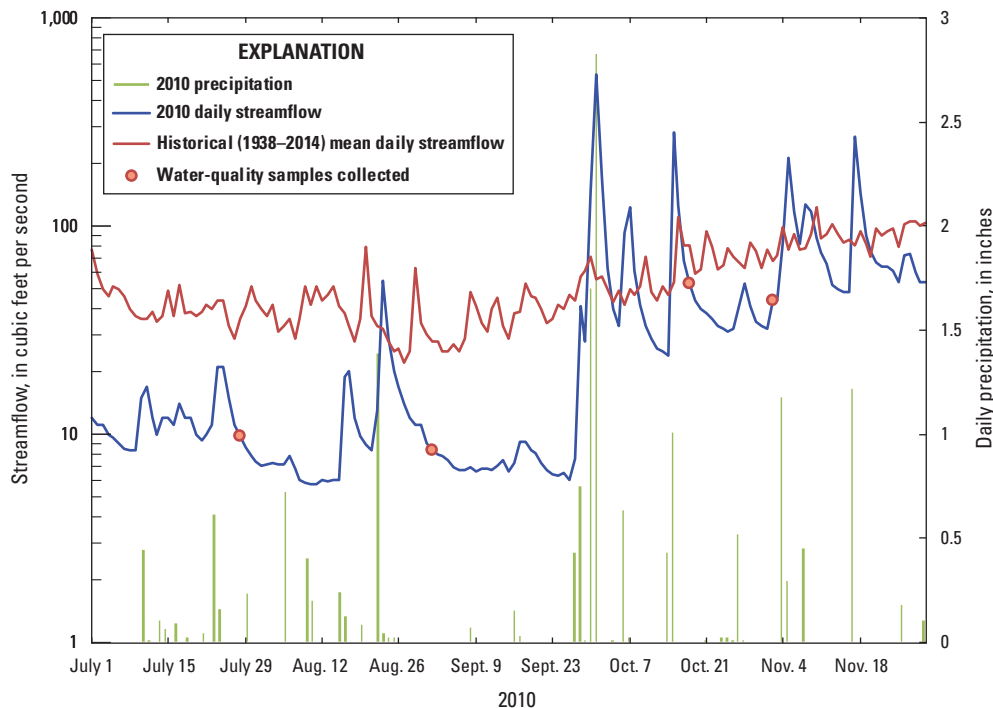

Figure 4. Precipitation near the Mill River recorded at Westover Air Reserve Base, Massachusetts, sample dates for the period from July 1, 2010, through November 30, 2010, daily streamflow at the nearby Mill River in Northampton, Massachusetts (USGS streamgage 01171500), during this period, and historical mean daily streamflow for the period November 1938 to September 2014.

collected from the Mill River on March 17, 2011. One sample was collected from the site upriver and one from the site downriver of the Mill Street CSO site (fig.1). The Springfield Water and Sewer Commission reported that the CSO had been triggered by a rain/snowmelt event on March 16, 2011, but had ended and was not flowing into the Mill River at the time of sampling (0800 March 17, 2011). The raw sewage sample was collected for comparison of its results with those of the samples collected from the Mill River, to determine whether the pharmaceutical compounds and fluorescence whitening agents (FWAs) that were assessed in the Mill River samples were present in raw sewage, and to determine if constituents from the CSO release were detectable downstream from the outfall.

Analysis for Sewage Constituents

Field parameters were measured on each day when water samples were collected for laboratory analyses of wastewater constituents. Water samples were collected and analyzed for several indicators of anthropogenic wastewater contaminants, including pharmaceutical compounds, fecal coliform bacteria, and FWAs. Fecal coliform bacteria were enumerated and analyzed for host-specific genetic markers to determine whether they were of human or nonhuman origin. For quality assurance, water samples were split and analyzed independently for *E. coli* bacteria by USGS and by WES.

Pharmaceutical Compounds

Water samples were collected and analyzed for pharmaceutical compounds by using methods developed by USGS (Furlong and others, 2008). Mill River water samples were filtered through a 0.47-micrometer glass-fiber filter on a free-standing aluminum filter plate placed inside an isolation chamber. A Teflon diaphragm pump with C-Flex tubing was used to force the raw-water sample through the filter into 1-liter baked amber-glass sample bottles. Samples were analyzed by NWQL for 14 pharmaceutical compounds and metabolites (table 1) by solid-phase extraction onto chemically modified styrene-divinylbenzene resin, followed by high-performance liquid chromatography/mass spectrometry (Furlong and others, 2008). In this report, analytical results are presented if the concentration was greater than the NWQL reporting level (RL) or as detected if they were observed at concentrations less than the RL. The RL is defined as two times the long-term analytical method detection limit observed by the laboratory (Furlong and others, 2008). Adjustments to RLs are commonly made by the NWQL and are based on statistical quantification of the analytical-method performance. An explanation of how RLs are determined is available elsewhere (Childress and others, 1999).

Table 1. Human-health pharmaceutical compounds measured in water from the Mill River at three sites from Watershops Pond to the mouth above the Connecticut River, Springfield, Massachusetts, 2010–11.

Compound name	Chemical Abstracts Service Registry Number (CASRN)[1]	Primary use
1,7-dimethylxanthine	611-59-6	Caffeine metabolite
Acetaminophen	103-90-2	Antipyretic
Albuterol (Salbutamol)	18559-94-9	Antiasthmatic
Caffeine	58-08-2	Stimulant
Carbamazepine	298-46-4	Antiepileptic
Codeine	76-57-3	Analgesic
Cotinine	486-56-6	Nicotine metabolite
Dehydronifedipine	67035-22-7	Antianginal
Diltiazem	42399-41-7	Antihypertensive
Diphenhydramine	147-24-0	Antihistamine
Sulfamethoxazole	723-46-6	Antibiotic
Thiabendazole	148-79-8	Antifungal, antihelmintic
Trimethoprim	738-70-5	Antibiotic
Warfarin	81-81-2	Anticoagulant

[1]This report contains CAS Registry Numbers®, which is a registered trademark of the American Chemical Society. CAS recommends the verification of the CASRNs through CAS Client Services℠.

Field Parameters

Measurements of field parameters (temperature, specific conductance, pH, and dissolved oxygen) were made with a multiparameter water-quality-monitoring instrument that was calibrated before each use on the day of sampling in accordance with manufacturer's instructions and USGS protocols (Wilde and others, 2004). During deployment at all sample sites, the instrument was lowered into the thalweg to a depth of about 0.3 meter below the water surface (table 2).

Fecal Coliform Bacteria and Host-Specific Genetic Markers

Three types of bacteria were assessed for potential human sewage origin in this study: *Bacteroidetes, Enterococcus*, and *E. coli*. All three are common bacteria associated with fecal contamination from humans and warm-blooded animals; *Enterococcus* and *E. coli* are also indicator organisms that are widely used in testing and regulating water quality. *E. coli* and enterococci bacteria were enumerated in Mill River samples by using modified mTEC agar and membrane filtration

Table 2.   Field parameters measured in river water and bacteria results from samples collected intermittently at four sites at the Mill River, Springfield, Massachusetts, from Watershops Pond to the mouth above the Connecticut River, July 2010 through March 2011.

[*Escherichia coli* (*E. coli*) samples were independently analyzed by U.S. Geological Survey (USGS) and Massachusetts Department of Environmental Protection Wall Experiment Station (WES) personnel. Results for *E. coli* replicate samples are in parentheses. °C, degree Celsius; µS/cm at 25 °C, microsiemens per centimeter at 25 °C; mg/L, milligram per liter; CFU/100 mL, colony forming unit per 100 milliliters; --, not sampled; <, less than; >, greater than]

Sample site	Temperature (°C)	Specific conductance (µS/cm at 25 °C)	pH (standard units)	Dissolved oxygen (mg/L)	E. coli (USGS) (CFU/100 mL)	E. coli (WES) (CFU/100 mL)	Enterococci (WES) (CFU/100 mL)
July 28, 2010							
Mill River at Watershops Pond— 01177500	25.4	321	8.83	10.3	--	19	14
Mill River at Hancock Street—01178000	24.9	326	8.31	6.93	2,500	2,200 (1,800)	1700
Mill River at Mouth—420519072345701	--	--	--	--	--	--	--
September 1, 2010							
Mill River at Watershops Pond—01177500	24.7	307	9.69	14.2	<10	<5	14
Mill River at Hancock Street—01178000	25.4	307	9.46	6.36	1,825	1,800 (2,100)	1200
Mill River at Mouth—420519072345701	--	--	--	--	--	--	--
October 18, 2010							
Mill River at Watershops Pond—01177500	12.7	263	6.81	6.36	23	10	3
Mill River at Hancock Street—01178000	12.6	266	7.28	9.24	157	120	210
Mill River at Mouth—420519072345701	12.4	267	7.66	9.82	230	210	260
November 2, 2010							
Mill River at Watershops Pond—01177500	10.7	284	7.17	7.22	12	10	3
Mill River at Hancock Street—01178000	10.5	287	7.52	10.6	78	57 (71)	78
Mill River at Mouth—420519072345701	10.3	289	5.63	11.3	180	150	110
March 17, 2011							
Mill River at Watershops Pond—01177500	--	--	--	--	--	--	--
Mill River at Hancock Street—01178000	3.52	224	6.85	13.53	160	120	190
Mill River at Mouth—420519072345701	3.59	226	7.11	13.64	157 (113)	120 (100)	180
Sewer at Mill River combined sewer overflow—420536072340901	--	--	--	--	47,000	>20,000	>1,300

according to EPA Methods 1603 and 1600, respectively (U.S. Environmental Protection Agency, 2000) in the WES laboratory. Quality-control samples (that is, sterile blanks, duplicates, and the raw sewage sample) were tested with each batch of samples analyzed by WES laboratory. In addition, the USGS analyzed replicate samples for enumeration of *E. coli* by following Myers and others (2007).

Analysis of human-host-specific genetic markers for *Bacteroidetes* organisms can be used to infer human sources of fecal coliform bacteria (Bernhard and Field, 2000a and 2000b). Additionally, techniques and quality-assurance procedures have been developed by the MassDEP that allow identification of human-sewage-derived fecal bacteria in water samples (Tang and others, 2006).

After enumeration, the enterococci samples were preserved for genetic speciation by two conventional polymerase chain reaction (PCR) assays. The *esp* gene assay is used to detect a genetic marker specific to human sewage (that is, a putative virulence factor, the *esp* gene coding for an exocellular surface protein) in *Enterococcus faecium*, an indicator of fecal contamination (Scott and others, 2005). In this assay, water samples were first analyzed for culturable enterococci by membrane filtration on mEI agar plates according to EPA Method 1600. The colonies that grew on the mEI plates were then harvested as a group, and associated DNA was

extracted. The extracted DNA was then analyzed for the presence of the *esp* gene according to the procedure of Scott and others (2005).

The second PCR assay used fecal *Bacteroidetes* for the detection of human-specific ribosomal DNA markers (Bernhard and Field, 2000a). *Bacteroidetes* is a group of anaerobic bacteria present in high concentrations in human and other animal feces. For this assay, water samples were filtered through polycarbonate filters (GE Osmonics, Inc., Minnetonka, Minnesota). DNA was extracted from the polycarbonate filters and tested for the presence of fecal *Bacteroidetes* by using a group-specific primer set to detect the human-specific genetic marker sequence *Bacteriodetes-Prevotella* (GB32) (Bernhard and Field, 2000a). The samples that contained the fecal *Bacteroidetes* group were further tested with human-specific primers (Bernhard and Field, 2000a) for the two genetic markers, HF134 and HF183, by methods described in Duerring and others (2010).

Fluorescent Whitening Agents

Fluorescent whitening agents, or optical brighteners (OBs), are commonly used additives to laundry detergents, fabrics, paper products, and other manufactured goods and therefore are frequently found in domestic sewage and can be used as indicators of wastewater in environmental samples. Fluorescent whitening agents (including FWA–1, FWA–2, FWA–4, OB–1 and OB–2) were quantitated in water samples in the WES laboratory by using a method of solid-phase extraction and high-performance liquid chromatography (Duerring and others, 2010), which is a procedure based on Poiger and others (1996).

Quality-Assurance and Quality-Control Procedures

For quality assurance, quality-control (QC) samples were collected in the field and produced in the laboratory to assess sampling and analytical procedures. QC samples helped verify analytical results and consisted of blank samples, replicate samples, and spike samples. Blank samples were collected to check for potential contamination in the process of sampling and laboratory analysis. The replicate sample was used to check precision of laboratory analyses, and the spike was used to assess recovery and analytical accuracy. Field blanks were collected in the same manner and setting as the environmental samples, except that deionized water was used in place of the river water.

Multiple QC procedures and methods were performed before and during the analysis of environmental samples and blank-water blind samples by the WES laboratory. During each round of sampling, 12 total samples were tested: 8 QC samples and 4 environmental samples, or a ratio of 2:1 QC to environmental. All QC samples were analyzed "blind" so the laboratory did not make assumptions about the quality of the

water or adjust the analytical method in the laboratory. For each of the four sample sites, WES analyzed 3 samples: 1 environmental sample of Mill River water, 1 trip blank, and 1 replicate or spike. Spikes and replicates were randomly assigned by the USGS and kept blind from WES staff. As a result, WES did not know the difference among samples prior to analysis to ensure every sample was handled in the same way.

Replicate samples are additional samples collected in the field and intended to be identical in composition to the environmental samples. Replicate samples provide a measure of precision that accounts for variability in sample collection and processing (filtering), and for possible effects such as in-bottle compound degradation prior to laboratory analysis (Smith, 2008).

An additional replicate sample was collected to produce the spike sample with the same matrix as a typical Mill River water sample. At the laboratory, the sample to be spiked was subsequently fortified at 0.25 microgram per liter (µg/L) for all pharmaceutical compounds analyzed for this study and for two additional surrogate compounds. The percent recovery for each target compound added to the environmental sample is used to determine bias and variability arising from (1) the degradation of target compounds during shipment to and holding by the laboratory, (2) the analytical method, and (3) interferences that mask or enhance determinations of the target compounds in the environmental sample as a result of matrix effects (Smith, 2008).

In addition to the types of QC samples collected during this project, the NWQL routinely analyzes other types of QC samples, including laboratory-reagent blanks, interference-check solutions, laboratory control samples, standard-reference materials, laboratory-reagent spike samples, and laboratory duplicate samples to test and track method performance (Garbarino and others, 2006; Furlong and others, 2008). The NWQL also adds two surrogate compounds (carbamazepine-d10 and ethyl nicotinate-d4) to all samples for routine determinations of percent recovery. Surrogate compounds are expected to react similarly to the targeted environmental compounds in the laboratory. Because these compounds are not normally found in the environment, the recovery of the surrogate compounds can be used to qualify the performance of the analysis (Smith, 2008).

Results for Field Parameters and Wastewater Constituents

The following sections describe the results of the analyses of field parameters, pharmaceutical compounds, fecal coliform bacteria, host-specific genetic markers in fecal coliform bacteria, and whitening agents. This section also describes the results of an analysis of the QC samples.

Field Parameters

Analyses of field parameters (temperature, specific conductance, pH, and dissolved oxygen) in the river water provided information on basic water-quality conditions at the time of sample collection. Temperatures ranged from a high of 25.4 degrees Celsius (°C) during July and September to a low of 3.5 °C in March and were similar among sites on each sample date (table 2). Water temperatures were in the mid-20s (°C) during the summer in the narrow river channel, where shaded conditions dominated because of the dense tree canopy and locally steep valley walls.

Specific conductance measurements were within a narrow range from 263 to 326 microsiemens per centimeter (μS/cm) from July through November in 2010 (table 2). Specific conductance was consistent among sites on each sample date. During March 2011, specific conductance was somewhat lower than during the summer and fall of 2010, and measurements were also similar between sites.

The pH measurements at Watershops Pond impoundment were relatively high during the summer months over the course of the study and ranged from a high of 9.69 pH units on September 1, 2010, to a low of 6.81 pH units on October 18, 2010 (table 2). Measurements of pH were near neutral at the Hancock Street site as compared with measurements from the upriver Watershops Pond site.

Dissolved oxygen was elevated in Watershops Pond during the summer months of July and September 2010 and coincided with high pH measurements (table 2). Elevated dissolved oxygen in Watershops Pond was likely the result of photosynthesis by green algae (fig. 5). Sunlight can cause rapid reproduction of green algae in waters with abundant nutrient loads. In turn, photosynthesis by the algae may cause dissolved oxygen concentrations to increase during daylight hours.

Pharmaceutical Compounds

None of the pharmaceutical compounds were detected at concentrations greater than their analytical reporting levels during the July through November sampling. Detections of caffeine; 1,7-dimethylxanthine, a metabolite of caffeine; carbamazepine, an antiepileptic; and cotinine, a metabolite of nicotine, were found at sub-part-per-billion concentrations (table 3), but they could not be reliably quantified because these concentrations were below their RLs.

The samples collected in March 2011 included two environmental samples and the grab sample of raw sewage from the sewer main near the Mill Street CSO along the lower reach of the Mill River. Four pharmaceutical compounds were detected in the diluted (50:1) raw sewage (table 3). After the dilution factor was applied, the estimated undiluted concentrations were caffeine at 31 μg/L, acetaminophen at 10 μg/L, and the caffeine metabolite 1,7-dimethylxanthine at 31 μg/L. The antibiotic trimethoprim was detected at a low estimated

Figure 5.   Green algae in Mill River water spilling from Watershops Pond impoundment under the mill at Watershops Pond, Springfield, Massachusetts, September 1, 2010. Photograph by Andrew J. Massey, U.S. Geological Survey.

concentration, well below the RL in the diluted sewage (table 3). No pharmaceutical compounds were detected in the environmental samples collected at Mill River sites upriver (Hancock Street) and downriver (at the mouth) of the CSO on March 17th except caffeine, which was detected in samples from both locations at estimated concentrations below the RL. Therefore, any potential residual contamination from the CSO release on March 16th had probably flushed through the lower reach to the Connecticut River, or was at such a diluted concentration that it was not detectable (table 3).

Fecal Coliform Bacteria and Host-Specific Genetic Markers

Concentrations of the fecal indicator bacteria *E. coli* were present in Mill River water at each site sampled and at all sample times (table 2). Concentrations of *E. coli* generally increased along the course of the river from Watershops Pond to the mouth and were greatest in samples collected during the summer (fig. 6). The highest *E. coli* concentration, 2,500 colony-forming units per 100 milliliters (CFU/100 mL), was measured in late July 2010 in samples of water collected at the Hancock Street site. *E. coli* concentrations in late July and early September were above the Massachusetts criterion for a single sample collected at a site far from bathing beaches (235 CFU/100 mL maximum). *E. coli* concentrations were lower during cooler months, but all samples of the river contained *E. coli*.

The results for enterococci bacteria were similar to the results of the *E. coli* analysis. All samples contained enterococci bacteria, and bacterial concentrations generally increased

Table 3. Concentrations of pharmaceutical compounds measured in samples from three sites at the Mill River, Springfield, Massachusetts, from Watershops Pond to the mouth above the Connecticut River, about monthly from July through November 2010.

[Additional samples were collected from two sites and from the Springfield municipal sewer at Mill Street combined sewer overflow on March 17, 2011. µg/L, microgram per per liter; <, less than noted value, which is the laboratory analytical reporting level; --, not sampled; E, estimated value]

Sample site	1,7-dimethylxanthine (µg/L)	Acetaminophen (µg/L)	Albuterol (µg/L)	Caffeine (µg/L)	Carbamazepine (µg/L)	Codeine (µg/L)	Cotinine (µg/L)	Dehydronifedipine (µg/L)	Diltiazem (µg/L)	Diphenhydramine (µg/L)	Sulfamethoxazole (µg/L)	Thiabendazole (µg/L)	Trimethoprim (µg/L)	Warfarin (µg/L)
July 28, 2010														
Mill River at Watershops Pond—01177500	<0.100	<0.120	<0.080	<0.060*	<0.060*	<0.046	<0.026*	<0.080	<0.060	<0.036	<0.160	<0.060	<0.034	<0.080
Mill River at Hancock Street—01178000	<0.100	<0.120	<0.080	<0.060*	<0.060*	<0.046	<0.026*	<0.080	<0.060	<0.036	<0.160	<0.060	<0.034	<0.080
Mill River at mouth—420519072345701	--	--	--	--	--	--	--	--	--	--	--	--	--	--
September 1, 2010														
Mill River at Watershops Pond—01177500	<0.100	<0.120	<0.080	<0.060*	<0.060	<0.046	<0.026*	<0.080	<0.060	<0.036	<0.160	<0.060	<0.034	<0.080
Mill River at Hancock Street—01178000	<0.100*	<0.120	<0.080	<0.060*	<0.060	<0.046	<0.026*	<0.080	<0.060	<0.036	<0.160	<0.060	<0.034	<0.080
Mill River at mouth—420519072345701	--	--	--	--	--	--	--	--	--	--	--	--	--	--
October 18, 2010														
Mill River at Watershops Pond—01177500	<0.100	<0.120	<0.080	<0.060	<0.060	<0.046	<0.038	<0.080	<0.020	<0.058	<0.091	<0.060	<0.034	<0.080
Mill River at Hancock Street—01178000	<0.100	<0.120	<0.080	<0.060	<0.060	<0.046	<0.038	<0.080	<0.020	<0.058	<0.091	<0.060	<0.034	<0.080
Mill River at mouth—420519072345701	<0.100	<0.120	<0.080	<0.060	<0.060	<0.046	<0.038	<0.080	<0.020	<0.058	<0.091	<0.060	<0.034	<0.080
November 2, 2010														
Mill River at Watershops Pond—01177500	<0.100	<0.120	<0.080	<0.060*	<0.060	<0.046	<0.038	<0.080	<0.020	<0.058	<0.091	<0.060	<0.034	<0.080
Mill River at Hancock Street—01178000	<0.100	<0.120	<0.080	<0.060*	<0.060	<0.046	<0.038	<0.080	<0.020	<0.058	<0.091	<0.060	<0.034	<0.080
Mill River at mouth—420519072345701	<0.100	<0.120	<0.080	<0.060	<0.060	<0.046	<0.038	<0.080	<0.020	<0.058	<0.091	<0.060	<0.034	<0.080
March 17, 2011														
Mill River at Watershops Pond—01177500	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Mill River at Hancock Street—01178000	<0.100	<0.120	<0.080	<0.060*	<0.060	<0.046	<0.038	<0.080	<0.020	<0.058	<0.091	<0.060	<0.034	<0.080
Mill River at Mouth—420519072345701	<0.100	<0.120	<0.080	<0.060*	<0.060	<0.046	<0.038	<0.080	<0.020	<0.058	<0.091	<0.060	<0.034	<0.080
†Sewer at Mill River CSO, Springfield, MA—420536072340901	0.2272E	0.6164E	<0.080	0.6252	<0.060	<0.046	<0.038	<0.080	<0.020	<0.058	<0.091	<0.060	<0.034*	<0.080

* Analyte detected at a concentration that could not be reliably quantified.
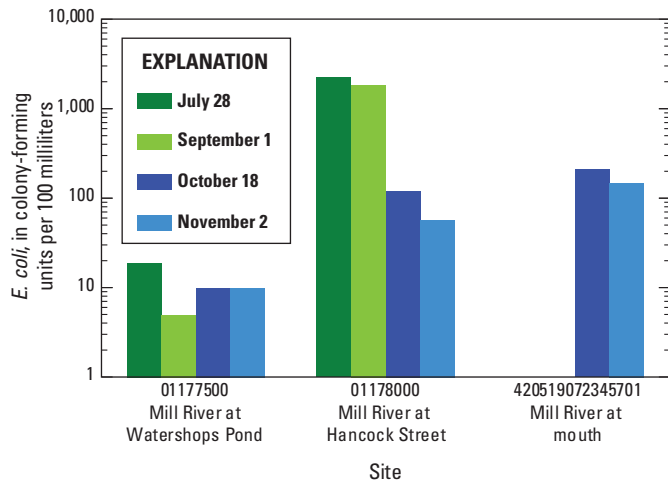
† Sewage sample diluted 50:1.

Figure 6. *Escherichia coli (E. coli)* concentrations measured in samples of Mill River water, Springfield, Massachusetts, collected from three sites and at four times during summer and fall 2010.

from Watershops Pond to the mouth (table 2) (fig. 7). One Mill River sample (collected at Watershops Pond on October 18, 2010) tested positive for the presence of the *esp* gene. Only three colonies were counted on the culture plate from this sample, so there was little material for the DNA analysis. By contrast, the cultures from the two downriver sites grew over 200 colonies of enterococci, and the genetic material collected from these cultures tested negative for the *esp* gene. The diluted raw sewage sample also grew robust cultures of enterococci, and this DNA material tested positive for the *esp* gene (table 2).
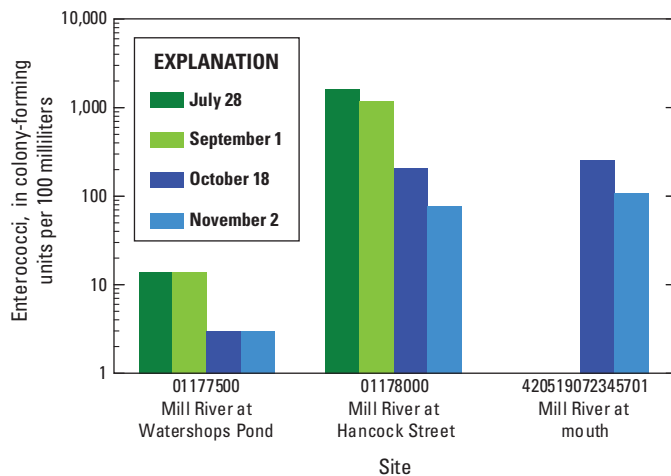

Figure 7. Enterococci concentrations measured in samples of Mill River water, Springfield, Massachusetts, collected from three sites and at four times during summer and fall 2010.

In July, the *Bacteroidetes* group marker (GB32) was absent from water samples. In September, GB32 was present in both samples, but the human-specific rDNA markers were absent. In October, GB32 was absent from the upriver site but present in water collected from the lower two sample sites. Further analysis for the human-specific markers determined a presence in only the downriver site (Mill River at mouth). November results were similar to October results except that the group marker was also present in the sample collected from the upriver location (Mill River at Watershops Pond). Samples from the Watershops Pond and Hancock Street sites tested negative in 2010 for two *Bacteroidetes* human-specific genetic markers, HF134 and HF183, but the sample from the site near the river mouth tested positive for both markers in October and November (table 4). Samples from all three sites tested positive in March 2011 for both genetic markers, HF134 and HF183.

Fluorescent Whitening Agents

The water sample collected at the upriver Watershops Pond site on July 28, 2010, contained 2.75 µg/L of FWA–1 (table 4). FWA–1 was also detected in a sample collected on the same day at the downriver Hancock Street site at a concentration below the minimum reporting level (table 4). FWA–1 and FWA–2 were detected in samples collected in October 2010, but the presence of these compounds in the Mill River is uncertain because they were also detected in the corresponding blanks (table 4). Concentration of fluorescent whitening agent-1 in the diluted sewage sample was 90,000 times greater than the median concentration in the Mill River samples.

Quality Assurance and Quality Control

No pharmaceutical compounds were detected in the blank samples (table 5), indicating that samples were not contaminated during sampling or laboratory analysis. There were no measurable differences in concentrations of any compounds between the replicate sample and its corresponding environmental sample (table 5). Caffeine and cotinine were detected in both the river-water sample and its QC replicate, but the concentrations were too small to be reliably quantified (1,7-dimethylxanthine was also detected in the environmental sample at an estimated concentration 3.6 times lower than the RL).

Percent recoveries in the spike sample ranged from 1.8 for sulfamethoxazole to 82.7 for dehydronifedipine (table 5). The percent recovery of carbamazepine-d10 in quality-assurance (QA) samples ranged from 43.9 to 113.3, whereas the percent recovery of ethyl nicotinate-d4 in QA samples ranged from 75.2 to 103.9 (table 5). The fact that the percent recoveries of the two added surrogate pharmaceutical compounds were consistently substantially higher in blank water than in river water indicates the likelihood of matrix effects masking or degrading pharmaceutical compounds in river water.

Table 4. Results of analyses performed by the Senator William X. Wall Experiment Station for detection of human-specific bacteria and anthropogenic materials in the Mill River, Springfield, Massachusetts, measured at three sites from Watershops Pond to the mouth above the Connecticut River, July through November 2010.

[Samples were collected about monthly; an additional set of samples was collected from two sites and from the Springfield municipal sewer at the Mill Street combined sewer overflow on March 17, 2011. PCR, polymerase chain reaction; °C, degree Celsius; FWA, fluorescence whitening agent; OB, optical brightener; P, analyte present; --, no sample collected; A, analyte absent; J, other quality-control criteria not met; NA, not analyzed; M, analyte concentration greater than method detection limit but less than the RL reporting level; ND, not detected; B, analyte detected in sample and in one or both of the filter blanks]

Sample site	PCR inhibition, positive control	Bacteroides group marker (GB32 at 55 °C)	Bacteroides human marker (HF134 at 68 °C)	Bacteroides human marker (HF183 at 68 °C)	Enterococcal human marker (esp gene)	FWA–1	FWA–2	FWA–4	OB–1	OB–2
July 28, 2010										
Mill River at Watershops Pond—01177500	P	A, J	NA, J	NA, J	A, J	2.75, J	ND, J	ND, J	ND, J	ND, J
Mill River at Hancock Street—01178000	P	A, J	NA, J	NA, J	A, J	0.0030, J, M	ND, J	ND, J	ND, J	ND, J
Mill River at mouth—420519072345701	--	--	--	--	--	--	--	--	--	--
September 1, 2010										
Mill River at Watershops Pond—01177500	P	P	A	A	A	ND	ND	ND	ND	ND
Mill River at Hancock Street—01178000	P	P	A	A	A	ND	ND	ND	ND	ND
Mill River at mouth—420519072345701	--	--	--	--	--	--	--	--	--	--
October 18, 2010										
Mill River at Watershops Pond—01177500	P	A	NA	NA	P	0.0047, M, B	0.057, M, B	ND	ND	ND
Mill River at Hancock Street—01178000	P	P	A	A	A	0.0046, M, B	0.530, M, B	ND	ND	ND
Mill River at mouth—420519072345701	P	P	P	P	A	0.0062, M, B	0.087, B	ND	ND	ND
November 2, 2010										
Mill River at Watershops Pond—01177500	P	P	A	A	A	0.0034, M, B	ND	ND	ND	ND
Mill River at Hancock Street—01178000	P	P	A	A	A	0.0033, M, B	ND	ND	ND	ND
Mill River at mouth—420519072345701	P	P	P	P	A	0.0078, M, B	ND	ND	ND	ND
March 17, 2011										
Mill River at Watershops Pond—01177500	--	--	--	--	--	--	--	--	--	--
Mill River at Hancock Street—01178000	P	P	P	P	P	0.0035, M, B	ND	ND	ND, J	ND
*Sewer at Mill River CSO, Springfield, MA—420536072340901	P	P	P	P	P	8.4, J	ND	ND	ND	ND
Mill River at mouth—420519072345701	P	P	P	P	A	0.0040, M	ND	ND	ND	ND

*Sewage sample diluted 50:1.

Table 5.   *A*, Concentrations of pharmaceutical compounds in quality-assurance samples and *B*, ranges of percent recoveries of known concentrations of compounds added to samples.

[µg/L, microgram per liter; <, less than; ---, not measured]

A

Quality-assurance sample type	Date and time	1,7-dimethylxanthine (µg/L)	Acetaminophen (µg/L)	Albuterol (µg/L)	Caffeine (µg/L)	Carbamazepine (µg/L)	Carbamazepine-d10 (percent recovered)	Codeine (µg/L)	Cotinine (µg/L)	Dehydronifedipine (µg/L)	Diltiazem (µg/L)	Diphenhydramine (µg/L)	Ethyl nicotinate-d4 (percent recovered)	Sulfamethoxazole (µg/L)	Thiabendazole (µg/L)	Trimethoprim (µg/L)	Warfarin (µg/L)
River water	9/1/2010 at 0900	<0.10*	<0.12	<0.08	<0.06*	<0.06	43.9	<0.046	<0.038*	<0.080	<0.060	<0.040	77.6	<0.160	<0.060	<0.034	<0.080
River-water replicate	9/1/2010 at 0903	<0.10	<0.12	<0.08	<0.06*	<0.06	46.6	<0.046	<0.038*	<0.080	<0.060	<0.040	75.2	<0.160	<0.060	<0.034	<0.080
Blank water	7/28/2010 at 0932	<0.10	<0.12	<0.08	<0.06	<0.06	105	<0.046	<0.038	<0.080	<0.060	<0.036	102	<0.160	<0.060	<0.034	<0.080
Blank water	3/17/2011 at 0802	<0.10	<0.12	<0.08	<0.06	<0.06	113.3	<0.046	<0.038	<0.080	<0.020	<0.058	103.9	<0.091	<0.060	<0.034	<0.080

*Analyte detected at a concentration that could not be reliably quantified.

B

Quality-assurance sample treatment	Date and time	1,7-dimethylxanthine	Acetaminophen	Albuterol	Caffeine	Carbamazepine	Carbamazepine-d10	Codeine	Cotinine	Dehydronifedipine	Diltiazem	Diphenhydramine	Ethyl nicotinate-d4	Sulfamethoxazole	Thiabendazole	Trimethoprim	Warfarin
Percent spike recovered from river water sample (%)	9/1/2010 at 0913	16.6	50.6	80.7	76.5	44.5	---	68.1	65.3	82.7	37.7	43.9	---	1.8	20.7	49.2	49.7

The variability in percent recovery observed among the 14 compounds analyzed in river water likely results from sample-specific matrix effects as well as losses during sample preparation and handling. The ranges of recoveries reported here are comparable to previously published observations for surface-water samples (Furlong and others, 2008).

Discussion of Wastewater Constituents in the Mill River

The concentration data for fecal indicator bacteria and pharmaceutical compounds were considered along with other data collected for this study, including the concentrations of FWA and human-specific bacterial genetic markers, to provide multiple lines of evidence with which to assess sewage presence in the Mill River during the low-flow study period. The use of multiple indicators of anthropogenic contamination is important in reliably assessing the potential presence of sewage in the environment because each method has limitations associated with detection limits and RLs. As the contaminant signal of each compound becomes more dilute, analytical methods become less effective at detecting the presence of these compounds at low concentrations.

Water samples for July and September were collected under low-flow conditions compared to historical average flows for a small nearby tributary in the Springfield area (fig. 4). When samples were collected during October and November, flows were estimated to be about five times higher than during the summer months based on the nearby Mill River streamgage in Northampton, Massachusetts, but even these flows were less than the historical averages for the fall period. If any wastewater constituent concentrations were most easily detected under low-flow conditions, then the likelihood of detection was relatively high in samples collected for this study in 2010.

None of the low-flow samples had concentrations of wastewater constituents above the laboratory RLs, and therefore the constituents were either not present or present at low concentrations. The absence of these compounds at reportable concentrations does not rule out their presence at trace levels. Recent advances in analytical methods have allowed quantification of pharmaceutical compounds at nanogram-per-liter concentrations, whereas the RLs in this study were quantified at tenths to hundredths of a microgram per liter. For example, Benotti and others (2009) reported concentrations of pharmaceutical compounds at nanogram-per-liter concentrations in drinking water. There may be no risk to human health from contaminants at low concentrations, but the effects of chronic exposure to a suite of these compounds is not well understood (Kumar and others, 2010).

Certain indicator compounds were detected at concentrations below the RLs, suggesting that trace amounts of sewage were present. For example, the water sample collected at the upriver Watershops Pond site on July 28, 2010, contained 2.75 µg/L of FWA–1, and the detection of caffeine below the RL points towards the possibility of sewage contamination at trace-level concentrations (tables 3, 4, and 5). On the same day, caffeine and FWA–1 were also detected at concentrations below the RL at the downriver Hancock Street site. In September, similar results were observed, indicating possible sewage contamination at low levels at both sites and the presence of the *Bacteroidetes* group marker and detection of caffeine at concentrations below the RL.

In this study, the indicators included testing for the presence of *E. coli* and enterococci bacteria, two *Bacteroidetes* human-specific genetic markers (HF134 and HF183), the human-specific genetic marker *Enterococcus faecium* (*esp*), a suite of FWAs (FWA–1, FWA–2, and FWA–4), and optical brighteners (OB–1 and OB–2) (table 4), along with concentrations of the pharmaceutical compounds (table 3). Quality-assurance samples and the raw sewage sample collected from the sewer on March 17, 2011, helped to ensure reliability of the results.

An additional site at the mouth of the Mill River was sampled in October and November to expand the scope of the assessment. The addition of this site and the data collected there contribute to a more complete understanding of the origin of potential sewage contaminants between Watershops Pond and the mouth of the river. There are three CSOs between the sites at Hancock Street and the river mouth. No acetaminophen or caffeine was detected in the October samples, but the sample collected at the mouth tested positive for the presence of both *Bacteroidetes* human-specific markers (table 4). FWA–1 and FWA–2 were detected in samples collected in October, but these results were too low to be quantified (table 4).

November 2010 results included detections of caffeine at the upriver and mid-reach sample sites, but the detections were at concentrations below the RL and therefore were not reliably quantified (table 3). Additionally, the *Bacteroidetes* group marker was detected, but other indicators were not detected (table 4). The samples collected from the mouth did not contain any detectable concentrations of any of the pharmaceutical compounds (table 3) but tested positive for the presence of the *Bacteroidetes* group marker and both *Bacteroidetes* human markers in PCR assays.

Indicator bacteria in Mill River samples collected during this low-flow study are likely the result of fecal contamination from warm-blooded animals. Avian species including pigeons, ducks, and geese were observed upriver from and around the sample sites. Other animal sources are likely and include wildlife and pet wastes. The increase in bacterial concentrations (CFU/100 mL) moving downriver from Watershops Pond to the mouth of the river indicate contamination along this reach of the river. The bacteria likely entered the river from nonpoint sources, which may include human sources. Additionally, wastewater constituents, including harmful bacteria, may enter the river from CSOs during storms and remain in the downriver environment for an extended period (Flint, 1987; van Elsas and others, 2011; Byappanahalli and others, 2012).

Conclusions

The Mill River component of the Tri-State Connecticut River Targeted Watershed Initiative was performed by the U.S. Geological Survey in cooperation with the Pioneer Valley Planning Commission and the U.S. Environmental Protection Agency. A longitudinal approach was used to address water quality under low-flow conditions at three sites along the lower, urban reach of the Mill River in Springfield, Massachusetts, during summer and fall 2010 and March 2011. The highly urbanized tributaries in this river system are typical of those in cities along main stems of major rivers in the eastern United States. In past studies of similar locations, undocumented sources of wastewater were found to contribute measurable amounts of sewage constituents to rivers even during low-flow conditions.

However, the results of this study do not support the hypothesis that aging sewer lines or combined sewer overflow (CSO) infrastructure leak into the Mill River as tested during the low-flow conditions during sampling for this study. None of the results from Mill River samples offer conclusive evidence of the presence of sewage. Some low-level detections of pharmaceutical compounds, other man-made chemicals, and bacteria suggest an upstream, nonpoint source.

Bacteria in the Mill River upriver of CSO sites likely enter the river as nonpoint source contamination from mammalian and avian species but may also come from effluent from onsite septic systems or leakage from sewer lines. Indicators of sewage detected at low concentrations below CSOs during low-flow conditions may be the result of (1) contaminants lingering in the river after CSO discharge events have ended, (2) wastewater from leakage of untreated sewage from aging infrastructure or onsite septic systems, and (3) undocumented nonpoint sources such as wildlife or domestic animals.

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