Variability of *E. coli* density and sources in an urban watershed

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ABSTRACT

The objective of this study was to characterize the variability of *Escherichia coli* density and sources in an urban watershed, particularly to focus on the influences of weather and land use. *E. coli* as a microbial indicator was measured at fourteen sites in four wet weather events and four dry weather conditions in the upper Blackstone River watershed. The sources of *E. coli* were identified by ribotyping. The results showed that wet weather led to sharp increases of *E. coli* densities. Interestingly, an intense storm of short duration led to a higher *E. coli* density than a moderate storm of long duration ($p < 0.01$). The ribotyping patterns revealed microbial sources were mainly attributed to humans and wildlife, but varied in different weather conditions and were associated with the patterns of land use. Human sources accounted for 24.43% in wet weather but only 9.09% in dry weather. In addition, human sources were more frequently observed in residential zones (>30% of the total sources), while wildlife sources were dominant in open land and forest zones (54%). The findings provide useful information for developing optimal management strategies aimed at reducing the level of pathogens in urban watersheds.

Key words | *E. coli*, land use, microbial source tracking, urban watershed, variability, wet weather

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INTRODUCTION

Pathogens in contaminated water sources pose a significant threat to human health. For the purpose of human health, it is essential to protect watershed-based source waters. In an urban watershed with a high population density, knowledge about the location and extent of the region vulnerable to pathogen contamination is critical for establishing Total Maximum Daily Loads (TMDLs) and developing microbial load reduction plans to attain water quality standards (Smith & Perdek 2004). Aside from this, identification of faecal contamination sources is necessary for hazard analysis and accurate assessment of the risk posed by pathogens. Once hazards are identified within a watershed, strategies can be developed to reduce faecal contamination thereby reducing the risk to human health (Meays *et al.* 2006).

Impairment of water quality due to the presence of pathogens is typically assessed by monitoring microbial indicators (such as *E. coli* and enterococci), which have been associated with enteric pathogens present in water from human or animal faecal contamination (e.g. Hörman *et al.* 2004). Therefore, understanding the levels and sources of indicator organisms is critical for the management of watershed pathogen contamination. To date, a number of studies have been conducted to describe the variability of microbial indicator concentrations or sources in watershed scale. For example, Reeves *et al.* (2004) carried out a series of field studies to identify the spatial distribution of faecal indicator bacteria in dry conditions and wet weather run-off from the Talbert watershed. In the Hoosic River watershed, bacterial levels were found to be higher in summer than in winter, and higher during storms rather than during baseflow conditions (Traister & Anisfeld 2006). In contrast, a study in a heavily impacted watershed showed that pathogen levels could

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decrease to undetectable levels sometimes during storms (Dorner *et al.* 2007). By studying the density and sources of *E. coli* in multiple watersheds, Meays *et al.* (2006) found that the faecal coliforms (FC) counts varied by year, month and site, for each of the watersheds, and the main sources of *E. coli* tended to be similar between watersheds but changed in different years.

There exists an association between extreme weather and outbreaks of waterborne diseases (Curriero *et al.* 2001), for example, the outbreak caused by the protozoan parasite *Cryptosporidium parvum* in Milwaukee in 1993 (Mackenzie *et al.* 1994) and the outbreak caused by *E. coli* O157:H7 and *Campylobacter jejuni* in 2000 in Walkerton, Ontario, Canada (Auld *et al.* 2004). Both outbreaks followed periods of heavy rainfall (Hrudey *et al.* 2002; Hunter 2003). Studies showed that precipitation played an important role in microbial contamination in urbanized watersheds (Shehane *et al.* 2005; Dorner *et al.* 2007). During wet weather events, pathogens from a variety of sources, including wildlife, agriculture, combined sewer overflows and run-off, are mobilized and enter receiving rivers (Marsalek & Rochfort 2004). In addition, pathogens may be released from sediments to the water column (Hansen & Ongerth 1991; Wu *et al.* 2009). These factors can give rise to sharp increases of pathogens in water (Hansen & Ongerth 1991; Kistemann *et al.* 2002).

Knowledge of the influences of land use on source water quality, especially on sources of faecal contaminants, is also critical for efforts towards proper management within a watershed. The management based on land use can be effective for mitigating rainfall impacts to the water body of watersheds with appropriate water quality data (Long & Plummer 2004). The degree of microbial contamination may be predictable according to the types of land use of a watershed. A study revealed that bacteria in storm water run-off varied from higher to lower concentrations by the following order of land use types: recreational, agricultural, urban and open space (Tiefenthaler *et al.* 2008). In addition, analysis of microbial water quality contamination based on land use can help to make optimal practices for watershed development to minimize conflicts between agricultural and urban interests (Fisher *et al.* 2000). Land use information is also useful for determining sources of faecal contamination. For example, using multiple antibiotic resistance methods and land use characteristics, Kelsey *et al.* (2003) found that the majority of faecal pollution in Murrells Inlet, a small estuary in South Carolina, was not from human sources.

To date, however, few studies have illustrated how weather and land use influence the temporal dynamics of sources of microorganisms in urbanized watersheds. The primary hypothesis tested in this study is that the increase in *E. coli* density is influenced by the intensity and duration of precipitation, and the sources of microbial contaminants are related to the types of land use. The major objectives of this research were to: 1) characterize the variability of *E. coli* density and sources in an urban watershed; 2) illustrate the influences of weather and land use on the density and sources of microbial contaminants.

MATERIALS AND METHODS

Study area description

This study was conducted in the upper Blackstone River watershed (Figure 1). The Blackstone River originates in central Massachusetts and ultimately flows into Narragansett Bay passing by Rhode Island. The Blackstone River watershed comprises 966 square kilometres in south central Massachusetts and 272 square kilometres in northern Rhode Island (Shanahan 1994). The historic industrial city of Worcester, Massachusetts, is located in the upper Blackstone River watershed. Several major tributaries, such as the Quinsigamond, West, Mumford, Mill and Peters Rivers also flow through this area. The area is densely populated and, according to the 2000 census data, the population density of the whole watershed is approximate 386 persons per square kilometre. Forest and residential zones are the two major types of land use, which account for 41.5 and 29.9%, respectively.

The water quality of the Blackstone River watershed has historically been impaired by intensely industrial development and urbanization. Significant improvements in the overall water quality of the Blackstone River have been made in the past 30 years as a result of the Clean Water Act and other pollution reduction initiatives. Currently, the Blackstone River, as well as its tributaries, is commonly used for recreation, such as fishing, wildlife viewing, swimming,

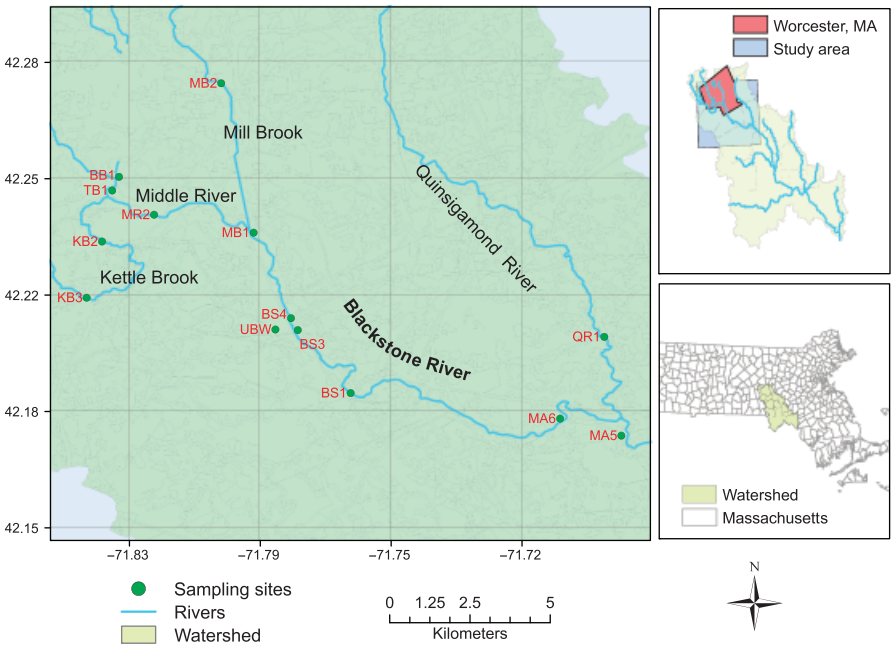

Figure 1 | The upper Blackstone River watershed and sampling sites.

boating, and habitat for aquatic life. The Blackstone River also supplies a large proportion of freshwater to Narragansett Bay, an estuary in Rhode Island used for fishing and tourism (Mangarillo 2006).

Water samples collection

Two microbial indicators, *E. coli* and enterococci, were investigated from May to November, 2006, involving four wet weather events (May 9–14, June 26–27, October 11–18 and November 8–15) and several dry days (May 17, September 7 and 27, October 19). In total, 14 monitoring sites were distributed along the Blackstone River and its tributaries (Figure 1). The descriptions of the locations of these sites are listed in Table 1. Water samples were collected once (occasionally twice) per day. At each site, duplicate surface water samples were collected in mid-stream using 500 mL sterilized plastic bottles. A few of the sediment samples were taken at BS1, BS4 and MR2 for *E. coli* source tracking. The details for sediment sampling and analysis were provided by Wu *et al.* (2009). All samples were kept in an ice cooler and returned immediately for analysis within 4 hours.

Microbial and physiochemical water quality analysis

For microbial analysis, water samples were measured directly or diluted with PBS (phosphate-buffered saline) solution (pH = 7.2) by 10 or 100 times before measurement. *E. coli* was quantified by the Colilert method using Quanti-Tray®/2000, and enterococci were measured by the Enterolert® method (IDEXX, Atlanta, GA) following instructions provided by the supplier.

Besides microbial indicators, other water quality variables were also measured. Temperature, specific conductivity, pH and dissolved oxygen (DO) were measured by YSI sondes (YSI Integrated Systems & Services, USA) in real time. Ammonium-N ($N\text{-}NH_3$), nitrate-N and nitrite-N ($N\text{-}NO_2$/NO_3), total phosphorus (TP) and total suspended solids (TSS) were analysed at an accredited laboratory following the Standard Methods for the Examination of Water and Wastewater (APHA *et al.* 2000).

Ribotyping for microbial source tracking

Escherichia coli at three sites, BS1, BS4 and MR2, was isolated for microbial source tracking. BS1 is located at the

Table 1 | Descriptions of monitoring sites

Site	Waterbody	Location	Rationale
BB1	Beaver Brook	Upstream of confluence of Beaver Brook and Tatnuck Brook at Scrub a Dub Carwash on Park Ave	To compare with Tatnuck Brook just above confluence
BS1	Upper Blackstone River	USGS[1] gauge 1109730 off of Elm Court	USGS streamflow gauging site at Millbury and downstream extent of the Upper Blackstone River
BS3	Upper Blackstone River	Near footbridge at the end of Mass Highway access road off Greenwood Street. Upstream of confluence with unnamed tributary	Downstream of UBWPAD[2] discharge
BS4	Upper Blackstone River	Upstream of confluence of UBWPAD discharge and Blackstone River	Upstream of UBWPAD discharge
KB2	Leesville Pond	20 ft downstream of dam at USGS gauge 1109500	Downstream of Leesville Pond
KB3	Kettle Brook	Rockland Rd Bridge near USGS Gauge 1109439	Downstream of confluence with Chapin Brook and Dark Brook Upper and effect of Stoneville Reservoirs
MA5	Blackstone Mainstream	Route 122A bridge, Grafton, MA	Blackstone River below Fisherville Pond
MA6	Blackstone Mainstream	North Pleasant Street bridge and intersection with Fitzpatrick Road	Blackstone River above Fisherville Pond
MB1	Upper Blackstone River	Downstream of confluence of middle river and mill brook, upper Blackstone River	Downstream of confluence of middle river and mill brooks
MB2	Salisbury Pond Outlet	North arched dam on Humboldt Ave	Output from Salisbury Pond and inlet of Mill Brook into culvert
MR2	Middle River	In St. John's Cemetery at bridge from east bank	To compare with Tatnuck, Beaver and Kettle Brooks
QR1	Quinsigamond River	Millbury Street in Grafton, MA	Upstream of confluence of Quinsigamond River with Fisherville Pond
TB1	Tatnuck Brook	Upstream of confluence of Beaver Brook and Tatnuck Brook	To compare with Beaver Brook just above confluence
UBW	Point Source	UBWPAD discharge channel above inlet of highway run-off drainage	UBWPAD discharge channel

[1]USGS: US Geological Survey
[2]UBWPAD: The Upper Blackstone Water Pollution Abatement District

Blackstone River, close to the US Geological Survey Millbury Gauge. BS4 is located at the Blackstone River immediately upstream of the Upper Blackstone Water Pollution Abatement District (UBW), a large wastewater treatment plant. MR2 is located on the Middle River, which later becomes the Blackstone River, upstream of both BS1 and BS4. These three sites represent the downstream, the middle stream and the upper reaches of the Blackstone River, respectively.

After detection using the Colilert method, *E. coli* strains were randomly picked out and isolated using LB-agar (Luria-Bertani agar) plates, and confirmed following the method of Feng & Hartman (1982). *E. coli* ATCC 29194 (Remel Europe, Ltd, Dartford, UK) and *Klebsiella pneumoniae* ATCC® 33495™ (Quality Technologies, Ltd, Newbury, CA, USA) were used as positive controls and negative controls for *E. coli*, respectively. A purified *E. coli* colony was inoculated in an LB agar tube, and made into an *E. coli* slant. The slants were kept at 4°C and sent to the New York City Department of Environmental Protection (DEP) for ribotyping analysis.

A RiboPrinter® Microbial Characterization System (DuPont Qualicon, Inc.) was used to identify the sources of

E. coli based on its riboprint pattern, which was generated by restriction fragment length polymorphisms (RFLP) of 16S ribosomal RNA genes from tested *E. coli*. By comparing riboprint patterns (band position, weight and intensity) of tested *E. coli* strains with those from known hosts in the DuPont Identification library and an additional custom library (DEP library), the plausible sources of the tested strains were presumed. The protocol for ribotyping analysis was described elsewhere (Parveen *et al.* 1999; Kelsey *et al.* 2008; Wu *et al.* 2009). Briefly, after *E. coli* was lysed by heating, the DNA was digested using restriction enzymes EcoR1 and PvuII. Then the digested DNA was separated by electrophoresis and subsequently transferred to a nylon membrane and immobilized. Chemically labelled *E. coli* rRNA was applied to hybridize to the denatured DNA fragments on each membrane. Then, the membrane was rinsed by blocking buffer and treated with an anti-sulfonated DNA antibody/alkaline phosphatase conjugate. A chemiluminescent substrate was added after unbound conjugate was removed. Images from the membrane were captured and processed to generate a set of riboprints. The riboprint for tested *E. coli* was compared with the known patterns in the Custom Identification library. Similarity between isolates and library cases was calculated based on band position, weight and intensity. The sources of the isolates were assumed to be identified if their genetic patterns (riboprint) matched the riboprints in the library with a similarity of 90% or greater. The chemicals and materials used for ribotyping were also supplied by DuPont Qualicon, Inc.

Watershed land use analysis

ArcGIS software (ArcMap 9.2; ESRI, Redlands, CA, USA) was used for land use analysis. The GIS layer for the Blackstone River watershed and the land use data were obtained from the MassGIS website. The projection of GIS layers was transferred into GCS-WGS-1984 from NAD-1983-state-plane-Massachusetts-mainland-FIPS-2001. To learn about the types of land use around each sampling site, a buffer with radius of 1 km around each sampling site was clipped from the watershed layer. Though choosing 1 km as radius is arbitrary, it is reasonable. First, 1 km is the basic unit of our study area. Second, the patterns of land use within 1 km more than a longer distance around a site are related to the sources of *E. coli* at that site. The percentages of the types of land use in each buffer were calculated by GIS tools.

Statistical analysis

To examine the relationship between *E. coli* density and some environmental variables, Spearman correlation and multiple linear regression analyses were conducted. The difference of land use patterns among the sampling sites was examined using the paired samples t test. One-way ANOVA and chi-squared test were used to compare the differences of *E. coli* densities and sources in different weather conditions as well as in different types of land use. The significance level was set at 0.05, and 95% confidence intervals (95% CI) were calculated using the following equation: 95% CI = Mean ± 1.96 × Standard error. All statistical analyses were carried out with SPSS 16.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Variability of *E. coli* density and sources

Escherichia coli density in water samples collected from the upper Blackstone River watershed was measured in four wet weather events (May, June, October and November, 2006) and four dry days. Overall, *E. coli* density ranged from 1 to 9061 MPN/100 mL (MPN: most probable number, the unit of *E. coli* counts) and 32.9% of the data were below 235 MPN/100 mL, the state standard for microbial pollution in freshwater (USEPA 1986). As expected, the density of *E. coli* displayed remarkable spatial and temporal variability. Temporally, in the May event, the peak *E. coli* densities occurred on May 12 and 14, which were 1647 (95% CI: 328–2966) and 1388 (95% CI: 1251–1525) MPN/100 mL, respectively. *E. coli* density was 1214 MPN/100 mL on June 26 but decreased to 545 MPN/100 mL the next day. During the October event, the density was the highest on October 12, which was 2361 MPN/100 mL. *E. coli* densities were higher in the November event than the previous three wet events, which was up to 4719 MPN/100 mL (November 8), and maintained these high densities for five consecutive days (Figure 2). Spatially, *E. coli* density was the highest at BB1, which was 3768 (95% CI: 2633–4902) MPN/100 mL on

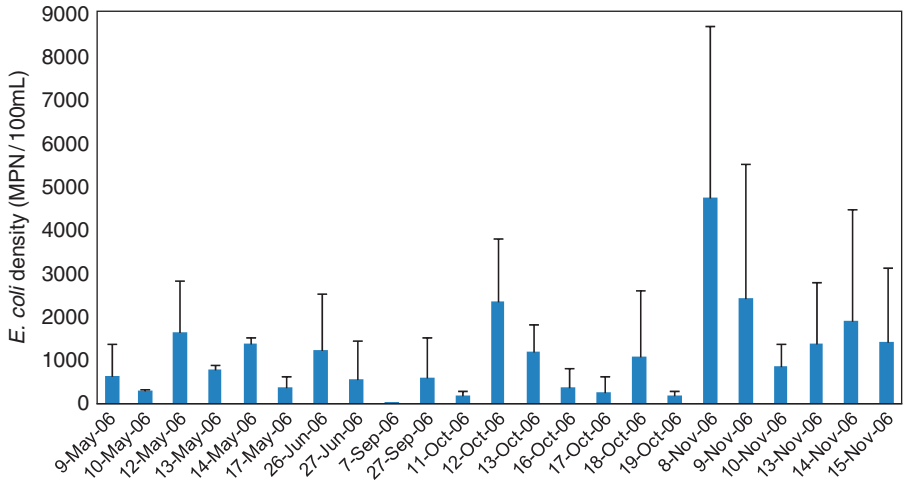

Figure 2 | Temporal variability of *E. coli* density in the upper Blackstone River watershed.

average. The following were sites TB1, MR2, BS2 and MB1, where the densities were larger than 1000 MPN/100 mL. Lower densities of *E. coli* were found at sites KB2, KB3, MA5, MA6, QR1 and UBW. Among these sites, UBW had the lowest density, which was 11 (95% CI: 3–20) MPN/100 mL on average, followed by QR1 and KB3, for which the densities were less than 235 MPN/100 mL (Figure 3).

Seventy-one *E. coli* isolates were analysed by the riboprinting system using restriction enzyme EcoR1 and PvuII, and the ribotyping patterns of sixty-two isolates were generated to identify *E. coli* sources. For one ribotype corresponding to multiple sources, the probability of each source was calculated. By comparison of the patterns analysed with the known-host patterns in the database, 10 isolates were indigenous (bacteria for which the riboprint patterns only appeared in the Blackstone River watershed); about 14 isolates were associated with human sources, including from human faeces and sewage treatment plants; and about four isolates originated from dogs (Table 2). The probable sources for the remaining majority of isolates were from wildlife, including many species of birds and animals. Canada geese (*Branta canadensis*), gull (*Larus*), whitetail deer (*Odocoileus virginianus*) and cliff swallows (*Hirundo rustica*) were potentially the major contributors. The sources of *E. coli* were much different across the three events. Gulls (10%), humans (9%) and cliff swallows (9%) were the major sources during the May event, followed by whitetail deer (5%), Canada geese (4%) and deer mice (4%). However, in October and

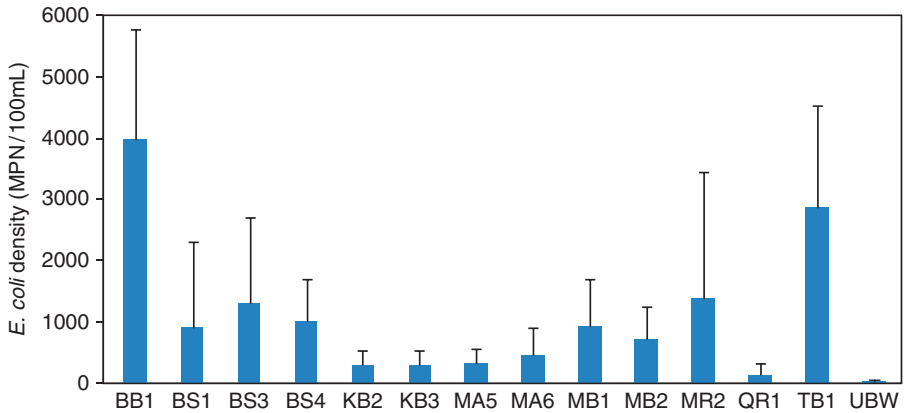

Figure 3 | Spatial variability of *E. coli* density in the upper Blackstone River watershed.

Table 2 | Neighbourhood land use at each sampling site

Site	Neighbourhood land use (radius = 1 km)
BB1	Residential (52%), commercial (18%), industrial (9%), water (8%)
BS1	Residential (42%), forest (24%), open land (13%), commercial (10%)
BS3	Forest (28%), residential (21%), transportation (18%), open land (8%), recreational (7%)
BS4	Forest (26%), transportation (24%), residential (17%), waste disposal (12%), open land (6%)
KB2	Residential (38%), open land (35%), water (7%), forest (6%), recreational (6%)
KB3	Residential (50%), forest (20%), open land (8%)
MA5	Forest (37%), residential (24%), wetland (10%), water (8%), open land (8%)
MA6	Forest (38%), residential (36%), open land (10%)
MB1	Residential (29%), forest (24%), industrial (22%), open land (12%)
MB2	Residential (36%), open land (21%), industrial (15%), commercial (10%)
MR2	Open land (34%), residential (28%), industrial (18%), transportation (8%), forest (7%)
QR1	Residential (41%), forest (33%), open land (9%)
TB1	Residential (38%), commercial (18%), open land (16%), indusial (11%), water (9%)
UBW	Forest (33%), residential (20%), transportation (18%), waste disposal (10%), open land (7%)

November, humans (36%) and dogs (14%) were the dominant sources; the next most important sources were cliff swallows (6%), Canada geese (3%) and whitetail deer (3%) (Table 2). The percentages of the sources of *E. coli* at three sites were illustrated in Figure 4. At MR2, human sources only accounted for 8%, while wildlife sources accounted for 54%. However, at BS1 and BS4, human sources accounted for 36 and 31%, respectively.

Relationship between *E. coli* and other water quality variables

Determination of the relationship between microbes and physiochemical water quality variables can help to understand the variability and source of microorganisms. In addition to *E. coli*, other water quality variables, such as enterococci, TSS, TP and inorganic nitrogen, were measured simultaneously. As with *E. coli*, enterococci concentrations were highly variable in wet weather, with an average density of 1063 MPN/100 mL and a standard deviation of 2416 MPN/100 mL. The relationship between *E. coli* and enterococci was defined by a simple linear regression model: $C_{EC} = 1.2672 \times C_{ENT}$, $R^2 = 0.5495$, where C_{EC} is *E. coli* density and C_{ENT} is enterococci density (Figure 5). Water temperature and pH varied little during the sampling events, with average values of 12.61 °C and 7.2, respectively. Specific conductivity and dissolved oxygen had average values of 0.37 mS/cm and 0.6 mg/L, respectively. Total phosphorus, inorganic nitrogen and total suspended solids varied in space and time, of which the 95% confidence intervals were 0.21–0.36 mg/L, 1.52–2.27 mg/L and 8.82–14.60 mg/L, respectively.

Multiple linear regressions were conducted to examine the associations between *E. coli* density and some environmental variables. Besides TP, the other three variables were positively associated with the log-transform of *E. coli* density (Table 4). However, all the associations were not significant ($p > 0.05$). Spearman correlation analysis showed that only TSS was significantly correlated with *E. coli* ($r = 0.299$, $p = 0.022$) and enterococci ($r = 0.404$, $p = 0.006$), which suggested that TSS might have a better relationship than other variables (such as TP, N-NO_2/NO_3) with *E. coli* density. It is reasonable because bacteria are likely to attach to the fine particles (<2 μm) and move rapidly to receiving waters during rainfall (Muirhead *et al.* 2006). An early study (Davis *et al.* 1977) showed that microorganisms and suspended solids in stormwater run-off were significantly correlated. Recently, some studies also found that *E. coli* level was significantly correlated with turbidity (Dorner *et al.* 2007) and total suspended solids (Tiefenthaler *et al.* 2008) in watersheds in wet weather.

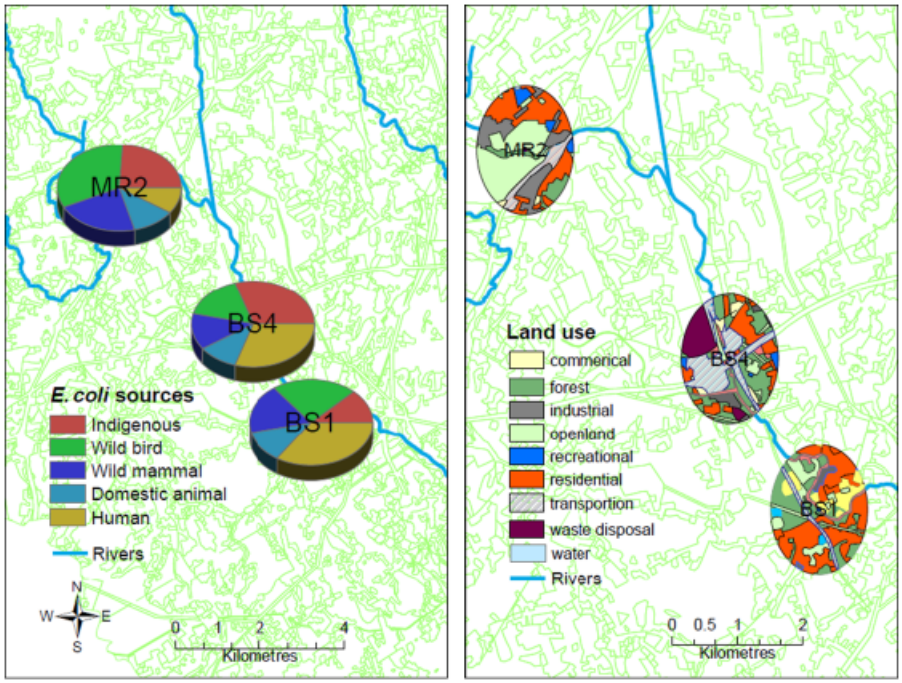

Figure 4 | *Escherichia coli* sources and land use at the three representative sites.

Influences of weather and land use on *E. coli* density

The daily precipitation during sampling periods was obtained from the NOAA's National Weather Service website (http://www.weather.gov/climate/) and are shown in Figure 6. In the May event, light rain (0–10 mm/day) that started on May 9 became moderate (10–30 mm/day) on May 12. In the October event, moderate rain occurred on October 11 and became heavy rain on October 12. In the November event, light rain initiated on November 7 and suddenly became a heavy storm (45 mm/day) on November 8. The heavy rain only lasted one day and then stopped on November 10. No rain fell on May 17, September 6 and 11, and October 18. Following a day of precipitation, *E. coli* densities varied sharply. Based on the results of spearman correlation analysis, daily precipitation was significantly correlated with daily average density of *E. coli* (the average of 14 sites) ($r = 0.532$, $p = 0.013$) and *E. coli* density at BS4 ($r = 0.645$, $p = 0.004$). During dry weather, 68.8% of the samples were below 235 MPN/100 mL. However, in wet weather, only approximately 32% of samples attained this standard. Higher densities of *E. coli* in wet weather were expected, since similar phenomena have been observed in previous studies (Traister & Anisfeld 2006). However, it is unknown how wet weather influences *E. coli* density in an urban watershed. In this study, we observed four wet weather events, from which some similar influences on *E. coli* densities were found: (1) the peak of rainfall corresponded to the highest density of *E. coli* for each event; (2) in the day following the peak, the rainfall decreased or stopped, and the density of *E. coli* also decreased significantly but was still much higher than normal. Since the intensity and duration of rainfall in

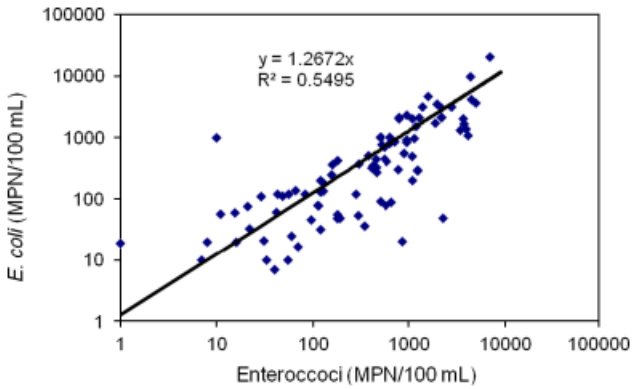

Figure 5 | Relationship between *E. coli* and enterococci in water samples from the upper Blackstone River watershed.

Table 3 | Possible sources of *E. coli* in different weather events

Possible sources	Total (n = 62)	Dry weather (n = 11)	Wet weather (n = 51)	Spring (n = 37)	Autumn (n = 25)
Canada geese	3.65%	7.45%	4.22%	3.92%	3.24%
Cliff swallows	7.95%	18.18%	5.75%	9.00%	6.40%
Cottontail rabbits	1.27%	0.00%	1.82%	1.32%	1.20%
Dogs	8.42%	4.73%	9.33%	3.24%	16.08%
Grey squirrels	1.32%	0.00%	1.61%	2.22%	0.00%
Gulls	8.08%	7.09%	8.61%	12.14%	2.08%
Humans	22.74%	9.09%	24.43%	7.89%	44.72%
Indigenous	22.58%	18.18%	23.53%	32.43%	8.00%
Opossums	1.69%	6.36%	0.49%	0.81%	3.00%
Deer mice	2.60%	0.00%	3.16%	4.35%	0.00%
Raccoons	0.26%	0.00%	0.31%	0.43%	0.00%
Red squirrels	1.45%	0.00%	1.76%	1.46%	1.44%
White deer	2.81%	5.45%	3.63%	2.84%	2.76%

each event were quite different, there were also some different influences on *E. coli* densities. In the May event, moderate rain lasted four days, in the June and October events, the moderate rain lasted one or two days, while in the November event, the strong storm lasted only one day. However, *E. coli* density was much higher in the November event than in the previous three events ($p < 0.01$). The results revealed that the intense participation of short duration gave rise to higher *E. coli* densities and loadings than moderate storms over a longer period, suggesting that *E. coli* densities were associated with the patterns of precipitation.

The neighbourhood land use at each site is shown in Table 2. The major types of land use around these sites were residential, forest, open land, commercial and industrial zones. Residential zoning was the most common type of land use among these sites, and was dominant at BB1, BS1, KB3, QR1 and TB1. Forest was the second most common type of land use, which accounted for a large percentage of land use at sites BS3 (28%), BS4 (26%), MA5 (37%), MA6 (38%) and UBW (33%). Open land was present at most sites, but its percentage was smaller in contrast to residential ($p < 0.01$) and forest zones ($p = 0.03$) except at MR2 (34%) and KB2 (35%). Commercial zones were located at BB1, MR2 and TB1 with a small percentage of overall land use (10–18%). A waste disposal area was only found at site UBW. The highest density of *E. coli* was observed at sites BB1 and TB1. These two sites were very close to each other, and residential and commercial zones were the two major types of land use. As a result, human activities would be expected to predominate around these sites, which led to higher densities of *E. coli* in wet weather. *E. coli* density was the lowest at UBW, which was attributed to disinfection processes employed during wastewater treatment. BS3 and BS4 were near UBW, BS1 was downstream of BS4 and BS3. The densities of *E. coli* at these three sites were at a moderate level, which might have been caused by the overflow of wastewater during wet weather. MA5, MA6 and QR1 were far from other sampling locations. The lower densities of *E. coli* at these three sites might be explained by the fact that forest was the major type of land use, implying less population density and fewer human activities.

Table 4 | Multiple linear regression analysis of the associations between *E. coli* density and some environmental variables

Independent variables	Beta	Standard error	95% confidence intervals	t	p
TP	−0.692	0.508	(−1.688–0.304)	−1.363	0.181
TSS	0.004	0.006	(−0.008–0.016)	0.731	0.469
N-NO_2/NO_3	0.094	0.184	(−0.267–0.455)	0.512	0.612
N-NH3	0.061	0.127	(−0.188–0.310)	0.476	0.636

Dependent variable: log-transform of *E. coli* density; R-square of the whole model is 0.161.

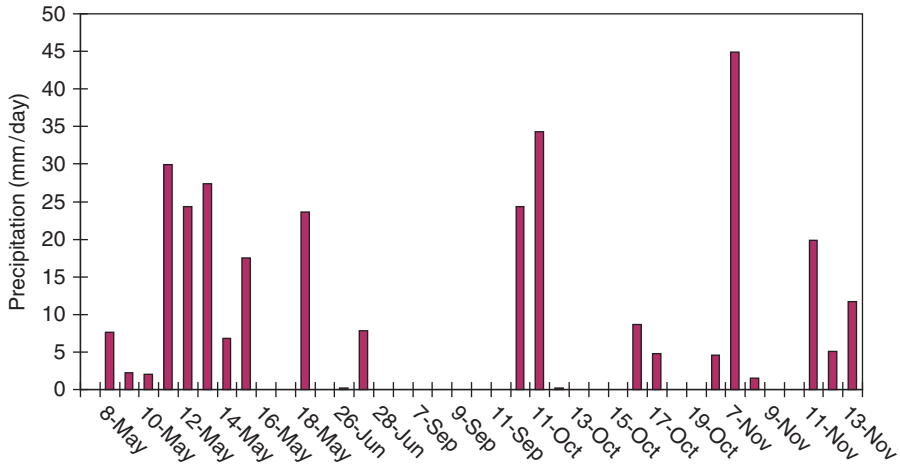

Figure 6 | Daily precipitation in the Blackstone River watershed during sampling event.

It is unclear what factors were related to the lower densities of *E. coli* at KB2 and KB3.

Influences of weather and land use on *E. coli* sources

Escherichia coli exists in the intestinal tracts of humans and other warm-blooded animals. The presence of *E. coli* in the water column represents the existence of faecal pollutants and possibly the presence of pathogens. Our results revealed that both human and wildlife were the primary sources of *E. coli* in the Blackstone River watershed. No livestock sources were identified, as would be expected for an urban watershed with virtually no agricultural activities.

We identified *E. coli* sources in dry weather conditions as well as wet weather events (Table 3). Similarly, Canada geese, cliff swallows, dogs, gulls, humans, opossums and white deer were possible sources in both dry and wet weather. In addition, the indigenous strains accounted for a large proportion of isolates. In contrast, a greater variety of sources were found in wet weather. In addition to the sources mentioned above, sources from cottontail rabbits, grey squirrels, deer mice, raccoons and red squirrels were also found in wet weather. Furthermore, the percentages of each source were much different for different weather conditions ($p < 0.01$). For example, human sources accounted for 24.43% in wet weather but only 9.09% in dry weather ($p < 0.01$). Since the potential sources that contribute to *E. coli* loadings in the Blackstone River watershed in wet weather include cross connected storm sewers, combined sewer overflows, failing septic systems, stormwater drainage systems and storm water run-off, human sources would be expected more frequently in wet weather.

Wildlife contributed to the highest percentage (40%) of *E. coli* in the river during the May event. The likely species included Canada geese, gulls, whitetail deer, cliff swallows and grey squirrels. However, during the October and November sampling events, humans became the dominant source (36%). This is reasonable since the activities of wild animals become more frequent in the late spring and summer but less frequent in the late fall and winter. It is also possible that migration from the area played a role. The results suggested that non-point-source contributions from wildlife had substantial effects on the microbial pollution in the urban watershed but was strongly influenced by seasonal changes.

The composition of the identified sources was quite different ($p < 0.01$) at site MR2 from sites BS1 and BS4. At MR2, sources from wild animals accounted for 56%, and human sources only accounted for 8%. In contrast, human sources contributed to a large proportion at sites BS1 (36%) and BS4 (31%). The difference in *E. coli* sources is likely associated with the patterns of land use in these areas. In the BS1 neighbourhood, the major land uses are designated as residential (42%), forest (24%), open land (13%) and commercial areas (10%); and around BS4, forest (26%), transportation (24%), residential (17%) and waste disposal (12%) were the major types of land use. It is not surprising that humans are the major probable source of *E. coli* at these two

sites, since *E. coli* from human sources is usually associated with human faeces which are discharged by septic systems or wastewater discharges. In contrast, around MR2, open land is the major land use (34%), which raises the potential for inputs from wildlife. It should be mentioned that wildlife sources could exist in a variety of types of land use, such as forest, wetland and open land, even in agriculturally dominated watersheds (Somarellia *et al.* 2007).

Limitations of microbial source tracking methods

Though intensive research has been conducted and many techniques have been applied for microbial source tracking (MST), whether a single method can accurately identify a microbial source is still being debated. Ribotyping is considered one of the most reproducible of the molecular typing methods to identify *E. coli* from human or animal sources (Carson *et al.* 2001). In contrast to other MST methods (antibiotic resistance analysis, carbon utilization profiling, REP-PCR, BOX-PCR, pulsed-field gel electrophoresis), ribotyping with EcoRI and PvuII approached 100% correct classification but only 6% of isolates were classified (Stoeckel *et al.* 2004). It was also effective for the identification of *E. coli* collected from a large geographic region (Scott *et al.* 2003) as well as in a small-scale (less than 1 km^2) watershed (Kelsey *et al.* 2008).

However, this method is library-dependent and the accuracy is restricted by the database of riboprint patterns. Some studies doubted the accuracy of ribotyping methods. For example, when Moore *et al.* (2005) analysed HindIII ribotypes of 997 *E. coli* isolates, only 27% isolates were assigned to the correct sources, which suggested that the library-based MST methods, such as ribotyping, might not be suited to identifying the sources of faecal pollution in large urban watersheds (about 347 km^2 in the study). Our study area of the upper Blackstone River Watershed is of moderate scale (about 200 km^2). Whether ribotyping is a good method for this scale of watershed needs further discussion. Given these reasons, this study only attempted to describe the general trends of *E. coli* sources in the watershed, instead of accurately identifying sources to the species level. In addition, in our study, ten isolates were regarded as indigenous to the Blackstone River because their ribogroups did not match any pattern in the database. In the future, they could be matched with isolates identified from other sources as the database of known-host patterns is expanded.

Implication to watershed management

Wet-weather pollution is recognized as a major source of impairment of water quality in urban watersheds, which impacts on water quality in many ways. Among these, pathogens from multiple sources are one of the contaminants most difficult to control. As an urban watershed, the Blackstone River watershed is subjected to impairment by pathogens according to the 2002 303(d) List of Impaired Waters prepared by the Rhode Island Department of Environmental Management (RIDEM). It is unsurprising that *E. coli* density could be ten times higher than normal during wet weather events. However, our results revealed that a short-term intense precipitation gave rise to higher *E. coli* densities than a moderate storm over a long period ($p < 0.01$), suggesting that *E. coli* densities were influenced by the intensity and duration of precipitation. This interesting finding provides useful information to watershed managers that particular attention should be paid to heavy storms, even those of short duration.

Section 303(d) of the Clean Water Act requires that total maximum daily loads (TMDLs) must be established for impaired waters. As for watersheds impaired by faecal contamination, TMDL studies need be conducted to help restore water quality and reduce the risk of waterborne diseases, which involve the identification of microbial sources. Using ribotyping methods, we traced microbial sources from both humans and wildlife in an urban watershed. However, the percentages of human sources and wildlife sources varied in different space and time, which were influenced by weather conditions and land use. Human sources were more frequently observed in wet weather and residential areas. This finding suggests controlling pollution from human sources is critical in urban watersheds and the patterns of land use are very important for developing remedial strategies.

CONCLUSION

The study examined the influences of weather conditions and land use on *E. coli* densities and sources in the upper

Blackstone River watershed in Massachusetts. *E. coli* density was strongly influenced by the intensity and duration of precipitation. A short-duration intense storm led to higher *E. coli* densities than a moderate storm over a long duration ($p < 0.01$). *E. coli* densities were also impacted by the patterns of land use. The sites near residential and commercial zones had higher densities. Wildlife and humans were two major sources of microbial contamination in the upper Blackstone River watershed. Weather conditions and the patterns of land use played a remarkable role in the variability of microbial sources in the watershed. Human sources were more frequent in wet weather events than in dry weather conditions ($p < 0.01$), and more frequently observed in residential and commercial zones than in forest and open land ($p < 0.01$). Overall, the study provides useful information for developing optimal management strategies aimed at reducing the level of pathogens in urban watersheds.

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