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Hydrometeorological and Physicochemical Drivers of Fecal Indicator Bacteria in Urban Stream Bottom Sediments

Hehuan Liao,* Leigh-Anne H. Krometis, W. C. Hession, Leanna L. House, Karen Kline, and Brian D. Badgley

Abstract

High levels of fecal indicator bacteria (FIB) are the leading cause of surface water quality impairments in the United States. Watershed-scale models are commonly used to identify relative contributions of watershed sources and to evaluate the effectiveness of remediation strategies. However, most existing models simplify FIB transport behavior as equivalent to that of dissolved-phase contaminants, ignoring the impacts of sediment on the fate and transport of FIB. Implementation of sediment-related processes within existing models is limited by minimal available monitoring data on sediment FIB concentrations for model development, calibration, and validation purposes. The purpose of the present study is to evaluate FIB levels in the streambed sediments as compared to those in the water column and to identify environmental variables that influence water and underlying sediment FIB levels. Concentrations of *Escherichia coli* and enterococci in the water column and sediments of an urban stream were monitored weekly for 1 yr and correlated with a variety of potential hydrometeorological and physicochemical variables. Increased FIB concentrations in both the water column and sediments were most strongly correlated with increased antecedent 24-h rainfall, increased stream water temperature, decreased dissolved oxygen, and decreased specific conductivity. These observations will support future efforts to incorporate sediment-related processes in existing models through the identification of key FIB relationships with other model inputs, and the provision of sediment FIB concentrations for direct model calibration. In addition, identified key variables can be used in quick evaluation of the effectiveness of potential remediation strategies.

Elevated levels of fecal indicator bacteria (FIB) are the leading cause of surface water quality impairments in the United States (USEPA, 2012a). Under the 1972 Clean Water Act, states are required to address identified impairments through the development of basin-specific total maximum daily load (TMDL) restoration plans. A TMDL is a "pollutant budget" that quantifies the maximum loadings of a contaminant that a water body can receive while still safely meeting applicable water quality standards (USEPA, 2012a). Because of the technical difficulties and considerable expense associated with monitoring FIB levels, water quality models are commonly used in the TMDL process to estimate the relative contributions of various watershed sources of FIB and to evaluate the effectiveness of potential load-reduction strategies. The ability to develop robust and effective TMDLs is therefore directly dependent on model accuracy. However, efforts to model FIB fate and transport are notoriously plagued by uncertainty, largely associated with current limitations in understanding the relative importance of various microbial behaviors in receiving waters (Benham et al., 2006; Shirmohammadi et al., 2006).

In most existing microbial water quality models, FIB are assumed to behave in a manner similar to dissolved-phase contaminants (Jamieson et al., 2004). However, an increasing number of laboratory- and field-scale studies suggest that FIB fate and transport is much more complex (Fig. 1). For example, a significant fraction of waterborne bacteria are associated with settleable particulates in stream sediments, which can be resuspended into the water column if subsequently disturbed (Jamieson et al., 2005; Krometis et al., 2009; Rehmann and Soupir, 2009). Bacteria associated with particulates can persist in the environment and may pose human health risks for an extended period of time (Droppo et al., 2009), further emphasizing the need to consider sediment-related processes in modeling in-stream FIB concentrations.

To better understand the dynamics of FIB fate and transport, several previous studies used existing water quality monitoring datasets to correlate environmental variables and

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*Corresponding author (hehuan86@vt.edu).

H. Liao, L.-A.H. Krometis, W.C. Hession, and K. Kline, Dep. of Biological Systems Engineering, Virginia Tech, Blacksburg VA 24061; L.L. House, Dep. of Statistics, Virginia Tech, Blacksburg VA 24061; B.D. Badgley, Dep. of Crop & Soil Environmental Science, Virginia Tech, Blacksburg VA 24061. Assigned to Associate Editor Wei Zheng.

Abbreviations: FIB, fecal indicator bacteria; gds, gram dry sediment; QQ, quantile–quantile; StREAM Lab, Stream Research, Education, And Management Laboratory; TMDL, total maximum daily load; TSS, total suspended solids; VADEQ, Virginia Department of Environment Quality.

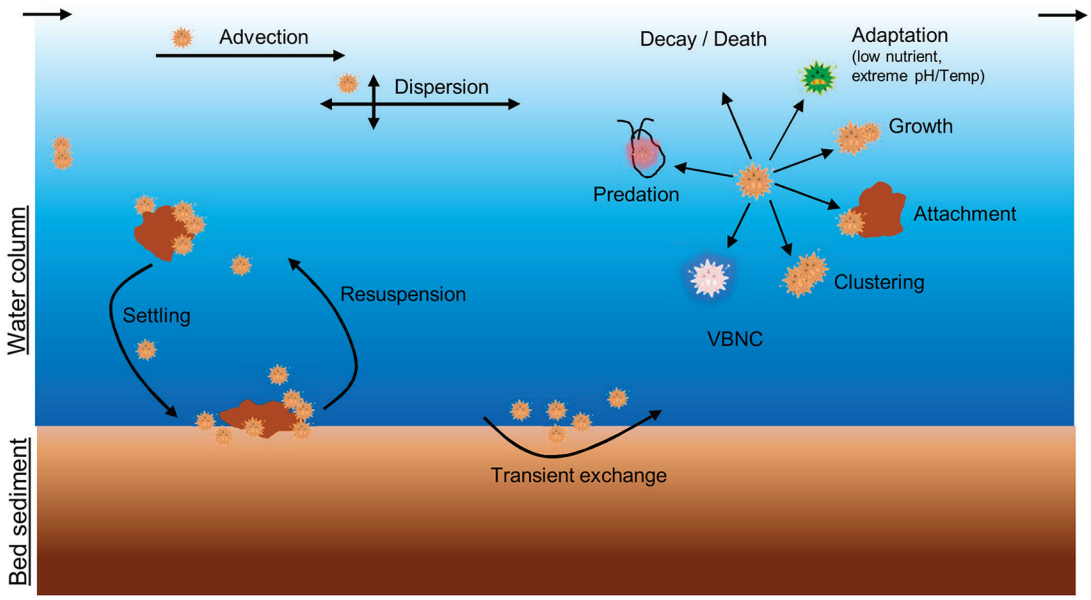

Fig. 1. Major in-stream bacterial fate and transport processes. Current watershed-scale models generally only account for advection, dispersion, and first-order decay, although several studies have developed models by including other fate and transport processes (Kim et al., 2010; Russo et al., 2011; Wilkinson et al., 2011). VBNC = viable but not culturable.

FIB concentrations in the water column. These studies largely associated water FIB levels with measures of precipitation in watersheds; for example, rainfall/antecedent rainfall generally appear positively correlated with FIB concentrations (Gentry et al., 2006; Hathaway et al., 2010; Mallin et al., 2001; Reeves et al., 2004; Vidon et al., 2008). This is not surprising, as many studies noted that increases in water FIB concentrations were associated with runoff-generating storm events (Gaffield et al., 2003; Krometis et al., 2007). A separate study of two midwestern U.S. watersheds observed a significant positive relationship between water FIB concentrations and average daily discharge (Vidon et al., 2008), which may reflect the "flushing" of sediment-associated FIB concentrations. With respect to related water quality metrics, increases in turbidity have been correlated with increases in water FIB concentrations in both coastal (Mallin et al., 2001) and inland (Gentry et al., 2006; Huey and Meyer, 2010; Vidon et al., 2008) waters. Although total suspended solids (TSS) concentrations have been positively correlated with water FIB concentrations in several studies of inland freshwaters (Huey and Meyer, 2010; McCarthy et al., 2007), no statistical significant correlations were observed between TSS and water FIB concentrations in a study of the Neuse River Estuary by Fries et al. (2006). This may reflect the existence of different relationships between FIB and TSS in different types of watersheds or geographic regions.

Unlike water column levels of FIB, FIB concentrations in natural streambed sediments are not typically monitored by environmental agencies. Available datasets for the development of models incorporating fluxes of microorganisms between the water column and sediments are therefore quite limited. Previous efforts to incorporate sediment-related processes in modeling in-stream FIB concentrations relied on theoretical estimates or very limited streambed FIB monitoring data for model formulation, calibration, or validation purposes, which introduced considerable uncertainty (Bai and Lung, 2005; Pandey et al., 2012; Russo et al., 2011).

The overall goal of this study is to improve the current understanding of patterns of FIB concentrations in streambed sediments and to inform future monitoring efforts and subsequent model development. This effort focuses on an impaired stream within a highly urbanized watershed in the Virginia Ridge and Valley ecoregion, where TMDL development is required by the Virginia Department of Environment Quality (VADEQ) under the U.S. Clean Water Act's sections 305(b) and 303(d). Specific objectives include (i) quantification and comparison of water and sediment FIB concentrations and (ii) identification of key hydrometeorological and physicochemical water quality variables associated with FIB levels in the water column and streambed sediments. To our best knowledge, this is the first study investigating correlations between sediment FIB concentrations and other environmental variables. As many microbial fate and transport processes are difficult to measure in natural conditions but are highly coupled to a variety of more readily measurable hydrometeorological and physicochemical factors (Vidon et al., 2008), identifying the dominant factors influencing FIB levels in both the water column and underlying sediments of receiving waters will assist with incorporation of sediment-related processes in model development.

Materials and Methods

Study Area Description

The present study is centered within the Virginia Tech Stream Research, Education, and Management Laboratory (StREAM Lab) along Stroubles Creek in Blacksburg, VA (Thompson et al., 2012; Fig. 2). Stroubles Creek flows approximately 15 km from northeastern Blacksburg, through the Virginia Tech campus, and into the New River, which has served as the region's main source of drinking water supply and home to a variety of recreational activities since the early 1950s (Parece et al., 2010). The 31-km^2

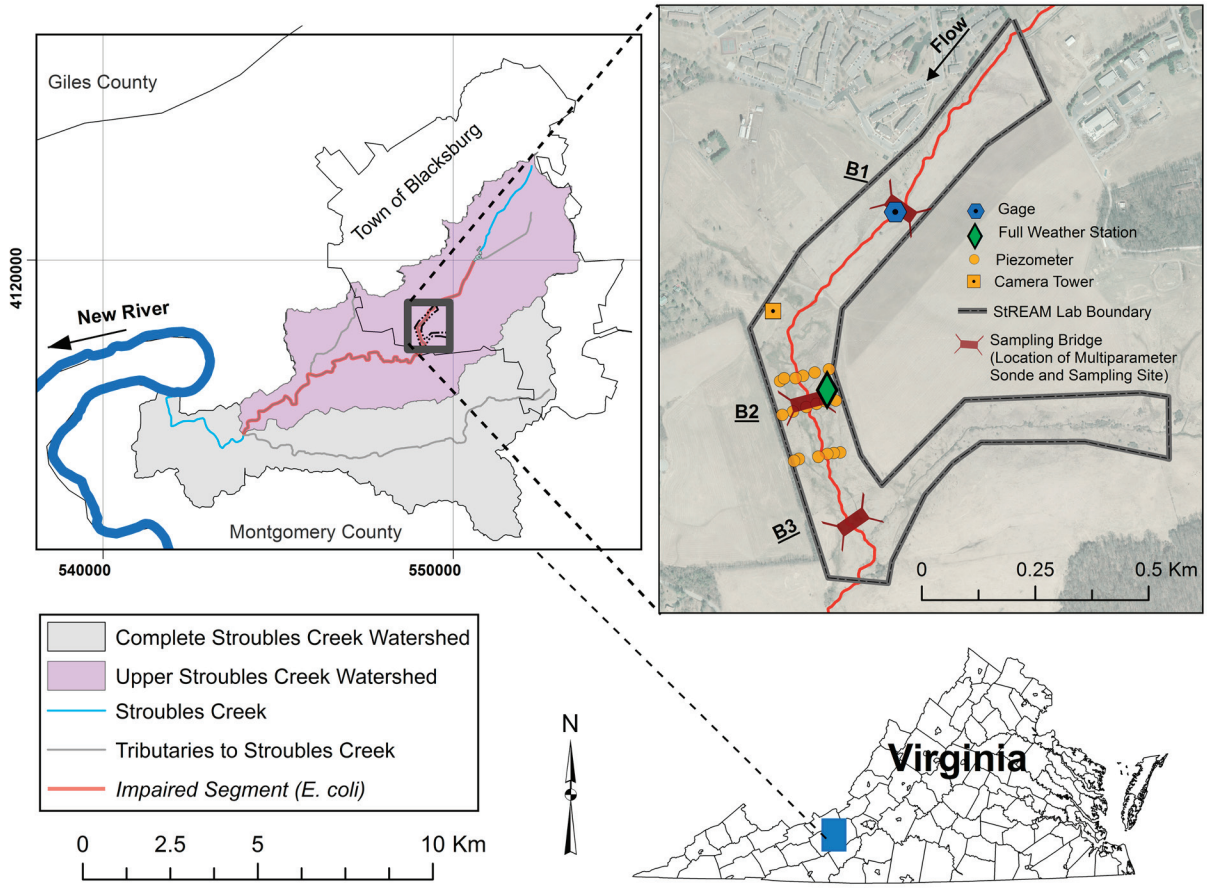

Fig. 2. Location of Stroubles Creek watershed, StREAM Lab (Stream Research, Education, and Management Laboratory), and monitoring sites (B1: sampling bridge 1; B2: sampling bridge 2; B3: sampling bridge 3).

Upper Stroubles Creek watershed is within the Ridge and Valley ecoregion, which is characterized by dolomite and limestone formations with numerous sinkholes and natural springs. The streambed is composed of alluvial-floodplain deposits of stratified unconsolidated silt, clay, and sand with lenses and beds of cobbles and pebbles (Parece et al., 2010). A TMDL to address high sediment loads affecting stream benthic macroinvertebrate communities was developed for the stream in the early 2000s (VT-BSE, 2003; VT-BSE and VWRRC, 2006). The 10-km benthic-impaired segment draining the highly urbanized Upper Stroubles Creek watershed (approximately 50% residential; 34% impervious surfaces) is also impaired by fecal contamination, indicated by elevated *Escherichia coli* concentrations. The benthic TMDL assumed that microbial loadings would also be substantially reduced through sediment control practices, as past experimental and field studies reported significant associations between suspended sediments and indicator organisms (Cizek et al., 2008; Jeng et al., 2005; Krometis et al., 2010; Ling et al., 2002).

Sampling Design

Water and underlying sediment samples were taken from three sites within the StREAM Lab corresponding to the existing sampling access bridges. In Fig. 2, from upstream to the downstream, the three sampling sites are designated as B1, B2, and B3. Site B2 is located approximately 550 m downstream of B1, and B3 is approximately 330 m downstream of B2. Both banks are covered with thick grassy vegetation. At baseflow conditions, the water depth of this segment varied from 13 to 33.5 cm, and the channel width varied from 2.7 to 4.0 m (Abel, 2012). Physicochemical water quality variables (e.g., stream water temperature, specific conductivity, pH, turbidity, and dissolved oxygen) are continuously monitored by multiparameter water quality sondes (YSI Inc.) at all three sites. Stage is monitored via a gauge (Campbell Scientific, Inc.) at B1, which was converted to flow rate using an existing stage-discharge equation developed by the StREAM Lab via the velocity-area method (NRCS, 2014). Hourly rainfall is recorded by a full weather station (Campbell Scientific, Inc.) near B2.

Grab samples of water and sediment from the three sampling bridges were collected weekly for 1 yr (February 2012–January 2013), a total of 50 samples per sampling site. Samples were collected from the most downstream to the most upstream site to avoid contamination via upstream resuspension. Water samples were collected in sterile 250-mL wide-mouth polypropylene bottles. Although studies have shown that FIB can penetrate the hyporheic zone even in fine-grained bedded streams (Drummond et al., 2014), it is largely assumed that most FIB are retained in the upper layer of bed sediment (i.e., the top few centimeters associated with sedimentation and resuspension) (Jamieson et al., 2005; Kim et al., 2010; Pandey et al., 2012). Therefore, in this study, sediment samples were collected from approximately the upper 1 cm of bed sediment, and triplicate sediment samples from each site were composited

in a sterile Whirl-Pak bag as recommended previously by Myers et al. (2007). The time of day was recorded following sampling, and samples were transported to the laboratory on ice within 1 h and promptly analyzed.

Laboratory Analysis

Two FIB targets, *E. coli* and enterococci, were analyzed in this study. Although only *E. coli* is currently monitored by the VADEQ to ensure standards compliance (State Water Control Board, 2010), both *E. coli* and enterococci are recommended by the 2012 USEPA Recreational Water Quality Criteria for freshwater monitoring (USEPA, 2012b).

Water Samples

Concentrations of *E. coli* and enterococci were determined via the Colilert and Enterolert defined substrate techniques, respectively (IDEXX Laboratories, Inc.). Quantification through incubation in the 97-well Quanti-tray/2000 permitted calculation of most probable number–based concentrations (Hurley and Roscoe, 1983). Colilert trays were incubated at 37°C for 24 h, and Enterolert trays were incubated at 41°C for 24 h per manufacturer recommendations. Following incubation, fluorescent wells within each Quanti- tray/2000 were enumerated under a 6-W, 365-nm handheld UV lamp Model UVL-56 (UVP LLC). The bacteria concentrations in water samples were expressed as number of organisms per 100 mL water (i.e., *E. coli*/100 mL, enterococci/100 mL, FIB/100 mL).

Bottom Sediment Samples

Sediment-microbial aggregates were dispersed before quantification by votexing with Tween-85 (Fisher Scientific), which disperses sediment-microbial aggregates as effectively as sonication without measurable microbial die-off (Krometis et al., 2009). For each sample, a wet weight of 3.0 g sediment was mixed with 200 mL of distilled water. Forty drops of Tween-85 (approximately 400 μL) were added to the solution, followed by 10-s vortex mixing to produce a homogeneous slurry. After allowing the slurry to settle for 30 min, the supernatant was analyzed using the same defined substrate techniques (i.e., Colilert, Enterolert) used in the water sample analysis. Potential quantification errors due to particle-bound organisms during Quanti-tray analysis (e.g., multiple cells per well) have been determined to be minor in most scenarios (Fries et al., 2006). To determine the dry weight of sediment samples, 3.0-g subsamples for each site were dried at 40°C for 24 h. Concentrations of FIB in the bed sediments were expressed as number of organisms per gram dry sediment (gds) (i.e., *E. coli*/gds, enterococci/gds, FIB/gds).

Hydrometeorological and Physicochemical Variables

Continuous monitoring records for hourly rainfall, water temperature, stage, specific conductivity, pH, dissolved oxygen, and turbidity were available for the February 2012–January 2013 monitoring period from the StREAM Lab (http://www.bse.vt.edu/site/streamlab/). Flow rate was calculated using an existing stage-discharge equation developed by the StREAM Lab. Cumulative antecedent rainfall (Rain x, where x = 12 h, 24 h, 48 h, or 72 h) was calculated through summation of the total rainfall in the time x before the sampling event.

Statistical Analysis

Fecal indicator bacteria density values were log10 transformed to reduce skewness, as visually confirmed by a quantile–quantile plot (QQ plot) of the standardized data against the standard normal distribution (for normal data, the points plotted in the QQ plot should fall approximately on a straight line). Spatial variations in FIB concentration between sites were examined via one-way analysis of variance (ANOVA). The Pearson product-moment correlation coefficient (r) was used to test for correlations between FIB in the water column and FIB in bed sediment (log10 transformed data were used). Because the skewness of other physicochemical variables was not reduced sufficiently by log10 transformation for parametric Pearson product-moment correlation analysis, non-parametric Spearman's rank correlation coefficient analysis was used to test the monotonic relationships with FIB. All statistical analyses in this study were performed in R version 2.15.3 (R Development Core Team, 2013).

Results and Discussion

Differences between observations of FIB concentration were not statistically significant for any of the three sampling bridges (ANOVA test, $p > 0.2$). Therefore, FIB concentrations from the three sites in each sampling event were pooled to compare with the water quality standards. The geometric mean of the FIB concentrations corresponding to the three sampling bridges in each sampling event was used in the correlation analysis with discharge and rainfall, as there is only one stage recorder at B1 and one full weather station close to B2 (Fig. 2). Because there are no significant flow inputs between the three sampling bridges, it appears reasonable to assume the flow rates are similar.

Statistics of Fecal Indicator Bacteria Concentrations in the Water Column and a Comparison with Multiple Standards

The distribution of FIB concentrations in water samples by month (February 2012–January 2013) is shown in Fig. 3. The observed monthly geometric mean of *E. coli* concentrations in the water column ranged from 70 *E. coli*/100 mL (November) to 1000 *E. coli*/100 mL (September), with a median value of 290 *E. coli*/100 mL (Fig. 3a; Table 1). The monthly geometric mean of enterococci concentrations ranged from 4 enterococci/100 mL (March) to 290 *E. coli*/100 mL (September), with a median value of 40 enterococci/100 mL (Fig. 3b; Table 1).

It is worth noting that the decision to include Stroubles Creek on the Virginia 303(d) list of impaired waters was made based on the observation that approximately 8 of 32 samples (25%) collected by the state exceeded the 235 colony forming units (CFU) *E. coli*/100 mL single sample maximum criterion during any 2-yr period from 2006 to 2012 (VADEQ, 2012). In 2012, the USEPA released revised recreational water quality standards based on the use of two bacterial indicators (*E. coli* and enterococci) and two magnitude measures (geometric mean and statistical threshold value) (Table 2). Although adherence to the new water quality guidelines is not required, they are intended as guidance to each state in establishing new standards. Figure

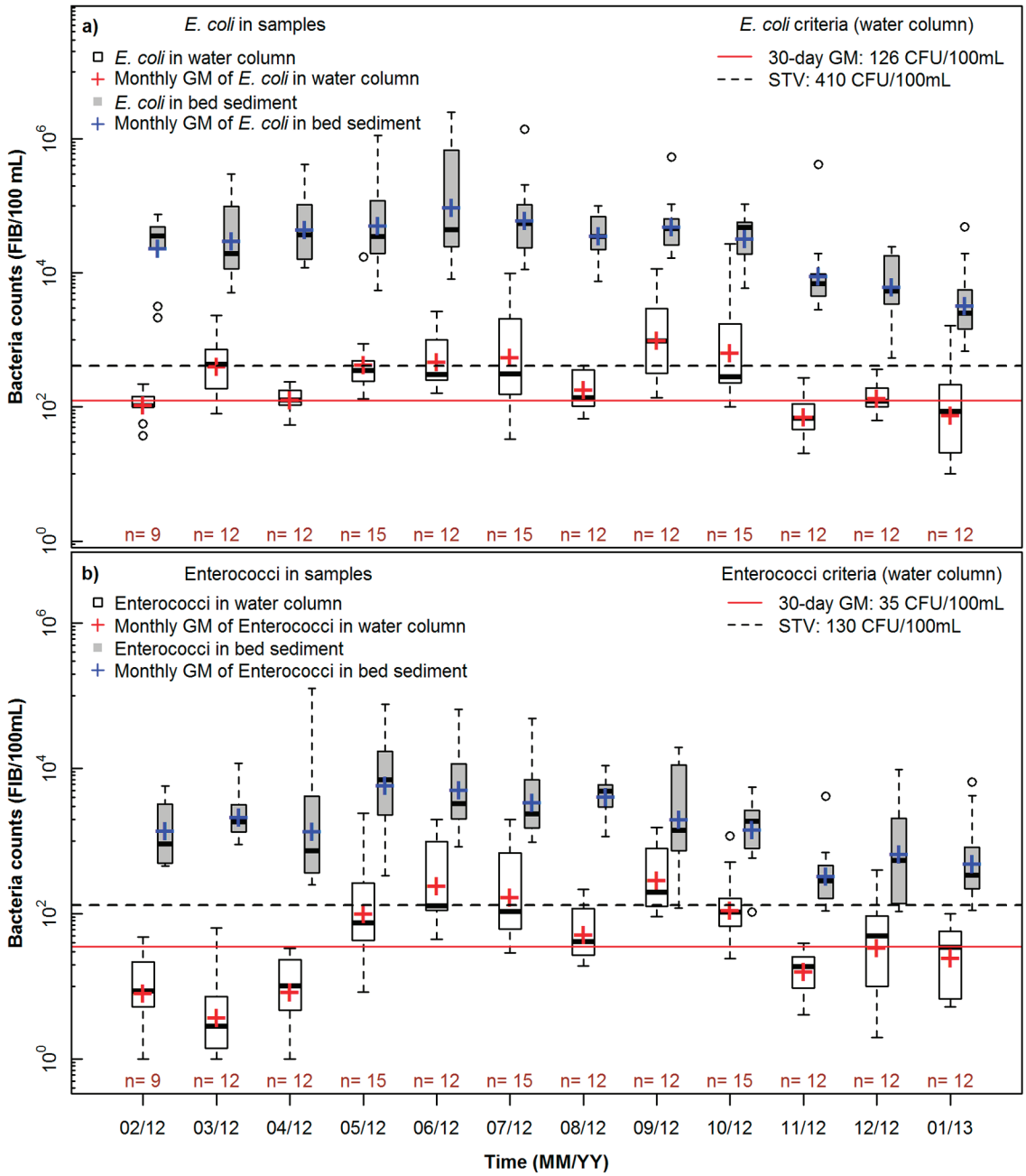

Fig. 3. Monthly distribution of (a) *Escherichia coli* concentrations in the water column and streambed sediments, and (b) enterococci in the water column and streambed sediments. The upper and lower whiskers represent 90th and 10th percentiles, respectively; the box shows 75th, 50th, and 25th percentile; circles represent outliers. CFU, colony forming units; FIB, fecal indicator bacteria; GM = geometric mean; STV = statistical threshold value.

3 therefore illustrates a comparison of FIB measurements with both the existing state standard and the 2012 USEPA revised standard. Overall, similar exceedance rates (9 out of 12 mo) were observed by using the state standard and the revised USEPA guidelines (Fig. 3).

Statistics of Fecal Indicator Bacteria Concentrations in the Streambed Sediments and a Comparison with Overlying Water FIB Concentrations

Concentrations of FIB in the streambed sediments were compared with FIB concentrations in the water column via volumetric ratios. Given uncertainty regarding the homogeneity of FIB concentrations in sediments, this is an imperfect measure, but it allowed comparison with previous work. The sediment FIB concentrations were converted from FIB/gds to FIB/100 mL by sediment density as described previously (Buckley et al., 1998). The monthly geometric mean of *E. coli* concentrations in the bed sediment during the period of study ranged from 3300 *E. coli*/100 mL (January) to 95,000 *E. coli*/100 mL (June), with a median value of 34,000 *E. coli*/100 mL (Fig. 3a; Table 1). The monthly geometric mean of enterococci concentrations in the sediment ranged from 330 enterococci/100 mL (November) to 5900 enterococci/100 mL (May), with a median value of 1700 enterococci/100 mL (Fig. 3b; Table 1).

Table 1. Summary of statistics of monthly geometric mean of fecal indicator bacteria concentrations in the bed sediment, water column, and the ratio.

		Min.	Max.	Median; arithmetic mean
Monthly geometric mean of *Escherichia coli* concentrations in	sediment (*E. coli*/100 mL)	3,300 (Jan.)	95,000 (Jun.)	34,000; 37,000
	water (*E. coli*/100 mL)	70 (Nov.)	1,000 (Sept.)	290; 350
	sediment/water	40 (Dec.)	350 (Apr.)	110; 130
Monthly geometric mean of enterococci concentrations in	sediment (enterococci/100 mL)	330 (Nov.)	5,900 (May)	1,700; 2,400
	water (enterococci/100 mL)	4 (Mar.)	290 (Sept.)	40; 90
	sediment/water	7 (Sept.)	580 (Mar.)	20; 100

Ratios of the monthly geometric mean of *E. coli* concentrations in the bed sediments to that in the water column ranged from 40 to 350 (Table 1), while the ratio of the monthly geometric mean of enterococci concentrations in the bed sediments to that in the water column ranged from 7 to 580 (Table 1). While the present study represents an initial attempt to include sediment samples in a regular FIB monitoring effort for an inland urban stream, these results agree with previous studies of streambed sediment grab samples that observed higher FIB concentrations per unit volume as compared to overlying water column concentrations. Buckley et al. (1998) observed that total coliform concentrations in bottom sediments were on average 1000 times higher than in the overlying water column in a subtropical rainforest conservation reserve, where sediment samples were taken on 12 occasions from November 1993 to April 1994. An et al. (2002) reported that *E. coli* concentrations in sediments were more than 10000 times higher than in the water column based on a 2-d observation in an inland lake, and Pandey et al. (2012) documented that concentrations of *E. coli* in streambed sediments were 2 to 90 times higher than concentrations in the water column during a 1-d observation of different locations along an agricultural creek in summer. Comparisons between these limited references may suggest that ratios of FIB concentrations in bottom sediments to those in the water column in urban streams are lower than lakes or reservoirs but are higher than agricultural streams. It is worth noting that the transport of sediment-associated FIB in urban streams can have more severe impacts on downstream water quality compared with agricultural streams due to the intense hydrologic alterations in urban areas, which results in both increased frequency and magnitude of high-flow events (Wenger et al., 2009).

Correlations with Hydrometeorological and Physicochemical Factors

A summary of available hydrometeorological and physicochemical variables corresponding to the sampling time over the study period, their sources, and statistics is provided in Table 3. The Spearman rank correlation coefficient (ρ) was used to identify key hydrometeorological and physicochemical water quality variables that correlated with water and sediment FIB concentrations (Table 4). Statistically significant correlation coefficients ($\alpha = 0.05$) are discussed below.

Fecal Indicator Bacteria Concentrations in the Water Column

Overall, both increases in *E. coli* and in enterococci concentrations in the water column were strongly correlated with increases in antecedent 24-h rainfall, increases in stream water temperature, and decreases in dissolved oxygen (Table 4; Spearman's correlation coefficient $\rho >= 0.35$). While several previous studies also observed positive correlations of water FIB concentrations with 24-h antecedent rainfall (Mallin et al., 2001) and stream water temperature (He et al., 2007), these relationships do appear to vary. Gentry et al. (2006) observed negative correlations between stream water temperature and water FIB concentrations in a southeastern stream within a mixed land use watershed with karstic features, whereas Vidon et al. (2008) found no correlation between stream water temperature and water FIB concentrations in midwestern streams, and Kelsey et al. (2004) found antecedent 48-h rainfall correlated better with water FIB concentrations than antecedent 24-h rainfall in a

Table 2. Bacteria criteria to protect waters designated for primary contact recreational uses: Virginia surface water quality criteria as established in January 2011, and USEPA (2012b) recreational water quality criteria recommendations for protecting human health in all coastal and noncoastal waters. (Standards from Recommendation 1 are plotted to evaluate the Stroubles Creek microbial water quality.)

Fecal indicator bacteria	VA bacteria criteria		USEPA 2012 recommendations			
			Recommendation 1†		Recommendation 2‡	
	30-d GM§	Single-sample¶	30-d GM	STV#	30-d GM	STV
	CFU/100 mL††					
Escherichia coli (fresh)	126	235	126	410	100	320
Enterococci (marine & fresh)	35 (transition and saltwater)	104 (transition and saltwater)	35	130	30	110

† Recommendation 1 based on an estimated illness rate of 36/1000 primary contact recreators.

‡ Recommendation 2 based on an estimated illness rate of 32/1000 primary contact recreators.

§ GM = geometric mean.

¶ No more than 10% of the total samples in the assessment period shall exceed the single-sample criteria.

STV = statistical threshold value. Approximates the 90th percentile of the water quality distribution, and it is intended to be a value that should not be exceeded by more than 10% of the samples taken.

†† CFU, colony forming units.

Table 3. A summary of data statistics and their sources (data corresponded to the sampling time).

Variables		Sources	Monitoring frequency	Maximum	Minimum	Median	Arithmetic mean
Hourly rainfall (mm)		StREAM Lab†	1 h	0.51	0	0	0.02
Antecedent rainfall (mm)	12 h	Aggregated		34	0	0	2
	24 h			34	0	0	4
	48 h			34	0	2	6
	72 h			349	0	6	15
Water temperature (°C)		StREAM Lab	15 min	25.5	−0.1	14.9	13.6
Dissolved oxygen (mg/L)		StREAM Lab	15 min	15.5	6.3	9.8	10.1
Specific conductivity (mS/cm)		StREAM Lab	15 min	0.86	0.001	0.49	0.40
Flow (m^3/s)		Computed	10 min	0.26	0.03	0.08	0.09
Turbidity (NTU)‡		StREAM Lab	15 min	992	0.2	5.3	25.4
pH		StREAM Lab	15 min	8.1	5.9	7.7	7.7

† StREAM Lab = Stream Research, Education, and Management Laboratory.

‡ NTU, nephelometric turbidity unit.

high-salinity estuary draining an urbanized watershed in South Carolina coast. Therefore, consistent correlations between these variables and water FIB concentrations are not well established, which is probably due to the temporal and spatial complexity of different watersheds. It is likely necessary to establish correlations between water FIB concentrations and environmental variables for a specific site or a watershed type to facilitate long-term predictive modeling purposes.

Fecal Indicator Bacteria Concentrations in the Streambed Sediments

As FIB concentrations in the streambed sediments are not currently monitored or regulated by environmental agencies, long-term monitoring data of sediment FIB concentrations are rare. Comparison of these observations with correlations observed for water FIB concentrations does provide insight into key hydrometeorological and physicochemical variables, which should be the focus of later model refinements or remediation efforts.

Hydrometeorological Variables

Increases in hourly rainfall (i.e., total rainfall in the 1 h preceding a sampling event), although not correlated with overlying water FIB concentrations, were significantly correlated with decreases in sediment FIB concentrations ($p < 0.05$). Increases in antecedent 24-h rainfall were significantly correlated with increases in both water and sediment FIB concentrations ($p < 0.05$). This may suggest antecedent accumulative 24-h rainfall is generally a good indicator of FIB wash-off from upland watershed surfaces and sedimentation.

Increases in streamflow, although correlated with increases in water *E. coli* concentrations ($p < 0.05$), were not correlated with sediment FIB concentrations in this study ($p > 0.05$). Although previous studies indicated that FIB loadings to receiving waters were dominated by hydrological events (Davies-Colley et al., 2008; Muirhead et al., 2004; Wilkinson et al., 2011), it is important to note that in this study, samples were mostly taken during dry-weather (i.e., low-flow) conditions. The absence of correlation between flow and sediment FIB concentrations could therefore be a result of relatively stable flow conditions. Examining how sediment FIB respond to a wider range of flow conditions is an important need for future model development.

Table 4. Summary of Spearman's rank correlation ρ ($\alpha = 0.05$).

		FIB concentrations in water†		FIB concentration in sediment†	
		waterEC	waterENT	sedEC	sedENT
		FIB/100 mL		FIB/g dry sediment	
Hourly rainfall (mm)		–‡	–	−0.33	−0.29
Antecedent rainfall (mm)	12 h	0.34	–	–	–
	24 h	0.40	0.35	0.28	0.30
	48 h	0.33	–	–	0.39
	72 h	–	–	0.30	0.52
Water temperature (°C)		0.47	0.53	0.48	0.50
Dissolved oxygen (mg/L)		−0.51	−0.73	−0.35	−0.36
Specific conductivity (mS/cm)		–	−0.43	−0.26	−0.24
Flow (m^3/s)		0.38	–	–	–
Turbidity (NTU)§		0.19	–	–	–
pH		–	-0.31	–	–

† FIB = fecal indicator bacteria; waterEC = *Escherichia coli* in the water column; waterENT = enterococci in the water column; sedEC = *E. coli* in the streambed sediment; sedENT = enterococci in the streambed sediment.

‡ For cells marked with –, $p > 0.05$; all other cells, $p < 0.05$.

§ NTU, nephelometric turbidity unit.

Physicochemical Variables

Stream water temperature was positively correlated with FIB concentrations in both the water column and bottom sediments ($p < 0.05$). Water temperature has long been recognized as a critical environmental factor in the survival of FIB (Bradford et al., 2013; Ferguson et al., 2003; Hipsey et al., 2008). Under laboratory conditions with other variables (pH, salinity) held constant at ideal conditions, coliform bacteria survival was inversely proportional to water temperature, that is, cells remained culturable longer at lower temperatures (McFeters and Stuart, 1972). In the natural environment, however, water temperature can be a surrogate for multiple environmental and land use factors that affect FIB inputs and survival. While higher water temperatures may be more likely to increase bacterial inactivation and die-off rates, they also generally signal greater potential for fecal inputs (e.g., more livestock, greater wildlife and pet activity) and regrowth. There is no consensus from previous studies regarding temperature impacts; negative (Gentry et al., 2006), positive (He et al., 2007), and no (Vidon et al., 2008) correlation between water temperature and water FIB concentrations have been observed. In the Virginia Ridge and Valley region, more frequent storm events during the warmer seasons results in greater fecal, nutrient, and sediment loadings to the stream, which may create a more favorable environment for bacteria survival and potential growth in both the water column and bed sediments. It is possible that more fecal inputs into the stream during warmer months simply offset the rapid inactivation and die-off rates at higher water temperatures.

Increases in specific conductivity and dissolved oxygen were significantly correlated with decreases in FIB concentrations in bed sediments, in a manner similar to that of water enterococci concentrations. Previous studies observed decreased water FIB concentrations in laboratory conditions when specific conductivity rose from 1.2 to 6.7 mS/cm (Singleton et al., 1982), in natural ponds when specific conductivity rose above 5 mS/cm (He et al., 2007) and in streams when specific conductivity increased (value unreported) (Gentry et al., 2006), suggesting high salt concentrations accompanied by high levels of specific conductivity can inhibit microbial growth or even damage cells (Bradford et al., 2013; Gentry et al., 2006; He et al., 2007; Singleton et al., 1982). In this study, no correlation was observed between specific conductivity and *E. coli* concentration in the water column, which is not surprising as a previous study reported minimal photooxidative damage to *E. coli* at low salinity conditions (the specific conductivity levels observed at this site ranged from 0.001 to 0.86 mS/cm; Table 3) (Sinton et al., 2002). Negative correlations between sediment FIB concentrations and specific conductivity were unexpected, as specific conductivity was previously identified as a factor encouraging microbial-sediment attachment (Bradford et al., 2013). It is quite possible that other factors dwarfed this effect; for example, increases in specific conductivity have been found to be concurrent with rainfall events in this site (data not shown), which may disturb streambed sediments and release attached FIB.

The negative correlations between FIB concentrations and dissolved oxygen levels may be the result of increased waste inputs to the stream, which would be expected to simultaneously increase bacteria growth and biological oxygen demand. In addition, increases in dissolved oxygen levels can result from lowered water temperature due to substantial underground water recharge at this site, which may dilute the FIB concentrations. It is also possible that sunlight-induced inactivation (i.e., photooxidative effects) of FIB in the water column increased with sufficient dissolved oxygen in the stream (Hipsey et al., 2008; Sinton et al., 2002), which may affect the FIB in the bed sediment given high clarity of the creek when the level of dissolved oxygen is high and the creek is shallow (i.e., <0.23 m) at dry-weather conditions.

No significant correlations were found between sediment FIB concentrations and pH over the course of this study. This may be due to relatively stable pH values (range: 5.6–8.1) within Stroubles Creek (Table 3); in addition, previous studies suggested minimal effect of pH on bacteria survival at levels between 6 and 8 (Bradford et al., 2013; Foppen and Schijven, 2006; Hipsey et al., 2008). Correlations between turbidity and sediment FIB concentrations were also not significant in this study. Although turbidity is sometimes regarded as a general measure of nonpoint-source contamination to urban streams, observations of relationships between FIB and turbidity levels in previous studies were mixed. Whereas several studies found positive correlations between turbidity and water FIB concentrations at low-flow conditions (He et al., 2007; Mallin et al., 2001; Reeves et al., 2004) and over hydrograph events (Davies-Colley et al., 2008), other studies observed no significant correlation (Gentry et al., 2006; He et al., 2007; Vidon et al., 2008). These observations suggest that caution is needed before uncritically using turbidity as an indicator of fecal contamination.

Conclusions

Observations of streambed sediments harboring 40 to 350 times the *E. coli* concentrations of the overlying water column and 7 to 580 times the enterococci concentrations of the overlying water column are in agreement with many previous studies highlighting the potential for FIB storage in sediments. Ratios in this study were generally higher than those previously reported for an agricultural stream, which suggests the importance of considering watershed-specific land use when modeling FIB behavior.

While streamflow would be expected to be a strong predictor of in-stream FIB concentrations, given the importance of upland contributions of fecal loadings to urban receiving waters (Gaffield et al., 2003; Hathaway et al., 2010; Krometis et al., 2007), flow was only correlated with water *E. coli* concentrations in this study. Antecedent accumulative 24-h rainfall and water temperature were correlated with both water and sediment FIB concentrations. Increases in antecedent accumulative 1-h rainfall were associated with decreases in sediment FIB concentrations, supporting previously presented studies suggesting that initial storm surges "flush" (resuspend) sediment bacterial reservoirs (Ghimire and Deng, 2013; Jamieson et al., 2005; Pandey et al., 2012), although higher sediment FIB concentrations were associated with greater antecedent 24-hour rainfall amounts, suggesting that over time, these sediments may be

"reseeded" by further FIB loadings, as suggested previously (Russo et al., 2011). It is important to recognize, however, that while these results might support the previous findings of other researchers as discussed, because these samples were taken weekly rather than throughout a single storm, they cannot confirm this behavior to be the cause of the observed fluctuations in FIB concentrations. It would be essential for future intrastorm studies to quantitatively estimate the degree to which resuspended sediment FIB contributes to downstream concentrations. Understanding the relative importance of these two competing factors (sediment "flush" vs. sediment "seeding") would likely greatly improve the understanding of FIB transport that underlies model development.

Comparing the observations in this study to previously reported studies indicates that factors commonly associated with general nonpoint-source pollution (e.g., turbidity, flow) may not be universally associated with FIB levels in all watershed types. This may be the result of differences in water chemistry (fresh vs. salt water), geographical region (patterns of baseflow and storm magnitude), and/or upland land use. Further refinement of models should consider whether or not the sediment-related transport processes in FIB simulations are broadly applicable beyond the watershed-specific datasets used in model development.

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