Spatial and temporal variation of fecal indicator organisms in two creeks in Beltsville, Maryland

M. D. Stocker, J. G. Rodriguez-Valentín, Y. A. Pachepsky and D. R. Shelton

ABSTRACT

Evaluation of microbial water quality is commonly based on monitoring populations of fecal indicator organisms (FIO) such as *Escherichia coli* (EC) and enterococci (ENT). The occurrence of elevated FIO concentrations in surface waters after storm events is well documented and has been attributed to runoff and sediment resuspension. The reasons for FIO concentration variation under baseflow conditions are less clear. The objective of this study was to quantify the variability of EC and ENT in two small streams running through agricultural land use areas. FIO concentrations were measured at upstream and downstream locations under baseflow conditions. Concentrations were not significantly different along cross-sections of the streams. Diurnal concentration trends were observed at each of the sampling locations. Significant differences in concentrations between upstream and downstream locations were noted for both creeks during baseflow periods when no runoff or sediment resuspension occurred. A hypothetical explanation is that indicator organisms are released from sediments during baseflow conditions due to the effect of groundwater influx into streams or due to the motility of indicator organisms. If confirmed, this hypothesis may affect our understanding of the role of sediments in the microbial quality of surface waters.

Key words | diurnal variation, hyporheic exchange, indicator organisms, water monitoring, water quality

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INTRODUCTION

Microbiological monitoring of surface waters is mandated by the USEPA and serves to indicate health risks associated with the contamination of water by fecal sources (USEPA 1986). Contamination of surface waters can result from the influx of runoff from agricultural fields after manure applications, faulty septic systems near water bodies, waste water effluent or overflow from treatment plants, and/or direct deposition of fecal material from wildlife, domestic animals and humans (Byappanahalli *et al.* 2012; Sanborn & Takaro 2013). The use of contaminated surface waters for irrigation and recreation poses risks to human health. A 2013 study that analyzed data from 1998 to 2008 on 13,352 foodborne disease outbreaks attributed 46% of all illnesses to produce (Painter *et al.* 2013), which is predominantly irrigated. Between 2007 and 2008 there were 134 recreational water-associated disease outbreaks, of which the majority of cases were associated with pathogens known to be present in feces (Hlavsa *et al.* 2011). It is expected that water and foodborne-related illness will become more prevalent in the coming years as climate change occurs and enhances pathogen transport and survival in the environment (Karl *et al.* 2009). It is therefore imperative to gain a better understanding of the fate and transport of fecal pathogens in the environment in order to minimize economic and human health risks.

Due to the difficulties with detecting and enumerating fecal pathogens, microbial monitoring of surface waters is conducted via the study of fecal indicator organisms (FIO). FIO are nonpathogenic strains of fecal microorganisms

that are easier to detect and enumerate than their patho-genic counterparts (Indest 2003). Additional characteristics of FIO are that they are usually found wherever enteric pathogens are present, are useful for all types of water and survive longer than hardy enterobacterial pathogens (Bitton 2005). The most common FIO are *Escherichia coli* (EC) and enterococci (ENT), which are primarily used for freshwater and marine waters, respectively, although they are often used in conjunction for many water, soil, and sedi-ment studies (USEPA 1986; Cools *et al.* 2001; Indest 2003; Bai & Lung 2005; Haller *et al.* 2009; Bradshaw *et al.* 2013). The total coliform (TC) group have also been used in water quality assessments; they have recently been ruled to have no direct public health implication but can still serve as indicators of potential pathways for contamination into water distribution systems (USEPA 2013).

Factors that influence levels of FIO in surface waters have been well documented. In general, non-point urban and agricultural land use zones contribute largely to the degradation of surface water quality (Tong & Chen 2002; Traister & Anisfeld 2006; Wang *et al.* 2013). Seasonal vari-ation of FIO typically displays a pattern of highest concentrations in the summer months and lowest concen-tration in the spring and winter (Rodgers *et al.* 2003; Traister & Anisfeld 2006; Koirala *et al.* 2008). Additionally, precipitation, storm events, and wet seasons are associated with higher concentrations of FIO in surface waters (Bolsad & Swank 1997; Schilling *et al.* 2009; Chu *et al.* 2014). Physicochemical factors such as water and air temp-erature, insolation, turbidity, pH, dissolved oxygen, and nutrient concentrations have also been used to study indi-cator and pathogenic bacteria in surface waters (Shibata *et al.* 2004; McEgan *et al.* 2013). While these factors are often applied to larger scale water systems (rivers, water-sheds, coastlines) or greater temporal scales (day, month, season, year), it is relatively unknown what conditions and variability exist in small spatial scale settings such as creeks and streams and at finer temporal measurements. Smaller creeks and streams are typically viable sources for farmers when drawing water for produce irrigation and are often the center of recreational areas, and thus it is imperative to gain a better understanding of the nature of these waterways as it applies to recreational water and food safety.

The objective of this study was to observe and compare the spatio-temporal variability of two creeks having different upstream land-use, and both flowing through agricultural land use areas.

MATERIALS AND METHODS

Locations and land use

Little Paint Branch Creek (LPBC) and Beaverdam Creek Tributary (BDCT), two creeks in Beltsville, Maryland, were chosen for the study. The two creeks were chosen based on ease of access and because both contained portions adja-cent to agricultural land use zones (Figure 1). This allowed us to evaluate the effect of the agricultural land use on water quality within the creek. For LPBC, a third order stream, the reach used in this study started from the upstream sampling point (S1 U) located in Little Paint Branch Park, a forested recreational park, and flowed south for an approximate distance of 2.1 km through agricul-tural land of the USDA's Beltsville Agricultural Research Center West (BARC-W) until it finally reached a down-stream sampling point (S1D) in Cherry Hill Road Park, another forested recreational park. The surrounding land use is approximately 45% agricultural, 25% residential, 20% deciduous forest, and 10% commercial. The agricul-tural fields adjacent to the creek (~270 ha) are mostly (70–90%) under corn-soybean-wheat rotations, with the remain-der used for tomatoes, strawberries, potatoes, and squash. The area receives approximately 300–600 kg ha^{-1} of plant compost annually and receives no manure treatments. For BDCT, a first order stream, the observation reach was located on the OPE3 experimental site on BARC East (BARC-E). The upstream sampling site (S2 U) had minimal interaction with agricultural land, after which the creek flowed through a riparian zone flanked by several agricul-tural fields on OPE3 for an approximate distance of 0.5 km, until reaching the downstream sampling site (S2D). The surrounding land includes agricultural fields (approximately 75%) and deciduous forest (15%). The four fields within OPE3, which are adjacent to BDCT, have been under continuous corn production for 12 years. The north-most field receives 70,000 kg ha^{-1} of dairy manure
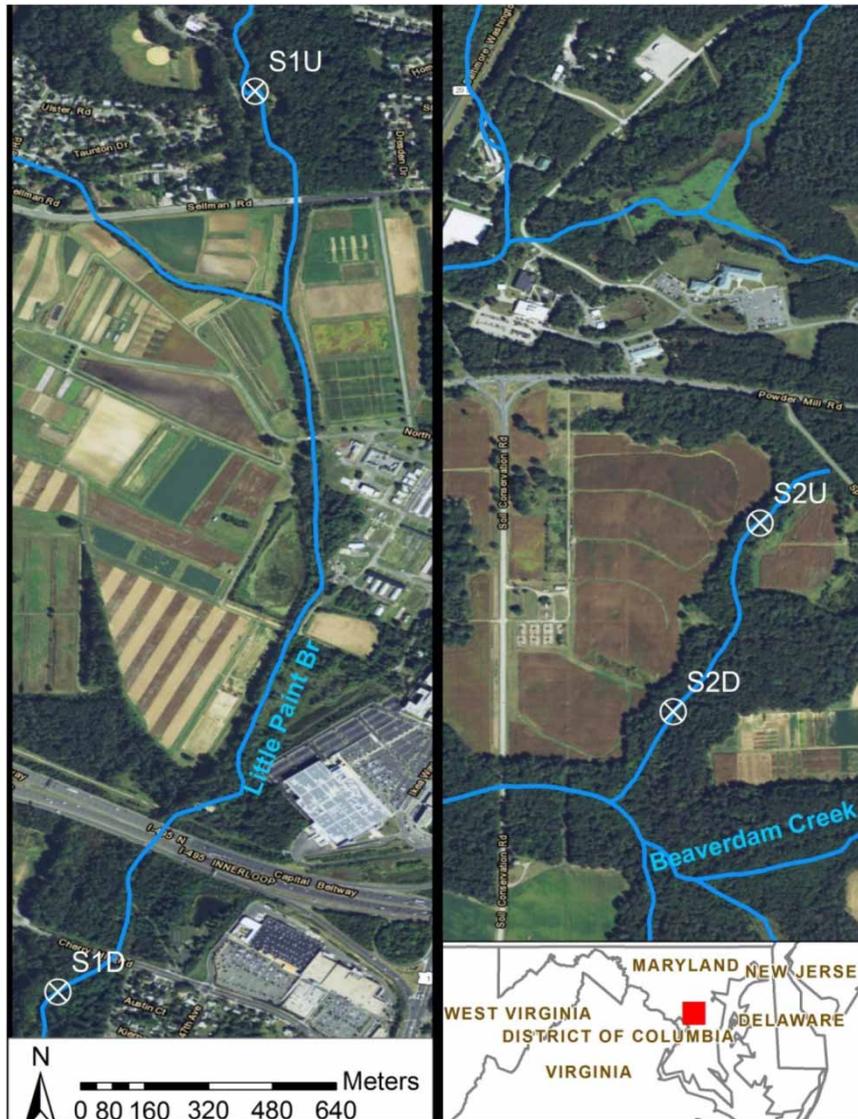
Figure 1 | LPBC (Site 1) on the left and BDCT (Site 2) on the right. Upstream and downstream locations relative to agricultural land are marked with U and D, respectively.

annually in April, while the other three fields receive only chemical fertilizers.

Sample collection, handling, and storage

The sampling method was based on that used in Microbiological Methods for Monitoring the Environment: Water and Wastes, Part II, Section A (USEPA 1978). Both streams were sampled simultaneously using the same procedure. The experiment was conducted throughout June and July of 2014. Samples were taken on Tuesdays and Thursdays each week

unless a rain event occurred, in which case sampling would be conducted on the day of or one day after rainfall to assess the effect of precipitation on indicator concentrations in the two creeks. Conditions were considered baseflow if no rainfall had occurred within 48 hours prior to the sampling date and were considered stormflow if samples were taken during or within 24 hours of rainfall. Samples were taken daily at 08:00, 12:00, and 16:00 and were collected in three locations within the creeks: one sample taken at one bank, one sample taken directly in the middle of the stream, and one sample taken on the opposite bank (Figure 2). Sampling
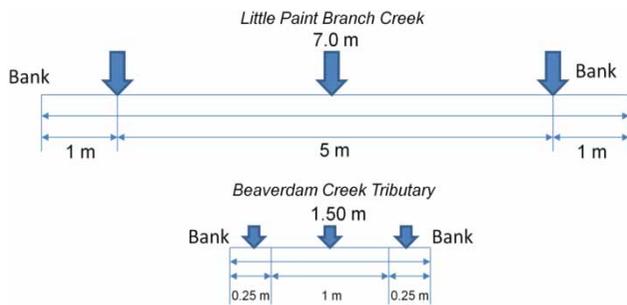
Figure 2 | A schematic of the cross-sectional sampling locations (arrows) within studied streams.

depth was 0–15 cm. Upstream and downstream sampling occurred within a 20-minute window. Samples were collected with 1-litre rod-mounted sample grabbers that were field disinfected with 70% ethanol followed by three rinses with creek water prior to the collection of each sample. Approximately 400 mL of each grab sample was transferred to a sterile Whirl-Pak bag for transport to the laboratory. Samples were kept on ice and shielded from light until being processed within 1 hour of collection.

Physiochemical measurements

Turbidity measurements were performed by thoroughly shaking samples within the Whirl-Pak bags they were collected with, and then transferring 13 mL of sample into special glass turbidity vials for reading. A turbidity meter (LaMotte Company, Chestertown, Maryland) was calibrated prior to 8 am, 12 pm, and 4 pm sample readings. Measurements were displayed in EPA mode, which rounded to the nearest whole number of nephelometric turbidity units (NTU). pH readings were taken at the same time as turbidity with a day-of calibrated meter (PerpHecT Log R meter Model 350). Both pH and turbidity were measured in a pooled sample created from the cross-sectional samples taken at each sampling location and time. Fifteen minute interval precipitation and solar radiation data were provided by BARC Farm Operations and taken with a tipping rain gauge and a pyranometer.

EC and TCs

A range of dilutions was used throughout the experiment to account for an ever shifting fluctuation of concentrations of target bacteria within the creeks. On baseflow days, culturing of EC and TCs consisted of spread plating 250 μL of sample on to *E. coli* and coliform (ECC) agar (Chromagar, Paris, France) and incubating plates for 24 hours at 37 °C. On days with or closely following precipitation events, samples were diluted 1:10 and 1:100 parts sample to sterile D.I. water and spread plated in 100 μL aliquots. After incubation the blue colonies on the ECC plates were counted as presumptive EC and the red or mauve colored colonies were counted as TC.

Enterococci

Two separate methods of enumeration were used for ENT. The first and primary method was membrane filtration on baseflow days. The dilutions for this varied from the start of the experiment to the end as concentrations of bacteria increased or decreased in the creeks throughout the course of the summer. At the start of the experiment 25 mL of sample was membrane filtered through a 0.45 um filter, which was then carefully transferred onto an m-Enterococcus agar plate and incubated shortly after for 24–48 hours at 37 °C. As the experiment progressed, lesser volumes of sample (20, 15, and 10 mL) were needed to achieve the desired range of colonies per plate without overloading (20–80 CFU). On or closely after days when precipitation events occurred, portions of sample were spread plated onto the same agar in aliquots of 250 or 500 μL and incubated as described above. All red colonies for both methods that developed after incubation were counted as presumptive ENT.

Samples were collected in triplicate. Various dilutions of the same EC, TC, and enterococci samples were averaged given they were within an acceptable range of colony forming units per plate (ideally 20–80 per membrane filter and 30–300 per spread plate). Stormflow and baseflow concentrations were analyzed independently. All values reported are per 100 mL.

Data analysis

Data were analyzed using the statistical software PAST v 2.17 (Hammer *et al.* 2001). One-way analysis of variance (ANOVA) tests were performed to compare concentrations at different times of day. Welch's ANOVA was used in cases of unequal variance. Statistical significance was

determined using P (same) as $P < 0.05$. The hypothesis of the equality of concentrations across creeks at the same sampling position was tested with the Friedman test.

RESULTS

Physicochemical characteristics

LPBC had consistently higher water temperatures than BDCT that were on average 4.2 °C warmer. Temperatures ranged from 21.3 to 25.7 °C in LPBC and from 18 to 21.8 °C in BDCT throughout the study. Temperature was found to have a very weak correlation with indicator organism concentrations. Non-parametric Spearman rank correlations for a number of variables can be seen in Table 1. Due to the relatively close proximity of the streams to one another (6.6 km), amounts of precipitation received on sampling dates were very similar ($P = 0.88$) (Supplementary Figure S1, available with the online version of this paper). The creeks received 12.3 and 16.2 cm of precipitation for LPBC and BDCT throughout the study, respectively. Precipitation was generally positively correlated with indicator organism concentrations in both creeks. Turbidity levels also showed a generally strong positive correlation with

Table 1 │ Non-parametric Spearman rank correlations (r_s) between environmental variables and fecal indicator organism concentrations in each stream

	n	TCs, r_s	EC, r_s	ENT, r_s
LPBC				
Temperature (°C)	26	0.36	–0.67	–0.21
pH	138	–0.07	**–0.21**	–0.35
Radiation (W/m²)[a]	10	–0.14	0.54	–0.14
Precipitation (cm)[a]	10	0.7	**0.89**	0.51
Turbidity (NTU)	138	**0.39**	**0.73**	**0.67**
BDCT				
Temperature (°C)	29	0.23	0.14	0.46
pH	144	0.06	0.11	–0.03
Radiation (W/m²)[a]	10	–0.2	–0.2	–0.43
Precipitation (cm)[a]	10	**0.63**	**0.85**	**0.76**
Turbidity (NTU)	144	**0.41**	**0.57**	**0.67**

Values in bold represent statistically significant ($P < 0.05$) correlations.
[a]Correlation made between daily averages of organism type and environmental variable. All other correlations were calculated using values measured in each sample.

indicator concentrations (Supplementary Figure S2, available with the online version of this paper). Turbidity varied from 2 to 170 NTU in LPBC and from 4 to 180 NTU in BDCT. The pH of water in the two creeks was very similar: LPBC had an average pH of 6.35, while BDCT had an average of 6.24. Both creeks shared the same pH range of 6.1–6.7 with an average of 6.35 and 6.24 for LPBC and BDCT, respectively. Solar radiation weakly correlated with concentrations of indicator organisms in all instances.

Variation of indicator organism concentrations across streams

To test the effect of sampling position within the creek sites, concentrations of indicator organisms were compared at the inner bank, middle, and outer bank of the creeks using the Friedman test. The sampling position in both creeks was found to be largely irrelevant. Concentrations were different in only 8.3 and 0% of cases ($n = 18$ for both) for LPBC and BDCT, respectively, showing insignificant impact of position for baseflow and stormflow measurements (data not shown). Due to this finding, it was decided that the samples taken at the same time across creeks would be considered replicates for further analysis.

Variation of indicator organism concentrations along streams

FIO showed inconsistent differences between sampling sites located upstream and downstream of agricultural land use zones and between organism groups (Figure 3(a) and 3(b)). Table 2 shows Wilcoxon signed rank test P values generated when comparing spatial variation at the two creeks used in this study. EC spatial variation differed the least of the three organism types; there were no statistical differences in LPBC at any sampling time, while BDCT EC concentrations differed statistically in half of the cases. Significant differences in TC concentrations were consistent in the LPBC at baseflow conditions at all sampling times. ENT showed the most consistency in spatial variation in that all baseflow observations for different sampling times were significantly different. In almost all cases, concentrations were greater after passing through agricultural land use zones than they were at reach inlets.
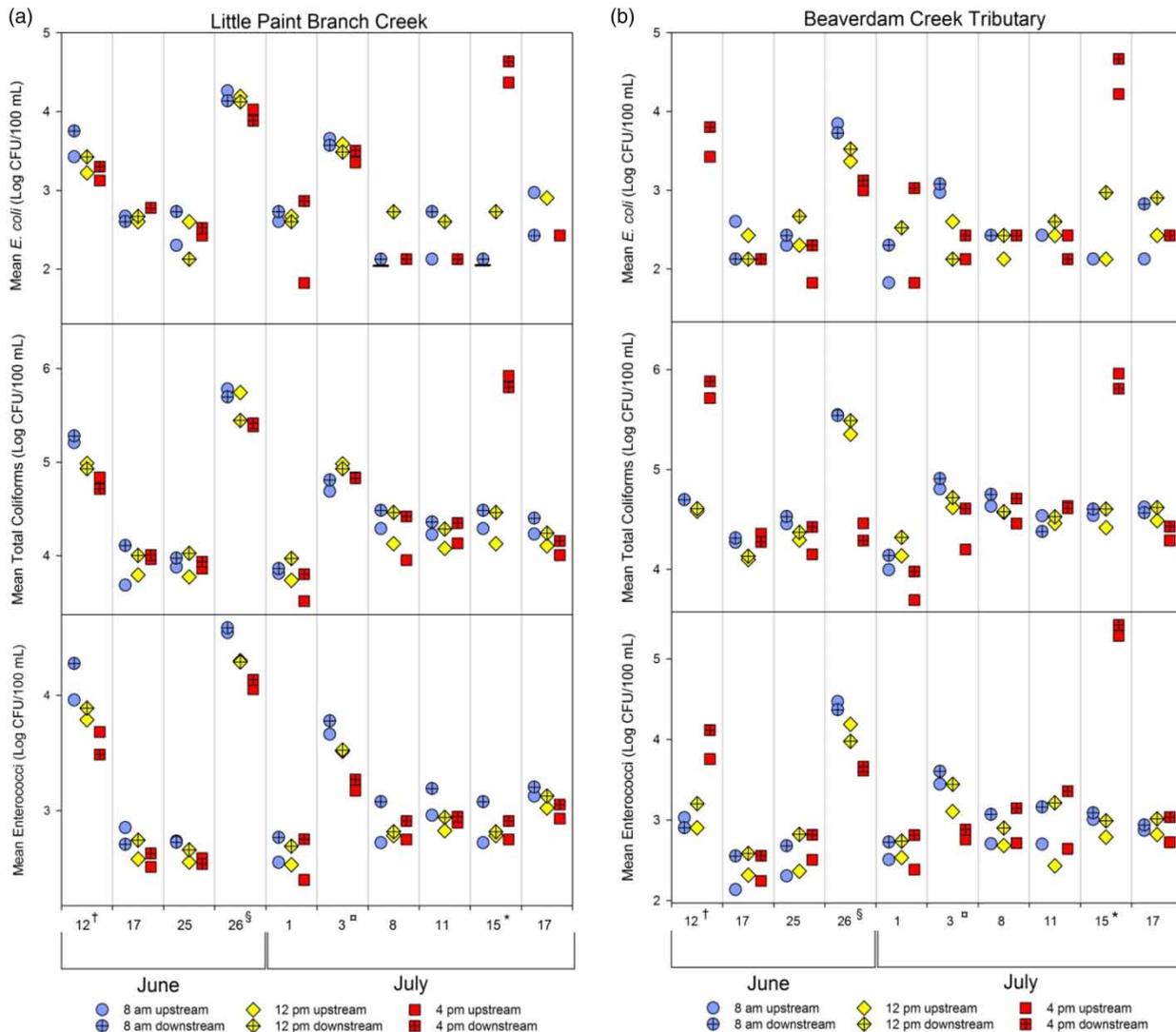
Figure 3 | (a) Spatial and temporal variation of indicator organisms in LPBC. The 10 sampling days are separated by vertical lines. [†]Where light rainfall occurred throughout the day with a heavy downpour at the 4 pm sampling time, [§]during the night prior to sampling, [□]during the night prior to sampling, and [*]during the 4 pm sampling time only. Absent symbols correspond to non-detectable concentrations of indicator organisms. Underlined symbols represent identical upstream and downstream concentrations at that time point. (b) Spatial and temporal variation of indicator organisms in BDCT. The 10 sampling days are separated by vertical lines. [†]Where light rainfall occurred throughout the day with a heavy downpour at the 4 pm sampling time, [§]during the night prior to sampling, [□]during the night prior to sampling, and [*]during the 4 pm sampling time only. Absent symbols correspond to non-detectable concentrations of indicator organisms. Full data set available from corresponding author upon request.

A comparison was made to determine if the land use adjacent to the streams affected the average indicator organism concentrations; LPBC creek flows through a developed area while BDCT flows through a forested area. Daily average concentrations of EC in LPBC were greater than those of BDCT in 9 out of 10 of the sampling days. Conversely, TC populations were greater in BDCT in 8 out of 10 of the sampling days. ENT populations were greater in LPBC in 6 out of 10 of the sampling days. Although the daily population means in each

stream were found to be higher or lower than one another on certain days throughout the experiment, none of these differences were significant (data not shown).

Temporal variation of indicator organisms

In almost all cases time of day did not play a significant factor in population means of the three studied organism groups (Table 3). The three exceptions were TCs during

Table 2 │ Results of Wilcoxon signed rank test on median bacterial concentrations at sampling sites before and after creeks pass through agricultural land use areas, P (same)

Bacteria	Time	LPBC		BDCT	
		Baseflow	**Stormflow**	**Baseflow**	**Stormflow**
EC	8:00	0.68	0.84	0.77	1.00
	12:00	0.43	0.55	**0.01**[b]	0.65
	16:00	0.53	0.25	**0.05**[b]	**0.05**[b]
TCs	8:00	**< 0.000**[b]	0.71	0.50	0.74
	12:00	**< 0.000**[b]	**0.04**[a]	**0.02**[b]	0.25
	16:00	**< 0.000**[b]	1.00	**0.02**[b]	0.70
ENT	8:00	**0.001**[b]	**0.01**[b]	**< 0.000**[b]	0.44
	12:00	**< 0.000**[b]	0.78	**< 0.000**[b]	0.82
	16:00	**0.001**[b]	0.46	**< 0.000**[b]	**< 0.000**[b]

[a]Median group value significantly greater prior to agricultural land use.
[b]Median group value significantly greater after agricultural land use. Significant differences ($P = <0.05$) are marked in bold.

Table 3 │ P-values from ANOVA testing on mean bacteria concentrations between morning, afternoon, and evening sampling events, P (same)

Bacteria	Location	LPBC		BDCT	
		Baseflow	**Stormflow**	**Baseflow**	**Stormflow**
EC	Up	0.21	0.86	0.48	0.19
	Down	0.93	0.41	0.13	0.13
TCs	Up	**0.03**	0.84	0.21	0.11
	Down	0.22	0.58	0.83	0.15
ENT	Up	0.25	0.05	0.29	0.14
	Down	**0.03**	**0.01**	0.36	0.09

Significant differences ($P = <0.05$) are marked in bold.

baseflow and ENT during storm and base flow in the up and down sections of LPBC, respectively. The upstream ENT populations were also largely different; however, these values were not significant. ANOVA results can be seen in Table 3. While in most instances population differences were not significant ($P > 0.05$), in many cases P values were low indicating populations were largely different. Some diurnal trends can be seen in the data with higher concentrations in the morning hours and a gradual decrease as the day goes on (Figure 3(a) and 3(b)). In both creeks, TCs exhibited a trend of greatest concentrations in the morning followed by a decrease in the afternoon and a further reduction by the evening sampling time. ENT behaved in the same manner for all but the site 2 downstream location in which the inverse trend of lowest in the morning to highest in the afternoon was observed. EC displayed no discernible diurnal trend and experienced

site-specific concentration changes throughout the day at each sampling site.

The relative spatial and temporal variability between the three different FIO groups in both streams demonstrated a similar pattern. The coefficient of variation (CV) during baseflow conditions for both TCs and ENT was around 50% (Table 4). This is in stark contrast to EC concentrations

Table 4 │ CV (%) for baseflow concentrations of FIO

	Time	Upstream			Downstream		
		E.C.	**T.C.**	**E.N**	**E.C.**	**T.C.**	**E.N**
LPBC	8:00	102.5	48.7	59.1	83.1	51.1	45.3
	12:00	85.8	46.8	42.8	80.1	49.4	41.6
	16:00	127.2	39.1	45.8	122.6	54.2	43.1
BDCT	8:00	129.5	39.5	61.1	124.9	44.8	48.2
	12:00	104.3	41.1	45.6	76.8	38.2	51.1
	16:00	162.6	60.9	38.7	114.8	50.2	62.3

in each stream, which demonstrated consistently higher relative variation in the range of 80.1 to 129.5%.

Synchronous concentration changes of both streams

A Spearman's rank coefficient was used to determine how strongly changes in bacterial concentrations occurred together between the two streams through the same times of day. EC concentrations between the two streams throughout time weakly correlated (Spearman's rho = 0.31, $P = 0.014$). Conversely TCs and ENT concentrations strongly correlated throughout time (Spearman's rho = 0.74, $P = <$ 0.001 and 0.693, $P = <0.001$, respectively).

Rainfall events

Rainfall events occurred on the 1st, 4th, 6th, and 9th sampling days (Figure 3(a) and 3(b)). LPBC exhibited mean EC, TC, and ENT concentrations during stormflow that were 26.2, 18.6, and 14.8 times greater, respectively, than concentrations during baseflow. Storm concentration numbers for BDCT were at 18.8, 8.4 and 47.8 greater for mean EC, TC, and ENT than baseflow, respectively. Stormflow concentrations were extremely variable, but were clearly related to the amount of precipitation received. The largest concentrations recorded in the study occurred during the largest precipitation event on the ninth sampling day at the 4 pm sampling (Figure 3(a) and 3(b)).

DISCUSSION

Physicochemical properties of water have been examined in the past to compare with microbial indicator organism concentrations. In the present study, we examined water temperature, pH, turbidity, solar insolation, and rainfall. The weak correlation between indicator organism concentration and temperature could be due to the relatively small temperature variation among sampling days, which allowed other factors to have a greater effect on concentrations. Weak temperature dependence was also noted by a number of other researchers (Francy *et al.* 2000; Hennani *et al.* 2012; McEgan *et al.* 2013). The acidity of the water shows a very low correlation with indicator organism

concentrations. McFeters & Stuart (1972) found that the optimal pH for EC immersed in deionized water was between 5.5 and 7.5, with accelerated decline when above and below these values. When these researchers looked at EC in water from two rivers with pHs of 8.37 and 8.10, there was no significant difference in survival. This is very similar to the findings in the present study, which observed mean pHs of 6.35 and 6.24 that have less difference than the values reported in McFeters & Stuart (1972). When observing *Salmonella* concentrations at 18 different field sites, McEgan *et al.* (2013) reported very weak correlations between *Salmonella* concentrations and pH at all sites, with most sites' pH being between 6 and 8. This finding is similar to McFeters & Stuart (1972), and seems to confirm that if pH does not considerably change in a water body than it may be ignored as a factor. However, if large drops in pH are recorded then this may be caused by hydrolysis of acidic materials from microorganisms performing anaerobic fermentation due to low dissolved oxygen levels, which can indicate the presence of fecal pathogens (Juahir *et al.* 2011). While turbidity has been noted to be a weak indicator of microbial water quality (McCoy & Olson 1986; Hörman *et al.* 2004; McEgan *et al.* 2013) the present study found generally moderate to strong correlations between turbidity and indicator organism concentrations. Other researchers have also documented moderate to strong correlations of turbidity and indicator concentrations in water (Christensen *et al.* 2000; Rasmussen & Ziegler 2003; Dorner *et al.* 2007; Rao *et al.* 2015), which may support the use of turbidity as a supplemental or indirect indicator of fecal contamination. Solar radiation in the present study did not correlate strongly with indicator concentrations. This is contrary to a number of other studies (Whitman *et al.* 2004; Boehm 2007; Jenkins *et al.* 2011). A likely explanation for this is the primarily shaded nature of both creeks in this study, which may have provided adequate shielding from solar radiation.

The sampling position within the two creeks in the study played almost no factor in measurements of bacterial concentrations. This could be attributed to the small size of the creeks used in the study, at an average sampling site width of 7.0 and 1.5 meters wide for LPBC and BDCT, respectively. The fact that no significance of position was observed for stormflow events is most likely due to the

homogenization of the waterways during high and rapid flow conditions. Castillo *et al.* (2004) found that cross-sectional variation of planktonic bacteria production in both low and high water conditions did not significantly differ at either of the two clearwater or two blackwater rivers they used in their study. Additionally, Goyal *et al.* (1977) reported similar numbers of fecal coliforms at the water surface across the width of all six waterways used in their study, and Masopust (2005) reported the same uniformity of organisms across a river following two storm events of differing magnitudes. Buckalew *et al.* (2015) found that concentrations of EC were homogenously distributed both laterally and vertically in the water column of a freshwater stream when discharge did not exceed 245 cubic feet per second, which both creeks in the present study fall well below.

Agricultural land use had a noteworthy effect on indicator concentrations in the creeks in this study, although this was inconsistent between different organisms. The effect of agricultural land use on neighboring water quality has been well documented (Bolsad & Swank 1997; Tong & Chen 2002; Rodgers *et al.* 2003; Tetzlaff *et al.* 2012; Liang *et al.* 2013; Wang *et al.* 2013). Jokinen *et al.* (2012) found that indicator organisms, bacteroide marker detection rates, and pathogen isolation rates were highest at sites downstream of agricultural land use zones relative to upstream locations. The authors attributed this to intensive agricultural activities that included high livestock densities and the use of irrigation and manure spreading. Additionally, Fisher *et al.* (2000) compared creeks from wooded areas to those adjacent to pastures, and found higher concentrations of TCs and ENT in pastured creeks and a less clear result with EC, similar to the present study. Hurley & Mazumder (2013) found agricultural land use to be a factor in the increase of EC, but noted that this was only associated with land use at a local (5–10 km) spatial scale. Both creeks in the present study are within this spatial scale, and both had greater microbial concentrations at sites downstream of agricultural land use.

The effect of land use on water quality has been shown to vary depending on category. Traister & Anisfeld (2006) found median EC concentrations that were 14 times greater in more developed watersheds than in largely forested ones which was similar, to a lesser extent, to what was observed in the present study, with LPBC containing greater daily means of EC

than BDCT on all but one of the sampling days. Hennani *et al.* (2012) also found consistently higher fecal coliform levels during base and high flow periods in urban watersheds when compared to other land uses. Francy *et al.* (2000) found land use to have 'the most significant and discernible effect on concentration of bacterial indicators in streamwater', with agricultural-urban (similar to LPBC in this study) settings having greater median TC, EC, and *C. perfringens* concentrations than in agricultural, forest-urban (similar to BDCT in this study), or forest categorized zones. Stream order may also play a factor in higher EC concentrations in LPBC. EC and fecal coliform concentrations have been observed to increase with increasing stream order (Bolsad & Swank 1997; Byappanahalli *et al.* 2003), which was also seen in the present study with LPBC having a Strahler number of three and BDCT being a first-order creek.

While runoff is likely the primary cause of increased indicator concentrations in waters downstream of agricultural land use zones during high or stormflow events, the cause of increased FIO concentrations along stream reaches during baseflow conditions is less clear. It was noted that there apparently exists FIO flux to stream water from the bottom sediment during baseflow periods. This flux appears to be independent of rainfall hydrology (Muirhead *et al.* 2011). A probable explanation for this is the occurrence of hyporheic exchange. Grant *et al.* (2011) suggested hyporheic exchange to be the greatest process controlling nutrient and fecal indicator organism sequestration and remobilization between sediments and the overlying water column. High nutrient inputs to streams have been observed at agricultural land (Hooda *et al.* 2000; Tong & Chen 2002; Ahearn *et al.* 2005), and thus the sediments in these areas are likely habitable for bacteria. Water flowing through hyporheic sediments travels at low velocities and comes in close contact with biofilms growing on sediment surfaces (Wondzell 2012). Sediment biofilms have been documented to harbor allochthonous EC and ENT, which can coexist in smaller concentrations with autochthonous biofilm organisms (Balzer *et al.* 2010). These indicator bacteria can be released into the water column via hyporheic exchange either due to convective transport with groundwater, or through chemotactic motility, or both. This would help explain the statistically significant increase in mean TC and ENT concentrations in creek water at both LPBC and BDCT at

downstream locations relative to the upstream sites. This observation was less consistent with EC concentrations, however while rarely significantly different, concentrations were greater in downstream locations 70% of the time. Additionally, the high variability of EC concentrations (Table 4) could hinder the statistical significance of the 'upstream–downstream' differences. The hypothesis for the effect of hyporheic exchange on FIO concentrations in surface waters is relatively unexplored, and thus would benefit from future research that aims to better quantify the effect for the improvement of both monitoring and predictive modeling applications (Oliver *et al.* 2016).

The present study found very few instances of statistically significant differences in indicator populations by time of day, although certain groups of data were largely different. A closer look into some of these groups revealed that a number of trends exist within the data. During baseflow, most of the time if not always, the TC and ENT groups were greatest in the morning and least in the evening with the afternoon being a transitional period between the two times of day. This finding, despite the concentrations not being statistically significant in our study, is consistent with what others have observed. Traister & Anisfeld (2006) observed consistent diurnal oscillations of EC concentrations, with very early morning hours exhibiting the highest concentrations and a steady decrease throughout the day before rising again at night during their summer sampling. The authors largely attributed this to sunlight-induced die off. Enterococci populations have also been observed to exhibit diurnal trends for the same reason (Boehm 2007). The Office of Water for the USEPA concludes that when culture methods are used for indicator bacteria enumeration, the highest densities will be observed at least until 8 am with the lowest concentrations occurring between 2 and 3 pm dependent on insolation at any given site or day (USEPA 2010).

Concentrations of ENT and TCs in LPBC and BDCT were found to strongly correlate according to Spearman's Rho, which indicates that these two organism groups have similar hourly population dynamics in the two observed creeks. The parallel diurnal changes of concentrations could be due to similar solar irradiance because of the proximity of the two creeks to one another. EC concentrations between the two creeks weakly correlated. This may be due to the finding that LPBC creek has greater EC concentrations than BDCT, and may be subjected to more dynamic inputs of fecal contamination that act to mask diurnal trends. The relative variation of EC concentrations was roughly double that of TCs and ENT (Table 4) which may also help explain why concentrations of EC between the two streams did not shift in unison.

Substantially greater concentrations of indicator organisms in creek water during precipitation or increased discharge events were observed, which is consistent with findings from similar studies (Bolsad & Swank 1997; Clark & Norris 2000; Rodgers *et al.* 2003; Schilling *et al.* 2009; Cho *et al.* 2010a; Hennani *et al.* 2012; Chu *et al.* 2014). Cho *et al.* (2010b) stated that this occurs for two primary reasons, which are the addition of overland runoff into the waters and resuspension of bacteria from the streambed. During medium and low flow conditions, bacteria live in stream sediments and can be easily released during high flow events (Pachepsky & Shelton 2011; Chu *et al.* 2014).

CONCLUSIONS

The monitoring study carried out at two creeks running through agricultural arable lands showed that turbidity and precipitation events were the strongest covariates for bacterial concentrations whereas temperature, pH, and solar radiation were poorly correlated with indicator bacteria concentrations. Cross-sectional creek variability of indicator organism concentrations was not significant. Diurnal concentration variation was found to be minimal in the present study. Waters downstream of agricultural areas generally had higher mean indicator concentrations than upstream locations during baseflow conditions. As the sediment-water column exchange processes appear to be a probable reason for that, future research is needed to better understand and quantify the effect of hyporheic exchange on indicator concentrations in surface waters.

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First received 5 December 2015; accepted in revised form 4 March 2016. Available online 8 April 2016