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# A Watershed Analysis of Seasonal Concentration- and Loading-based Results for *Escherichia coli* in Inland Waters

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**Abstract** Fecal indicator bacteria, such as *Escherichia coli*, are frequently monitored in recreational waterbodies as indicators of potential fecal pathogen presence and exposure. The present watershed analysis investigated the influence of season on *E. coli* concentration (MPN/100 mL) and loading (MPN/day) measurements for inland waters at baseflow conditions. The master dataset collected during 2007–2012 for three watersheds included 896 *E. coli* (Colilert) samples with simultaneous flow taken for a subset (39 %) of samples. The outcomes for grouped watersheds were reflected in most cases for individual watersheds. Concentration- and loading-based results were highest in summer and spring, and lowest in the winter and fall, respectively. The comparison of these two measurement techniques (concentration and loading) highlight the impact of flow data during baseflow conditions for inland waters and reveal that caution should be used when inferring one method's results from another.

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Protection and restoration of water resources are important worldwide to prevent human exposure to waterborne fecal pathogens. Singly or collectively, fecal sources such as combined sewer overflows, sanitary sewer overflows, septic tank failure, illicit sewer connection to stormwater infrastructure, bypass events from wastewater treatment plants, livestock and pastures, domestic pets, and wildlife have the potential to transfer fecal pathogens into recreational waterbodies (USEPA 2001). For example, Shuval (2003) estimated that worldwide 120 million gastrointestinal illnesses and 50 million respiratory cases per year are due to recreating in waterbodies influenced by municipal wastewater; in California similar illnesses were projected to cause a public health burden and subsequent economic loss of $3.3 million per year (Dwight et al. 2005).

Waterbodies are monitored for the presence of fecal contamination and possible waterborne pathogens by enumeration of *Escherichia coli*, which is used as a fecal indicator bacteria (FIB). Based on epidemiological studies at coastal and inland beaches where positive correlations between *E. coli* densities and gastrointestinal illnesses were found (USEPA 2003, 2010), many states now include *E. coli* sampling in their water quality monitoring programs regardless of waterbody type (e.g., lakes, inland streams, rivers, estuaries, oceans) or climate (e.g., temperate, arid, tropical).

Results from *E. coli* monitoring are typically analyzed either on a concentration-based or loading-based approach. Concentration-based analysis is utilized because of the ease of sample collection and established water quality criteria that allow for the evaluation of human health risk to fecal contamination (Hörman et al. 2004; Marion et al. 2010; Amorim et al. 2014). Loading-based analysis takes into account time specific water flow conditions allowing for the determination of relative contribution of a stream to a larger system (e.g., watershed) (Gentry et al. 2006), but at

a cost of increased time and expense. Both concentration- and loading-based analyses have been used to investigate fecal inputs (Stumpf et al. 2010; Gentry-Shields et al. 2012; Rowny and Stewart 2012); but simultaneous comparisons are not prevalent (Converse et al. 2011), especially for inland waters (Dorevitch et al. 2010).

*Escherichia coli* monitoring has been shown to be influenced by changes in season (Traister and Anisfeld 2006; Converse et al. 2011; Amorim et al. 2014; North et al. 2014). However, the results from previous researchers have shown differing patterns depending on the type of analysis performed (concentration- vs loading-based analysis). For example, Traister and Anisfeld (2006), utilizing a concentration-based analysis, found that *E. coli* concentrations increased from spring to summer and decreased in the winter during a year-long study in a forested and urban watershed in the Hoosic River Basin in the northeastern United States. This is in contrast to Converse et al. (2011) who showed the highest loading values for *E. coli* in November during a coastal storm water study using a loading-based analysis.

Despite the wide use of *E. coli* as an FIB, very little literature exists directly comparing the results of common approaches to *E. coli* analyses. The goal of the present study was to perform a simultaneous analysis on the influence of season on *E. coli* concentration and loading measurements for inland waters. Specifically, the objectives were to (1) compile a dataset that would allow for the direct comparison of *E. coli* concentrations and loadings; (2) determine the influence of season on *E. coli* concentrations and loadings independent of one another; and (3) compare the results of these independent analyses to one another.

## Materials and Methods

A master dataset was compiled from multiple studies by Nashville Tennessee's Metro Water Services, Stormwater Division/National Pollutant Discharge Elimination System Office. The master dataset included *E. coli* concentrations from three watersheds (Browns, Richland, and Mill) in Nashville TN, USA over a period of 2007–2012 (total of 896 samples). A subset of the *E. coli* data in the master dataset also had corresponding flow measurements recorded (~39%). These three watersheds (Browns, Richland, and Mill) were identified as ideal candidates for the present study because they were frequently monitored, had the most complete data, and were listed on the 303(d) list as impaired due to pathogens (TDEC 2014). All data included in the master dataset was sampled following Tennessee Department of Environment and Conservation (TDEC) *Standard Operating Procedure for Chemical and Bacteriological Sampling of Surface Water* (TDEC 2009). Briefly, all samples were collected during baseflow stream conditions (<0.25 cm rainfall

within last 72 h). Stream velocity (ft/s) was measured using a Swoffer Model 3000 Current Velocity Meter-Flowmeter and was used along with stream cross sectional area to calculate flow (cubic feet per second, CFS). All samples were analyzed for *E. coli* within 6 h of collection using the EPA-approved Colilert® method (TDEC 2009) (IDEXX Laboratories, Westbrook, Maine), in which a 100 mL water sample is distributed into a series of aliquots. The presence or absence of metabolic activity among the aliquots is used to derive the maximum likelihood estimate of *E. coli* concentration, reported as most probable number per 100 mL (MPN/100 mL) in the sample. Loadings were calculated by multiplying *E. coli* concentration (MPN/100 mL) by simultaneous flow measurements and were reported as MPN/day.

Five individual sites within each watershed were utilized, except Browns, in which six sites were utilized. Sampling seasons were defined as June, July, August (summer); September, October, November (fall); December, January, February (winter); and March, April, May (spring). Due to a catastrophic flood event, May 2010 was excluded from analysis. Yearly data were combined by season, across years, to incorporate a wide range of site, seasonal, and yearly variation to provide a robust estimate of FIB concentration and loadings. Sample sizes varied by year and season and can be found in Figs. 1 and 2.

The three impaired watersheds lie within a temperate climate (annual average 15°C) with warm summers (July average 26°C) and mild winters (January average 2°C) (https://ag.tennessee.edu/climate/Pages/climatedatatn.aspx). The watersheds are classified as part of the Outer Nashville Basin Level IV ecoregion. No wastewater treatment plants directly impact these watersheds nor does any concentrated animal feeding operation exist in these areas. Size, land use, population, and imperviousness of the watersheds are presented in Table 1.

One-way Analysis of Variance (ANOVA) tests were performed on the three watersheds grouped together as well as on each watershed individually to assess seasonal differences for both concentrations and loadings. If ANOVAs indicated significant seasonal differences, Tukey's post-hoc tests were performed to detect differences among seasons. Normality and equality of variances were assessed before statistical analyses were performed and bacteria concentrations and loadings were $\log_{10}$ transformed if assumptions were not met. An alpha ($\alpha$) = 0.05 was used as the significance level for all statistical analyses. IBM SPSS Ver 20 (Armonk, NY: IBM Corp) was used for all statistical analyses.

## Results and Discussion

Monitoring for fecal indicator bacteria, specifically *E. coli*, is a common approach for water quality regulators to assess human health risks from fecal contamination.
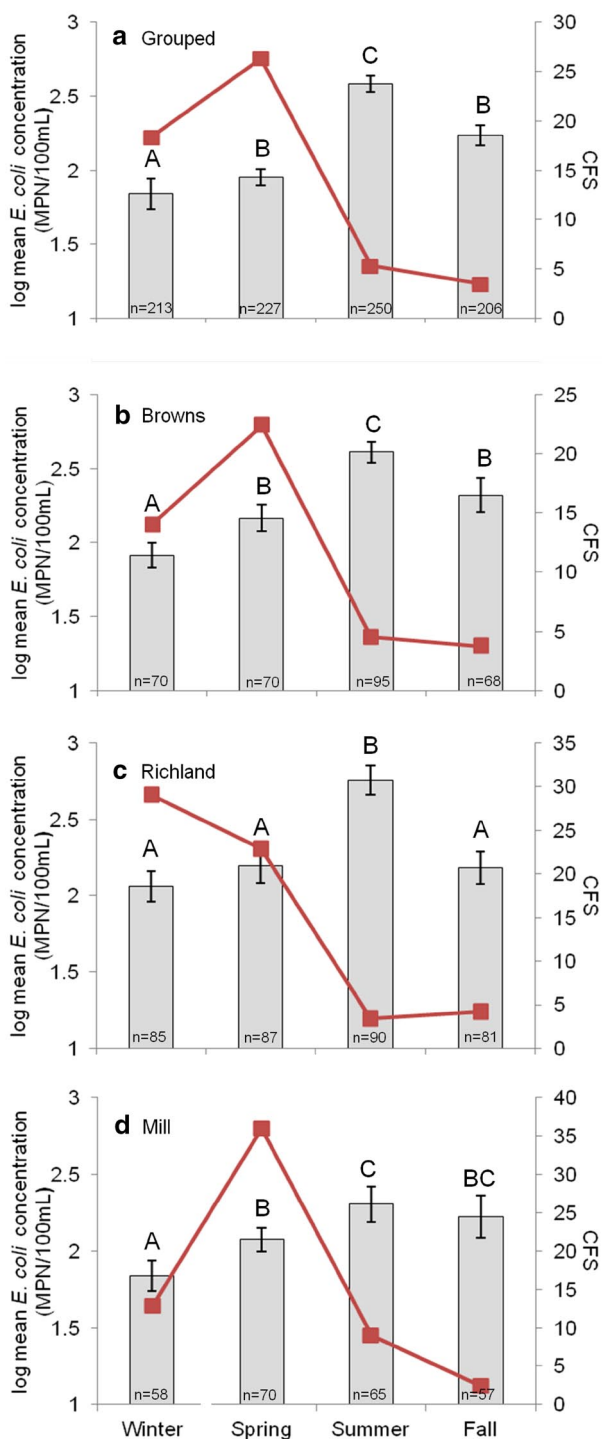
Fig. 1 a–d Seasonal comparisons for *Escherichia coli* concentrations (*bars*) and average flow in grouped (**a**) and in Browns (**b**), Richland (**c**), and Mill (**d**) watersheds. Seasons not sharing similar letters are significantly different from each other. Data presented as mean ± 95 % confidence intervals. *CFS* cubic feet per second

The employment of *E. coli* as a monitoring tool is useful in a variety of water quality programs, such as stormwater runoff monitoring for watershed studies (Jamieson et al. 2003; Converse et al. 2011), risk assessment to beach-goers

(USEPA 2012), gauging effectiveness of best management practices to minimize fecal inputs (Leisenring et al. 2012), and incorporation into total maximum daily loading calculations (USEPA 2001). The present watershed assessment was a unique opportunity to evaluate whether the effect of season influenced concentration- and loading-based analyses the same way, both at the individual watershed level and after combining multiple watersheds together.

Significant differences using concentration-based analyses were observed by season when watersheds were analyzed collectively ($F_{3, 892} = 81.169$, $p < 0.01$) and individually (Browns: $F_{3, 299} = 46.785$, *$p < 0.01$; Richland*: $F_{3, 339} = 36.506, p < 0.01$; *Mill*: $F_{3, 246} = 13.764, p < 0.01$) and showed summer concentrations being the highest and statistically greater than winter for both grouped and individual watershed analyses (Fig. 1a–d). Previous researchers have shown a similar seasonal trend (Traister and Anisfeld 2006; Koirala et al. 2008; Wilkes et al. 2009; North et al. 2014). For example, in a coastal urban bathing area in Portugal, mean *E. coli* concentrations were statistically highest in the summer for three of the four beaches studied (Amorim et al. 2014). The high *E. coli* concentrations observed both by previous researchers and in the present study are likely due to either lowered water levels and flow in the summer (Fig. 1a–d) causing *in situ* and imported *E. coli* to become more concentrated (Cha et al. 2010) or increased *E. coli* replication due to increased temperature (North et al. 2014) or both. Another potential factor influencing the observed seasonal concentration results could be the integration of *E. coli* from sediment or the riparian soil matrix (i.e. naturalization) into water. Concentrations of naturalized soil *E. coli* inputs from three temperate watersheds in the US were reported to be the highest in summer and fall and lowest in winter and spring months (Ishii et al. 2006). Regardless of the cause, the pattern of higher *E. coli* concentrations in summer months appears to be rather consistent and the results of the present study support previous findings.

Significant differences were also observed using loading-based analyses when watersheds were analyzed collectively ($F_{3, 343} = 30.635$, *$p < 0.01$*) and individually (Browns: $F_{3, 128} = 11.055, p < 0.01$; Richland: $F_{3, 108} = 16.018, p < 0.01$; Mill: $F_{3, 99} = 9.726$, *$p < 0.01$*) and showed significantly higher loadings in the spring compared to fall in all analyses (Fig. 2a–d). These results (seasonal *E. coli* loadings during baseflow conditions) are unique to the literature and fill a critical knowledge gap. Previous research by Converse et al. (2011) showed increased loads in fall compared to all other months tested, however this research was investigating *E. coli* loadings in storm water samples, not baseflow conditions. Likewise, adequate previous research exists on baseflow seasonal patterns of *E. coli* concentration (Traister and Anisfeld 2006; Wilkes et al. 2009; Amorim et al. 2014), but few studies take into account *E. coli* loading (Gentry et al.
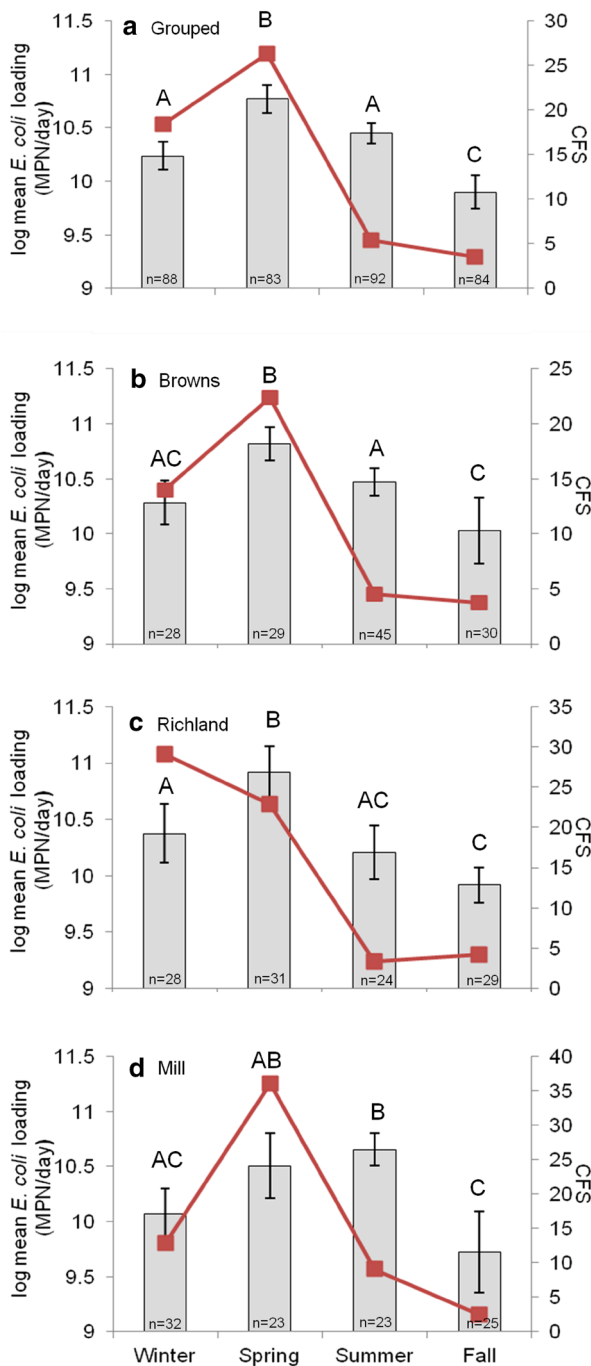
**Fig. 2 a–d** Seasonal comparisons for *Escherichia coli* loading (*bars*) and average flow in grouped (**a**) and in Browns (**b**), Richland (**c**), and Mill (**d**) watersheds. Seasons not sharing similar letters are significantly different from each other. Data presented as mean ± 95 % confidence intervals. *CFS* cubic feet per second

**Table 1** General land use and watershed features for three central Tennessee impaired watersheds

| Land use (%) | Watershed | | |
|---|---|---|---|
| | Browns | Richland | Mill |
| Residential | 49 | 56 | 41 |
| Commercial | 10 | 11 | 20 |
| Industrial | 9 | 3 | 9 |
| Transportation | 8 | 7 | 7 |
| Open | 24 | 23 | 23 |
| Watershed features | | | |
| Size (hectares) | 3237 | 5868 | 5261 |
| Imperviousness (%) | 30 | 19 | 40 |
| Est. 2010 population | 51,370 | 29,995 | 52,571 |

These results are in agreement with reports of significantly higher *E. coli* loads in the winter/spring than the summer/fall for streams in agriculturally drained watersheds (Vidon et al. 2008). Though flow appears to be the driving force for the loading increases observed in the spring (Fig. 2a–d), other factors such as sediment resuspension and increasing water temperatures (North et al. 2014) may also have contributed. It is also interesting to note that the high loading values observed in summer were not due to high flow, but instead were concentration-driven (Fig. 2a–d).

Concentration analysis is typically used for comparison to predetermined water quality criteria to assess exposure of humans to fecal pathogens, whereas loading analysis is used in the relative partitioning of fecal loads from point and nonpoint sources in a watershed for total maximum daily load (TMDL) programs (USEPA 2001). These two analysis types, although both focused on *E. coli*, have two different goals and the results of the present study show that the use of results from one analysis type should not be used as a surrogate for the other. More specifically, since loading data takes into account concentration, these results highlight the impact of flow data during baseflow conditions for inland waters and the necessity of obtaining flow data to accurately predict loading values. One technique used as a replacement for site-specific flow data has been the use of modeling programs; though concerns of inherent error have been reported (Shirmohammadi et al. 2006), such as use of limited data to model spatially and temporally variable parameters (e.g., sediment characteristics and flow patterns).

The analysis of both grouped and individual watersheds for the effect of season allowed for the incorporation of a wide variety of data across years and sites, while still allowing for watershed-specific analyses. Using these types of analyses in the future to develop background loading and/or concentration values would provide a means to better understand the impact of storm events, assess best management practice effectiveness, and elucidate long-term changes in

2006; Vidon et al. 2008; Jamieson et al. 2003). Results from the present study, showing high loadings in the spring compared to all other seasons (except in the Mill watershed) were not surprising given that spring is historically the rainiest season in central Tennessee and more frequent storm events lead to increased baseflow conditions (Wittenberg 2003).

land use or hydrological dynamics of the watershed. Additionally, it is recommended that larger scale analyses of *E. coli* be performed that take into account other factors such as geographic region and climate.

In summary, the approach used in this study proved to be a useful tool for determining seasonal effects at both a large scale and a watershed-specific scale. Concentration- and loading-based results for *E. coli* were highest in summer and spring, and lowest in the winter and fall, respectively. Given that these two commonly used techniques showed different results, care should be taken to not infer data gathered from one analysis technique to the other.

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