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Spatiotemporal Variability Comparisons of Water Quality and *Escherichia coli* in an Oklahoma Stream

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Abstract: Fecal indicator bacteria, *Escherichia coli*, for primary body contact recreation (PBCR) in Oklahoma waterbodies, is defined as the geometric mean of 10 samples from the recreation season, May 1 to September 30, with an impairment threshold of 126 colony forming units (cfu) per 100 mL. However, the water quality standards provide limited guidance on spatiotemporal and environmental factors that could influence samples collected and analyzed. In this study, two stream cross sections under baseflow conditions in a central Oklahoma urban perennial stream, Spring Creek, were densely sampled to investigate temporal and spatial variability of *E. coli* concentrations and water quality parameters across the stream channel. Water quality parameters (specific conductivity, temperature, dissolved oxygen, pH, turbidity, and total suspended solids (TSS)), stream discharge, and bacteria samples were collected simultaneously at equal intervals across the two cross sections in the morning and afternoon during one summer day with sunny, dry, and hot weather conditions. Results indicate a significant difference between time-of-day samples and water quality parameters and *E. coli* concentrations. Strong correlations between temperature, dissolved oxygen, and time versus *E. coli* concentrations were observed, while location, turbidity, and TSS were not significant or correlated to measured values. Furthermore, *E. coli* concentrations were highly variable spatially across each stream cross section, regardless of time of day or location. Results from this study provide an initial indication that stream water quality, spatial cross section sample location, and diurnal variations may be influencing factors on bacteria concentrations.

Keywords: *Escherichia coli, fecal indicator bacteria, sampling, freshwater, stream*

Fecal indicator bacteria (FIB) such as *Escherichia coli* in freshwater waterbodies are frequently monitored to assess potential human health risk from pathogen contact in recreational waters. The State of Oklahoma and U.S. Environmental Protection Agency water quality standard criteria for FIB for primary body contact recreation (PBCR) in waterbodies is defined as the geometric mean of 10 samples from the recreation season, May 1 to September 30, with an impairment threshold of 126 colony forming units (cfu) per 100 mL for *E. coli* (OWRB 2017). Thresholds were derived from epidemiology studies in freshwater and marine swimming beach areas in lakes and oceans where subjects contacted potential contaminated water and incidents of gastrointestinal illness occurred (USEPA 1986; 2012).

E. coli has been studied extensively for fecal source tracking, pathogenic strains, waterbody conditions, and other associated research questions related to human health risk and fecal water quality indicators for PBCR (Gitter et al. 2020). However, water quality standards provide limited guidance of how samples should be collected during the recreation season. State agencies and other entities that collect samples and make assessments often develop their own sampling metrics, but are not standardized to sampling protocols (USEPA 2012). The U.S. Environmental Protection Agency and others recognize that temporal and spatial factors could play significant roles in bacteria concentrations within a stream (USEPA 2010; Muirhead and Meenken 2018). Recent studies found that sampling location and frequency were significant factors when developing a

Research Implications

- Sample location and time can influence *E. coli* concentrations in streams and rivers.
- Environmental parameters can be used to develop relationships to predict bacteria concentrations.
- Monitoring approaches should consider sampling location, time, and other environmental conditions when sampling for fecal indicator bacteria.
- An improved understanding on how, when, where, and why to sample fecal indicator bacteria is needed to ensure representativeness of stream conditions for impairment determination for recreational waters.

monitoring plan to obtain representative samples for the evaluation of potential fecal contamination (Crosby et al. 2019; Stocker et al. 2019). In addition, previous research has indicated that sample type and technique when monitoring a stream should be considered to reduce uncertainty in analyses (Harmel et al. 2016). Gregory et al. (2019) determined there was a significant difference between streamflow thresholds (i.e., baseflow, floods) and *E. coli* concentrations, and indicated that specific hydrologic factors may provide stronger relationships to FIB stream concentrations and associated human health risk. Therefore, given the number of temporal and spatial factors within a stream sample reach, determining sample representativeness could be an important consideration for waterbody impairment designation.

Stream characteristics and environmental conditions have been shown to influence FIB and have been used to develop relationships between parameters and FIB concentrations (Dwivedi et al. 2013). Particularly, suspended solids, turbidity, water temperature, and habitat have previously been used as predictors for *E. coli* densities (Desai and Rifai 2010; Petersen and Hubbart 2020). Others have found significant relationships between nutrients, turbidity, and FIB in streams that can be used to predict bacteria concentrations (Christensen et al. 2002). Furthermore, discharge and precipitation, along with turbidity, have been found to strongly correlate with *E. coli* concentrations in streams (Hamilton and Luffman 2009). Comparison of stream reaches within similar land use segments has been explored with differentiating results for variable fecal indicator concentrations and environmental conditions (Stocker et al. 2016). Results indicated that there were significant differences between stream sampling locations, and that more research is needed to understand stream dynamics that may affect FIB. Diurnal variation and sunlight are also important considerations for evaluating FIB in streams and rivers (Desai and Rifai 2013). Previous research has indicated that FIB concentrations in waterbodies are cyclical, with decay shown during high sunlight periods and increases in bacteria concentrations during low light periods (Whitman et al. 2004; Schultz-Fademrecht et al. 2008). Hydrologic extremes such as floods and droughts can increase variability within stream reaches due to external bacterial inputs from stormwater conveyance, wastewater overflows, and non-point sources (Vogel et al. 2009; McKergow and Davies-Colley 2010; Sanders et al. 2013; Verhougstraete et al. 2015; Rochelle-Newall et al. 2016; Stocker et al. 2018). Furthermore, Piorkowski et al. (2014) showed a variable spatial distribution of FIB in stream sediments under different flow conditions and sampling location. Sediment type and stream habitats have also shown to be *E. coli* reservoirs within streams (Brinkmeyer et al. 2015; Devane et al. 2020). Stream bed sediments have the potential to provide a consistent source of resuspended FIB in the stream water column due to dynamic hydrologic conditions and can create variable sampling conditions (Jamieson et al. 2005; Haller et al. 2009; Bradshaw et al. 2016).

While environmental and hydrologic conditions have been extensively studied to develop relationships between these factors and *E. coli* within streams and rivers, limited information exists to understand the variability of bacteria concentrations within the longitudinal and cross-section profiles of streams. The objectives of this study were to 1) investigate spatial and temporal variability in two stream cross sections, 2) evaluate physical and chemical factors for correlations between variables and evaluate statistical trends, and 3) provide preliminary information for future research targeting specific environmental and spatiotemporal factors that may influence bacteria concentrations in streams and rivers, and ultimately, drive impairment criteria for water quality monitoring.

Methods

Two stream cross sections in a central Oklahoma urban perennial stream, Spring Creek, under baseflow conditions (less than 2.54 mm precipitation in previous seven days) were densely sampled during a seasonally average dry and hot, central Oklahoma summer day (Figure 1). Additionally, in-situ water quality parameters were collected across the stream channel sections at sampling points. Spring Creek is located in northwest Oklahoma City, OK at 35° 36' 18.7" N and -97° 36' 29.3" W, and the site location has an approximate drainage area of 30 km^2 as calculated in StreamStats (Smith and Esralew 2010). The land use category of the watershed is highly urban (>90%) with silty clay to clay loam soil types (USDA NRCS 2023). Potential bacteria inputs are primarily from non-point sources from urban runoff, as no septic tanks, wastewater discharges, or agriculture are located in the watershed. Stream cross sections were evaluated at two daily time periods, morning (0800) and afternoon (1500), at two locations. The two measured cross section stream feature morphologies were a pool (upstream) and a run (downstream) and were separated by 200 m of a series of riffles, glides, pools, and runs. The upstream cross section had a width of 6.7 m and downstream location had a cross section width of 7.3 m.

Factors investigated included *E. coli* concentration, dissolved oxygen (DO), specific conductivity (SC), total suspended solids (TSS), turbidity, water temperature (T), stream velocity and flow, channel depth, stream location and cross section, and time. Water quality samples and parameters were collected across the cross section simultaneously by our sampling team for evaluation of spatial variability (Figure 2). Grab samples were collected at evenly spaced 1.2 m cross section locations (minimum of six sampling locations) at mid-depth in sterile 1 L polypropylene bottles and split into respective subsamples for bacteria (*E. coli*), water quality parameters (turbidity, pH, conductivity), and sediment (TSS) analyses (Figure 2). Sampling protocols adhered to the U.S. Geological Survey sampling methods (USGS 2014). Discharge measurements were collected using a Sontek Flowtracker2® handheld-ADV (acoustic Doppler velocimeter) at each cross section, following collection of water quality samples. At each time period, samples were first collected at the downstream location to

Figure 1. Site sampling locations at Spring Creek in central Oklahoma.

Figure 2. Cross-section water quality sampling at the "run" location at Spring Creek.

minimize disturbance of the water column from the upstream location. *E. coli* concentrations in water were analyzed using IDEXX Quantitray Colilert (SM9223-B) to determine most probable number (MPN) per 100 ml (Baird and Bridgewater 2017). TSS analyses were completed using SM 2540-D and turbidity was measured using a Hach® portable turbidity meter. Water temperature, pH, DO, and SC were measured using a ThermoFisher Scientific Orion Star A329 multiparameter meter.

Data Analysis

Data were analyzed using Microsoft Excel® and R statistical software. Differences in means were evaluated using a two-sample t-test with unequal variances. A Pearson correlation test with a two-sample t-test with unequal variances was performed to determine significant linear relationships between variables. An F-test was used to evaluate variance of water quality data collected from each stream section. All statistical figures were generated using R and Excel®.

Results and Discussion

Stream flow characteristics were measured at both the morning and afternoon sampling periods. Stream locations had mean column depths of 0.35 m at the pool and 0.15 m at the run. Discharge during the morning and afternoon periods (measurement was within ± 0.01 m^3s^{-1} at both the upstream and downstream locations) was 0.08 m^3s^{-1} and 0.04 m^3s^{-1}, respectively, which is within range of the estimated 50% flow-duration for Spring Creek in July (0.05 m^3s^{-1}) (Smith and Esralew 2010). The drainage area is characterized as highly urban, silty clay soils (hydrologic soil group D), which could increase the potential for anthropogenic influences and explain the higher discharge in the morning period when lawn irrigation is most common. No measurable precipitation (>2.54 mm) was recorded at the nearest Oklahoma City East Mesonet station for the preceding seven days (Brock et al. 1995; McPherson et al. 2007).

From a two-sample t-test with unequal variances, *E. coli* concentrations between the upstream (pool) and downstream (run) were not significantly different between the means for each location for all time periods ($p=0.23$). However, a significant difference ($p<0.001$) between time periods (morning and afternoon) was shown between each location for *E. coli* densities. The geometric mean in the morning for *E. coli* was 664 MPN/100 ml (SD ± 116) and was 137 MPN/100 ml (SD ± 108) in the afternoon. Results from a t-test comparing Pearson correlation coefficients between factors indicate that time, DO, SC, and T were significant ($p<0.05$) for *E. coli* concentrations. Furthermore, DO was significantly higher ($p<0.01$) in the morning than afternoon and displayed a strong positive correlation of 0.69 to *E. coli* concentrations. Conversely, a very strong negative correlation (-0.93) of T was shown and a strong positive relationship with SC (0.78) was found versus *E. coli* concentrations ($p<0.01$). The mean DO and T for both locations was 9.05 mg/L (SD ± 0.12) and 26.63°C in the morning, and 7.02 mg/L (SD ± 0.42) and 29.14°C in the afternoon. When comparing SC to *E. coli* concentrations, a significant difference was statistically determined, however, the means of SC for the morning and afternoon were 1167 (SD ± 1.72) and 1171 µS/cm (SD ± 4.59), respectively, which provides limited inference for interpretation given the minute difference between time points. However, the flow was a factor of two higher in the morning than in the afternoon and could suggest that more flow slightly altered the water chemistry through dilution. Significant differences ($p<0.01$) were found from the Pearson correlation coefficient t-test when comparing *E. coli* concentrations from both sampling locations to water quality parameters (DO, T), water column depth, and time. However, no significant differences were shown ($p>0.05$) for TSS, turbidity, and stream velocity. Boxplots of water quality parameters are shown in Figure 3.

Previous research has indicated that sediment parameters are strong predictors for FIB sampling (Stocker et al. 2019). However, our results from the Pearson correlation indicated high variability and no significant relationship between turbidity, TSS, and *E. coli* for each cross section and location. Stream cross section versus TSS is presented in Figure 4, and visually demonstrates the variability of suspended sediments at time points and cross section location. Stream cross sections at both locations were evaluated using a two-sample F-test to determine if variability exists across the lateral

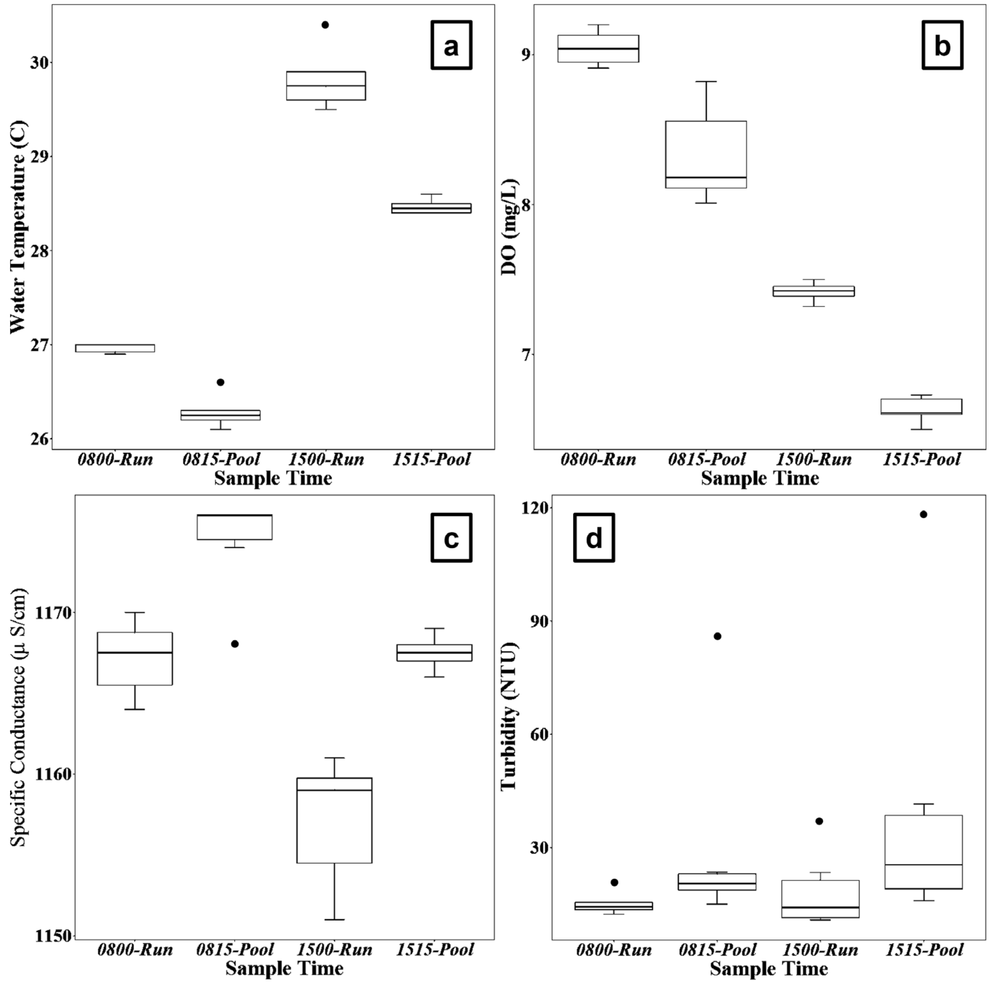

Figure 3. Standard box plots of a) Water Temperature, b) Dissolved Oxygen (DO), c) Specific Conductance (SC), and d) Turbidity, showing the median (line in box), lower (Q1) and upper (Q3) (T bars outside of box) and outlier values (points) grouped by sample time at each of the two Spring Creek sampling locations.

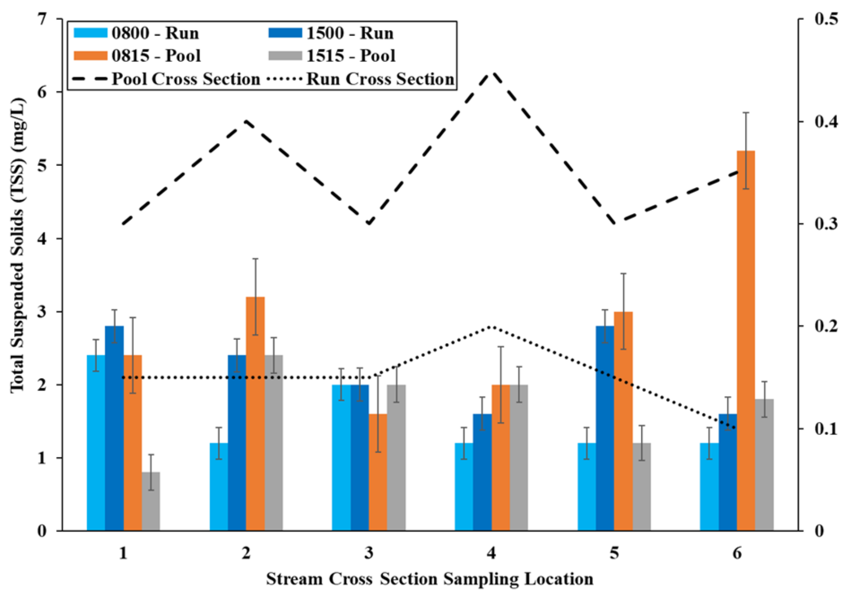

Figure 4. Combination of plot of morning and afternoon total suspended solids (TSS) at the pool and run cross sections. Cross section depth for each location is indicated by the dashed lines. Standard error is represented by the error bars.

profile of the stream for *E. coli* concentrations. Results show significant high variability between the pool and run locations ($p<0.01$) at both times, where the standard deviation was approximately a factor of three lower in the run location than the pool location. No significant difference in variability was found when comparing two time periods for the pool location ($p=0.44$), whereas a significant difference was indicated for the run location ($p=0.038$) when comparing two different time periods. *E. coli* stream cross section concentrations for two time periods and locations are displayed in Figure 5.

While sediment is generally highly correlated to *E. coli* concentrations, variability between sample times has been shown to skew results while monitoring (Crosby et al. 2019). Results from our cross-section study comparing stream location indicate that variability of FIB concentrations, specifically *E. coli*, can be reduced if samples are collected in a well-mixed stream reach, such as from the stream run location, with consideration that variability can occur across the cross section even when hydrologic conditions and other factors are considered. Others have indicated that composite samples may be a better representation of stream water quality parameters when compared to other sample types (e.g., grab samples) (Harmel et al. 2016). In our preliminary research, water quality parameters (DO, T, and SC) were better predictors for *E. coli* than sediment, which may be related to time-of-day conditions within the stream since T can influence DO, SC, and *E. coli* concentrations. Diurnal variation and percent sunlight at each location were not measured for this study, but when comparing to previous research, this variable may be an important consideration of where and when to sample. More research is needed in various stream types, geographic locations, and spatial and temporal resolutions to validate the variability within stream cross sections and longitudinal segments.

Conclusions

Sampling FIB for water quality impairment determination is important to evaluate recreational waterbodies for potential pathogen presence that can affect human health. However, the water quality standards do not provide detailed guidance of the spatial and temporal distribution of water samples at a point of interest in a waterbody. Our research provides initial evidence that sampling methods should be investigated further to

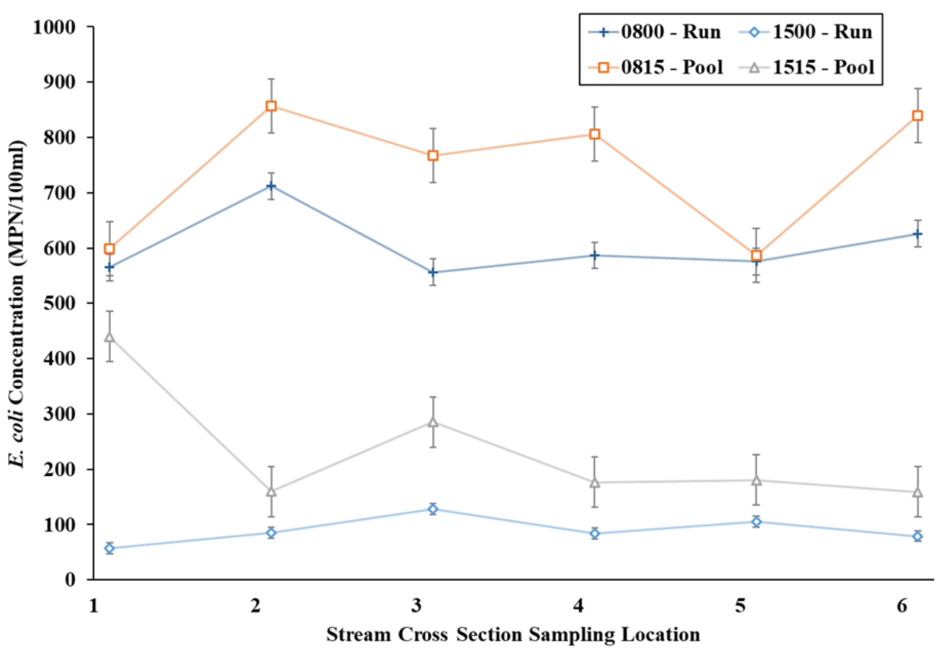

Figure 5. *Escherichia coli* concentrations at two cross section locations (pool and run) at the Spring Creek study site for two time periods, morning (0800 and 0815) and afternoon (1500 and 1515). Standard error is represented by the error bars.

properly evaluate streams for water quality fecal indicators. We demonstrated that high spatial variability of bacteria concentrations across both stream reaches was shown regardless of time of day or other waterbody conditions. Furthermore, basic water quality parameters (DO, T, and SC), time of day, and stream section locations may be useful predictors when selecting a representative location. This proof-of-concept study indicates that more emphasis should be placed on selecting site conditions that are representative (e.g., sampling reach) of the waterbody being sampled, with spatial and temporal considerations. Furthermore, other water quality and hydrologic factors could potentially be used to target stream reaches that are impaired and improve sampling protocols by understanding stream dynamics to obtain quality samples. Future work in this research area is needed to improve the water science community's approaches to enhance our understanding of streams and rivers and use our resources effectively.

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