Fecal Indicator Bacteria Transport from Watersheds with Differing Wastewater Technologies and Septic System Densities

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Abstract: Wastewater contains elevated concentrations of fecal indicator bacteria (FIB). The type of wastewater treatment technology and septic system density may influence the FIB concentration and exports at the watershed scale. The goal of this study was to gain a better understanding of FIB concentrations and exports from watersheds served by conventional septic (CS) systems, sand filter (SF) septic systems, and a municipal sewer (SEW) system. Seven watersheds (3 CS, 3 SF, and 1 SEW) were monitored to quantify FIB concentration and export monthly from April 2015 to March 2016. The type of wastewater treatment did not yield significant differences in FIB concentration or exports when pooling watersheds using similar wastewater treatment. Watersheds with the highest septic densities (approximately 0.4 systems ha\(^{-1}\)) contained greater FIB concentrations and exports than watersheds with the lowest (approximately 0.1–0.2 systems ha\(^{-1}\)), but only FIB concentrations significantly differed. These findings suggest that when the septic system density exceeds 0.4 systems ha\(^{-1}\), water quality degradation from septic leachate may be observable at the watershed scale, especially in watersheds dominated by residential development. More research is recommended to determine if this density threshold is similar for other water pollutants and/or in watersheds with differing hydrogeological, land use, and wastewater characteristics.

Keywords: bacteria; transport; wastewater; sand filter; septic; fecal contamination

1. Introduction

Elevated concentrations of pathogens in water resources are one of the most commonly cited reasons for water use impairment [1]. Fecal waste from humans and animals may contain various types of pathogens, including viruses, protozoa, and bacteria, that can cause infections, illnesses, or death if exposure via consumption, inhalation, or skin contact occurs [2]. Testing for each of these pathogens in surface water or groundwater is time consuming and expensive. Instead, fecal indicator bacteria (FIB) are often used to assess risk associated with exposure to contaminated water, because elevated concentrations of FIB may indicate the presence of harmful pathogens [3,4]. The United States Environmental Protection Agency (US EPA) [5] recommends that geometric mean values of Escherichia coli (E. coli) and Enterococcus spp. (henceforth called enterococcus/enterococci) should not exceed 126 and 35 most probable number (MPN) 100 mL\(^{-1}\), respectively, in recreational
water. Furthermore, no more than 10% of samples should exceed statistical threshold values (STVs) of 410 and 130 MPN 100 mL$^{-1}$ for *E. coli* and enterococci, respectively. Prior studies have shown that FIB in surface waters can be linked to waste material from a variety of sources, including wildlife [6], pets [7], livestock [8], and improperly treated domestic wastewater from cities and rural areas. In North Carolina, regulations have been enacted to reduce non-point source pollution via implementation of agricultural best management practices, stormwater control measures, and improvements in centralized wastewater infrastructure [9]. Efforts to improve water quality via improvement of septic system treatment efficiency were not initiated, partly due to a lack of information regarding their watershed contributions.

Septic systems are commonly used in rural or suburban communities where it may not be economically feasible to extend municipal sewer lines. In the United States, approximately 25% of Americans rely on septic systems for their primary means of wastewater treatment and 30% of new construction uses septic systems [10,11]. Previous studies have found greater concentrations of FIB in waters downgradient from septic systems relative to waters downgradient of municipal sewers [12–16]. Water quality and/or aquatic habitat degradation from septic-derived FIB may be exacerbated during periods of septic system malfunction, which release partially treated or untreated wastewater directly to groundwater or surface water [17]. Most of these studies have focused on conventional septic (CS) systems that collect, treat, and discharge wastewater effluent to subsoils beneath drainfield trenches. Alternative septic technologies exist that are generally used at sites that fail to meet the minimum soil requirements for CS outlined in state regulations. Sand filter (SF) systems are one example of commonly used alternative septic systems. SFs have a septic tank and effluent from the tank is piped to the SF where aerobic treatment occurs, and the SF effluent is discharged directly to drainageways or streams. Other research showed SF effluent contained FIB concentrations of up to 5000 MPN 100 mL$^{-1}$ [18,19]. These studies suggest that wastewater treatment efficiency and water quality adjacent to septic systems may be influenced by system type, but more information is needed to determine if differences in water quality are observed at larger spatial scales.

Municipal sewer (SEW) systems may also act as a significant source of FIB to surface waters. Elevated FIB concentrations were found in a stream that drains a watershed dominated by urban lands compared to a stream that drains forested lands [20]. Increased FIB loads to the environment from SEW systems can occur from excessive inflow and infiltration, which can result in poor system performance and possibly overflow [21]. Additionally, leakage from wastewater pipes can discharge FIB to groundwaters and/or surface waters [22]. During times of overflow or spills, the FIB concentration in and bacterial loadings to surface water can be substantially greater than recreational water quality standards, thus posing significant environmental and public health risks [23]. More research is needed to quantify and compare FIB concentration and exports from watersheds that predominantly utilize SF to watersheds that predominantly use CS and SEW.

The goal of this study was to compare the FIB concentration and exports from watersheds using various wastewater technologies. The specific objectives were to: (i) Quantify the septic system density within watersheds using CS and SF systems and determine at what threshold densities of systems influence watershed-scale FIB concentrations and exports; and (ii) compare FIB concentrations and exports from watersheds using CS, SF, and an SEW to elucidate spatiotemporal trends related to the wastewater treatment approach. Previous research has suggested that when the septic system density exceeds 1 system ha$^{-1}$, septic leachate can substantially influence FIB concentrations and/or yields [12,14,16]; however, in watersheds with mixed land uses (e.g., forested and agriculture), septic system density differences may become masked by wildlife and livestock signatures [24]. The current study analyzed FIB concentrations and loads to determine if differences existed between watersheds with septic system densities of <1 system ha$^{-1}$ and based on wastewater treatment approach (CS, SF, and SEW). Furthermore, few studies have included FIB transport data from SF type systems at the watershed scale.
2. Materials and Methods

2.1. Study Area and Site Selection

The study area consisted of 7 watersheds located in Durham County that drain to Falls Lake in the Piedmont geologic region of North Carolina (Figure 1). Each watershed was grouped and classified according to the dominant wastewater treatment approach (Table 1). Watersheds served by septic systems are numbered in order of increasing septic system density: 1 being the lowest (approximately 0.2 systems ha\(^{-1}\)) and 3 being the highest (approximately 0.4 systems ha\(^{-1}\)). Watershed boundaries were delineated using StreamStats 4.0, which is a web-based tool developed by the United States Geological Survey (https://streamstats.usgs.gov/), and land cover data were also complied from StreamStats for each watershed (Table 1). The CS1, CS3, and SEW watersheds are tributaries or segments of Lick Creek; the CS2 watershed drains the headwaters of Laurel Creek; and the SF watersheds discharge to Little Lick Creek. Laurel Creek is considered a tributary of Lick Creek for watershed management purposes. Each of these watersheds were analyzed independently based on septic system densities (Table 1). Most of the septic systems in the studied watersheds were originally permitted between the 1950s and 1970s based on public record via City of Durham’s interactive Go Maps (https://maps.roktech.net/durhamnc_gomaps4/).

![Figure 1. Watershed delineation map for the 3 conventional septic (CS), 3 sand filter (SF), and sewered (SEW) watershed. LC = Lick Creek; LLC = Little Lick Creek; and LaC = Laurel Creek.](image)

Falls Lake serves as the main water supply for the City of Raleigh and provides primary and secondary water-based recreational activities for the region. Raleigh serves as the county seat for Wake County, which ranks among the top 50 counties in the United States for population growth and it gained approximately 65 new residents each day from 2010 to 2017 [25]. Sustainable management of water resources is vital for continued growth and economic development in the region. Falls Lake and many of its tributaries, including Lick Creek and Little Lick Creek, are designated as impaired waters [26]. Lick Creek and Little Lick Creek have watershed restoration plans designed to improve water quality within their respective watersheds. These plans suggested that failing septic systems and surface discharging systems (i.e., SF-type systems) could be significant sources of wastewater constituents (e.g., nutrients, bacteria, oxygen-demanding substances) and contributors to aquatic habitat degradation in Lick Creek and Little Lick Creek [27,28]. The technology used for wastewater treatment (e.g., SF, CS, SEW) varied in different sub-watersheds of Falls Lake. Some areas were served mostly by CS, some mostly by SF, and others by SEW. Water quality monitoring data were not available for many of the sub-watersheds served by these different technologies, so more information was needed to determine if the wastewater treatment approach and/or septic system density affected water quality.
Table 1. Watershed characteristics, septic system density, and proximity of septic systems to watershed outlets. CS = conventional septic; SF = sand filter; SEW = sewer.

| Watershed | Stream Gradient | Imperviousness (%) | Area (ha) | Septic Systems (#) | Septic System Density (# ha⁻¹) | Septic System Distance to Outlet (Number of Systems) | Land Cover Data | Forestry | Agriculture | Developed |
|-----------|-----------------|-------------------|----------|--------------------|-------------------------------|-------------------------------------------------|----------------|----------|-------------|-----------|
|            |                 |                   |          |                    |                               | <0.2 km | 0 | 1 km | >1 km | 60.3% | 7.5% | 16.3% |
| CS1        | 0.002           | 4                 | 2283     | 280                | 0.12                          | 1       | 2 | 278  |         | 60.3% | 7.5% | 16.3% |
| CS2        | 0.005           | 1                 | 184      | 48                 | 0.26                          | 0       | 3 | 45   |         | 74.6% | 7.7% | 7.7%  |
| CS3        | 0.008           | 13                | 19       | 7                  | 0.37                          | 5       | 2 | 0    |         | 25.7% | 4.5% | 63.3% |
| SF1        | 0.003           | 9                 | 335      | 75                 | 0.22                          | 0       | 20 | 55   |         | 45.2% | 0.5% | 48.9% |
| SF2        | 0.007           | 6                 | 40       | 15                 | 0.37                          | 7       | 8 | 0    |         | 31.2% | 0.0% | 53.7% |
| SF3        | 0.005           | 7                 | 83       | 35                 | 0.42                          | 14      | 21 | 0    |         | 44.7% | 0.0% | 52.3% |
| SEW        | 0.002           | 7                 | 1128     | NA                 | NA                            | NA      | NA | NA   |         | 53.0% | 8.0% | 26.9% |

¹ = Percentages collected from StreamStats 4 based on 2011 National Land Cover Database data.
Lick Creek drains an area of 5690 hectare (ha) comprised mostly of unmanaged rural lands (37%), forest (21%), and protected natural area (10%). The Lick Creek watershed is currently undergoing a transitional period shifting from rural management and eventually to suburban/urban land uses [27]. The Little Lick Creek watershed is slightly smaller, draining an area of 4450 ha consisting mostly of mixed density residential (49%), vacant (18%), and parks and open space (15%). Nearly half (47%) of the watershed lies within the city limits of Durham, NC. High-density residential, industrial, and low-density residential land uses in the watershed are expected to increase by 3, 3, and 2 times, respectively. Meanwhile, agriculture, vacant, and parks and open spaces are expected to see declines of up to 100%, 100%, and 22%, respectively, over the next 20 years within the Little Lick Creek watershed [28]. Both the Lick and Little Lick Creek Watersheds are expected to see growth, which may result in permitting of additional septic systems, thus increasing system densities in these watersheds.

The study area generally receives 122 cm of precipitation per year (https://www.usclimatedata.com/climate/durham/north-carolina/united-states/usnc0192). Mean monthly high temperatures in warmer months (June–September) routinely exceed 27 °C. During the winter months (December–March), mean monthly high temperatures peak at approximately 16 °C and mean monthly low temperatures of approximately −2 °C. The geology of the study area is generally characterized by Triassic basin clays and in some areas Carolina Slate Belt and felsic crystalline rock. The White Store soil series was the dominant soil unit for subsoils of the studied sub-watersheds of Little Lick Creek (>80%), Lick Creek (>60%), and Laurel Creek (>30%) [29].

2.2. Sampling Protocol

Water quality assessment occurred at each of the 7 watershed outlets (Figure 1). Physicochemical parameters, including specific conductance, temperature, dissolved oxygen, and oxidation reduction potential, were measured in the field using a YSI-556 MultiProbe meter. A Hach turbidimeter was used to measure turbidity. Stream discharge was determined by multiplying the cross-sectional area of a transect by the mean velocity. Stream velocity was measured using a Global Water FP101 flow meter or the floating object method. The floating object method was used in scenarios when stream velocity was too low or the water not deep enough to engage the flow meter propeller [30]. Water samples were collected from watershed outlets monthly beginning in April 2015 until March 2016 (n = 84, 12 samples per watershed). Sterile 100-mL bottles were immersed in streams until bottles reached 100 mL. Samples were stored in an iced cooler and transported to East Carolina University where they were analyzed for FIB concentrations within 6 hours of sampling. Additionally, 250-mL bottles were used to collect water samples for chloride analyses. These samples were also stored on an iced cooler until they were vacuum filtered and analyzed using a Unity SmartChem 200 autoanalyzer.

E. coli and enterococci concentrations in streams were quantified using QuantiTray2000® trays via the IDEXX™ method (IDEXX Laboratories, Inc., Westbrook, Maine). Some samples were diluted to allow for enumeration of FIB beyond maximum concentrations of 2419.6 MPN 100 mL−1. Dilution factors generally ranged from 2 to 10. FIB concentrations were determined based on the MPN 100 mL−1 associated with the number of fluorescent wells using a chart provided by IDEXX.

2.3. Statistical Analysis

The exports of FIB were estimated for each watershed by multiplying the FIB concentration (MPN 100 mL−1) by stream discharge (L s−1). Normalization by watershed area was performed to account for variability in discharge that may be related to differences in watershed size. Thus, stream discharge and FIB export reported in the current study was area normalized; henceforth, “normalized exports” and “normalized discharge” will be referred to as “exports” and “discharge”, respectively. Concentrations and exports of FIB were compiled into comparison groups based on septic system density and wastewater treatment approach. FIB concentrations may vary seasonally due to changes in temperature [15,24,31–33], thus watersheds were grouped by summer (June–September) and winter (December–March) seasons (n = 56, 8 samples per watershed) to compare data based on the largest
differences in temperatures, which may affect FIB survivability. Summer and winter months are henceforth referred to as “warm” and “cold”, respectively. Data figures were developed using the “ggplot2” and “cowplot” packages developed for R software [34].

A Shapiro–Wilks normality test was employed to determine if data exhibited normality. Data were not normally distributed, even after transformation. Non-parametric statistics were employed to determine if significant differences (p ≤ 0.05) existed between CS, SF, and SEW watersheds based on the wastewater treatment approach or septic system density. Kruskal–Wallis H tests were employed to test if significant differences existed in FIB concentrations and exports between: (i) pooled data from watersheds using CS, SF, or SEW wastewater treatment; and (ii) septic system density regardless of the type of septic system. If a significant p-value was determined via Kruskal–Wallis, post hoc Mann–Whitney rank sum tests with Bonferroni correction were used to isolate differences among comparison groups. Statistical tests were conducted in the R statistical framework [34].

3. Results and Discussion

3.1. Septic System Density

Concentrations of FIB were greatest in watersheds that contained the highest septic system densities (Figure 2; Table 2). Differences in the median E. coli concentrations between the higher septic system density watersheds (SF3 and CS3) were statistically different (E. coli: p < 0.01; enterococci: p = 0.05) from the lower density watersheds (SF1 and CS1) but not for the intermediate density watersheds (E. coli: p = 0.12; enterococci: p = 0.10). Watersheds served by SF systems demonstrated clear differences in FIB concentrations based on septic system density. The SF3 watershed contained median concentrations of E. coli and enterococci of 1203 MPN 100 mL−1 and 253 MPN 100 mL−1, respectively. Median E. coli and enterococci concentrations were approximately 18 and 5 times greater than the SF1 watershed. Both E. coli and enterococci concentrations in the SF3 watershed were approximately 5 times greater than the SF2 watershed (Figure 2; Table 2). Furthermore, E. coli and enterococci concentrations in the SF3 watershed exceeded concentrations in the SF1 91% and 100% of the time, respectively. While median differences between SF3 and SF2 were not statistically significant, concentrations of E. coli and enterococci in SF3 exceeded concentrations in SF2 73% and 82% of the time, respectively. Watersheds served by conventional septic systems exhibited a similar trend for E. coli but not for enterococci (Figure 2). The CS3 watershed contained the greatest median E. coli concentration (526 MPN 100 mL−1) and was approximately 3 and 7 times greater than the CS1 and CS2 watersheds, respectively (Figure 2; Table 2). The CS3 and CS1 watersheds contained similar median concentrations of enterococci of 101 and 105 MPN 100 mL−1, respectively, and were approximately 1.3 times greater than the CS2 watershed (Table 2).

Warmer months tended to exhibit greater FIB concentrations and variability for all watersheds. When pooling data from all watersheds based on season, warm months contained median values of E. coli and enterococci of 725 and 253 MPN 100 mL−1, respectively; meanwhile, the median concentration of E. coli and enterococci was 149 and 70 MPN 100 mL−1, respectively, during cold months. These differences were statistically significant for both E. coli (p = 0.03) and enterococci (p < 0.01). Differences in the FIB concentration based on septic system density were most apparent when accounting for seasonality (Figure 2). When comparing seasonal trends based on septic system density, the lower density watersheds (CS1 and SF1) exhibited statistically significant differences based on season for E. coli (p = 0.04) and enterococci (p < 0.01) concentrations. Differences in median concentrations of enterococci (p = 0.03) were statistically significant for intermediate density watersheds (CS2 and SF2), but not for E. coli (p = 0.23). The higher density watersheds (CS3 and SF3) did not exhibit statistical differences for either E. coli (p = 0.52) or enterococci (p = 0.69). Past studies have found seasonal variability in FIB communities within surface waters due to wildlife and pet activity [7,35], sediment agitation via recreation or storms [36], snowmelt [37], and/or rainfall [36,38]. In the current study, elevated FIB concentrations in warmer months were likely due to increased wildlife and pet activities.
The lack of a significant temporal trend in the higher density septic watersheds may suggest that spatial differences in septic system density exhibit a stronger influence on river FIB communities than temporal fluctuations. Past studies also found that spatial variability affected river bacterioplankton communities more than seasonal variation [39–43]. FIB communities may be affected by, but not limited to, changes in salinity [40], reduced river flow and sedimentation rates [42], and land use [24,40,41,43].

Land use is the most likely explanation for differences in FIB concentrations among the studied watersheds since river flow (Table 3) and salinity (all freshwater watersheds) were similar. Sowah et al. [24] found that watersheds with a low density of septic systems (<1 system ha$^{-1}$) exhibited more variability in FIB concentration due to variable sources of fecal pollution (e.g., livestock, wildlife, and septic systems), which they attributed to an even distribution of land use between agriculture, forested, and developed area. In the current study, forested and developed area accounted for >75% of land cover (Table 1). Forested areas provide a habitat for wildlife, which can be a significant non-point source of FIB to surface waters, especially during warmer months when they are most active and FIB survivability is greater [44]. These additional microbial inputs made it more difficult to differentiate trends in \textit{E. coli} and enterococci concentrations based on septic system density during warmer months (Figure 2). During colder months, both \textit{E. coli} (median: >700 MPN 100 mL$^{-1}$) and enterococci (median: >100 MPN 100 mL$^{-1}$) concentrations were elevated in the higher density watersheds (SF3 and CS3) (Figure 2). \textit{E. coli} concentrations in the SF2 watershed were also elevated during colder months (Figure 2). The SF3, CS3, and SF2 watersheds were predominantly residential (≥53%), with lesser percentages of forested and agricultural land classes ranging from 26–45% and 0–5%, respectively (Table 1). Median values of \textit{E. coli} and enterococci were <200 and <100 MPN 100 mL$^{-1}$, respectively, in the CS1, CS2, and SF1 watersheds. Forest accounted for ≥60% of land cover in CS1 and CS2, whereas forest and residential development in SF1 was approximately equal (Table 1). Thus, land cover data suggest that differences in FIB concentrations during colder months were likely due to differing septic system densities when other major non-point sources of FIB were limited.
Waters downgradient from wastewater inputs tend to contain elevated specific conductance and with elevated specific conductance [47,48]. Past studies [45,46,49] reported specific conductance and These di

Specific conductance and chloride data in the CS3 and SF3 watersheds were also elevated relative to the other watersheds (Figure 3; Table 3), potentially indicating wastewater effects [45,46]. These differences were statistically significant for both specific conductance and chloride at p < 0.01. Waters downgradient from wastewater inputs tend to contain elevated specific conductance and chloride [45,46]. Furthermore, elevated specific conductance in water may indicate a continuous source of dissolved ions, such as septic systems, and increased septic system densities are often associated with elevated specific conductance [47,48]. Past studies [45,46,49] reported specific conductance and chloride values ranging from approximately 500–2000 µS cm⁻¹ and 50–300 mg L⁻¹, respectively, in wastewater, which were similar to values reported in the CS3 and SF3 watersheds (Figure 3; Table 3). The other watersheds in the current study contained similar specific conductance (approximately 30–250 µS cm⁻¹) and chloride (approximately 10 mg L⁻¹) values to groundwater and surface water unaffected by wastewater inputs reported in Birch et al. [46]. Runoff from roads treated with salt to prevent ice accumulation during winter weather can contain specific conductance and chloride values

### Table 2. Median and geometric mean concentrations (MPN 100 mL⁻¹) and exports (MPN s⁻¹ ha⁻¹) of fecal indicator bacteria for the studied watersheds. Median values, the range is included in parentheses. For geometric mean values, the standard deviation is included in parentheses. Conc = concentration; CS = conventional septic; SF = sand filter; SEW = sewer.

| Watershed | Median | Geometric Mean |
|-----------|--------|----------------|
|           |        | E. coli         | Enterococci | E. coli | Enterococci |
| Conc | Export | Conc | Export | Conc | Export | Conc | Export |
| CS1 | 160 | (105–725) | 85 | (5–486) | 105 | (26–1088) | 23 | (5–479) | 195 | (207) | 60 | (149) | 107 | (330) | 33 |
| CS2 | 71 | (34–7068) | 35 | (1–1744) | 80 | (3–3434) | 18 | (1–847) | 168 | (1992) | 35 | (492) | 92 | (963) | 19 |
| CS3 | 526 | (16–12100) | 47 | (1–1672) | 101 | (19–6017) | 20 | (1–855) | 410 | (3367) | 5 | (723) | 125 | (1702) | 2 |
| SF1 | 67 | (26–1628) | 18 | (1–730) | 55 | (5–968) | 11 | (1–434) | 89 | (449) | 15 | (210) | 64 | (283) | 11 |
| SF2 | 232 | (21–5600) | 26 | (1–2023) | 48 | (2–5176) | 19 | (1–156) | 229 | (1743) | 12 | (597) | 75 | (652) | 3 |
| SF3 | 120 | (22–12098) | 151 | (1–2086) | 253 | (133–12100) | 66 | (1–3634) | 792 | (3416) | 81 | (602) | 603 | (3530) | 49 |
| CS | 160 | (16–12100) | 48 | (1–1744) | 96 | (3–6017) | 22 | (1–855) | 238 | (2276) | 21 | (524) | 107 | (1126) | 11 |
| SF | 223 | (21–12098) | 41 | (1–2086) | 126 | (5–12100) | 22 | (1–3634) | 245 | (2256) | 24 | (497) | 139 | (2134) | 12 |
| SEW | 170 | (86–1153) | 94 | (7–1117) | 121 | (10–657) | 39 | (4–879) | 233 | (370) | 89 | (319) | 115 | (219) | 44 |

### Table 3. Median (range) of environmental parameters and stream discharge at watershed outlets. SC = specific conductance; Cl⁻ = chloride; DO = dissolved oxygen; ORP = oxidation-reduction potential.

| Watershed | SC µS cm⁻¹ | CI- mg L⁻¹ | DO mg L⁻¹ | pH | Temperature °C | ORP mV | Turbidity NTU | Discharge L min⁻¹ ha⁻¹ |
|-----------|------------|------------|----------|-----|----------------|--------|--------------|------------------------|
| CS1 | 152 | (119–298) | 15.7 | (12.1–39.9) | 8.5 | (3.7–15.7) | 7.3 | (6.4–8.8) | 13.3 | (1.6–25.1) | −22.0 | (−136.8–163.9) | 39 | (9–120) | 3.6 |
| CS2 | 103 | (77–1069) | 6.8 | (5.0–305.7) | 7.8 | (3.7–14.3) | 7.3 | (6.6–8.4) | 12.8 | (3.2–23.1) | −24.5 | (−118.6–193.5) | 13 | (5–66) | 1.4 |
| CS3 | 418 | (105–598) | 50.9 | (10.2–109.0) | 6.3 | (1.3–15.1) | 7.3 | (6.3–8.5) | 12.4 | (1.9–23.6) | −31.7 | (−143.2–141.3) | 12 | (4–58) | 2.4 |
| SF1 | 189 | (75–818) | 19.0 | (4.5–209.7) | 5.9 | (2.9–14.5) | 7.1 | (6.6–8.2) | 13.8 | (2.2–23.9) | −19.4 | (−128.8–187.8) | 22 | (5–132) | 1.2 |
| SF2 | 214 | (69–585) | 17.7 | (13.1–67.2) | 6.0 | (1.6–14.3) | 7.2 | (6.3–8.5) | 13.7 | (3.0–22.6) | −18.4 | (−137.0–163.0) | 24 | (5–72) | 0.9 |
| SF3 | 346 | (166–774) | 48.1 | (10.3–186.5) | 5.9 | (1.4–15.8) | 7.3 | (6.5–9.3) | 13.0 | (2.0–23.5) | −27.0 | (−154.5–164.3) | 25 | (6–122) | 0.7 |
| CS | 151 | (77–1069) | 15.7 | (5.0–305.7) | 13.7 | (1.3–15.7) | 7.2 | (6.3–8.8) | 12.9 | (1.6–25.1) | −21.3 | (−142.1–193.5) | 14 | (4–120) | 7.2 |
| SF | 203 | (75–818) | 21.5 | (4.5–209.7) | 5.9 | (1.4–15.8) | 7.2 | (6.3–9.3) | 13.8 | (2.0–23.9) | −23.4 | (−154.5–187.8) | 24 | (5–132) | 0.7 |
| SEW | 204 | (146–461) | 18.7 | (13.9–79.7) | 8.7 | (3.5–20.2) | 7.4 | (6.4–8.0) | 12.5 | (1.1–26.5) | −14.7 | (−120.4–132.0) | 27 | (12–88) | 3.5 |

E. coli and Enterococci in the CS3 and SF3 watersheds were also elevated relative to the other watersheds (Figure 3; Table 3), potentially indicating wastewater effects [45,46]. These differences were statistically significant for both specific conductance and chloride at p < 0.01.
up to approximately 10,000 $\mu$S cm$^{-1}$ and 8900 mg L$^{-1}$, respectively [49]. Road salts are not routinely applied throughout winters in the study area due to milder winters yielding infrequent snow and ice storms. However, the January 2016 sampling event occurred several days after a snowstorm hit the area when road salts were likely used, thus explaining the larger variability and sudden increase in chloride and specific conductance in all watersheds (Figure 3).

Figure 3. Chloride (A,C) and specific conductance (B,D) values from watersheds grouped by wastewater treatment approach and increasing septic system density. Pooled data are shown for each group as CS = conventional septic; SF = sand filter; SEW = sewer. The asterisks (*) denote outliers.

Median $E. coli$ and enterococci loadings increased with septic system density for the SF watersheds (Figure 4; Table 2) but the same was not observed for the CS watersheds. Despite the observed differences in median values, FIB loadings data were too variable to find significant differences. When isolating density effects in the SF watersheds, statistical tests were nearly significant ($p = 0.053$) between the SF3 and SF1 watershed for $E. coli$ and enterococci loadings. These same trends were not observed in the conventional septic watersheds, likely due in part to a prolonged period of little to no flow in the CS3 watershed. From June–October 2015, the CS3 watershed was dry or contained disconnected pools of stagnant water, which explains the large variability in the FIB loading data for this watershed (Figure 4). Research in Georgia [24] and North Carolina [15,16] showed median FIB exports typically ranged from 191–1116 and 101–721 MPN s$^{-1}$ ha$^{-1}$ for $E. coli$ and enterococci, respectively, for watersheds with $\geq$0.9 systems ha$^{-1}$. In the current study, watersheds with septic systems contained densities that other studies [16,24] classified as low density ($<1$ system ha$^{-1}$). Sowah et al. [24] reported a mean density of 0.22 systems ha$^{-1}$ for low density watersheds, and these watersheds exported a mean value of approximately 200 and 450 MPN s$^{-1}$ ha$^{-1}$ of $E. coli$ and enterococci, respectively. Humphrey et al. [16] reported median values of 80 and 24 MPN s$^{-1}$ ha$^{-1}$ for $E. coli$ and enterococci from watersheds with a median septic system density of 0.15 systems ha$^{-1}$. In the current study, median $E. coli$ and enterococci exports ranged from 35–151 and 11–66 MPN s$^{-1}$ ha$^{-1}$, respectively (Table 2). These exports overlapped with FIB yields ($E. coli$: 0.8–2559 MPN s$^{-1}$ ha$^{-1}$;
enterococci: 2–5382 MPN s\(^{-1}\) ha\(^{-1}\)) reported in the aforementioned studies from watersheds with <0.9 systems ha\(^{-1}\) \[16,24\].

Previous studies have shown that watersheds with septic system densities greater than 1 system ha\(^{-1}\) can contain substantially elevated FIB concentrations and exports relative to watersheds with lower septic system densities or without septic systems \[15,16\]. However, Sowah et al. \[24\] reported similarities in FIB concentrations and exports at the watershed scale regardless of septic system density. As noted earlier, their low-density watersheds exhibited substantial variability in non-point sources of FIB because of land use, whereas high-density watersheds were mostly residential developed lands (approximately 70%) with less forest and agricultural cover. In the current study, results suggest that water quality impacts may be observed at lower densities than observed by previous studies. Watersheds with septic system densities of 0.4 systems ha\(^{-1}\) contained elevated FIB concentrations that were statistically different from watersheds containing densities of approximately 0.2 systems ha\(^{-1}\). In addition to density, watershed-scale FIB concentrations could be affected by the distance from the septic system to watershed outlets. In the SF3 and CS3 watersheds, all systems were located within either 0.2 or 1 km to the outlet, whereas most systems were located > 1 km from outlets in the SF1 and CS1 watersheds (Table 1). Therefore, quantifying the septic system density and distance to watershed outlets may be strong indicators of watershed-scale concentrations and exports of FIB.

3.2. Wastewater Treatment Approach and Broader Water Quality Implications

FIB concentrations and exports were similar when aggregating FIB data based on the wastewater treatment approach (e.g., CS vs. SF vs. SEW) (Figures 2 and 4; Table 2). SF watersheds contained slightly elevated FIB concentrations relative to CS and SEW watersheds (Figure 2; Table 2);
However, this difference was not statistically significant for either bacteria ($p > 0.84$). This result was expected since only the SF3 watershed contained median values that were greater than the SEW watershed. Differences between the SF3 and SEW watersheds were statistically significant ($p = 0.01$) for both *E. coli* and enterococci concentrations, whereas all other watersheds were similar to the SEW watershed. Median monthly exports of *E. coli* from CS and SF watersheds were lower relative to SEW exports 83% and 75% of the time (Figure 4), respectively. Median monthly exports of enterococci from the SEW watershed exceeded exports from the CS and SF watersheds 75% and 67% of the time, respectively. These differences were not significant for either *E. coli* ($p = 0.49$) or enterococci ($p = 0.54$) yields. The SF3 watershed yielded median FIB that were approximately 1.6 times greater than the SEW watershed. These differences were not significant for either *E. coli* ($p = 0.49$) or enterococci ($p = 0.44$). A study by Iverson et al. [15] found that septic watersheds contained substantially elevated FIB concentrations and yields relative to watersheds served by sewers. The current study did not find similar results, but this was likely due to differences in the septic system density. The watersheds in the Iverson et al. [15] study contained septic system densities of >1 system ha$^{-1}$. Findings by the current study suggest that the wastewater treatment approach alone may not be a predictor of water quality at the watershed scale, unless watersheds contain elevated septic system densities [12,14–16] and limited wildlife and livestock inputs [24]. Thus, significant differences between watersheds using different treatment approaches may be due to other factors that influence water quality (e.g., septic system density, distance of the septic system to the outlet, land use, number of malfunctioning systems/leaking sewer infrastructure, presence and condition of riparian buffers).

When considering the broader water quality implications of FIB transport from these watersheds, the data suggest there is cause for concern. The US EPA [5] recommends that recreational waters should not contain geometric mean values that exceed 126 MPN 100 mL$^{-1}$ and 35 MPN 100 mL$^{-1}$ for *E. coli* and enterococci, respectively. If recreational water exceeds these values, there is an estimated 36 illnesses per 1000 primary contact recreationists. All watersheds, excluding SF1, exceeded the recommended standard for both *E. coli* and enterococci regardless of the wastewater treatment approach (Table 2). Furthermore, the US EPA [5] recommends that less than 10% of samples should exceed the statistical threshold value (STV) of 410 MPN 100 mL$^{-1}$ and 130 MPN 100 mL$^{-1}$ for *E. coli* and enterococci, respectively. All watersheds exceeded the STV for enterococci concentrations more than 10% of the time. Excluding the SF1 watershed (8%), all watersheds also exceeded the STV for *E. coli* concentrations more than 10% of the time. These data suggest that watersheds served by septic systems and sewer systems can pose a risk to public health by exposing recreationists to FIB concentrations high enough to indicate fecal contamination, which may be accompanied by pathogenic microbes. Furthermore, FIB concentrations were greater and more variable during warmer months relative to colder months in all watersheds except SF3 (Figure 2), which overlaps with the peak tourism season when water-based recreation is more common. These findings were consistent with previous studies showing greater FIB concentrations during warmer summer months [15,24,31–33]. While the studied streams are not likely routinely used for recreational purposes, they are low-order streams that discharge to Falls Lake, which is a reservoir intended for water supply and recreation.

4. Conclusions

This study found that watersheds with septic system densities of approximately 0.4 systems ha$^{-1}$ contained elevated FIB concentrations (both CS3 and SF3) and yields (SF3 only) compared to other septic watersheds. These differences were only significant when compared to lower density watersheds (approximately 0.2 systems ha$^{-1}$). Past research suggests that watersheds with septic system densities >1 system ha$^{-1}$ may have a significant effect on watershed FIB [16,24]. The current study suggests that the septic system density threshold of watershed-scale FIB concentrations and/or transport may be <1 system ha$^{-1}$, especially for SF systems and other surface discharging systems, although more research is recommended to account for longer duration spatiotemporal fluctuations. Septic system density is an important management consideration during the planning phase of
residential development and may provide guidance for retroactive water quality improvements. Management agencies considering retrofit activities could incorporate spatial analysis to identify and focus water quality monitoring and remediation efforts based on septic system characteristics at the watershed scale. Tetzlaff et al. [43] suggest similar efforts as land-use-based models can be effective predictive tools of surface water quality. While more research is recommended to determine if similar septic system density thresholds (approximately 0.4 systems ha\(^{-1}\)) are observed in watersheds with differing hydrogeological, land use, and/or wastewater characteristics, this may be a soft threshold that management agencies can utilize in their efforts to manage recreational and water supply watersheds.

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**References**

1. US EPA. National Summary of Impaired Waters and TMDL Information. 2016. Available online: [https://iaspub.epa.gov/waters10/attains_nation_cy.control?p_report_type=T](https://iaspub.epa.gov/waters10/attains_nation_cy.control?p_report_type=T) (accessed on 18 August 2020).
2. US CDC. Recreational Water Illnesses. 2017. Available online: [https://www.cdc.gov/healthywater/swimming/swimmers/rwi.html](https://www.cdc.gov/healthywater/swimming/swimmers/rwi.html) (accessed on 18 August 2020).
3. Payment, P.; Locas, A. Pathogens in Water: Value and Limits of Correlation with Microbial Indicators. *Groundwater* 2011, **49**, 4–11. [CrossRef] [PubMed]
4. USGS. Bacteria and *E. coli* in Water. 2016. Available online: [https://www.usgs.gov/special-topic/water-science-school/science-and-e-coli-water?qt-science_center_objects=0#qt-science_center_objects](https://www.usgs.gov/special-topic/water-science-school/science-and-e-coli-water?qt-science_center_objects=0#qt-science_center_objects) (accessed on 18 August 2020).
5. US EPA. Recreational Water Quality Criteria and Methods. 2012. Available online: [https://www.epa.gov/wqc/recreational-water-quality-criteria-and-methods](https://www.epa.gov/wqc/recreational-water-quality-criteria-and-methods) (accessed on 18 August 2020).
6. de Brauwere, A.; Ouattara, N.K.; Servais, P. Modeling Fecal Indicator Bacteria Concentrations in Natural Surface Waters: A Review. *Crit. Rev. Environ. Sci. Technol.* 2014, **44**, 2380–2453. [CrossRef]
7. Wright, M.E.; Solo-Gabriele, H.M.; Elmir, S.; Fleming, L.E. Microbial load from animal feces at a recreational beach. *Mar. Pollut. Bull.* 2009, **58**, 1649–1656. [CrossRef] [PubMed]
8. Scott, E.E.; Leh, M.D.K.; Haggard, B.E. Spatiotemporal variation of bacterial water quality and the relationship with pasture land cover. *J. Water Health* 2017, **15**, 839–848. [CrossRef] [PubMed]
9. NC DEQ. Nutrient Sensitive Waters and Special Watersheds. 2019. Available online: [https://deq.nc.gov/about/divisions/energy-mineral-land-resources/nsw-special-watersheds](https://deq.nc.gov/about/divisions/energy-mineral-land-resources/nsw-special-watersheds) (accessed on 18 August 2020).
10. Gelting, R. A public health perspective on onsite wastewater systems. *J. Environ. Health* 2007, **69**, 62–63. [PubMed]
11. Zarate-Bermudez, M.A. Contributions to enhancing the public health perspective on onsite wastewater management. *J. Environ. Health* 2014, **77**, 32–33. [PubMed]
12. Cahoon, L.B.; Hales, J.C.; Carey, E.S.; Loucaides, S.; Rowland, K.R.; Nearhoof, J.E. Shellfishing Closures in Southwest Brunswick County, North Carolina: Septic Tanks vs. Storm-Water Runoff as Fecal Coliform Sources. *J. Coastal Res.* 2006, **22**, 319–327. [CrossRef]
13. Meeroff, D.; Bloetscher, F.; Bocca, T.; Morin, F. Evaluation of Water Quality Impacts of On-site Treatment and Disposal Systems on Urban Coastal Waters. *Water Air Soil Pollut.* 2008, **192**, 11–24. [CrossRef]
14. Mallin, M.A.; McIver, M.R. Pollutant impacts to Cape Hatteras National Seashore from urban runoff and septic leachate. *Mar. Pollut. Bull.* 2012, 64, 1356–1366. [CrossRef]

15. Iverson, G.; Humphrey, C., Jr.; Postma, M.; O’Driscoll, M.; Manda, A.; Finley, A. Influence of Sewered Versus Septic Systems on Watershed Exports of E. coli. *Water Air Soil Pollut.* 2017, 228, 1–12. [CrossRef]

16. Humphrey, C.P.; Sanderford, C.; Iverson, G. Concentrations and Exports of Fecal Indicator Bacteria in Watersheds with Varying Densities of Onsite Wastewater Systems. *Water Air Soil Pollut.* 2018, 229, 1–16. [CrossRef]

17. Ahmed, W.; Neller, R.; Katouli, M. Evidence of septic system failure determined by a bacterial biochemical fingerprinting method. *J. Appl. Microbiol.* 2005, 98, 910–920. [CrossRef] [PubMed]

18. Gold, A.J.; Lamb, B.E.; Loomis, G.W.; Boyd, J.R.; Cabelli, V.J.; McKiel, C.G. Wastewater Renovation in Buried and Recirculating Sand Filters. *J. Environ. Qual.* 1992, 21, 720–725. [CrossRef]

19. Kauppinen, A.; Martikainen, K.; Matikka, V.; Veijalainen, A.; Pitkänen, T.; Heinonen-Tanski, H.; Miettinen, I.T. Sand filters for removal of microbes and nutrients from wastewater during a one-year pilot study in a cold temperate climate. *J. Environ. Manag.* 2014, 133, 206–213. [CrossRef]

20. Gotkowska-Płachta, A.; Goła´ s, I.; Korzeniewska, E.; Koc, J.; Rochwerger, A.; Solarski, K. Evaluation of the distribution of fecal indicator bacteria in a river system depending on different types of land use in the southern watershed of the Baltic Sea. *Environ. Sci. Pollut. Res.* 2015, 23, 4073–4085. [CrossRef]

21. Cahoon, L.B.; Hales, J.C.; Carey, E.S.; Loucaides, S.; Rowland, K.R.; Toothman, B.R. Multiple modes of water quality impairment by fecal contamination in a rapidly developing coastal area: Southwest Brunswick County, North Carolina. *Environ. Monit. Assess.* 2016, 188, 89. [CrossRef]

22. Jefferson, F.; Lawrence, B. Cahoon. Risks to Coastal Wastewater Collection Systems from Sea-Level Rise and Climate Change. *J. Coastal Res.* 2011, 27, 652–660.

23. Mallin, M.A.; Cahoon, L.B.; Toothman, B.R.; Parsons, D.C.; McIver, M.R.; Ortwine, M.L.; Harrington, R.N. Impacts of a raw sewage spill on water and sediment quality in an urbanized estuary. *Mar. Pollut. Bull.* 2007, 54, 81–88. [CrossRef]

24. Sowah, R.; Zhang, H.; Radcliffe, D.; Bauske, E.; Habteselasie, M.Y. Evaluating the influence of septic systems and watershed characteristics on stream faecal pollution in suburban watersheds in Georgia, USA. *J. Appl. Microbiol.* 2014, 117, 1500–1512. [CrossRef]

25. US Census. Annual Estimates of the Resident Population for Counties in the United States: April 1, 2010 to July 1, 2019 (CO-EST2019-ANNRES). 2020. Available online: https://data.census.gov/cedsci (accessed on 18 August 2020).

26. NC DEQ. 2018 North Carolina 303(d) List of Impaired Waters. 2019. Available online: https://files.nc.gov/ncdeq/Water%20Quality/Planning/TMDL/303d/2018-2018-NC-303-d--List-Final.pdf (accessed on 18 August 2020).

27. UNRBA. Lick Creek Watershed Restoration Plan. 2009. Available online: https://files.nc.gov/ncdeq/Mitigation%20Services/Watershed_Planning/Neuse_River_Basin/Lick_Creek/UNRBA_Lick%20Creek_LWP.pdf (accessed on 18 August 2020).

28. City of Durham. Little Lick Creek Watershed Plan. 2016. Available online: https://durhamnc.gov/960/Little-Lick-Creek-Watershed-Improvement- (accessed on 18 August 2020).

29. USDA. Web Soil Survey. 2017. Available online: https://websoilsurvey.sc.egov.usda.gov/App/HomePage.htm (accessed on 18 August 2020).

30. Rantz, S.E. *Measurement and Computation of Streamflow: Volume 1. Measurement of Stage and Discharge;* USGS Geological Survey Water-Supply Paper 2175: Washington, DC, USA, 1982.

31. Young, K.D.; Thackston, E.L. Housing Density and Bacterial Loading in Urban Streams. *J. Environ. Eng.* 1999, 125, 1177–1180. [CrossRef]

32. Frenzel, S.A.; Couvillon, C.S. Fecal-indicator bacteria in streams along a gradient of residential development. *J. Amer. Water Res. Assoc.* 2002, 38, 265–273. [CrossRef]

33. Schilling, K.E.; Zhang, Y.; Hill, D.R.; Jones, C.S.; Wolter, C.F. Temporal variations of Escherichia coli concentrations in a large Midwestern river. *J. Hydrol.* 2009, 365, 79–85. [CrossRef]

34. R Core Team. *R: A Language and Environment for Statistical Computing.* 2018. Available online: https://www.r-project.org/ (accessed on 17 September 2020).

35. Hathaway, J.M.; Hunt, W.F.; Simmons, O.D. Statistical Evaluation of Factors Affecting Indicator Bacteria in Urban Storm-Water Runoff. *J. Environ. Eng.* 2010, 136, 1360–1368. [CrossRef]
36. Crabill, C.; Donald, R.; Snelling, J.; Foust, R.; Southam, G. The impact of sediment fecal coliform reservoirs on seasonal water quality in Oak Creek, Arizona. *Water Res.* 1999, 33, 2163–2171. [CrossRef]

37. St Laurent, J.; Mazumder, A. Influence of seasonal and inter-annual hydro-meteorological variability on surface water fecal coliform concentration under varying land-use composition. *Water Res.* 2014, 48, 170–178. [CrossRef]

38. Anderson, C.W.S.A. Rounds. *Phosphorus and E. coli and Their Relation to Selected Constituents During Storm Runoff Conditions in Fanno Creek, Oregon*, 1998–99; USGS: Portland, OR, USA, 2003.

39. Kubera, Ł.; Malecka-Adamowicz, M.; Jankowiak, E.; Dembowska, E.; Perlinski, P.; Hejze, K. Influence of Environmental and Anthropogenic Factors on Microbial Ecology and Sanitary Threat in the Final Stretch of the Brda River. *Water* 2019, 11, 922. [CrossRef]

40. Ma, L.; Mao, G.; Liu, J.; Gao, G.; Zou, C.; Bartlam, M.G.; Wang, Y. Spatial-Temporal Changes of Bacterioplankton Community along an Exhorheic River. *Front. Microbiol.* 2016, 7, 250. [CrossRef]

41. Nguyen, H.T.M.; Le, Q.T.P.; Garnier, J.; Janeau, J.; Rochelle-Newall, E. Seasonal variability of faecal indicator bacteria numbers and die-off rates in the Red River basin, North Viet Nam. *Sci. Rep.* 2016, 6, 21644. [CrossRef]

42. Gazzaz, N.M.; Yusoff, M.K.; Juahir, H.; Ramli, M.F.; Aris, A.Z. Water Quality Assessment and Analysis of Spatial Patterns and Temporal Trends. *Water Environ. Res.* 2013, 85, 751–766. [CrossRef]

43. Tetzlaff, D.; Capell, R.; Soulsby, C. Land use and hydroclimatic influences on Faecal Indicator Organisms in two large Scottish catchments: Towards land use-based models as screening tools. *Sci. Total Environ.* 2012, 434, 110–122. [CrossRef]

44. Plummer, J.D.; Long, S.C. Monitoring source water for microbial contamination: Evaluation of water quality measures. *Water Res.* 2007, 41, 3716–3728. [CrossRef] [PubMed]

45. Katz, B.G.; Griffin, D.W.; Davis, J.H. Groundwater quality impacts from the land application of treated municipal wastewater in a large karstic spring basin: Chemical and microbiological indicators. *Sci. Total Environ.* 2009, 407, 2872–2886. [CrossRef] [PubMed]

46. Birch, A.L.; Emanuel, R.E.; James, A.L.; Nichols, E.G. Hydrologic Impacts of Municipal Wastewater Irrigation to a Temperate Forest Watershed. *J. Environ. Qual.* 2016, 45, 1303–1312. [CrossRef] [PubMed]

47. Hatt, B.E.; Fletcher, T.D.; Walsh, C.J.; Taylor, S.L. The Influence of Urban Density and Drainage Infrastructure on the Concentrations and Loads of Pollutants in Small Streams. *Environ. Manag.* 2004, 34, 112–124. [CrossRef]

48. Landers, M.N.; Paul, D. Ankcorn. Methods to Evaluate Influence of Onsite Septic Wastewater-Treatment Systems on Base Flow in Selected Watersheds in Gwinnett County, Georgia, October 2007; U.S. Geological Survey: Reston, VA, USA, 2008.

49. Kelly, W.R.; Panno, S.V.; Hackley, K.C.; Hwang, H.; Martinsek, A.T.; Markus, M. Using chloride and other ions to trace sewage and road salt in the Illinois Waterway. *Appl. Geochem.* 2010, 25, 661–673. [CrossRef]