

RELATIONSHIPS AND TRENDS OF *E. COLI*, HUMAN-ASSOCIATED BACTEROIDES, AND PATHOGENS IN THE PROCTOR CREEK WATERSHED

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Abstract. Urban surface waters can be impacted by anthropogenic sources such as impervious surfaces, sanitary and storm sewers, and failing infrastructure. Fecal indicator bacteria (FIB) and microbial source tracking (MST) markers are common gauges of stream water quality, however, little is known about their relationship with public health. In this study, we measured culturable and molecular concentrations of *E. coli*, a human-associated *Bacteroides* marker (HF183MGB), and selected waterborne pathogens and toxins (*Salmonella* sp. and Shiga-toxin (Stx2)) in surface water throughout a highly urbanized watershed near downtown Atlanta, GA to determine if any relationships exist between these parameters.

The Proctor Creek watershed, a tributary to the Chattahoochee River, consistently fails fecal coliform standards and is therefore, listed on the Environmental Protection Agency's (EPA) 303(d) impaired waters list. Water grab samples were taken at 12 locations throughout the watershed every two weeks for one year. Culturable *E. coli* concentrations varied greatly throughout the watershed (24 to 101,330 MPN/100 mL). The human associated marker was widespread throughout the watershed and correlated highly with molecular *E. coli* ($R^2 = 0.63$).

Salmonella was present at all mainstem and half of the tributary sites, whereas Stx-2 was widespread throughout the entire watershed but was most frequently found at headwater locations where storm water inlets and outfalls were more prevalent. Based on their high correlation, *E. coli* could be a useful parameter in predicting human-associated *Bacteroides* in this watershed. Our results also indicate that major and consistent human sources of fecal contamination are present throughout the watershed suggesting that sanitary sewer leaks could be widespread across the region.

INTRODUCTION

Fecal indicator bacteria (FIB) such as *E. coli* are used as an indicator for fecal contamination in freshwater ecosystems. These organisms are ideal fecal indicators as they live in the gastrointestinal tract of warm blooded animals

and are quickly identified using laboratory techniques. Their detection in water suggests contamination from fecal sources, which can endanger public health since feces can harbor pathogenic bacteria, viruses, and protozoans (Wade et al. 2006). Although FIB presence in a body of water generally indicates fecal contamination, it cannot identify the source. Microbial source tracking (MST) markers are an important water quality tool as they can identify the origin of fecal pollution in water using host-specific or host-associated microbial indicators (Meays et al. 2004, Seurinck et al. 2005).

It has also been shown that MST markers are more reliable in detecting fecal contamination than FIB (Sauer et al. 2011). Identification of fecal contamination sources allows for a more efficient development and/or establishment of best management practices (BMP) to reduce or eliminate the source of these pollutants, which in turn protects the public from pathogen exposure. For example, sources of human fecal contamination can be accurately identified using the human-specific *Bacteroidales* HF183MGB (Green et al. 2014). Identification of this marker can point to issues within the watershed such as decaying septic systems and infrastructure, municipal wastewater systems, or combined sewer overflows (CSO). Once the source of the problem is identified, they can be managed and remediated.

Detection of MST markers, particularly of human associated *Bacteroidales*, is especially important in urban watersheds. Sources of fecal pollution such as municipal wastewaters, faulty combined sewer overflows, and storm-water drains contaminated with sanitary sewers are common issues in urbanized watersheds that can adversely affect human health. Sauer et al. (2011) determined that out of 45 stormwater outfalls in Milwaukee sampled over a four-year period, all were positive for the human associated *Bacteroides* HF183 genetic marker at least once throughout sampling, concluding that urban stormwater systems can act as a conduit for human sewage.

In the City of Atlanta, Georgia, Proctor Creek is an example of a heavily urbanized watershed and is on the state's 303(d) impaired waters list (USEPA 2015). Proctor Creek is listed as impaired due to not meeting water quality

standards for fecal coliforms with the most probable causes of this being urban runoff, decaying infrastructure, and the presence of a combined sewer overflow. Flooding also plagues the watershed during high intensity rain events. In an attempt to alleviate current stormwater management problems, Proctor Creek watershed is being considered for a “Green Street Project” where green infrastructure such as in-ground planter boxes, permeable pavement, and rain gardens will be installed (USEPA 2015).

Poor water quality and high fecal coliform counts are one of the factors why the Proctor Creek watershed was subjected to a health impact assessment (HIA) by the Environmental Protection Agency (USEPA 2015). The main goal of this assessment was to provide science-based resources and inform the City of Atlanta Department of Watershed Management (DWM) on decisions moving forward with green infrastructure projects.

Results from the HIA determined that it was highly likely that water quality would be positively impacted by the proposed project, however, the strength of evidence to support these claims was listed as “limited.” It has been observed that the waters of Proctor Creek have exceeded fecal coliform water quality standards for water bodies used for fishing (1,000 CFU/100 mL (30 day geometric mean) between November and April and 200 CFU/100 mL from May to October) (GA-EPD 2013) and that these exceedances can endanger public health due to increasing likelihood of waterborne illnesses (Arnone and Walling 2007). Although high concentrations of fecal coliforms exist within Proctor Creek, there is not enough direct evidence to associate these concentrations with human sewage contamination.

In this study, we monitored surface water at various locations within the Proctor Creek watershed for culturable and molecular *E. coli*, human-associated *Bacteroides* (HF183MGB), the pathogen *Salmonella* and the virulence gene for Shiga-toxin (Stx-2). Here we present results that begin to address if human fecal contamination as well as human pathogens are present in the watershed.

METHODS

Watershed Study Area and Sampling

Proctor Creek is a tributary to the Chattahoochee River with headwaters originating in downtown Atlanta, GA and has a watershed area of 10,198 acres. Twelve sampling locations were selected throughout the Proctor Creek watershed (Fig. 1): six mainstem and six tributary sites. One-liter grab water samples were collected using sterilized Nalgene bottles every two weeks beginning November 23, 2015 through April 4, 2017. Dissolved oxygen (D.O.), conductivity, pH, temperature, and turbidity were measured at each sampling outing. Water samples were transported on ice to the EPA laboratory facilities in Athens, GA.

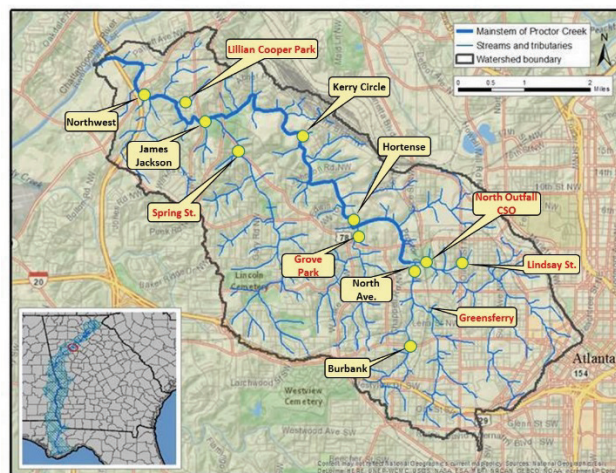


Figure 1. The Proctor Creek watershed. Yellow dots are sampling locations.

Laboratory Methods

Upon arrival in lab, samples were processed for culturable *E. coli* using the IDEXX Colilert assay (IDEXX Laboratories, Westbrook, ME). Water samples were processed for culturable *E. coli* within 6 hours of collection.

Fifty mL of sample were filtered through a 0.4 µm Isopore polycarbonate filter (EMD Millipore, Billerica, MA) for subsequent DNA analysis. Filters were stored at -80°C until DNA extraction was performed. DNA extraction of filters was done using MoBio PowerLyzer PowerSoil DNA extraction kits (MoBio Laboratories, Inc, Carlsbad, CA). Once extracted, DNA was stored at -80°C until quantitative polymerase chain reaction (qPCR) analysis.

To analyze for *Salmonella* and Stx-2, 200 mL of each sample was filtered through 0.45 µm S-pak filters (EMD Millipore, Billerica, MA) and placed in 20 mL of peptone enrichment broth. Samples were incubated at 37°C for 24 h and then centrifuged at 5,000 x g for 5 min. Supernatant was discarded and filters were rinsed with 20 mL of sterile phosphate buffer and centrifuged for 5 min at 5,000 x g. Supernatant was discarded and the remaining pellet was collected using 250 µl of phosphate buffer and stored at -80°C until DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA). Samples were stored at -80°C until subsequent qPCR analysis.

All qPCR analyses were conducted using a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA).

RESULTS

Culturable *E. coli*

Average *E. coli* was calculated for each site with standard deviation bars (Fig. 2). *E. coli* concentrations ranged from 2.2 to 4.1 log MPN/100 mL. Highest average concentrations on the mainstem were observed at the two locations nearest the headwaters of Proctor Creek, Burbank (3.3 log₁₀ MPN/100 mL) and North Ave (3.7 log₁₀ MPN/100 mL). These locations were well above the EPA water quality criteria for *E. coli*, 2.1 log₁₀ CFU/100 mL (USEPA 2012). *E. coli* at the pour point of the watershed, Northwest (2.9 log₁₀ MPN/100 mL), was also consistently above the criteria.

Greensferry, a decommissioned CSO in the watershed was a constant source of culturable *E. coli* (4.1 log₁₀ MPN/100 mL) and was consistently above the EPA recreational water quality standard limit. The influence of this tributary is also observed at the downstream mainstem location, North Ave, which had the highest culturable *E. coli* concentrations of any mainstem site. The North Ave. CSO had the lowest concentration of any site monitored in the watershed. Although this is a functioning CSO, the low concentration is likely due to the fact that samples were generally taken under minimal flow conditions and hence, the CSO was not discharging.

Molecular *E. coli* and HF183MGB

Molecular *E. coli* and HF183MGB were analyzed from November 2015 through June 2016. Trends observed thus far for *E. coli* have followed similar trends to what was observed for culturable *E. coli*. Greensferry displayed consistently high concentrations with the downstream, mainstem site of North Ave. displaying the highest mainstem site concentrations (Fig. 3). Aside from these sites, molecular *E. coli* concentrations were relatively constant throughout the watershed, with the exception of North Ave. CSO, which was substantially lower on average and likely due to low flow and no discharge being present.

The human-associated *Bacteroides* marker, HF183MGB, was widespread throughout the watershed and consistently detected at all sites, with the exceptions of North Ave. CSO and Lillian Cooper Park (Fig. 3). As was the case with *E. coli*, HF183MGB was considerably higher at the Greensferry and North Ave. locations. Burbank, the site nearest the headwaters of the mainstem, had high concentrations of HF183MGB (5.0 log gene copies/100mL). Remaining locations in the mainstem displayed similar concentrations of HF183MGB throughout. Particularly low concentrations of HF183MGB were observed at the North Ave. CSO and Lillian Cooper Park.

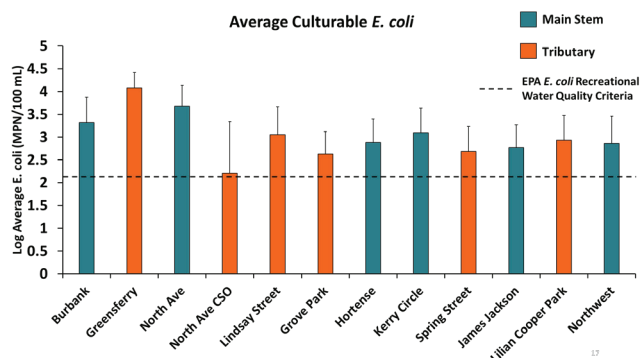


Figure 2. Average culturable *E. coli* concentrations (log₁₀ MPN/100mL) of mainstem (blue) and tributary (orange) sites from November 2015 through April 2017. Sites are arranged from headwaters to pour point (left to right). The black dashed line is the EPA *E. coli* recreational water quality criteria. Error bars represent standard deviation.

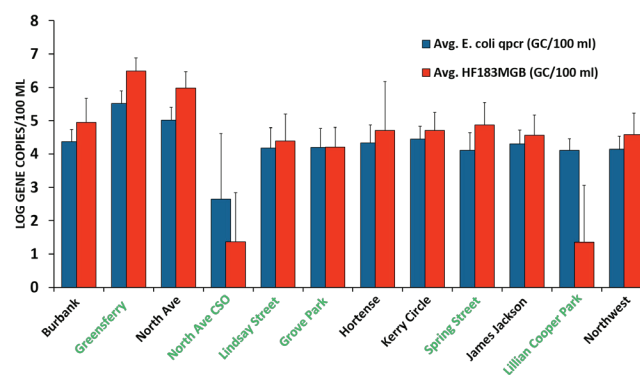


Figure 3. Average molecular *E. coli* and HF183MGB (log₁₀ gene copies/100 mL) concentrations for tributary (green font) and mainstem (black font) sites from November 2015 through June 2016. Sites are arranged from headwaters to pour point (left to right). Error bars represent standard deviation.

Correlations

Significant, positive correlations existed between the molecular markers and culturable *E. coli* (Table 1). Unsurprisingly, culturable and molecular *E. coli* demonstrated the highest correlation ($r^2 = 0.76$). Human-associated *Bacteroides* and molecular *E. coli* also displayed a relatively strong predictive relationship with r^2 of 0.63.

Table 1. Correlations (r^2) of molecular markers for culturable *E. coli* (MPN/100 mL), HF183MGB (GC/100mL), and molecular *E. coli* (MPN/100 mL). Data from November 2015 through June 2016.

	Culturable <i>E. coli</i>	HF183MGB	Molecular <i>E. coli</i>
Culturable <i>E. coli</i>	-	0.49	0.76
HF183MGB	0.49	-	0.63
Molecular <i>E. coli</i>	0.76	0.63	-

Salmonella

Salmonella was detected intermittently at all mainstem sites, with the majority having a frequency of 40% presence (Fig. 4). A slightly lower frequency of 30% was observed at the North Ave. and Hortense locations.

Salmonella was detected at half of the tributary sites. Lindsay Street had the highest occurrence (80%) in the entire watershed. *Salmonella* went undetected at the Greensferry, North Ave. CSO, and Spring Street locations.

Stx-2

The Shiga-toxin, Stx-2, was widespread throughout the watershed and was detected at all locations (Fig. 5). Burbank and Grove Park had the highest frequency of Stx-2 (80%). In most mainstem sites, Stx-2 was present 20-30% of the time. North Ave., however, had 10% occurrence of Stx-2. This was likely influenced by Greensferry, which displayed the lowest occurrence of Stx-2 (9%) throughout our sampling period.

DISCUSSION

The results presented here indicate that the Proctor Creek watershed is highly impacted by human-associated *Bacteroides*. Major and consistent sources of human fecal contamination are common throughout the watershed, particularly in the upper reaches of the watershed. The tributary site at Greensferry was a constant source of HF183MGB that was impacting downstream mainstem locations. This suggests that illicit sewage connections or failing infrastructure are present in the headwaters of the watershed.

Significant, positive correlations existed for HF183MGB and molecular *E. coli* and to a lesser extent, culturable *E. coli*. This suggests that some of the *E. coli* detected is likely originating from human sources. In addition, molecular *E. coli* could also be used as a predictor for human contamination in this watershed. This information could be useful for future modeling of the watershed, or helpful in determining which markers are most pertinent for monitoring.

In addition, preliminary results suggest that *Salmonella* and Shiga-toxin are present in the watershed, and along with the human-associated *Bacteroides*, are particularly abundant in the upper reaches of the watershed. However, the location that displayed the highest concentrations of *E. coli* and HF183MGB, Greensferry, showed little to no *Salmonella* and Shiga-toxin. These preliminary results suggest that although both *Salmonella* and Shiga-toxin are present in the watershed, they may not originate solely from human sources.

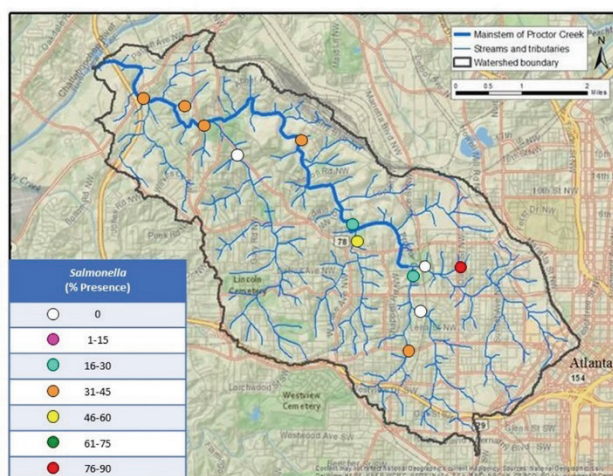


Figure 4. *Salmonella* frequency at all sites from November 2015 through April 2016.

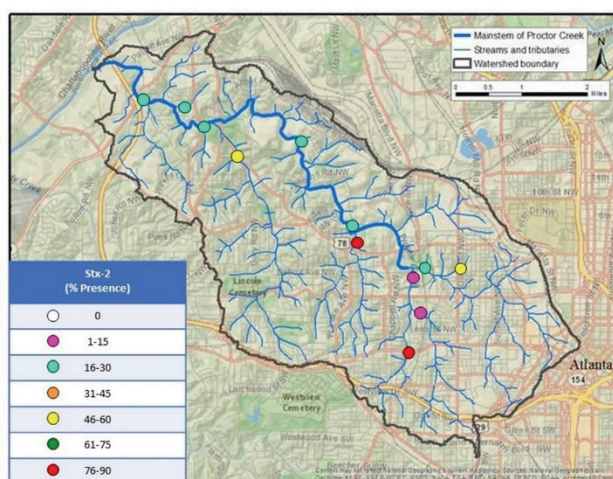


Figure 5. Stx-2 frequency at all sites from November 2015 through April 2016.

One of the issues addressed in the EPA's HIA was the lack of direct evidence of human impact on water quality. High concentrations of fecal coliforms have been observed in the watershed, but direct evidence of human fecal contamination has been limited. Results from this study show that the Proctor Creek watershed is highly impacted by human fecal contamination. Evidence from this study suggests that stream quality is directly being impacted by anthropogenic factors.

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