ASSESSING THE IMPACT OF SEPTIC SYSTEMS ON MICROBIAL WATER QUALITY OF STREAMS IN URBANIZING WATERSHEDS OF METROPOLITAN ATLANTA, GEORGIA

by

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(Under the Direction of Mussie Habteselassie)

ABSTRACT

Septic systems, also known as onsite wastewater treatment systems (OWTS), are used widely across the southeast of U.S. As widely as these systems are used in the southeast and other parts of the U.S., their impact on microbial water quality has not been elucidated to allow for their proper management at the watershed level. The overall goal of this study was to isolate septic system impact at the watershed level by using multiple approaches including targeted monitoring of fecal indicator bacteria (FIB) and microbial source tracking (MST) markers as well as watershed scale modeling. Twenty four urbanizing watersheds impacted by a gradient of septic system density and land use characteristics were monitored over a three year period for water quality parameters including the FIB *E. coli* and enterococci, human-specific *Bacteroidales* genetic markers and standard water quality parameters (pH, dissolved oxygen, temperature, specific conductance). Septic system influence on fecal bacteria loads at the watershed level was also modeled with the soil and water assessment tool (SWAT). Correlation and multivariable regression analysis indicate that septic systems, specifically the density of septic systems and the proximity of septic systems to streams, were significant drivers of fecal

pollution in urbanizing watersheds of metropolitan Atlanta. The influence of septic systems was seasonally dependent with the strongest impact observed in spring season. Analysis of the human-associated marker showed strong negative correlation (r = -0.64) to the proximity of septic systems to streams during the spring season. Additionally, the human marker was significantly higher in high density watersheds compared to low density areas overall. SWAT model results show septic system influence as a result of the proximity of septic systems to local streams, with the most significant influence observed when septic systems are less than 10 m from nearby streams. This study provides tools that can be used at the watershed level to understand the impact of septic systems on microbial water quality. The study findings can be used to support decisions regarding septic system management to protect water resources.

INDEX WORDS: Septic systems, Fecal pollution, Fecal indicator bacteria, Microbial source tracking, *Bacteroidales*, Host specific marker, SWAT, SWAT-CUP

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DEDICATION

I dedicate this dissertation to my wonderful wife, Cynthia Sowah, for your love and unflinching support throughout my Ph.D. program. I would like to also dedicate this work to my sons Aaron, Jeremy and Nathan; you have been my motivation in the low points of this journey. I couldn't have achieved this without your love, understanding and support.

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CHAPTER 1

INTRODUCTION

Septic systems have been reported to impact the quality of groundwater and surface water resources in the United States and other parts of the world (Carroll et al., 2005; Gerba and Smith Jr, 2005; Scandura and Sobsey, 1997; Whitlock et al., 2002). Water quality impairments of concern include pathogens, elevated nitrate, and organic enrichment. These ongoing pollution incidents have raised serious questions about the effectiveness of septic systems. In recent years, growing concerns over public health and environmental impacts have resulted in improved controls over point sources of contaminants (Kramer et al., 2006). Control measures have been developed based on improved characterization of point sources and their impacts on water quality. In contrast, controls over non-point sources (e.g. septic systems) are either non-existent or where they exist have proved to be inadequate because of limited data on the sources and fate of pollutants. It is therefore vital, in the interest of public health and environmental protection, to evaluate the contribution of non-point sources such as septic systems in the context of developing improved management controls.

Fecal pathogens account for the majority of assessed water quality impairments in the United States (Benham et al., 2006). Over 157,289 miles of rivers and streams in the United States are listed as impaired by fecal pathogens under Section 303 (d) of the Clean Water Act (USEPA 2016). Potential sources of pathogens identified in the report include septic systems, run-off from impervious surfaces and unspecified non-point sources. While a great deal is known about the contributions of septic systems to nutrient loads in groundwater and surface waters, the

same cannot be said of septic systems' contribution to pathogen loads in groundwater and surface waters. Very little is known about the relative contributions of septic systems, storm-water run-off, livestock and wild animals to the total pathogen load at the watershed scale to enable effective control of sources (Cahoon et al., 2006; Carroll et al., 2005; Lipp et al., 2001). Furthermore, the relationship between contaminant load, sources and septic system density has not been clearly established.

Several approaches have been used to implicate septic systems in pathogen contamination. A couple of studies employed direct measurements of FIB to link septic systems to the occurrence of pathogens in groundwater and surface waters (Arnade, 1999; Atoyan et al., 2011; Cahoon et al., 2006; Lipp et al., 2001). The presence of FIB such as fecal coliforms, *Clostridium perfringes*, *Escherichia coli* (*E. coli*) and fecal enterococci is used as an indicator of contamination with other pathogenic bacteria, protozoa and viruses of fecal origin (Field and Samadpour, 2007). The FIB paradigm is a useful tool that signifies the presence of pathogens of fecal origin but tells us very little about the sources of these pathogens (Bernhard and Field, 2000).

Even though FIB monitoring is mandatory under current water quality monitoring regimes, the effectiveness of this approach has been questioned because of the isolation of infectious pathogens in water bodies showing low concentrations of FIB (Plummer and Long, 2007). Others have used source tracking or tracer techniques to implicate septic systems in pathogen contamination (Dickerson Jr et al., 2007; Habteselassie et al., 2011; Knee et al., 2008). Microbial source tracking (MST) methods are currently the gold standard for tracking the source(s) of fecal pollution at the watershed level (Dickerson Jr et al., 2007; Griffith et al., 2003; McQuaig et al., 2006). In the long-term, several authors (Boehm et al., 2003; Field and

Samadpour, 2007; Habteselassie et al., 2011; Noble et al., 2006) have suggested multiple approaches utilizing targeted pathogen monitoring, FIB for baseline monitoring coupled with MST to determine pathogen sources.

Other studies have reached contrasting conclusions about the contribution of septic systems to pathogen loads in groundwater and surface waters (Thompson et al., 2012; Mallin et al., 2000; Young and Thackston, 1999). Research by Thompson et al. (2012) showed no correlations between septic systems and pathogen levels in groundwater and surface waters even though septic systems significantly contributed to nutrient loads in groundwater and surface waters. Inconsistencies in research results can be attributed to differences in local conditions of soil, precipitation, temperature, solar radiation and hydrologic regimes that influence pathogen dynamics in soil and water. Variability in the above controls affects sorption, predation and die-off of pathogens at the drainfield and watershed scales (Habteselassie et al. 2011; Beal et al., 2005). Understanding the interrelationships between local controls and septic system performance is therefore critical to our search for local solutions to protect public health and the environment from elevated pathogen concentrations in water.

It has been widely reported that increasing septic system density has a direct impact on pathogen loads in nearby surface waters (Atoyan et al., 2011; Cahoon et al., 2006; Lipp et al., 2001). The authors correlated increasing septic system density to high pathogen concentrations in surface waters. This assertion is debatable because of poor definition of what constitutes high or low density systems. Such inconsistencies have led to non-comparable results; hence little from previous studies can be extrapolated to other areas. The situation is complicated by septic systems failures which can affect contaminant loads significantly (Habteselassie et al., 2011). For example, a failed septic system in a low density impacted watershed can increase the

discharge of pollutants several fold. Previous studies have also largely focused on the impacts of high density of septic systems on coastal waters, with little known about the potential impacts of varying watershed sizes and land-use distribution on water quality in streams and rivers.

A better understanding of septic system impacts at the watershed scale, and watershed characteristics influencing contaminant fate and transport will be instrumental to the development of watershed management programs. Significantly, after years of extensive research in urban watersheds there is still inadequate information about the dynamics of pollutant transport and fate in the environment (Carey et al., 2013). The identification of pollutant sources has become vital because of the requirements to develop total maximum daily load (TMDL) to facilitate the restoration of impaired water bodies (Habteselassie et al., 2011). In Georgia, where over 600 stream segments are listed as impaired by fecal coliforms (GDNR, 2011), TMDL development is crucial for the restoration of these water bodies to water quality standards. Water bodies that are not listed as impaired under Section 303 (d) of Clean Water Act will also benefit substantially from management programs based on improved characterization and quantification of contaminant sources.

Objectives of study

The overall goal of this study was to determine the impact of septic systems on microbial water quality of streams in watersheds impacted by a gradient of septic system density. The major hypothesis to be tested is that areas of high septic system density are at increased risk of fecal pollution compared to areas of low septic system density. This hypothesis depends on the proper identification of the contribution of septic systems to fecal contamination in streams – previous studies have not clearly established the contribution of septic systems to fecal pollution at the watershed scale. This study utilizes multiple approaches consisting of targeted monitoring

of FIB, MST and watershed scale modeling, to evaluate the impact of septic systems on microbial water quality at the watershed scale. To achieve the project goal, the following objectives have been set;

1. Identify the influence of septic systems and land use characteristics on stream fecal pollution at the watershed level
2. Isolate and quantify the impact of septic systems in streams of watersheds with variable density of septic systems using bacterial and viral genetic markers
3. Model septic system impact on microbial water quality with the soil and water assessment tool (SWAT)

Three approaches were employed to address the objectives outlined above. First, we monitored FIB numbers in streams of well-characterized urbanizing watersheds with varying septic system density. This study, which is covered in Chapter 3, addresses the question of fecal pollution sources outlined in Objective 1. Chapter 4 of this dissertation research presents data on the use of MST methods to isolate the influence of septic systems in our study watersheds. This will fulfill Objective 2 of our study. Finally, we used the SWAT watershed scale model to predict the impact of septic systems on stream fecal pollution loads in our study area. This information is covered in Chapter 5 of this dissertation.

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CHAPTER 2

LITERATURE REVIEW

Status of septic systems in the United States

Septic systems play an important role in the wastewater management infrastructure in the United States. According to the United States Census Bureau (2009), approximately 25% of housing units in the U.S. depend on septic systems for the treatment of wastewater. Recent trends indicate a rise in the use of septic systems in response to rapid population growth and the concomitant increase in housing. The USEPA (2002) estimates that ~33% of all new built homes use septic systems. Moreover, septic systems are now considered as a viable low-cost, long-term, decentralized approach to wastewater treatment if they are planned, designed, installed, operated and maintained properly (USEPA, 2002). This is in contrast to previous thinking which considered septic systems as temporally installations to be replaced eventually by centralized wastewater treatment systems.

The U.S. Census Bureau (2009) estimates that approximately 26 million homes, businesses and recreational facilities nationwide use septic systems as the primary method of wastewater treatment. These systems collect, treat, and release about 4 billion gallons of treated effluent daily (USEPA, 2002). The majority of septic systems are found in small communities (those with fewer than 10,000 people) and in these communities greater than 61% of housing units rely on septic systems for their wastewater treatment needs (U.S Census Bureau, 2009). Nationally, the distribution and density of housing units with septic systems varies widely across regions and states. According to the USEPA (2002), greater than one third of homes in the

Southeast use septic systems, including 48% in North Carolina, 43% in Alabama, 40% in South Carolina and about 36% in Georgia. Additionally, one third of new homes nationwide rely on septic systems, emphasizing its importance and highlights the significance of septic systems in meeting current and future wastewater treatment needs.

Septic systems impact on water quality

It has long been known that improperly functioning or poorly maintained septic systems are sources of pollution that contaminate surface and ground waters (USEPA, 2002). Even properly functioning systems have been reported to contribute significantly to pollutant loads in surface waters after high precipitation events (Arnade, 1999; Habteselassie et al., 2011). Several studies have implicated septic systems in the impairment of ground water and surface water resources with contaminants including pathogens, organic pollutants and nutrients (Badruzzaman et al., 2012; Lapworth et al., 2012; Mallin and McIver, 2012; Weiskel et al., 1996). Over the last couple of decades, nutrients such as nitrogen and phosphorus have received the most attention due to apparent public health and environmental impacts of elevated levels of these contaminants. Human and environmental health impacts such as eutrophication leading to reductions in fish populations have combined to drive efforts at the local and national levels to reduce nutrient concentrations in water bodies (Badruzzaman et al., 2012; Kramer et al., 2006). The impacts of pathogens until recently, have received very little attention at the local and national level.

Septic systems and surface water quality

Microbial contamination of surface waters is commonly associated with septic systems. Atoyan et al. (2011) evaluated microbial water quality of the Pettaquamscutt River, Rhode Island, and observed high counts of fecal coliform and enterococci bacteria. The authors

attributed contamination to the high density of septic systems around the monitoring stations. Cahoon et al. (2006) reports of long-term closures of shellfish harvesting areas in coastal waters in Southwestern Brunswick County, North Carolina. The authors attributed this phenomenon to fecal coliform contamination. Evaluation of water quality data from sampling locations pointed to septic systems as the major contributor to fecal coliform bacteria loads. Comparisons of septic systems to storm water runoff to determine sources of fecal coliform showed that even in instances where storm water runoff was influential, the source of the pathogens was traceable to wastewater originating from failing septic systems.

Coastal water contamination with *E. coli*, enterococci and Bifidobacteria (Conn et al., 2012; Thompson et al., 2012; Plummer and Long, 2007), Cryptosporidium and Giardia (Lipp et al., 2001a, b) originating from septic systems has also been reported. According to Conn et al. (2012) coastal areas with shallow groundwater and sandy soils are at most risk from septic system failure and hence contamination by total coliform bacteria. A combination of high rainfall, high septic system density and the frequent occurrence of episodic precipitation events such as hurricanes and tropical storms in coastal climates have been linked to septic system failures (Habteselassie et al., 2011). In North Carolina, approximately 429,000 acres of potential shell-fishing waters have been permanently closed due to fecal coliform impairments (Conn et al., 2012). Fecal bacteria impairments also impact the recreational use of coastal water resources. Frequent closures of public beaches in Virginia have been attributed to high enterococci counts that exceeded regulatory thresholds (Dickerson Jr et al., 2007).

Human enteric viruses have been detected in surface waters in watersheds impacted by effluent from septic systems (Lipp et al., 2001a, b). Frequently, enteric viruses have been detected in water samples even though FIB concentrations have been within threshold values

(Harwood et al., 2005; Hörman et al., 2004). Viruses have also been shown to migrate long distances away from their origin and show remarkable survivability; remaining viable for long periods of time (Lipp et al., 2001a). Lipp et al. (2001b) isolated enteroviruses in Sarasota Bay, Florida with virus concentrations ranging from 0.17 to 0.77 infectious units 100 l^{-1}. These viruses are known to be infectious at relatively low concentrations (Rodríguez-Lázaro et al., 2012). Paul et al. (2000) studied viral fate and transport in Florida Keys and found that viral pathogens seeded into septic tanks migrated at rates ranging from 1.7 m hr^{-1} to an astounding 57.5 m hr^{-1}. Viral particles were also detected in adjacent canals within 3 hrs and 15 min after seeding under local conditions. Additionally, some enteric viruses are thought to be zoonotic and differ in persistence, pathogenicity and infectivity (Rodríguez-Lázaro et al., 2012).

Septic systems and groundwater quality

The relationship between septic systems and groundwater quality has been widely studied with evidence of water quality impairments reported. The contributions of septic systems to groundwater recharge (Bremer and Harter, 2012; Landers and Ankcorn, 2008; Burns et al., 2005) signifies the potential for transfer of contaminants from septic system drainfield to underlying groundwater resources. Bremer and Harter (2012) concluded, based on detailed groundwater flow and transport modeling, that areas with high spatial septic density are more susceptible to groundwater contamination with leachate from septic systems compared to low density septic areas. Arnade (1999) reported significant fecal coliform contamination of groundwater in wells located 12 m to 36 m away from septic tanks. The results showed a strong correlation between increasing fecal coliform contamination and decreasing distance between wells and septic tanks. Also, the results indicated a strong influence of seasons on contaminant loads, with samples

showing twice as much fecal coliform contamination in the wet season compared to the dry season.

Other pathogenic bacteria and viruses have been detected in groundwater near septic systems. In most instances the degree of contamination is related to the density of septic systems, status of septic systems i.e. whether systems are functioning properly or failing, and the hydrologic, soil and climatic factors which control the fate and transport of contaminants (Cahoon et al., 2006; Habteselassie et al., 2011; Lipp et al., 2001a; Lipp et al., 2001b). Direct monitoring of groundwater wells revealed significant loads of total coliforms, *E. coli* and enterococci in groundwater near septic systems (Conn et al., 2012; Habteselassie et al., 2011). High contaminant loads in monitoring wells was attributed to hydraulic and treatment failures of septic systems.

Approaches to fecal source tracking

The identification of sources of pathogens and other contaminants in water is driven by the need to quantify the relative contributions of sources in order to develop best available management techniques to protect and improve water quality (Plummer and Long, 2007). The identification of pollutant sources has become vital due to the requirements to develop total maximum daily load (TMDL) under section 303(d) of the Clean Water Act to facilitate the restoration of impaired water bodies (Habteselassie et al., 2011). Typically, standard methods of monitoring FIB have been used to assess the quality of water and to implement control measures necessary to protect public health and the environment (Harwood et al., 2014). Although successful at identifying fecal contamination in water bodies, FIB monitoring do not identify the sources of contaminants (Cahoon et al., 2006; Field and Samadpour, 2007; Habteselassie et al., 2011; Plummer and Long, 2007). Multiple approaches utilizing targeted pathogen monitoring,

FIB for baseline monitoring coupled with MST has been suggested (Field and Samadpour, 2007; Habteselassie et al., 2011).

The presence of diverse non-point sources of fecal contamination at the watershed scale presents challenges to water resource management (Carroll et al., 2005). Carroll et al. (2005) observed that the numerous possible sources of pathogens at the watershed scale make it difficult to isolate septic systems as a prominent source of fecal pollution. In a comparative study of failing and properly functioning septic systems, Habteselassie et al. (2011) suggested the contribution of other fecal sources to *E. coli* and enterococci contamination. Non-point sources that have been widely implicated at the watershed scale include storm-water run-off (Cahoon et al., 2006), wildlife (Habteselassie et al., 2011) and livestock (Parajuli et al., 2009). Uncertainty in source discrimination can be reduced through the development of new methods and approaches for identifying and quantifying fecal sources at the watershed scale (Griffith et al., 2010).

Conventional and emerging approaches for MST at the watershed scale have been previously described (Tran et al., 2015; Wuertz et al., 2011; Field and Samadpour, 2007). MST techniques are divided into library-dependent and library-independent methods depending on whether the method requires a library (a database of bacterial isolates or patterns or traits from fecal samples of known origin) (Plummer and Long, 2007; Stoeckel and Harwood, 2007). Library-dependent methods are most often culture-based, requiring the culture of environmental isolates from water samples (Field and Samadpour, 2007; Stoeckel and Harwood, 2007). Library-independent methods on the other hand are based on sample-level detection of source-specific microbial genetic markers (Roslev and Bukh, 2011). The use of host-specific genetic markers in source tracking studies has increased in recent years due to improvements in molecular methods leading to rapid turn-around for genetic assays (Field and Samadpour, 2007).

Comparative studies of source tracking methods found host-specific molecular assay to be best at differentiating between human and non-human fecal sources (Griffith et al., 2003).

MST methods employing *Bacteroidales* genetic markers have shown remarkable promise for rapid source identification and quantification of fecal sources in environmental water samples (Ahmed et al., 2016; Stoeckel and Harwood, 2007; Griffith et al., 2003). Several studies have applied these markers for source identification in the U.S. (Boehm et al., 2013; Stewart et al., 2013; Shanks et al., 2010), Europe (Gawler et al., 2007; Gourmelon et al., 2007) and other parts of the world (Ahmed et al., 2009; Jenkins et al., 2009). Moreover, *Bacteroidales* markers have showed high sensitivity and specificity in comparative studies (Griffith et al., 2003; Stoeckel and Harwood, 2007). Viral genetic markers have also been employed successfully to track the sources of enteric viruses in water samples (Wong et al., 2012). Noble et al. (2006) used PCR to detect and quantify markers of human fecal contamination including a human-specific *Bacteroides* marker and enteroviruses in surface water near Santa Monica Bay, California. Boehm et al. (2003) also employed human specific *Bacteroides* sp. and enterovirus to implicate human sewage in fecal contamination in coastal surface waters and groundwater of the California coast. Some genetic markers including *Bacteroidales* are geographically stable and generally persist long enough to be detectable in natural water samples (Field and Samadpour, 2007).

Modeling bacteria loads at the watershed level

Watershed models have been used as part of an integrated watershed management approach to forecast peak flow, assess the effect of land use change, identify options for reduction of non-point sources of pollution, model source-specific pollution, analyze causes of nutrient loss and assess climate change impacts among others (Yang et al., 2008). Distributed

watershed models such as SWAT (Soil Water Assessment Tool) are designed to predict the impact of management on water, sediment, microbial loads and agricultural chemical yields in ungaged watersheds (Gassman et al., 2007). Few studies have used SWAT to model the contribution of septic systems to fecal contamination at the watershed scale (Niazi et al. 2015; Frey et al., 2013; Parajuli et al., 2009; Coffey et al., 2010). Parajuli et al. (2009) used FIB and antibiotic resistance analysis to model source specific fecal bacteria. This model which was first calibrated for flow and sediment, was used to first model single sources of bacteria (livestock or human or wildlife) and then the combined effects of two sources were also evaluated. Results indicated poor agreement between simulated and observed results for single sources whilst the combined sources showed results ranging from unsatisfactory to good. The authors recommended further research to address uncertainties in results stemming from bacteria source tracking (BST) uncertainty and spatial variability.

In other studies, Frey et al. (2013) estimated that approximately 4 septic systems were failing in an agricultural watershed in the 178 km^2 Payne River watershed in Ontario, Canada. The contribution of these failing systems was treated as a point source input in the model. Niazi et al. (2015) treated input from failing septic systems as a fertilizer management operation in a rural watershed in Salem County, New Jersey. Similar to other work in the literature, the study by Niazi et al. (2015) focused on a rural watershed dominated by agricultural land use. Septic systems' influence on fecal bacteria loads in areas not dominated by agricultural land use has received very little attention. The limited studies that have modeled septic system impact at the watershed level shows promise, however further studies are needed to assess the performance and reliability of these models for predicting septic system influence in mixed use watersheds.

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CHAPTER 3

INFLUENCE OF SEPTIC SYSTEMS AND LAND USE CHARACTERISTICS ON STREAM FECAL POLLUTION AT THE WATERSHED LEVEL[1]

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ABSTRACT

This study aims to identify the sources of fecal pollution in suburban watersheds and in so doing determine the impact of septic systems on microbial water quality. Water samples were collected from streams in 24 well characterized watersheds during baseflow to analyze for the levels of fecal indicators *E. coli* and enterococci. The watersheds represent a gradient of land use conditions from low to high density of septic systems, as well as developed to undeveloped uses. Our findings indicate statistically significant interaction between septic density and season for enterococci count ($p = 0.005$) and stream yield ($p = 0.04$). Seasonal variations in bacterial count and stream yield were also observed, with significant differences between spring-fall and summer-fall. Results from multiple linear regression models suggest that land use (including septic system density, average distance of septic systems to streams, percent developed area and forest cover) and water temperature could explain approximately half ($R^2 = 0.50$) of the variability in bacterial count and yield in spring and summer. There is a significant positive relationship between septic system density and fecal pollution levels. However, this relationship is season dependent and is influenced by watershed level characteristics such as average distance of septic systems to streams, percent developed area and forest cover. This study confirms the significant impact of septic systems on fecal pollution during baseflow and provides the tools that will enable effective pollution monitoring at the watershed scale.

INTRODUCTION

As widely as septic systems are used for wastewater treatment in the U.S., their impact on water quality have not been clearly elucidated. This scenario is nowhere more evident than in southeastern U.S. where approximately 40% of residential housing units use septic systems for wastewater treatment (U.S. EPA, 2002). There is no doubt that septic systems serve an important wastewater treatment need in suburban and rural settings where centralized wastewater treatment facilities are non-existent. Of significant concern, however, are recent reports that implicate septic systems and non-point sources in general in widespread fecal pollution of surface waters (U.S. EPA, 2014; U.S. EPA, 2013). It is generally agreed that non-point sources such as septic systems, agricultural runoff and wildlife have become the greatest threat to surface water quality in the wake of stringent controls over point sources of contamination under the Clean Water Act (Conn et al., 2012; Carroll et al., 2005). The public health risks, ecological implications and socio-economic impact associated with fecal pollution of surface waters are well documented in previous studies (Field and Samadpour, 2007; McQuaig et al., 2006; Gerba and Smith Jr, 2005; Borchardt et al., 2003). These concerns have heightened the need to identify the impact of septic systems at the watershed level to aid management efforts.

Identifying the sources of fecal pollution at the watershed level is an important element in current water quality management strategies such as the total maximum daily load (TMDL) (Ahmed et al., 2012; Habteselassie et al., 2011). However, source identification has proved challenging due to the presence of multiple non-point sources of fecal pollution at the watershed level (Carroll et al., 2005). It is therefore not surprising that most watershed management programs have failed to account for septic systems as a prominent source of fecal pollution. This

is of concern as several researchers have implicated septic systems in fecal pollution of surface water resources (Habteselassie et al., 2011; Cahoon et al., 2006; Carroll et al., 2005).

In recent years, multiple approaches encompassing FIB monitoring, targeted pathogen monitoring and MST techniques have been suggested as a long-term solution to the challenges arising from the diverse sources of fecal pollution at the watershed level (Habteselassie et al., 2011; Field and Samadpour, 2007; Noble et al., 2006; Boehm et al., 2003). In the short-term, FIB monitoring, which is an established water quality assessment tool, can be combined with analysis of watershed level characteristics to identify the sources of fecal pollution. A number of studies have used this approach to identify the sources of fecal pollution at the watershed level (Sauer et al., 2011; Burns et al., 2005; Hatt et al., 2004; Mallin et al., 2000). Burns and co-workers assessed the effects of impervious area on pollutant levels in a suburban catchment near New York City. The authors reported that the levels of pollutants in developed areas were elevated relative to an undeveloped watershed. Mallin et al. (2000) examined the effect of land use factors on fecal pollution and noted that percentage impervious coverage explained as much as 95% of the variation in FIB abundance in estuarine systems.

Other studies have considered the influence of septic systems on microbial quality of surface waters (Atoyan et al., 2011; Lipp et al., 2001a; Lipp et al., 2001b; Young and Thackston, 1999). There is anecdotal evidence that increased septic system density leads to increased pollutant levels in surface waters (Mallin and McIver, 2012; Lipp et al., 2001a). In contrast, Young and Thackston (1999) noted that the true impact of septic systems at the watershed level may be masked by the complexity of sources in a mixed use watershed. In a similar study, Thompson et al. (2012) found no association between septic system density and FIB levels in an island community off the coast of Florida. Available studies on septic system impacts at the

watershed level have focused on large catchments and predominantly estuarine or marine waters (Mallin and McIver, 2012; Carroll et al., 2005; Lipp et al., 2001a). Thus, the results from these studies are not easily applicable to small watersheds and the streams that drain these watersheds. Typically, runoff-induced septic system impacts have received the bulk of attention with little emphasis on septic systems' impacts during baseflow. In an attempt to bridge this knowledge gap, Landers and Ankcorn (2008) evaluated the influence of septic systems on baseflow quantity and specific conductance over one dry season in 2007. The authors, who investigated the same watersheds used in the present study, observed significant increases in baseflow quantity and specific conductance with increasing septic system density. The present study would address the impact of septic systems on microbial water quality as well as capture any seasonal patterns in septic system impacts.

The overall goal of our study was to understand the dynamics of septic systems' impact at the watershed level as influenced by land use characteristics. To the best of our knowledge, no published work has considered the impact of septic systems on microbial water quality across a broad septic density gradient. Also, the relationships between septic system density and fecal pollution, and the influence of land use factors on this interaction must be explored further to identify trends. The working hypothesis in the present study was that increasing septic system density increases fecal pollution levels in streams under baseflow conditions. Our objectives included the evaluation of temporal and spatial variations in fecal pollution levels in streams of urbanizing watersheds with varying septic density. Also, we intended to isolate septic system and watershed level factors that influence microbial water quality under baseflow conditions.

MATERIALS AND METHODS

Site characterization

The study area is in Gwinnett County, northeast of Atlanta, GA and has a mean annual precipitation of about 1245 mm (National Weather Service; http://www.nws.noaa.gov/climate/xmacis.php?wfo=ffc). The study area consists of 24 watersheds which range in size from 0.18 to 8.81 km^2. A map of the study area and watershed boundaries, modified from Landers and Ankcorn (2008), is presented in Figure 3.1. The selected watersheds are in the Ocmulgee and Oconee River basins, which drain to the Altamaha River and ultimately into the Atlantic Ocean. The selected watersheds are typical of urbanizing watersheds along the Interstate 85 corridor in the southeastern Piedmont region of the U.S. This region, which has seen rapid population growth over the past two decades, depends largely on surface water for more than 65% of public water supply. The watersheds represent a gradient of land use conditions from low to high density of septic systems, as well as developed to undeveloped uses. Watersheds 1 – 11 and 15 are characterized as having low density of septic systems (LD) with the remaining twelve characterized as having high density of septic system (HD). An arbitrary threshold of less than 38 septic systems per km^2 was set for LD watersheds and greater than 77 septic systems per km^2 for HD watersheds (Table 3.1). These thresholds took into consideration the U.S. EPA's designation of areas with greater than 15 units/km^2 as regions of potential groundwater contamination. Considering that the EPA's recommendation was published over three decades ago (U.S. EPA, 1977) and the intervening years, especially the past 20 years have seen improvements in septic system technology and regulation, we decided to raise the low density threshold to 38 units/km^2.

Watershed characteristics were determined from spatial datasets processed in geographic information systems. Watershed characteristics described by Landers and Ankcorn (2008) and land uses determined using the StreamStats interactive map of Georgia (http://water.usgs.gov/osw/streamstats/georgia.html) are shown in Table 3.1. The average septic density (units km^{-2}), percent impervious coverage and percent agricultural land use are 22, 6.7 and 32.5 respectively for LD, and 216, 18.3 and 4.2 respectively for HD watersheds. Other watershed selection criteria in addition to septic density included similar geological setting, precipitation, climate, accurate base-flow measurement locations and available spatial datasets of natural, infrastructure and water-use characteristics. Weather data for the area was collected from the Georgia Automated Environmental Monitoring Network (http://www.georgiaweather.net/). Additional information on site characteristics can be found in Landers and Ankcorn (2008).

Sample collection

Surface water samples from streams in the 24 watersheds were collected during baseflow on 7 sampling events spanning November, 2011 to November, 2013, creating a data set with two spring samples, two summer samples and three fall samples. Baseflow conditions were determined using long-term discharge measurements at two USGS stream gages (http://waterdata.usgs.gov/ga/nwis/uv/?site_no=02205522; http://waterdata.usgs.gov/ga/nwis/uv/?site_no=02207385) near the study site. Also, baseflow sampling coincided with periods of zero precipitation 72 hours prior to the sampling event. Baseflow sampling (n = 168) coincided with the spring (n = 48; March 2012 and April 2013), summer (n = 48; July 2012 and 2013) and fall (n = 72; November 2011, 2012 and 2013) seasons. At each monitoring station, samples were collected in duplicate in 1 L sterile high-density polypropylene, screw-capped bottles. Samples were kept on ice and transported to the laboratory for analysis (usually within 6 hours of sample

collection). Sample collection and analysis followed guidelines of the National Field Manual for the Collection of Water-Quality Data (USGS, variously dated). Baseflow discharge (m^3 sec^{-1}) was measured at each monitoring point during sampling events by our project partners at the United States Geological Survey (USGS) Georgia Water Science Center in Atlanta. The velocity-area method (Rantz, 1982) was used for discharge measurements. Environmental water quality parameters including pH, temperature, dissolved oxygen and specific conductance were measured during sampling with a calibrated Quanta multi-parameter probe (HYDROLAB, Loveland, CO).

Water quality analysis

Water samples were analyzed for the FIB *E. coli* and enterococci using the Colilert-18 and Enterolert kits (IDEXX Laboratories Inc., Westbrook, ME). The Colilert-18 and Enterolert kits are defined substrate methods for *E. coli* and enterococci respectively and are U.S. Environmental Protection Agency (U.S. EPA) approved and are included in Standard Methods for Examination of Water and Wastewater. Each sample was diluted (10–fold dilution based on previous analysis of samples from the monitored streams) to 100 ml volume using sterile deionized water. The Colilert-18 and Enterolert substrates were then added to the 100 ml dilution to dissolve. The samples were then poured into a 97 well tray, sealed and incubated for 18 hours and 24 hours for *E. coli* and enterococci respectively. The number of positive wells, based on UV fluorescence, was used to estimate the MPN of *E. coli* and enterococci using manufacturer supplied MPN tables. All samples were run with negative controls (100 ml of diluent used to dilute samples) and followed manufacturer recommended quality control procedures.

Data analysis

FIB levels in streams were expressed as stream yield (MPN sec^{-1} km^{-2}) by multiplying FIB count (MPN 100 ml^{-1}) by the stream discharge (m^3 sec^{-1}) to obtain the stream load. The load was then divided by the watershed area to give the stream yield. Both FIB count and stream yield were reviewed for normality and log-transformed to achieve normality prior to data analysis. Statistical analysis assessed the influence of environmental parameters and land use factors on microbial water quality in the watershed groups. The distribution free Kruskal-Wallis test was used to identify seasonal differences in physical and chemical water quality parameters. A distribution free Spearman's rank correlation test was also used to determine correlations between water quality parameters and land use factors. Correlation analysis was conducted for each watershed group on a seasonal basis as well as for the pooled dataset. In addition, multiple linear regression analysis was performed to determine the combination of environmental parameters and land use factors driving microbial water quality in urbanizing streams. Two-way analysis of variance (ANOVA) was used to test differences between watershed groups and seasons and to see if there was a significant interaction between the factors. Tukey pairwise comparison test was performed when treatment groups were significantly different. All data analysis was performed in SAS 9.3 (SAS Institute, Cary, NC) and statistical significance estimated at the 95% confidence level.

RESULTS

Physical and chemical water quality

The means and ranges for environmental parameters are summarized by season and for the pooled dataset in Table 3.2. For the pooled data, specific conductance in LD watersheds ranged from 25 to 85 µS cm^{-1} with a mean of 50.6 µS cm^{-1}. The mean specific conductance in

HD watershed was 83.6 µS cm^{-1} with a range of 39.5 to 193 µS cm^{-1}. The range of values for specific conductance, however, varied by season. Specific conductance was lowest during the fall months with a range of 26.5 to 65 µS cm^{-1} and mean of 46 µS cm^{-1}. In comparison, the mean values for the spring and summer seasons were 57 µS cm^{-1} and 52 µS cm^{-1} respectively. The data for spring and summer were also more variable, with data ranges of 37 to 85 µS cm^{-1} and 25 to 79 µS cm^{-1} respectively. In general, specific conductance was higher in HD watersheds than LD watersheds based on the pooled as well as the seasonal data. The average specific conductance (pooled data) for the HD watersheds was approximately 65% greater than specific conductance for LD watersheds. Seasonally, the mean specific conductance for HD watersheds was higher and the range of values more variable than LD watersheds regardless of season.

The main effects of density and season on specific conductance were statistically significant with $p < 0.001$. Pairwise comparison showed a statistical difference ($p < 0.001$) between LD and HD watersheds. Also, statistically significant differences were observed for spring-fall ($p < 0.001$), spring-summer ($p = 0.04$) and summer-fall ($p = 0.03$). There was not a statistically significant interaction between density and season ($p = 0.59$). Other environmental water quality parameters including pH, dissolved oxygen and temperature were not significantly different irrespective of septic density or season (Table 3.2).

Microbial water quality

Microbial water quality was assessed by enumerating *E. coli* and enterococci levels in stream water samples. Table 3.3 summarizes *E. coli* and enterococci counts and stream yields across watershed groups and over seasons. *E. coli* count (pooled) ranged from 15 to 2,739 MPN 100 ml^{-1} in LD watersheds and 10 to 1,643 in HD watersheds. Enterococci counts (pooled) were relatively higher, ranging from 20 to 11,401 MPN 100 ml^{-1} in LD watersheds and 31 to 5,963

MPN 100 ml^{-1} in HD watersheds (Table 3.3). The ranges of FIB counts were consistently wider in LD watersheds than HD watersheds. Bacterial stream yield, which reflects the influence of streamflow and watershed area, followed a similar trend (Table 3.3). The data also suggests widespread fecal pollution across both LD and HD watersheds (Figure 3.2). In LD watersheds, approximately 49% of all samples exceeded the one-time single sample *E. coli* action value for recreational water use of 235 MPN 100 ml^{-1}. The percentage of samples that exceeded the *E. coli* threshold in HD watersheds was 45% (Figure 3.2a). Enterococci counts exceeded the single sample action value of 70 MPN 100 ml^{-1} in 90% and 92% of all samples collected in LD and HD watersheds respectively (Figure 3.2b). The recommended action values are provided in the revised EPA recreational water quality criteria and are described as precautionary single-sample threshold values for recreational water use (U.S. EPA, 2012).

Seasonal variations in FIB count and stream yield were observed and the data is presented in Table 3.3 and Figures 3.2 and 3.3. Mean FIB count for a single sampling event was highest during summer in LD watersheds and during spring in HD watersheds (Figure 3.2a). Two-way ANOVA showed no significant interaction between septic system density and season for *E. coli* count and stream yield (Table 3.4). Detailed analysis of the effect of septic system density on *E. coli* count and stream yield showed no significant differences between HD watersheds and LD watersheds within season. The effect of season on *E. coli* count was varied, with significant differences observed for spring-fall (LD; $p = 0.04$), spring-fall (HD; $p = 0.044$), summer-fall (LD; $p = 0.008$) and summer-fall (HD; $p = 0.03$); spring-summer was however not statistically different in both watershed groups. The stream yield of *E. coli* showed a similar seasonal pattern, with significant differences between spring-fall (LD and HD; $p < 0.001$), summer-fall (LD and HD; $p < 0.001$). *E. coli* yield was not significantly different between spring

and summer in both watershed groups. The outcome of two-way ANOVA test (Table 3.4) show statistically significant interaction between septic system density and season for enterococci count ($p = 0.005$) and stream yield ($p = 0.04$). The main effect of season and watershed density was therefore difficult to generalize due to the significant interaction between the factors. Typically, FIB was lower in count and stream yield during the fall (Figures 3.2 and 3.3).

Land use/water quality relationships

The influence of land use factors on water quality indicators was evaluated through correlation analysis (Tables 3.5 and 3.6). Five main land use factors, namely septic systems density, average distance of septic to streams, percent agriculture land use, percent forest cover and percent developed area, were evaluated to determine their impact on fecal pollution dynamics in the monitored streams. Spearman rank correlation between microbial and physicochemical water quality/land use parameters by watershed density (Table 3.5) show a positive and moderate correlation ($r = 0.42$) between *E. coli* count and percent agricultural land use in LD watersheds. As expected, agricultural activities remain the dominant source of fecal pollution in LD watersheds. In HD watersheds, agricultural land use was negatively correlated ($r = -0.28$) with *E. coli* count as envisaged because other land use factors play a more significant role than agriculture. Septic system density, percent impervious area and average distance of septic to streams were negatively correlated with *E. coli* count in LD watersheds. Positive correlation, albeit weak correlation was observed between *E. coli* count and other land use factors including septic system density, percent impervious area and percent developed area in HD watersheds. *E. coli* stream yield showed a similar correlation pattern to *E. coli* count.

Enterococci count, similar to *E. coli*, was positively correlated with septic system density and percent developed area in HD watersheds. Percent forest cover and agriculture on the other

hand were negatively correlated with enterococci count in the HD watersheds. Land use factors did not show significant correlations with enterococci count in LD watersheds. Enterococci stream yield was not significantly correlated with any land use factor in HD watersheds but was correlated to percent impervious area in LD watersheds. In general, FIB count and stream yield were significantly correlated with physical and chemical water quality parameters in both watershed groups. Temperature, pH and specific conductance were overall positively correlated with FIB count and stream yield, whilst dissolved oxygen was largely negatively correlated with bacterial count and yield. Temperature showed the strongest correlation with FIB count and stream yield amongst the environmental parameters. Analysis of relationship between microbial water quality indicators showed moderately positive (r = 0.49) correlation between *E. coli* and enterococci counts in LD watersheds. The relationship was positive in HD watersheds, however, the Spearman correlation coefficient was lower (r = 0.38).

Relationships between FIB and land use/environmental parameters were also explored at the seasonal level (Data not shown). Average distance of septic systems to streams was the most important factor affecting microbial water quality in the spring. Our results demonstrate a negative correlation between average distance of septic to streams and bacterial count and stream yield in spring. Percent developed area, percent imperviousness and septic system density were all negatively associated with enterococci count in the summer whilst percent developed area was positively related to enterococci count during spring. With the exception of distance of septic system to stream, all land use factors were not significantly related to enterococci count during fall.

Multiple linear regression

Multiple linear regression (MLR) models were developed to assess the ability of land use characteristics and environmental water quality parameters to predict fecal pollution in streams. The backward elimination procedure was employed to identify the set of variables that best explained variations in FIB load. The criteria for including variables in the final model included a variance influence factor (VIF) of less than 10 and significance of each variable at $\alpha = 0.05$ (Gonzalez, 2012; Hathaway, 2010). Details of the reduced models for each microbial water quality parameter, predictive variables included, VIF values and p-values are presented in Table 3.6. Models were developed at the seasonal level due to the significant seasonal variations observed in microbial water quality. The reduced model for *E. coli* count in spring included percent forest cover, percent developed areas, septic system density and water temperature. The coefficient of determination (R^2) was 0.46 for this model. In general, septic system density and average distance of septic to streams were important predictors of microbial water quality in spring. Enterococci yield exhibited the highest R^2 of 0.50 in spring with average distance of septic to streams and septic system density being the important explanatory variables. Land use characteristics were not significant predictors of *E. coli* count and stream yield during the summer. Temperature appeared to play a significant role in microbial water quality during the summer. However, septic system density was an important predictor of enterococci count during the summer. The R^2 for this model, which also included water temperature, was a moderate 0.50. Water quality parameters and land use characteristics were generally not good predictors of microbial water quality during fall ($R^2 = 0.06$ to 0.23).

DISCUSSION

Although there is currently no U.S. water quality standard for specific conductance, it is generally considered a useful indicator of water quality (Plummer and Long, 2007). Specific conductance, which is a measure of the amount of dissolved solutes in water, was significantly different between LD and HD watersheds. Consistently higher specific conductance observed in HD watersheds indicates a continuous source of dissolved ions in these watersheds. This observation agrees with results from a study by Hatt et al. (2004) which showed that increasing septic system density was associated with elevated specific conductance under baseflow conditions. Moreover, Landers and Ankcorn (2008) found septic system density to be statistically significant in relation to specific conductance and opined that this may indicate the influence of treated wastewater in HD watersheds. In our study watersheds, the only likely source of treated wastewater, especially in HD watersheds, is septic systems.

FIB counts in monitored streams showed widespread fecal pollution with variations along temporal and spatial scales. Data (Figure 3.2) indicate that more than 49% and 90% of all water samples exceeded the single sample threshold for *E. coli* and enterococci respectively. This is a significant observation considering that the numbers of intestinal enterococci in feces are generally about an order of magnitude lower than those of *E. coli* (WHO, 2006). Differences in counts of *E. coli* and enterococci may be attributed to the fact that most enterococci species generally survive longer in water environments and are more resistant to drying (McFeters et al., 1974). Wider ranges of FIB values observed in LD watersheds are indicative of the presence of multiple sources of fecal pollution. On average the percent of forest, agriculture and developed areas in LD watersheds are evenly distributed in the ratio 37:32:23 whereas the same land uses in HD watersheds are disproportionate in the ratio 25:4:69. The observed range of FIB counts in

LD watersheds is therefore a reflection of the variable sources of fecal pollution in these watersheds. Moreover, the sources impacting water quality in LD watersheds may be unstable (for example the activities of livestock and wildlife within the watershed), explaining the wide variation in bacterial counts at different sites in LD watersheds. A single sample taken in a LD watershed may significantly over or under estimate FIB content. The sources of fecal pollution in HD watersheds, with their low percentage of agricultural and forest land uses, are less varied, explaining the smaller range of *E. coli* and enterococci counts.

Under baseflow conditions, elevated FIB counts may be indicative of continuous sources of fecal pollution at the watershed level (Carroll et al., 2005). Continuous sources, such as centralized wastewater treatment facilities are however insignificant in this study as the Georgia Permit Compliance System database (Georgia GIS Clearing House, https://data.georgiaspatial.org/index.asp) indicates no NPDES (National Pollutant Discharge Elimination System) discharge points within the study watersheds. In the absence of point sources, the FIB source could be polluted groundwater or it could be residual FIB that reached the streams in runoff during storms. The most likely groundwater source that is impacting the watersheds is septic systems. As noted by Landers and Ankcorn (2008), the annual contribution of septic systems to baseflow is constant, assuming steady-state water use and recharge to baseflow conditions. If FIB originates from runoff, then the FIB source could be a factor that is associated with developed areas such as pet waste or failing septic systems, especially in HD watersheds.

Our findings suggest seasonal variations in bacterial count and stream yield at the watershed level. In general, *E. coli* counts and stream yield did not vary significantly between LD and HD watersheds between and within season. *E. coli* count and yield were however higher in warmer periods (spring and summer) than fall. This outcome is consistent with other studies

that found higher fecal bacterial counts during summer and spring in comparison to fall (Hathaway et al., 2010; Selvakumar and Borst, 2006). Plummer and Long (2007) observed that increased fecal bacterial densities in streams during the warmer months could be attributed to increased inputs from livestock and increased survival times of FIB. Two-way ANOVA showed statistically significant interaction between septic system density and season for enterococci count and stream yield with p-values 0.005 and 0.04 respectively. This finding is important considering that several studies have found enterococci to be a better fecal indicator organism than coliforms (Savichtcheva and Okabe, 2006; WHO, 2006; Kinzelman et al., 2003). For future water quality analysis of fresh waters, complimentary monitoring of enterococci levels should be undertaken to provide confidence in analysis of absence/presence of fecal pollution and seasonal patterns of pollution levels as impacted by septic system density.

Correlation analysis indicated that agricultural activities played a significant role in fecal pollution levels in LD watersheds. Positive correlation with bacterial levels was observed as agriculture land use increased in LD watersheds. However, increasing agriculture land use resulted in decreased bacterial levels in HD watersheds indicating that other sources such as septic systems and other suburban features may be more significant indices of fecal pollution levels in HD watersheds. The percent developed area, percent impervious area and septic system density were all positively correlated with fecal pollution as expected. Although climatic factors are considered the main drivers of fecal pollution during storm flow, fecal pollution during baseflow seem to be primarily driven by land use factors (Hatt et al., 2004; Young and Thackston, 1999). At the seasonal level, average distance of septic to streams appears to be the most significant factor influencing indicator bacterial levels in streams. This effect is prominent during spring when the further away streams are from septic systems resulted in lower bacterial

count and stream yield. It was not surprising that the average distance of septic to streams was most influential during spring because higher precipitation during spring should lead to higher rates of microbial transport from septic drainfields to streams down gradient.

Statistical predictive models have been suggested as supplementary tools to improve fecal pollution monitoring (Gonzalez et al., 2012). Predictive models developed using generalized linear regression models have proved successful as an early warning system for recreational use of surface water (Francy, 2009; Telech et al., 2009). In the present study, MLR analysis of baseflow microbial water quality confirms that land use and water temperature explained approximately 50% of the variability in FIB count and yield in spring and summer. Land use factors that were instrumental included septic system density, average distance of septic to streams, percent developed area and forest cover. Land use factors and environmental parameters did not appear to influence variability in microbial water quality during fall with $R^2 < 0.25$. Additional sampling is needed or other variables should be considered to better explain variations in FIB content during the fall.

CONCLUSIONS

Our findings indicate statistically significant interaction between septic density and season for enterococci count and stream yield. Seasonal variations in bacterial count and stream yield were also observed, with significant differences between spring-fall and summer-fall. Results from multiple linear regression models suggest that land use (including septic system density, average distance of septic systems to streams, percent developed area and forest cover) and water temperature could explain approximately half ($R^2 = 0.50$) of the variability in bacterial count and yield in spring and summer. The results also show significant positive relationship between septic system density and fecal pollution levels. This relationship is, however, season

dependent and is influenced by watershed level characteristics such as average distance of septic systems to streams, percent developed area and forest cover. Understanding the seasonal changes in FIB counts and the relationship with land use characteristics such as septic systems can inform decisions to bring surface waters in line with water quality standards. This study also provides information that can be used in predictive models to greatly increase the predictive power of such models.

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TABLES AND FIGURES

Table 3.1. Septic system properties and land use characteristics in the study area

Watershed ID	Low density (LD) or high density (HD) of septic systems	Basin area (km^2)	Septic density (units/km^2)	Av. distance of septic to stream (m)	% Forest cover	% Agriculture land use	% Developed area	% Impervious area	% Slope
1	LD	8.39	8	163	51.0	27.7	9.4	4.2	8.8
2	LD	1.55	10	126	44.5	25.8	13.8	3.3	10.6
3	LD	2.67	14	163	48.8	32.9	10.2	4.3	8.5
4	LD	0.62	36	172	46.4	23.9	22.0	11.6	7.3
5	LD	1.48	20	86	32.6	45.5	17.8	5.4	5.8
6	LD	5.28	15	108	30.2	49.2	14.4	4.1	6.5
7	LD	1.11	18	90	43.7	29.4	18.5	6.3	10.6
8	LD	1.27	17	94	34.2	48.9	14.3	3	9.2
9	LD	2.95	27	159	26.4	42.6	23.4	7.8	7.7
10	LD	4.4	34	119	43.1	17.2	36.1	7.3	8.3
11	LD	4.2	25	119	31.8	36.3	25.6	7.6	7.8
15	LD	1.68	37	140	14.7	10.3	70.6	15.2	4.6
12	HD	3.29	115	105	44.1	10.6	41.8	12.3	9.1
13	HD	8.81	88	117	33.8	6.6	54.5	13.2	8
14	HD	1.74	141	104	26.0	6.1	60.7	16.1	8.5
16	HD	2.59	187	99	19.6	0.1	77.6	26.4	5.7
17	HD	1.68	230	138	21.0	12.0	66.8	20.1	7.5
18	HD	0.98	308	151	31.3	1.5	66.6	18.4	7.4
19	HD	0.18	373	105	11.4	0.0	88.2	20.3	7.8
20	HD	0.54	290	83	24.8	0.0	75.5	18.3	6
21	HD	1.14	214	63	22.2	5.0	70.7	17.5	8.6
22	HD	1.94	157	63	21.7	4.5	71.7	18.9	7
23	HD	0.52	233	65	22.9	3.1	73.9	18.4	7.3
24	HD	0.67	253	55	19.4	1.6	77.7	20	7.6
Mean LD		2.97	22	128	37.3	32.5	23.0	6.7	8
Mean HD		2.01	216	96	24.8	4.2	68.8	18.3	7.5

Table 3.2. Mean and ranges for environmental water quality parameters (Data summarized by season and watershed group; LD or HD = watersheds with low or high density of septic systems, respectively)

	LD watersheds			HD watersheds			LD watersheds			HD watersheds		
	Mean	Low	High	Mean	Low	High	Mean	Low	High	Mean	Low	High
pH	6.64	4.45	7.18	6.22	5.16	7.4	6.87	5.75	8.2	6.82	6.13	7.6
Dissolved oxygen (mg/l)	8.34	5.99	10.4	8.85	7.02	12	8.03	6.95	9.5	7.63	4.64	9.4
Temperature (^{o}C)	14.1	12.1	17.1	15.8	12.5	20	20.3	17.2	25	20.9	18.1	25
Specific conductance (µS/cm)	57	37	84.5	102	54	193	51.9	25.1	79	83.6	54	140
	Fall						Pooled dataset					
	LD watersheds			HD watersheds			LD watersheds			HD watersheds		
Parameter	Mean	Low	High	Mean	Low	High	Mean	Low	High	Mean	Low	High
pH	6.49	5.72	7.14	6.49	5.55	7.8	6.64	4.45	8.2	6.51	5.16	7.8
Dissolved oxygen (mg/l)	11.72	5.99	19.4	11.7	7.02	22	9.83	5.99	19	9.85	4.64	22
Temperature (^{o}C)	9.72	7.25	11.8	9.73	6.54	13	14	7.25	25	14.6	6.54	25
Specific conductance (µS/cm)	46.17	26.5	64.5	73.1	39.5	138	50.6	25.1	85	83.6	39.5	193

Table 3.3. Mean and ranges of microbial water quality parameters (Data summarized by season and watershed group; LD or HD = watersheds with low or high density of septic systems, respectively)

	LD watersheds			HD watersheds			LD watersheds			HD watersheds		
	Mean	Low	High	Mean	Low	High	Mean	Low	High	Mean	Low	High
E. coli count (MPN 100 ml^{-1})	485	41	2,739	413	10	1,643	533	28	1,970	313	68	985
E. coli yield (MPN sec^{-1} km^{-2})	35,937	2,456	255,944	33,900	542	164,293	22,563	1,066	83,628	24,382	892	100,495
Enterococci count (MPN 100 ml^{-1})	285	56	1399	759	54	5,635	2,238	259	11401	879	92	5963
Enterococci yield (MPN sec^{-1} km^{-2})	22,720	3,294	130,682	58,919	3,961	305,189	127,832	4,539	538,160	72,146	2,199	524,120

	Fall						Pooled dataset					
	LD watersheds			HD watersheds			LD watersheds			HD watersheds		
Parameter	Mean	Low	High	Mean	Low	High	Mean	Low	High	Mean	Low	High
E. coli count (MPN 100 ml^{-1})	261	15	1,181	238	10	1,358	404	15	2,739	309	10	1,643
E. coli yield (MPN sec^{-1} km^{-2})	7,383	75	55,017	5,719	125	28,595	20,029	75	255,944	19,103	125	164,293
Enterococci count (MPN 100 ml^{-1})	251	20	1,173	301	31	1,351	829	20	11,401	597	31	5,963
Enterococci yield (MPN sec^{-1} km^{-2})	6,210	234	29,484	6,432	565	41,515	45,677	234	538,160	40,204	565	524,120

Table 3.4. Two-way ANOVA results (p-values) for water quality parameters by septic system density and season

Parameter	*E. coli* count (MPN 100 ml^{-1})	*E. coli* yield (MPN sec^{-1} km^{-2})	Enterococci count (MPN 100 ml^{-1})	Enterococci yield (MPN sec^{-1} km^{-2})
Density	0.425	0.815	0.838	0.786
Season	<0.001	<0.001	<0.001	<0.001
Density * Season	0.808	0.853	0.005	0.040

Table 3.5. Statistically significant Spearman correlations (*r*) between microbial and physicochemical water quality parameters/basin characteristics (Dataset pooled by watershed group: LD and HD = watersheds with low or high density of septic systems, respectively)

	E. coli count (MPN 100 ml^{-1})		*E. coli* yield (MPN sec^{-1} km^{-2})	
Parameter	LD watersheds	HD watersheds	LD watersheds	HD watersheds
Temperature (^{o}C)	0.32	0.26	0.37	0.47
pH	0.25	ns	0.31	ns
Dissolved Oxygen (mg l^{-1})	-0.26	ns	ns	ns
Specific conductance (µS cm^{-1})	0.31	ns	0.46	0.23
Enterococci	0.49	0.38	0.48	0.36
% Forest cover	ns[§]	-0.24	ns	ns
% Agriculture land use	0.42	-0.28	0.33	ns
% Developed area	ns	0.28	ns	ns
Septic density	-0.25	0.22	-0.26	ns
% impervious area	-0.39	0.24	-0.36	ns
Av. distance from septic (m)	-0.49	ns	-0.39	ns

	Enterococci count (MPN 100 ml^{-1})		Enterococci yield (MPN sec^{-1} km^{-2})	
Parameter	LD watersheds	HD watersheds	LD watersheds	HD watersheds
Temperature (^{o}C)	0.58	0.26	0.59	0.50
pH	0.26	ns	0.32	ns
Dissolved oxygen (mg l^{-1})	-0.37	ns	-0.33	-0.23
Specific conductance (µS cm^{-1})	ns	0.22	0.36	0.28
E. coli	0.49	0.38	0.48	0.36
% Forest cover	ns	-0.23	ns	ns
% Agriculture	ns	-0.41	ns	ns
% Developed	ns	0.37	ns	ns
Septic density	ns	0.23	ns	ns
% impervious	ns	ns	-0.24	ns
Av. distance from septic (m)	-0.25	ns	ns	ns

[§]ns: not significant

Table 3.6. Multiple linear regression models of *E. coli* count (EC), *E. coli* yield (ECY), enterococci count (ENT) and enterococci yield (ENTY) (Data grouped by season)

	Spring				Summer				Fall			
	EC R^2 =0.46	ECY R^2 =0.42	ENT R^2 =0.36	ENTY R^2 =0.50	EC	ECY R^2 =38	ENT R^2 =0.49	ENTY R^2 =0.62	EC R^2 =0.21	ECY R^2 =0.23	ENT R^2 =0.06	ENTY R^2 =0.10
Intercept	2.75	7.68	4.85	6.91		7.52	6.05	7.71	4.77	6.59	2.57	3.78
Variable 1	% Forest cover	% Forest cover	Av. distance	Av. distance	No significant variable	Temperature	Temperature	DO‡	% Developed	% Impervious	Av. distance	% Developed
β1	-0.037	-0.029	-0.009	-0.0085		-0.16	-0.14	0.17	-0.0055	-0.04	-0.003	-0.012
VIF	3.37	3.56	1.12	1.17		na†	1.00	1.28	1.33	1.22	na	4.16
p-value	0.0025	0.029	0.0004	0.0017		<0.0001	<0.0001	0.02	0.02	0.0012	0.04	0.0063
Variable 2	% Developed	% Developed	Temperature	Septic density			Septic density	Temperature	Av. distance	Av. distance		Septic density
β2	-0.027	-0.029	-0.097	0.0014			-0.0015	-0.22	-0.0073	-0.0064		0.0025
VIF	7.87	7.84	1.12	1.19			1.00	1.28	1.56	1.42		4.16
p-value	0.0008	0.0016	0.035	0.04			<0.0016	<0.0001	0.0005	0.015		0.014
Variable 3	Septic density	Av. distance¶		Temperature					pH	pH		
β3	0.0032	-0.0074		-0.12					-0.41	-0.57		
VIF	4.25	1.23		1.21					1.38	1.39		
p-value	0.039	0.014		0.0092					0.0097	0.0074		
Variable 4	Temperature	Septic density							Temperature	Temperature		
β4	0.12	0.0058							0.12	0.18		
VIF	1.19	5.55							1.28	1.28		
p-value	0.007	0.0084							0.0076	0.002		
Variable 5		SC§										
β5		-0.014										
VIF		2.4										
p-value		0.029										

¶Av. distance: Average distance of septic to streams; §SC: Specific conductance; †na: not applicable; ‡DO: Dissolved oxygen

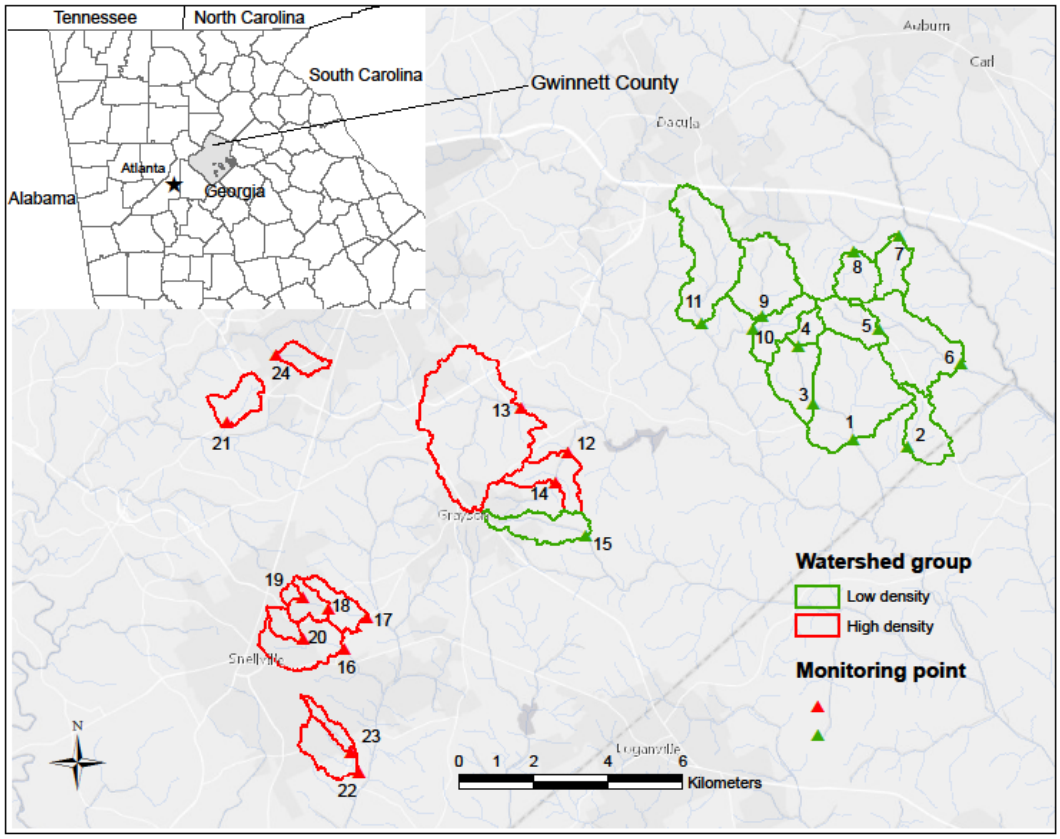

Figure 3.1. Location of the study site with boundaries for watersheds with low (LD) or high (HD) density of septic systems and monitoring stations in Gwinnett County, GA

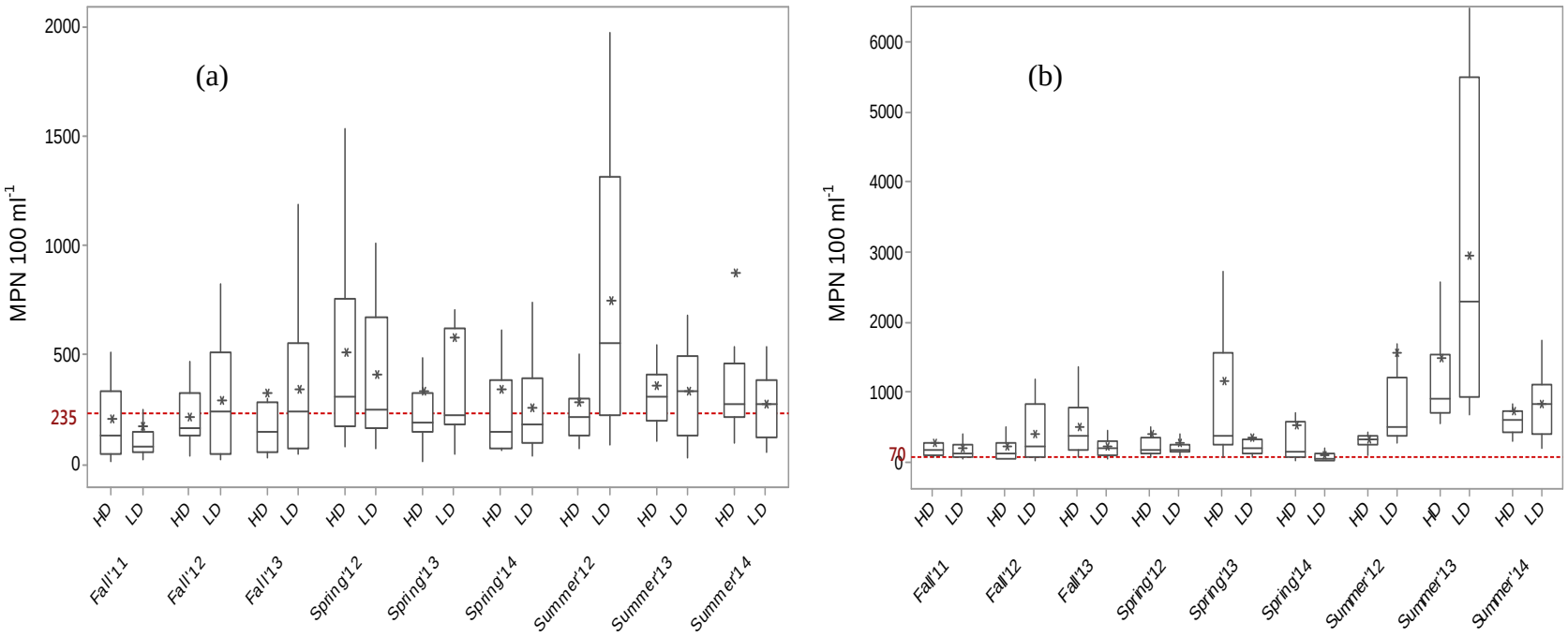

Figure 3.2. *E. coli* (a) and enterococci (b) counts in streams of watersheds with low (LD) or high (HD) density of septic systems over the sampling period. Broken line represents the single sample threshold value for *E. coli* and enterococci for recreational use. Mean bacterial count for each sampling event is represented by (*) on each boxplot.

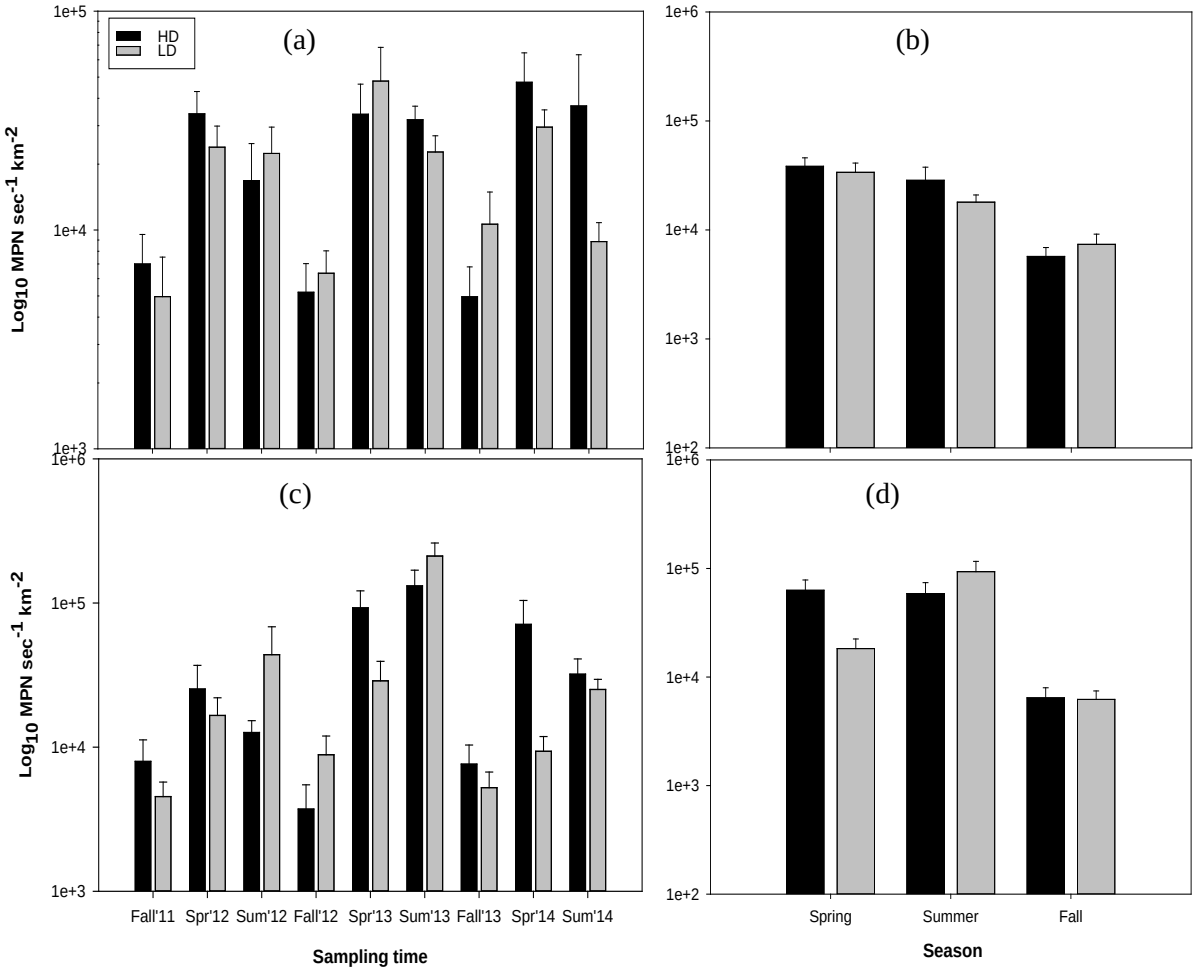

Figure 3.3. *E. coli* (a and b) and enterococci (c and d) stream yield grouped by sampling period and season in watersheds with high density (HD) and low density (LD) of septic systems.

CHAPTER 4

ISOLATING THE IMPACT OF SEPTIC SYSTEMS ON FECAL POLLUTION IN STREAMS OF SUBURBAN WATERSHEDS[2]

[2] Sowah, R. A., Habteselassie, M. Y., Radcliffe, D. E., Bauske, E. and Risse, M. 2016. *Water Research* http://dx.doi.org/10.1016/j.watres.2016.11.007. Reprinted here with permission of the publisher.

ABSTRACT

The presence of multiple sources of fecal pollution at the watershed level presents challenges to efforts aimed at identifying the influence of septic systems. In this study multiple approaches including targeted sampling and monitoring of host-specific *Bacteroidales* markers were used to identify the impact of septic systems on microbial water quality. Twenty four watersheds with septic density ranging from 8 – 373 septic units/km^2 were monitored for water quality under baseflow conditions over a 3-year period. The levels of the human-associated HF183 marker, as well as total and ruminant *Bacteroidales*, were quantified using quantitative polymerase chain reaction. Human-associated *Bacteroidales* yield was significantly higher in high density watersheds compared to low density areas and was negatively correlated (r = -0.64) with the average distance of septic systems to streams in the spring season. The human marker was also positively correlated with the total *Bacteroidales* marker, suggesting that the human source input was a significant contributor to total fecal pollution in the study area. Multivariable regression analysis indicates that septic systems, along with forest cover, impervious area and specific conductance could explain up to 74% of the variation in human fecal pollution in the spring season. The results suggest septic system impact through contributions to groundwater recharge during baseflow or failing septic system input, especially in areas with >87 septic units/km^2. This study supports the use of microbial source tracking approaches along with traditional fecal indicator bacteria monitoring and land use characterization in a tiered approach to isolate the influence of septic systems on water quality in mixed-use watersheds.

INTRODUCTION

Septic systems are used widely for wastewater treatment in southeastern United States. It has been reported that 37 – 48% of all housing units in North and South Carolina, Georgia and Alabama use septic systems for wastewater treatment (U.S. EPA, 2002). This usage rate exceeds the national average of 23% according to the same report. It is also estimated that more than 33% of new homes in the United States are on septic systems, which make septic systems second only to centralized systems, in terms of the number of households served, in the wastewater management infrastructure (U.S. EPA, 2002). A significant number of these septic systems are in suburban areas, with some reports showing that the majority of septic systems are now located in suburban communities compared to rural areas (MNGWPD, 2006; U.S. EPA, 2002). The upward trend in septic systems' use has coincided with widespread fecal pollution of surface waters across the United States, raising questions about the potential contribution of septic systems to water quality impairment at the watershed level (Verhougstraete et al., 2015; Sowah et al., 2014).

Data from the United States Environmental Protection Agency implicate fecal pathogens as the leading cause of water quality impairment in the United States (U.S. EPA, 2016). Frequently, the sources of fecal matter impacting surface water bodies have proved difficult to isolate especially in urbanizing areas with mixed land use (Liang et al., 2013). Typically, fecal pollution of surface water resources derives from two or more sources within a watershed (Jent et al., 2013; Chin et al., 2009). These sources may include wildlife, livestock, manure applications and human inputs (wastewater treatment facility discharges, faulty septic systems and leaky sewers). The inputs from these sources can also vary on both temporal and spatial scales which further complicate pollution management. The traditional approach of monitoring fecal indicator bacteria (FIB) such as *Escherichia coli* (*E. coli*), enterococci and fecal coliforms

is not capable of differentiating sources of fecal pollution at the watershed scale (Tran et al., 2015; Sauer et al., 2011; Field and Samadpour, 2007). This has led to new approaches that rely on FIB monitoring along with analysis of watershed characteristics (such as land use, imperviousness and septic density) and environment conditions (including precipitation and rainfall intensity) in addition to microbial source tracking (MST) with host-specific markers to discern fecal pollution sources at the watershed scale (Verhougstraete et al., 2015; Tran et al., 2015; Sowah et al., 2014; Peed et al., 2011).

Understanding the link between septic systems and fecal pollution of surface waters is critical to improving water quality. It is a well-known fact that individual septic systems can contribute to fecal pollution of ground and surface waters if the systems are not properly designed, sited and maintained (Schneeberger et al., 2015; Habteselassie et al., 2011; Humphrey et al., 2011). Until recently, there was limited information on the aggregate impact of septic systems at the watershed scale leading to inadequate regulation and improper management of septic systems throughout the United States. (Gregory et al., 2013; Carey et al., 2012; Farnleitner et al., 2011; Swann, 2001). Recent studies have attempted to bridge this knowledge gap by examining the relationships between septic system use and fecal bacteria levels at the watershed level (Verhougstraete et al., 2015; Sowah et al., 2014; Peed et al., 2011). Using host-specific MST markers in combination with low-order head-watershed sampling and precipitation information, Peed et al. (2011) observed a positive correlation between the concentration of human-specific marker and septic systems following precipitation. Predictive models developed by Sowah et al. (2014) indicated that septic system density and average distance of septic to streams were significant factors driving fecal pollution in suburban watersheds of Georgia. More recently, Verhougstraete et al. (2015) used classification and regression tree analysis (CART) to

demonstrate a link between increasing septic system numbers and bacterial concentrations. The findings of these studies show that MST methods employing human-associated *Bacteroidales* markers, when combined with land use information, can be a powerful tool for isolating septic system influence at the watershed level.

As promising as these studies are for water resources management, questions still remain about the relationship between septic system density and fecal pollution under different hydrologic and climatic conditions. The applicability of MST methods to different geographic areas and land use scenarios need to be assessed to improve confidence in this approach. This information is critical in suburban watersheds with mixed land use and multiple fecal sources including failing septic systems, leaky sewers, livestock, pets and wildlife that can present challenges to watershed managers. Also, previous studies utilizing MST methods have focused on relatively low to medium density septic impacted watersheds, that is, areas with <100 septic units/km^2 (Verhougstraete et al., 2015; Peed et al., 2011). The current study provides a comprehensive assessment of septic system impacts in watersheds with a gradient of septic system density ranging from 8 – 373 septic units/km^2. In addition to examining septic system impacts, this study also addressed concerns about the influence of leaky sewer pipes on fecal pollution loads.

The overarching goal of this study therefore was to evaluate the impact of septic systems on fecal pollution loads in suburban streams. Our main objective was to determine the relationship between septic system variables and fecal pollution loads at the watershed level. The present study used MST approaches together with targeted water quality monitoring to capture septic system influence across a wide range of septic densities typical of suburban areas in the southeastern United States. Additionally, seasonal and temporal trends in fecal pollution loads as

impacted by increasing septic density, land use characteristics and water quality parameters were examined. Moreover, the utility of enteric viruses for tracking human sources of fecal pollution will be explored as part of a comprehensive MST toolkit approach to evaluating septic system impacts at the watershed level. Finally, the utility of MST for identifying septic system influence on water quality was evaluated as part of a tiered approach to fecal source identification at the watershed level.

MATERIALS AND METHODS

Study Area

The study area is in Gwinnett County, northeast of Atlanta, GA and was previously described in Sowah et al. (2014) and Landers and Ankcorn (2008). It has a mean annual rainfall of about 1245 mm. The study area consists of 24 watersheds which range in size from 0.2 to 8.8 km^2. A summary of watershed characteristics is provided in Table 4.1. The selected watersheds are in the Ocmulgee and Oconee River basins, which drain to the Altamaha River, and ultimately into the Atlantic Ocean. A map of the study area and watershed boundaries is presented in Figure 4.1. The selected watersheds are typical of suburban watersheds along the Interstate 85 corridor in the southeastern piedmont region of the United States. The watersheds represent a gradient of land use characteristics from low to high density of septic systems representing low density residential to medium density residential land use. The watersheds were classified into two groups: low density (LD) watersheds and high density (HD) watersheds. An arbitrary threshold of <38 septic units/km^2 was set for LD watersheds and >77 septic units/km^2 for HD watersheds. The criteria for watershed classification was based on U.S. EPA's designation of areas with >15 septic units/km^2 as regions of potential groundwater contamination (U.S. EPA, 1977). The LD threshold was raised to account for improvements in septic system technology and regulation in

the last two decades. The predominant soil types in the study area are the moderately permeable Appling and Pacolet soil series (Web Soil Survey, 2016). The soils in this region are typically underlain by saprolite with saturated hydraulic conductivities (K_{sat}) in groundwater ranging from 0.7 – 62.4 cm/day (Amoozegar et al., 1993). Other watershed characteristics were determined from spatial datasets of land cover (Fry et al., 2011), septic systems (GCBC, 2013) and sewer lines (GCDPU, 2004).

Sample collection

Surface water samples from streams in the 24 selected watersheds were collected during baseflow on 9 synoptic sampling events spanning November, 2011 to July, 2014. Synoptic sampling coincided with the spring (n = 72; March 2012, April 2013 and March 2014), summer (n = 72; July 2012, 2013 and 2014) and fall (n = 72; November 2011, 2012 and 2013) seasons. Baseflow conditions were determined using antecedent precipitation and hydrograph from two USGS gage stations near the study area (USGS, 2016). Also, baseflow sampling coincided with periods of zero precipitation at least 72 hours prior to the sampling event. At each monitoring station, samples were collected in duplicate in 1-L sterile high-density polypropylene bottles. Samples were kept on ice and transported to the laboratory for analysis of FIB (usually within 6 hours of sample collection). Sample collection and analysis followed guidelines of the National Field Manual for the Collection of Water-Quality Data (USGS, variously dated). Baseflow discharge (m^3/s) was measured at each monitoring point during sampling events by our project partners from the United States Geological Survey (USGS) Georgia Water Science Center in Atlanta. The velocity-area method (Rantz, 1982) was used for discharge measurements.

Standard water quality analysis

Data for *E. coli* and enterococci concentrations and water quality parameters such as pH, temperature, dissolved oxygen and specific conductance which covers synoptic sampling from November 2011 – November 2013 were presented in Sowah et al. (2014). In this study, two additional synoptic samples (March 2014 and July 2014) were analyzed for *E. coli* and enterococci using the Colilert-18 and Enterolert kits (IDEXX Laboratories Inc., Westbrook, ME). The methods used followed similar protocols outlined in Sowah et al. (2014). Standard water quality parameters including pH, temperature, dissolved oxygen and specific conductance were also measured during sampling with a calibrated Quanta multi-parameter probe (HYDROLAB, Loveland, CO).

Bacterial and viral DNA extraction

Bacterial DNA was concentrated by filtering 100 ml of water sample through 0.40 µm polycarbonate filters (GE Whatman, Pittsburgh, PA). For virus concentration, the same volume of water was filtered through a 0.45 um HA mixed cellulose filter (EMD Millipore, Billerica, MA) pretreated with 2.0 ml of 250 mM $AlCl_3$ as suggested by Haramoto etal 2004. Filters were placed in microcentrifuge tubes and stored at -80^{o}C prior to DNA extraction. Bacterial and viral DNA were directly extracted from the filters using the PowerFecal and PowerWater DNA isolation kits (MOBIO Laboratories, Carlsbad, CA) respectively and followed manufacturer's recommendations. At least one extraction blank was processed along with each batch of synoptic surface water samples. The method blanks were extracted from sterile ultrapure water. DNA was eluted to a final volume of 50 µl and stored at -20^{o}C for use in qPCR assays.

Bacterial qPCR assays

To identify septic system influence in our watersheds, we performed qPCR assays targeting the HF183 human-associated *Bacteroidales* marker (Seurinck et al., 2005) in extracted water samples. A ruminant-associated marker BacR (Reischer et al., 2006) and total Bacteroidales marker AllBac (Layton et al., 2006) were also enumerated to identify drivers of fecal pollution in the watershed. The MST markers used in this study were selected on the basis of their widespread use and validation in different geographic regions. Method comparison studies in the United States indicate that the HF183 marker is one of the high performing markers in terms of specificity and sensitivity (Boehm et al., 2013; Layton et al., 2013; Shanks et al., 2010). Similar to the HF183 marker, the AllBac marker has seen widespread application in different locations and demonstrated high sensitivity to fecal material from a wide range of animals. Recently, the specificity of the AllBac and other generic Bacteroidales markers have been questioned due to their cross-reaction with environmental *Bacteroidales* strains (Vierheilig et al., 2012; van der Wielen and Medema, 2010). In the absence of new and more specific general *Bacteroidales* marker - the development of a new marker was outside the scope of this study, we believe the AllBac marker can provide useful information for MST analysis and furthermore the data generated in this study can be compared to previous studies that employed this marker. Finally, the BacR marker has proved effective for discriminating ruminant sources from other sources of fecal pollution as attested to by method validation studies in the U.S. (Boehm et al., 2013; Raith et al., 2013). Human and ruminant sources of fecal pollution were the focus of this study as land use information suggests that these sources were the likely contributors to total fecal pollution in streams.

These genetic markers target regions of the 16S rRNA gene of *Bacteroidales* in representative host groups. To achieve quantitation by qPCR, standards were developed for each

assay using previously described protocols (Okabe et al., 2007a; Ahmed et al., 2009). Standards were developed from *Bacteroides sp*. genomic DNA obtained from the American Type Culture Collection (ATCC) or extracted from fecal material sourced locally. Amplified DNA products were cloned into pGEM-T Easy Vectors (Promega, Madison, WI), transformed into competent *E. coli* cells (JM109 High Efficiency Competent Cells, Promega) and plated on LB agar plates containing IPTG/X-gal/Ampicillin. Recombinant plasmids were purified with a PureYield Plasmid Midiprep System (Promega, Madison, WI), quantified with NanoDrop 2000 spectrophotometer (NanoDrop Technology, Wilmington, DE) and serially diluted 10-fold to generate reaction standards. Standard curves ranged from 3×10^0 to 3×10^7 copies of plasmid DNA and were run in triplicate. The StepOnePlus Real-Time PCR System (Life Technologies, Grand Island, NY) was used for all qPCR reactions. Each qPCR reaction mixture contained 10 µl of SYBR Select Master Mix (Life Technologies, Grand Island, NY), optimized concentration of 150 nM for both forward and reverse primers and 2 ul of sample DNA in a final reaction volume of 20 µl. PCR conditions for HF183 marker consisted of hold of 10 min at 95^{o}C followed by 40 cycles of 30 s at 95^{o}C, 30 s at 53^{o}C and 1 min at 60^{o}C. The conditions for AllBac marker were 10 min at 95^{o}C followed by 40 cycles of 30 s at 95^{o}C and 45 s at 60^{o}C. Finally, PCR conditions for BacR marker consisted of hold for 10 min at 95^{o}C, 40 cycles at 95^{o}C for 15 s and 1 min at 60^{o}C. Primer sequences for each marker are detailed in Table 4.2.

Viral qPCR assay

The human adenovirus marker JTVX (Jothikuma et al.,2005) was quantified using TaqMan qPCR for confirmatory evidence of human source impact. Five sampling events (March 2012, July 2012, Nov. 2012, March 2014 and July 2014) that showed widespread distribution of the human HF183 marker were selected for adenovirus screening. This approach is in line with

suggestions by several authors for use of multiple MST markers to improve confidence in MST results (Lee et al., 2014; McQuaig et al., 2012; Ahmed et al., 2012). Adenovirus standards were developed from genomic DNA from human adenovirus strain ADV40 obtained from ATCC. Standards were generated from plasmid DNA following the same procedure outlined above. TaqMan qPCR assay was performed using the StepOnePlus Real-Time PCR System (Life Technologies, Grand Island, NY). Each 20 ul reaction mixture contained 1 x TaqMan Gene Expression Master Mix (Life Technologies, Grand Island, NY) with 0.9 uM forward and reverse primers, 150 nM of probe, and 2 ul of template DNA. PCR conditions for Adenovirus marker was slightly modified from Jothikuma et al. (2005) and consisted of hold at 95^{o}C for 10 min followed by 40 cycles at 95^{o}C for 15 s, 55^{o}C for 30 s and 72^{o}C for 30 s. Adenovirus primer and probe sequences are provided in Table 4.2.

qPCR inhibition test and lower limit of quantification (LLOQ)

The presence of humic acids and other organic substances in environmental samples have been noted to inhibit PCR (Opel et al., 2010; Field and Samadpour, 2007). A test for qPCR inhibition in water samples was performed using a previously described protocol (Ahmed et al., 2009). Briefly, 3 x 10^4 copies of the HF183 standard was spiked into undiluted (n = 18) and 10-fold diluted samples (n = 18) that tested negative for the HF183 marker and method control samples (n = 14) that were extracted from sterile ultrapure water. The cycle threshold (C_T) values of undiluted and diluted sample DNA was compared to the C_T values from method control samples using one way analysis of variance (ANOVA) to determine differences in C_T values. Significance was tested at 95% confidence level. Additionally, samples that showed a positive or negative signal for the *Bacteroidales* markers were also diluted 10 and 100-folds and run again to see if inhibition contributed to the negative signal. The LLOQ for the MST assays was

determined as the lowest concentration of the qPCR standards that was detected in all qPCR assays (Ahmed et al., 2015; Kildare et al., 2007; Okabe et al., 2007a; Reischer et al., 2007). Therefore the lowest concentration of the standards within the linear range of quantification was taken as the assay LLOQ which also represents the limit of detection in this study.

Quality control for qPCR

Melt-curve analysis was performed for SYBR Green reactions to distinguish specific PCR products from non-specific products. Melt-curve runs proceeded by increasing the temperature from 53^{o}C to 95^{o}C at 0.3^{o}C increments. The PCR products were also routinely verified on agarose gel to confirm specificity of amplified products. All qPCR runs also included positive controls (plasmid DNA) and negative controls (in triplicate) containing nuclease free water. Standard curves, which were calculated as simple linear regressions, were used to calculate amplification efficiencies (which ranged from 90 to 100% in this study) for each instrument run. Amplification efficiencies were calculated using the equation $E = 10^{\frac{1}{-s}} - 1$, where *E* is the amplification efficiency and *s* is the slope of the standard curve. In theory, 100% efficiency implies that the amount of PCR product doubles with each cycle. All standard curves generated for marker quantification in this study had a goodness of fit R^2 value above 0.98.

Data Presentation and Statistical Analysis

The data was summarized on a seasonal basis for presentation and statistical analysis. Data for spring, summer and fall represent the geometric mean of synoptic samples collected over the study period. For statistical analysis, samples that were below the limit of quantification and qPCR non-detects were replaced with values imputed using robust regression on order statistics (ROS). Regression on order methods of imputing non-detects are reported to provide better results compared to other methods such as the maximum likelihood elimination approach

or substitution with detection limits (Helsel, 2010; Wong et al., 2009; Helsel, 2005). The ROS method was used within the U.S. EPA's ProUCL Statistical tool to impute values of non-detects based on a log-normal distribution of the detected values (U.S. EPA, 2013). The copies of total and host-associated markers were expressed as marker yield in gene copies per second per square kilometer (copies s^{-1} km^{-2}) by accounting for streamflow and watershed area. Marker yield was log-transformed to achieve normality for use in statistical tests. A closer look at the watersheds in Figure 4.1 will show that nine of the watersheds (sites 3, 4, 5, 9 in LD watersheds and sites 14, 18, 19, 20, 23 in HD watersheds) were nested within larger watersheds. In order to meet independence assumption of statistical tests, we performed Durbin Watson test to check for autocorrelation of *Bacteroidales* markers in the nested watersheds (Little et al., 2008). The Durbin Watson test results showed that sites 3, 5, 14, 18 and 23 were auto-correlated with watersheds downstream and as such were excluded from statistical analysis.

Two-way analysis of variance (ANOVA) was performed to determine the effect of septic density and season on total and host-associated marker yield. The generalized linear model procedure (proc GLM) was used in SAS 9.3 (SAS Institute, Cary, NC) to examine variations in marker yield due to septic density and seasonal factors. The Tukey post-hoc multiple comparison test was used to determine main effects and simple main effects following statistically significant difference or interaction. Spearman rank correlations were performed to determine the relationships between total and host-specific marker and septic density, average distance of septic systems to streams, sewer line density, land use characteristics, FIB, and standard water quality parameters. Additionally, multivariable linear regression models were developed to determine the combination of land use and water quality variables driving human-associated *Bacteroidales* levels in suburban streams. Variable selection in the regression models was based

on the backward elimination method using the proc REG procedure in SAS. Inclusion of variables in the model depended on the variables meeting a significance threshold of $p = 0.05$ to avoid over-parameterizing the regression models, and a variance inflation factor of <10 to reduce multicollinearity of model variables (Gonzalez et al., 2012; Hathaway et al., 2010). Moreover, residuals for response and explanatory variables were plotted and checked to confirm that normality assumptions of the models were not violated. All statistical analyses were performed with SAS 9.3 (SAS Institute, Cary, NC) and statistical significance was defined at $p \leq 0.05$ unless stated otherwise.

RESULTS

Analysis of qPCR Inhibition and limit of quantification

Comparison of qPCR inhibition in water samples collected from the study area with sterile water showed no statistically significant difference in C_T values for the spiked human-specific marker (Table 4.3). Moreover, dilution of samples that tested positive and negative in qPCR tests did not result in significant changes in results following re-run of the samples. This leads us to conclude that qPCR inhibition did not significantly affect results from this study. Assay limits of quantification determined using the standards were 3 gene copies per reaction for the human marker and 30 gene copies per reaction for the total, ruminant and adenovirus markers.

Distribution of total and host-associated Bacteroidales markers

The *Bacteroidales* markers targeted in this study were widely distributed in streams impacted by LD to HD of septic systems. Figure 4.2 shows the distribution of total, human- and ruminant-associated markers grouped by season and septic density. The total *Bacteroidales* marker, which captures fecal inputs from human, bovine, canine and swine among others, was

detected in 100% (n = 216) of samples. Total *Bacteroidales* concentration ranged from 3.3 – 6.7 $\log_{10}$ copies/100 ml whilst the yield of the total marker was between 4.7 – 8.8 $\log_{10}$ copies/s.km^2. The highest and lowest concentrations of total *Bacteroidales* were recorded in fall in LD areas whereas the yield was highest in spring in LD watersheds and lowest in fall in HD areas (Figure 4.2). The human marker was quantifiable in 57% (n = 216) of the samples collected from the study area. The frequency of detection, based on the number of samples that were quantifiable, was 63% for HD watersheds and 51% for LD watersheds. The human marker varied from non-detect or below the limit of quantification to a maximum of 3.7 $\log_{10}$ copies/100 ml. The highest concentration was observed in spring in HD watersheds. Similarly, the maximum yield of human marker was observed in HD areas in the spring. The ruminant marker was quantifiable in 65% (n = 192) of surface water samples: 61% in HD watersheds compared to 68% in LD watersheds. A total of 192 samples (representing 8 synoptic sampling events) were included in the analysis of the ruminant marker due to low detection (3 out of 24 samples) of the marker in samples collected in November 2011. It is recommended that more than 50% of the samples have to be detected (ITRC, 2013). The ruminant marker varied from non-detect to maxima of 5.9 $\log_{10}$ copies/100 ml and 8.1 $\log_{10}$ copies/s.km^2 in concentration and yield respectively. The average concentration and yield of the total, human- and ruminant-associated markers in HD and LD watersheds followed a seasonal trend, with low levels of the markers observed in fall in comparison to spring and summer seasons. Statistical tests were performed with the yield of total and host-associated *Bacteriodales* markers since the yield provides a robust estimate of marker distribution, accounting for differences in hydrologic conditions and watershed area.

Results from two-way ANOVA tests show variations in the influence of septic system and season on the yield of total and host-associated markers (Table 4.4). Analysis of the effect of

septic density and season on total *Bacteroidales* yield showed no significant interaction between the factors on marker yield. Septic density, moreover, did not appear to influence total *Bacteroidales* yield in the watersheds. However, seasonal changes played a key role in the yield of total *Bacteroidales* with statistically significant differences ($p < 0.001$) between spring, summer and fall. A look at the main effect showed that total *Bacteroidales* yield was significantly higher in spring and summer compared to fall ($p < 0.001$). There was however no difference in marker yield between spring and summer. For the human-associated marker, there was no significant interaction between septic density and season. The main effect showed significance difference between levels of septic density ($p = 0.046$) and season ($p < 0.001$) for the human-specific marker. The results showed that the marker was significantly higher in HD watersheds compared to LD areas. Similar to the results for total *Bacteroidales*, the human marker yield was significantly higher in spring and summer compared to fall ($p < 0.001$), but not statistically different between spring and summer. Statistically significant interaction ($p = 0.002$) was observed between septic density and season for the ruminant-specific marker. Simple main effect analysis showed that the ruminant marker was significantly higher in LD watersheds in the spring and summer when compared to the fall season in HD watersheds.

Correlation analysis

Spearman rank correlation coefficients for total and host-associated marker yield as influenced by land use characteristics and standard water quality parameters are presented in Table 4.5. Results indicate that the human marker was negatively correlated with average distance of septic to stream ($r = -0.64$, $p = 0.003$) for samples collected in spring. Septic density and the average distance of septic to stream were not correlated with human marker yield in summer or fall. However, the pooled data showed a significant negative correlation between the

average distance of septic to stream and human marker yield (r = -0.52, p = 0.008). The relationship between septic density and ruminant marker, as expected, was negatively correlated (r = -0.76, p < 0.001) in fall. We also observed a strong positive correlation between total *Bacteroidales* and human marker yield (r = 0.65, p = 0.005) for the pooled data. However, total *Bacteroidales* was not correlated with ruminant marker in general. Sewer line density, a potential source of human fecal input into streams, showed no correlation with human marker yield for all seasons and the pooled dataset. In contrast, sewer line density was correlated with total *Bacteroidales* (r = 0.62, p = 0.006) in the summer. In general, *E. coli* and enterococci yield were positively correlated with human marker for all seasons and the pooled data. No correlations were observed between the total *Bacteroidales* marker and land use and environmental parameters in spring. Overall, the percent of agriculture land cover was a significant predictor of ruminant marker yield in this study (r = 0.57, p = 0.009).

Multivariable linear regression analysis

Multivariable regression models developed in this study were of the format $Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots \beta_n X_n$ where Y is the dependent variable, β_0 is the intercept, β_1 to β_n are parameter estimates and X represents the explanatory variables. Using the proc REG procedure in SAS, we examined the influence of land use characteristics and water quality parameters on human marker yield on a seasonal level. The results, summarized in Table 4.6, showed that septic density and average distance of septic to stream were important variables explaining variations in human marker yield in spring and fall. A strong septic system impact was detected in spring samples in line with the observed outcome from correlation analysis. Moreover, the regression model for the pooled data had septic system as the most critical factor driving human fecal pollution in the study area. The percent of impervious cover could also explain some of the

variation in human fecal pollution overall. Water quality parameters such as specific conductance and water pH were also significant explanatory variables for human fecal pollution in the study area. None of the land use characteristics and environmental variables examined in this study were predictors of human marker yield in the summer. The adjusted R^2 values for the seasonal models were 0.74, 0.31 and 0.47 in spring, fall and pooled data respectively.

Distribution of human adenovirus

Five sampling events that showed widespread distribution of human *Bacteroidales* marker were further assessed for molecular signature of human adenovirus pathogen. The detection frequency of adenovirus marker was comparable in HD and LD watersheds, with the highest detections observed in samples collected in spring. In HD watersheds approximately 85% of the samples (n = 60) were positive for human adenovirus compared to 78% (n = 60) of samples collected in LD watersheds. Approximately 56% (n = 120) of the samples analyzed were within the range of quantification of the Taqman qPCR. However, a significant number of the quantifiable samples were collected in spring season (66% of the samples) with 28% and 6% from summer and fall respectively. Of the samples that were quantifiable with qPCR, 58% were from HD watersheds and 53% from LD areas. Comparison of the average yield of adenovirus marker from HD and LD watersheds using One Way ANOVA shows that the marker yield was not significantly different ($p = 0.58$) between the watershed groups (Figure 4.3).

DISCUSSION

The overall goal of this study was to examine the influence of septic systems on fecal pollution in suburban watersheds by tracking the sources of human fecal input impacting streams of watersheds with varying septic density. In a previous study, Sowah et al. (2014) assessed septic system influence by monitoring the streams in the watersheds in this study for standard

FIB (Sowah et al., 2014). The results from the previous study which included comparison of FIB loads in HD and LD watersheds, correlation and multivariable regression analysis pointed to the contribution of septic systems to total fecal pollution in streams. Validation of the results from the previous study and similar studies implicating septic systems in water quality impairment require the use of multiple approaches as recommended in the literature (Noble et al., 2006; Boehm et al., 2003). The present study employs the widely used HF183 human-associated *Bacteroidales* marker to track the influence of septic systems on fecal pollution in streams. This marker has shown stability in different geographical locations with comparatively better specificity and sensitivity in method comparison studies here in the United States (Boehm et al., 2013; Stewart et al., 2013; Shanks et al., 2010;), Europe (Gawler et al., 2007; Gourmelon et al., 2007) and other parts of the world (Ahmed et al., 2009; Jenkins et al., 2009).

Seasonal trends in total and host-associated marker distribution in the study area compare to the observed distribution of FIB reported by Sowah et al. (2014). Overall, FIB and *Bacteroidales* marker yields were higher in samples collected in spring and summer compared to fall. The seasonal trends are also symptomatic of the underlying seasonal changes in hydrologic conditions. Table 4.7 shows the seasonal changes in climatic and hydrologic conditions in the study area. Average baseflow discharge in this study was relatively low in the fall compared to summer and spring (Table 4.7). This pattern in flow and fecal pollution indicators suggests that the sources of fecal pollution impacting the streams in the study area are both temporally and seasonally stable. This seasonal trend, in relation to human-associated *Bacteroidales*, is suggestive of a continuous source of fecal pollution such as wastewater treatment discharges, leaky sewer pipes, failing septic systems or septic effluent transported through groundwater into streams (Carroll et al., 2005). Our research, however, shows that none of the streams in this

study are impacted by permitted wastewater treatment discharges (GAEPD, 2016). Moreover, leaky sewers appear to be an insignificant source of human fecal input in the study area based on correlation and regression analysis. This leaves septic systems as the most likely continuous source of human fecal pollution in the study area.

Results of ANOVA and correlation analyses support our hypothesis that septic systems are a significant source of human fecal pollution in HD areas. The observed increase in human marker yield in streams of HD watersheds compared to LD areas, and strong correlation of human marker with average distance of septic to stream are suggestive of pronounced septic system impact in areas with septic densities above 87 septic units/km^2. The linear increase in human marker with decrease in septic distance to stream was particularly strong in spring which coincides with the seasonal shallow groundwater table in the study area. Combined with moderate to high hydraulic conductivity of saprolite in the saturated zone, the shallow groundwater can act as a conduit for the transport of effluent from the dense network of septic drainfields into nearby streams. The influence of septic systems on baseflow water quality has been the subject of recent studies including work by Peed et al. (2011) that found no correlation between septic systems and human-associated *Bacteroidales* marker under low flow conditions. This study however confirms that under baseflow conditions, the influence of septic systems depends on seasonal trends in hydrologic conditions in our study area. The marked septic system impact in spring is not surprising considering the reported interconnectivity of groundwater and surface water systems in the Southern Piedmont region (Clarke and Peck, 1991). Evaluation of the relationships between *Bacteroidales* markers showed a positive correlation between human and total *Bacteroidales* yield for the pooled data, suggesting the significant contribution of human sources to total fecal pollution in the study area. The relative contribution of the human

marker to total *Bacteroidales* yield in streams was not examined in this study due to the differential persistence of *Bacteroidales* markers in the aquatic environment, and inadequate information about the copies of *Bacteroidales* genetic markers in different host animals and humans (Tambalo et al., 2012; Dick et al., 2010; Walters and Field, 2009).

Our data shows significant difference in ruminant influence in LD and HD watersheds, with LD areas recording higher ruminant marker yield. This result was expected as stream walks of the watersheds in the summer of 2015 revealed that livestock, particularly cattle had access to the streams in watershed numbers 6, 7 and 8. The positive correlation of ruminant marker with total *Bacteroidales* in summer highlights the seasonal nature of animal impacts, especially in the LD watersheds with higher agricultural and forest cover. The prominent agricultural land uses include hay and pastures which are commonly grazed by cattle and horses. The strong negative correlation between the ruminant marker and septic density in fall was expected as increasing septic system footprint was associated with low agricultural land use. The diversity of animal hosts contributing to the ruminant signature in streams may explain the seasonal differences in the influence of land use factors such as forest cover and agricultural activities (Table 4). The pooled data in contrast, isolates agriculture as the predominant animal source of ruminant marker in the study area.

In general, poor correlations have been reported between human *Bacteroidales* markers and FIB levels in previous MST studies (Sauer et al., 2011; Edge et al., 2010; Okabe et al., 2007a). These studies have, however, been largely focused on pollution incidents arising from storm runoff. Storm induced pollution can originate from diverse sources at the watershed level which leads to confounding results. In this study we found moderate to strong positive correlations between human marker and *E. coli* and enterococci levels. The overall correlation

coefficients between the human marker and *E. coli* and enterococci were 0.71 and 0.57 respectively. It has been reported that FIB and *Bacteroidales* markers differ in their persistence profiles in the aquatic environment (Ballesté et al., 2010; Okabe et al., 2007b). The observed positive correlations between FIB and the human marker therefore suggest a continuous source (e.g. septic systems) of human fecal pollution in the study area. In addition to septic systems, forest cover was also significant in driving variations in host-associated marker yield in suburban streams. The effect of forest cover was particularly strong in the spring for both human and ruminant markers. With respect to the human marker, forest cover was negatively correlated, supporting our observation of increasing human input from areas with higher septic system density and low forest cover. Surprisingly, forest cover was negatively correlated with the ruminant marker in spring which suggests that ruminant animals that were not directly associated with forest cover may be contributing to ruminant input in the streams.

The regression models developed in this study provide further evidence of the significant contribution of septic systems to human fecal pollution at the watershed level. Combined with land use characteristics such as impervious cover and percent forest cover, the density of septic systems and average distance of septic to stream could explain a significant amount of human fecal pollution, especially in the spring. The contribution of septic systems to variations in human fecal pollution in fall and pooled data was also significant, stressing the apparent influence of septic systems on fecal pollution in suburban streams. Water quality indicators such as specific conductance and pH, which can affect microbial survival and persistent, were also important explanatory variables of human fecal pollution in the study area. However, the contribution of septic systems to human fecal pollution in summer was not obvious from correlation and multivariable statistical analysis.

Additionally, the distribution of human adenovirus marker was not sensitive to the density of septic systems based on the limited analysis. The viral marker, however, was most frequently detected and quantifiable in samples collected in spring compared to summer and fall. This finding suggests once again that human impact, in terms of fecal pollution of streams, was more elevated during the spring months in the study area. Due to the low detections and copies numbers of adenovirus marker in samples collected in summer and fall, a detailed analysis of seasonal changes of this marker was not possible. We suggest the use of larger volumes of water samples (>100 ml) for concentrating viral particles for MST studies in future to overcome low copy numbers of the marker in summer and fall.

CONCLUSIONS

The findings from this study suggest the influence of septic systems, specifically the density of septic systems and average distance of septic to streams, on fecal pollution at the watershed level in areas with >87 septic units km^{-2}. Our study also showed that septic systems were more likely to impact water resources in the study area during the spring season which is associated with shallow groundwater table and high baseflow conditions. Apparently, the density of sewer pipes in the study area did not affect the yield and distribution of human-associated *Bacteroidales* marker which makes septic systems the predominant source of human fecal pollution in the study area. This study supports the use of MST approaches together with traditional FIB monitoring and land use characterization in a tiered approach to isolate the influence of septic systems on water quality in mixed use watersheds. Future research should consider monitoring other human-associated markers and pathogens as multiple lines of evidence to elucidate septic system impacts. Finally, the findings of this study can be used by watershed managers and stakeholders to promote septic system management at the watershed level.

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TABLES AND FIGURES

Table 4.1. Average land use characteristics by watershed classification

Watershed group	HD watershed			LD watershed		
	Mean	Min	Max	Mean	Low	High
Watershed area (km^2)	2.01	0.2	8.8	3	0.6	8
Slope (%)	7.5	5.7	9	8	4.6	10.6
Septic density (units/km^2)	216	88	373	22	8	37
Sewer line density (m/km^2)	1298	0	3149	633	0	4119
Impervious cover (%)	18	12	26	6.7	3	15
Agricultural land use (%)	4	0	12	32.5	10	49
Forest cover (%)	24.8	11	44	37	14.7	50.9
Average distance of septic to streams (m)	96	55	151	128	86	172

Table 4.2. Primer and probe sequences for *Bacteroidales* and adenovirus markers analyzed in this study

Host-specific marker	Primer sequence (5' - 3')	Amplicon size (bp)	Source
Total *Bacteroidales*	F - GAGAGGAAGGTCCCCCAC R - CGCTACTTGGCTGGTTCAG	106	Layton et al., 2006
Human *Bacteroidales*	F - ATCATGAGTTCACATGTCCG R - TACCCCGCCTACTATCTAATG	82	Seurinck et al., 2005
Ruminant *Bacteroidales*	F - GCGTATCCAACCTTCCCG R - CATCCCCATCCGTTACCG	118	Reischer et al., 2006
Adenovirus marker	F - GGACGCCTCGGAGTACCTGAG R - ACIGTGGGGTTTCTGAACTTGTT FAM – TGGTGCAGTTCGCCCGTGCCA - TAMRA	96	Jothikumar et al., 2005

Table 4.3. Analysis of inhibition in extracted DNA spiked with human-specific marker

Samples	PCR cycle threshold (C_T ± margin of error at 95% confidence level)	
	Undiluted extract	10-fold diluted extracts
Sterile water	28 ± 0.6	
Stream water samples	28 ± 0.2 (p = 0.41*)	28 ± 0.2 (p = 0.35*)

* p-value represents statistical significance for comparison of CT values for diluted and undiluted stream water samples and sterile water

Table 4.4. ANOVA results showing *p*-values for human marker as impacted by septic density and season

Bacteroidales marker	Parameter	*p*-value ($\alpha = 0.05$)
Total *Bacteroidales*	Density	0.35
	Season	<0.001
	Density*Season	0.42
Human *Bacteroidales*	Density	0.046
	Season	<0.001
	Density*Season	0.76
Ruminant *Bacteroidales*	Density	0.004
	Season	0.006
	Density*Season	0.002

Table 4.5. Spearman rank correlation data for *Bacteroidales* markers as influenced by standard water quality parameters and land use characteristics

Bacteroidales Marker	Parameter	Spearman correlation coefficient (*r*)			
		Spring	Summer	Fall	Pooled Data
Total *Bacteroidales*	Percent impervious cover	-0.20	0.35	-0.19	-0.12
	Septic density	-0.05	0.41	0.02	0.1
	Percent forest cover	0.16	-0.51*	0.14	-0.01
	Percent agricultural land use	0.27	-0.39	0.11	-0.02
	Av. distance of septic to stream	-0.23	-0.25	-0.19	-0.29
	Sewer line density	-0.04	0.62*	0.13	0.36
	Dissolved oxygen	-0.07	0.44	0.02	-0.23
	pH	0.34	-0.04	0.15	0.15
	Water Temperature	-0.03	0.16	0.31	0.4
	Specific conductance	-0.30	0.51*	0.18	0.21
	E. coli	0.23	0.48*	0.63*	0.65*
	Enterococci	-0.10	0.17	0.6*	0.39
	Human *Bacteroidales*	0.25	0.31	0.77*	0.65*
	Ruminant *Bacteroidales*	0.35	0.52*	0.28	0.34
Human *Bacteroidales*	Percent impervious cover	0.27	0.07	-0.24	0.08
	Septic density	0.39	0.25	-0.11	0.29
	Percent forest cover	-0.57*	-0.02	0.24	-0.18
	Percent agricultural land use	-0.23	-0.12	0.18	-0.11
	Av. distance of septic to stream	-0.64*	-0.27	-0.23	-0.52*
	Sewer line density	-0.13	-0.07	-0.01	-0.09
	Dissolved oxygen	0.11	0.39	-0.05	0.26
	pH	0.04	-0.2	-0.14	0.04
	Water Temperature	0.54*	-0.08	0.34	0.35
	Specific conductance	0.44	0.36	0.24	0.41
	E. coli	0.4	0.79*	0.71*	0.71*
	Enterococci	0.52*	0.54*	0.63*	0.57*
	Ruminant *Bacteroidales*	0.43	0.32	0.38	0.4
Ruminant *Bacteroidales*	Percent impervious cover	0.29	-0.26	-0.75*	-0.34
	Septic density	0.37	-0.21	-0.76*	-0.33
	Percent forest cover	-0.53*	-0.03	0.54*	0.07
	Percent agricultural land use	-0.05	0.3	0.85*	0.57*
	Av. distance of septic to stream	-0.45*	0.08	0.51*	0.21
	Sewer line density	0.32	0.11	-0.41	-0.07
	Dissolved oxygen	0.11	0.23	0.09	0.18
	pH	0.12	0.14	-0.03	0.17
	Water Temperature	0.35	-0.15	0.4	-0.05
	Specific conductance	0.46*	0	-0.46*	-0.11
	E. coli	0.37	0.55*	0.54*	0.52*
	Enterococci	0.04	0.48*	0.37	0.07

* Significant at $p \leq 0.05$

Table 4.6. Output of multivariable linear regression models for human associated *Bacteroidales* marker yield for seasonal and pooled data

Season	Variable	Parameter estimate	Variance inflation factor (VIF)	*p*-value	Intercept	Adjusted R-square (R_a^2)
Spring	Impervious cover	-0.049	4.3	0.003	6.4	0.74
	Septic density	0.003	5.9	0.006		
	Forest cover	-0.029	2.5	0.0006		
	Dist. to stream[a]	-0.006	1.5	0.006		
	SC[b]	-0.01	3.5	0.018		
Summer	None[c]					
Fall	Impervious cover	-0.029	1.4	0.021	10.46	0.31
	Dist. to stream	-0.007	1.6	0.011		
	Water pH	-0.9	1.4	0.02		
Pooled data	Impervious cover	-0.025	3.2	0.025	4.2	0.47
	Septic density	0.002	1.3	0.017		
	Dist. to stream	-0.003	3.7	0.041		

[a] Average distance of septic systems to streams
[b] Specific conductance
[c] No variables met the significant threshold of $p \leq 0.05$

Table 4.7. Seasonal climatic and hydrologic conditions in the study area

Parameters	Spring	Summer	Fall
Average monthly ambient temperature (^{o}C)	16	27	11
Average monthly precipitation (cm)	11	10	10
Average baseflow during sampling (m^3/s)	0.025	0.012	0.008

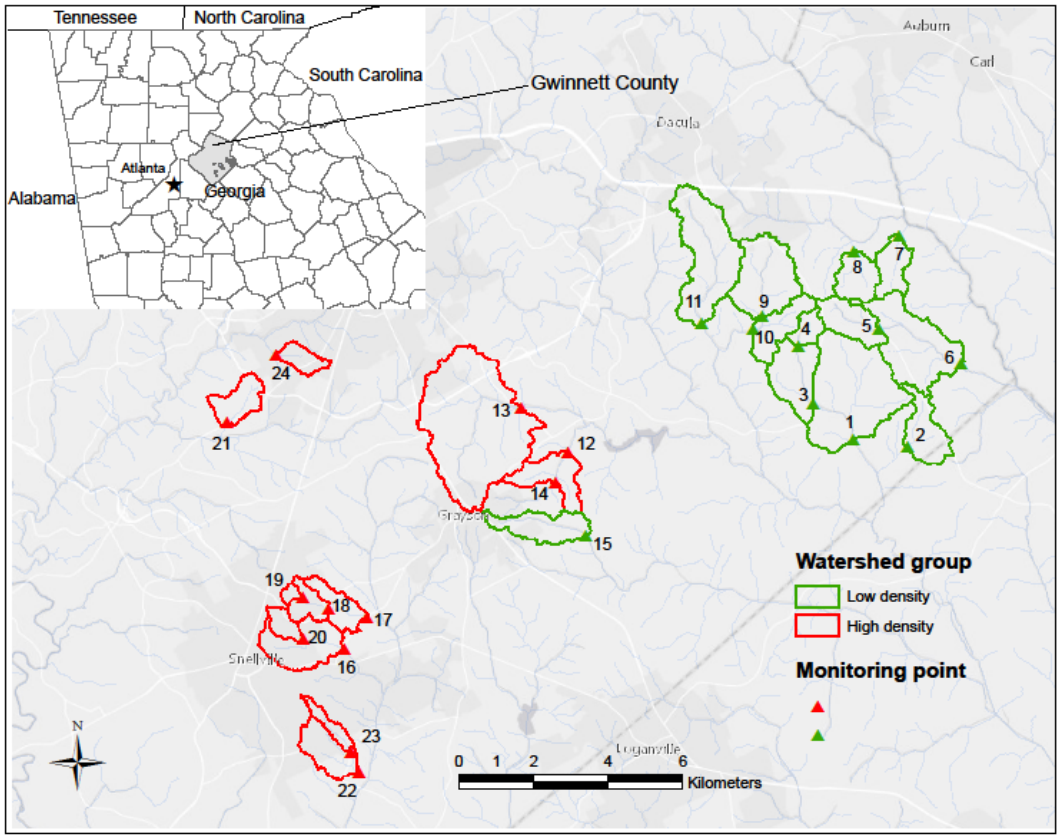

Figure 4.1. Location of the study site with boundaries and monitoring stations in Gwinnett County, GA

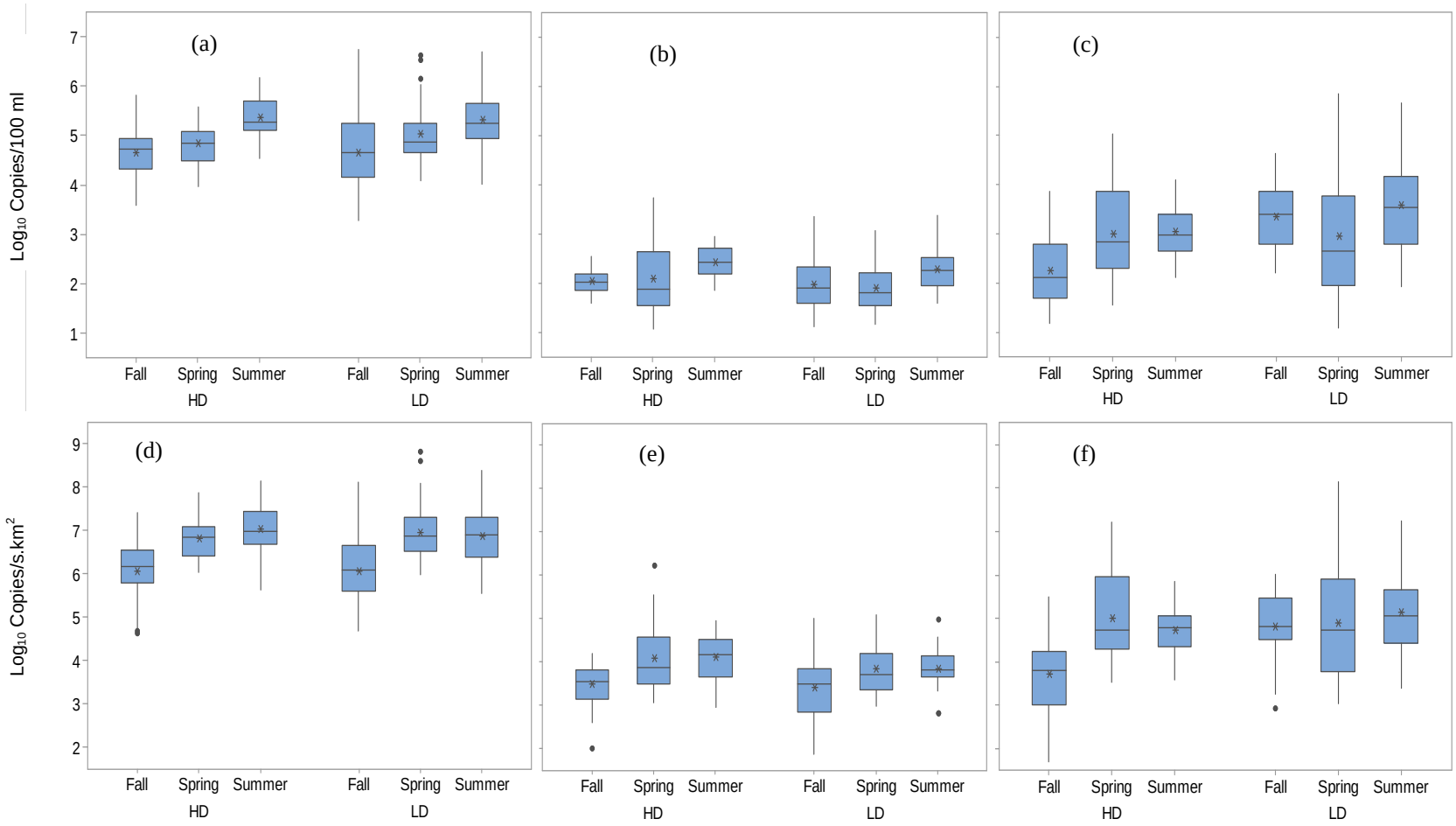

Figure 4.2. Distribution of total, human and ruminant markers grouped by septic system density and season. Figures (a), (b) and (c) shows concentrations of total, human and ruminant markers respectively, whilst (d), (e) and (f) represents the yield of total, human and ruminant markers respectively. The data includes imputed non-detect values and the (*) symbol represents the mean of the observations

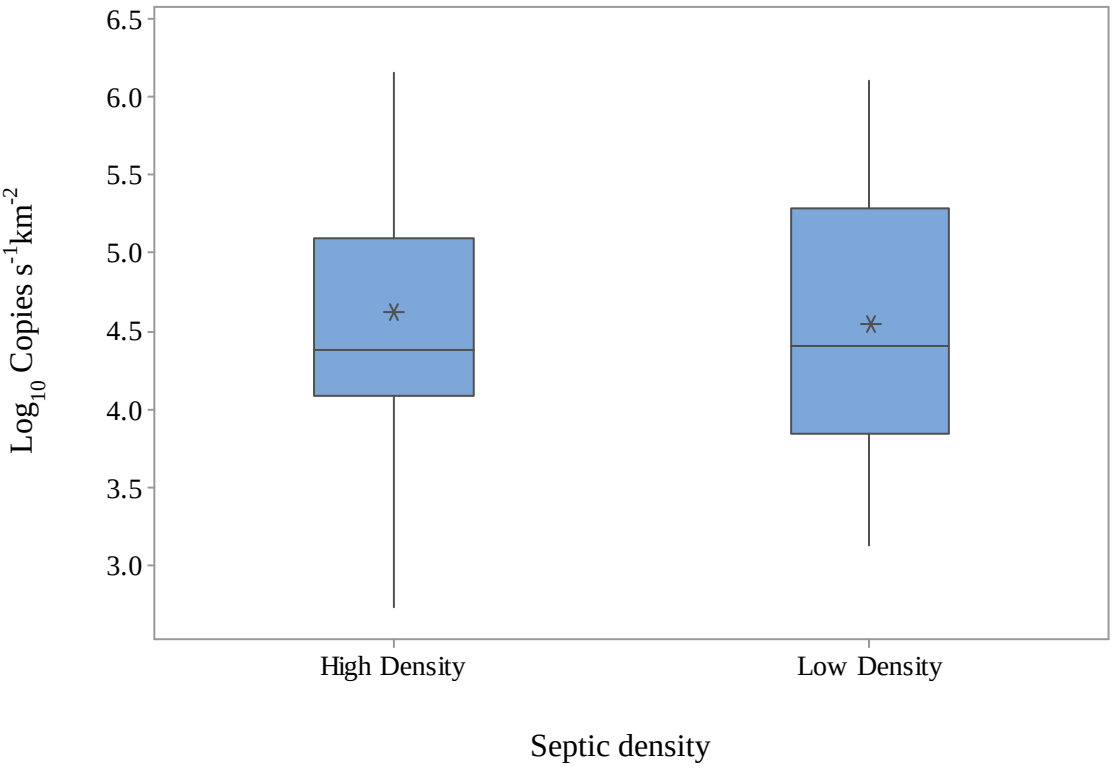

Figure 4.3. Plot of adenovirus marker distribution in high and low density watersheds for five selected sampling events. Bars represent interquartile range whilst horizontal line in bars and * symbol depicts the median and mean respectively.

CHAPTER 5

MODELING SEPTIC SYSTEM IMPACT ON MICROBIAL WATER QUALITY WITH THE SOIL AND WATER ASSESSMENT TOOL (SWAT)[3]

[3] Sowah, R. A., Radcliffe, D. E. and Habteselassie, M. Y. To be submitted to *Environmental Science and Technology*

ABSTRACT

Watershed scale models such as the soil and water assessment tool (SWAT) are promising tools for studying the impacts of septic systems on water quality and quantity. The goal of this study was to use SWAT to evaluate the impact of septic systems on bacterial loads in urbanizing watersheds. To achieve this objective, we modeled the flow regime and fecal bacterial loads in Big Haynes Creek watershed located in Gwinnett County, GA and a smaller sub-basin that is nested within the Big Haynes Creek watershed. Flow predictions in the study area shows that on average septic systems contribute approximately 7% to the total water yield annually. This observation is significant and contradicts suggestions that septic systems are 100% consumptive use. Model results also suggest that the distance of septic systems to streams in the study area can influence bacterial loads in streams. Bacterial source analysis points to septic systems contribution to microbial water quality when septic systems are less than 10 m from streams. This result suggests that the current mandated minimum distance of 15 m between septic system drainfields and streams in the state of Georgia may be adequate to protect water resources. However, the results from this study show that there are still local areas with septic systems within the minimum separation threshold which could present risk to water quality. The findings of this study provide the tools that can be used at the watershed level to assess septic system critical areas to support septic system management.

INTRODUCTION

There is a common perception that septic systems are contributing to widespread fecal pollution of streams and rivers in the U.S. (U.S. EPA, 2016). Recent studies by Verhougstraete et al. (2015) and Peed et al. (2011) have sought to address concerns about the potential impact of septic systems on microbial water quality in the U.S. Water quality assessments in the metropolitan Atlanta area by the present authors suggest the impact of septic systems on microbial water quality of streams (Sowah et al., 2014; Sowah et al., 2016). These studies by Sowah et al. monitored streams in 24 urbanizing watersheds for traditional fecal indicator bacteria (FIB) and microbial source tracking (MST) markers to characterize the influence of septic systems on microbial water quality. The findings from these studies show that FIB loads in streams can be partly explained by the density of septic systems and the average distance of septic systems to streams in the watersheds. Moreover, the human-specific *Bacteroidales* marker (HF183) was found to be significantly higher in watersheds impacted by high septic system density compared to low density areas and was negatively correlated with the average distance of septic systems to streams in the spring season. Understanding the contribution of septic systems to water quality at the watershed level will require multiple approaches including watershed scale modeling to support monitoring programs.

Watershed scale models such as HSPF (Hydrologic Simulation Program Fortran) and SWAT (Soil and Water Assessment Tool) have been used widely to evaluate the impact of land use and management on water quality in rural and urban watersheds (Baffaut and Sadeghi, 2010; Chin et al., 2009; Benham et al., 2006; Jamieson et al., 2004). The SWAT model was developed by the USDA Agricultural Research Service in the early 1990s to predict the impact of agricultural activities and management practices on water, sediment and chemical yields in

mixed-use watersheds (Arnold et al., 1998). The model has been updated over the years to include bacteria and septic system subroutines. The current version of the model (SWAT 2012), which incorporates a septic biozone algorithm, is a promising tool for evaluating septic systems' impact on water quality and quantity (Oliver et al., 2014b; McCray et al., 2009).

Although SWAT bacterial modeling has received considerable attention over the years, the contribution of septic systems to bacterial loading at the watershed level has not been adequately assessed. This has been partly attributed to limited information on the spatial distribution of septic systems at the watershed level to allow for accurate prediction of septic system impacts on bacterial loads (Benham et al., 2006; Jeong et al., 2011). Moreover, the few studies that have addressed septic impacts on bacterial loads at the watershed level using the modeling approach have mainly focused on rural/agricultural watersheds (Niazi et al., 2015; Frey et al., 2013; Coffey et al., 2010; Parajuli et al., 2009b). To the best of our knowledge no study has used SWAT to examine the impact septic systems on bacterial loading in watersheds with high septic system density.

Accurate prediction of septic system impact using watershed scale models depends largely on the availability of data on the distribution and condition of septic systems in the watershed. It has been suggested that the lack of precise information on the spatial distribution of septic systems can reduce the predictive power of watershed scale models (Jeong et al., 2011; Coffey et al., 2010). Previous studies have addressed the lack of spatial information on septic systems by assigning septic units to houses in rural settings only (Parajuli et al., 2009b; Niazi et al., 2015). This approach may overlook large areas of septic influence in suburban areas which currently have the majority of septic systems (U.S. EPA, 2002). In recent years, there has been a move toward better management of septic systems which holds promise for septic system

modeling in suburban watersheds. Other developments include the availability of water quality data required for model calibration and validation. These new developments should help reduce model uncertainties leading to improved prediction power of watershed scale models.

The traditional approach to assessing septic systems impacts on water quality has focused on failing septic systems. Three types of septic system failure are generally described (Swann, 2001). The first type of failure, which is widely reported, is hydraulic failure which results in sewage backing up into the house and ponding of septic effluent on the surface of the drainfield (also called soil absorption system). This failure can be caused by clogging of the distribution system and build-up of plaque in the biozone (biologically active layer in the soil absorption system) or installation in unsuitable soils (Jeong et al., 2011; Beal et al., 2005). Another type of failure occurs when plumes of inadequately treated effluent move through soil macropores and cracks due to poor soil conditions. This is known as subsurface failure and can result in the transport of pollutants into groundwater and nearby water bodies. Finally, treatment failure occurs when the septic system is not able to efficiently remove pathogens and nutrients from the wastewater prior to discharge into shallow groundwater. Most modeling applications usually consider failure due to hydraulic failure of the system (Jeong et al., 2011). Subsurface and treatment failures have received little attention in water quality studies due to difficulties in assessing their impact on water resources.

Typically, properly functioning septic systems are considered to be efficient in the removal of pollutants from sewage and as such their impact on water quality is not accounted for in most watershed scale models including SWAT. Properly operating septic systems have been reported to remove significant amount of contaminants from sewage including 99.9% of bacteria (Coffey et al., 2010). This treatment of properly operating septic systems may not hold true

under all conditions as several studies have demonstrated the movement of viral tracers from active septic systems through groundwater into receiving surface waters (Paul et al., 2000; Deborde et al., 1998; Scandura and Sobsey, 1997). It may also be true that above a given density of systems (number of systems per square km), properly functioning systems have a detrimental effect. Further studies are therefore needed to elucidate the fate and transport of fecal pollutants through properly operating septic systems.

In this study, we used the SWAT 2012 watershed scale model to evaluate the impact of septic systems on bacterial loads in urbanizing watersheds. The septic system subroutine developed by Jeong et al. (2011) and incorporated in SWAT 2012 was evaluated to assess model performance for flow predictions. Sensitivity of bacterial loads to failing septic systems was examined by modeling the effect of different septic failure rates at the watershed level. We also assessed model sensitivity to septic inputs applied under the following scenarios: as a land application, or as point source input discharged directly into streams as reported in previous studies (Coffey et al., 2010, Parajuli et al., 2009a, Niazi et al., 2015). Our objectives included a) determine the impact of septic systems on the flow regime in an urbanizing watershed; b) assess the effect of different septic failure rates on bacteria load and; c) examine watershed level factors and model parameters driving septic system impacts on microbial water quality. This study provides a modeling framework to address the poorly understood subject of septic system impacts on fecal pollution at the watershed scale.

MODEL SET-UP

Study Area

The study area was the Big Haynes Creek watershed located in Gwinnett County, Georgia, USA (Figure 5.1). The watershed, which has a USGS gage station at the outlet, is 44.77 km^2 in area and falls within the Altamaha River Basin in the Southern Piedmont physiographic region. Nested within the Big Haynes Creek watershed is a smaller watershed (sub-basin 7) of 2.1 km^2. This sub-basin has an automated ISCO sampler (Teledyne ISCO, USA) installed at the outlet (Figure 1). Sub-basin 7 is also one of the high density septic impacted watersheds (watershed #17) discussed in Chapters 3 and 4 of this dissertation. The predominant land use in the Big Haynes Creek watershed is residential (approximately 65% of the watershed area). Other significant land uses include forest (25%), pasture/hay (8.7%) and septic systems (0.9%) based on an assumed septic drainfield area of 100 m^2. Details of the land use distribution for Big Haynes Creek and Sub-basin 7 are presented in Table 5.1. The area has a mean annual rainfall of about 1245 mm and average slope of 7%. The population density is approximately 4403 km^{-2} according to the 2010 population census (U.S. Census Bureau, 2009). Observed flow and bacterial data were obtained from the USGS gage station at the outlet of Big Haynes Creek and from our ISCO sampler in sub-basin 7.

SWAT Model

The SWAT (ArcSWAT 2012) watershed model was used to predict flow and bacteria in the watershed. The model, which is a continuous-time, semi-distributed model, is interfaced with ArcGIS which provides capability to represent spatial data on land use, topography, climate and hydrology (Arnold et al., 2012). The SWAT model was selected for this study due to the incorporated septic system subroutine which allows the modeling of septic influence at the

watershed level. Other advantages of the SWAT model are the capability to simultaneously model persistent and less persistent bacterial populations within a watershed, and the direct simulation of bacterial die-off/regrowth with Chick's law (Chin et al., 2009). Major model components relevant to flow and bacteria include weather, hydrology, soil properties, agricultural operations, stream/pond/reservoir routing and septic systems (Gassman et al., 2007; Neitsch et al., 2011). The model works by dividing the watershed into sub-basins which are further divided into hydrologic response units (HRUs) (Coffey et al., 2010). These HRUs are unique combinations of soil, land use and slope within the sub-basins and have no spatial context. The use of HRUs allows the heterogeneity of the soil, topography and land use at the sub-basin level to be represented in the simulation (Douglas-Mankin et al., 2010).

Bacteria subroutine in SWAT

The bacterial module in SWAT covers key processes such as source loadings from livestock, wildlife, point sources and septic systems that contribute to bacterial loads in the watershed. Bacterial fate and transport processes in SWAT are represented by equations governing the movement of bacteria from land areas to the stream network and bacterial die-off and regrowth in each sub-basin reach (Neitsch et al., 2011). Bacterial movement from land areas can be in surface runoff or with sediments eroded into the stream network. The SWAT model assumes that only bacteria in the top 10 mm of the soil are available for transport with runoff. The bacteria that leaches out of the surface layer with the soil solution is assumed to be lost to the system and is not accounted for in shallow groundwater return flow into streams. The bacteria partition coefficient in manure (BACTKDDB [–]) and partition coefficient in soil (BACTKDQ [m^3 mg^{-1}]) are parameters that affect how much bacteria is transported to the reach

in any given day. Die-off and regrowth of bacteria in both soil and stream is described by Chick's first order decay equation below:

$$C_t = C_0 \, e^{-KtA^{(T - 20)}} \quad (1)$$

where C_t = concentration at time t, C_0 = initial concentration, K = decay rate (d^{-1}), t = time (days), A = temperature adjustment factor (THBACT), and T = temperature (^oC). In this study regrowth of bacteria is not modeled due to uncertainties in regrowth parameters.

The new septic system algorithm in SWAT is based on whether a septic system is active (properly functioning) or failing (altered by plaque build up to the point where hydraulic conductivity is compromised) (Jeong et al., 2011). Under active septic systems, septic effluent is treated within an active biozone layer that attenuates bacteria and other pathogens in the wastewater. Failing septic systems on the other hand have no biozone processes implemented in SWAT leading to ponding of septic effluent on the soil surface (Jeong et al., 2011). Although SWAT does not directly model bacteria and pathogens in septic effluent ponding on soil surface, it can be assumed that failing septic systems in close proximity to streams can potentially discharge effluent into nearby water bodies under the right conditions. This explains why previous studies have shown better SWAT bacteria model sensitivity when failing septic systems were treated as point source inputs (Parajuli et al., 2009a; Frey et al., 2013).

Data Acquisition

A 10 m digital elevation model (DEM) and national land cover dataset (NLCD) was obtained from the Natural Resources Conservation Service (http://datagateway.nrcs.usda.gov/). The Soil Survey Geographic Database (SSURGO) was downloaded from the SWAT website (http://swat.tamu.edu/software/arcswat/). A septic system shapefile was obtained from the Gwinnett County Geographic Information Systems database (GCBC, 2013). Climatic data for

the project was accessed from two sources: SWAT's Global Weather Database (http://globalweather.tamu.edu/) and Applied Climate Information System (ACIS) PRISM datasets (http://www.rcc-acis.org/). Daily precipitation and temperature values were obtained from 6 ACIS weather stations while information on wind speed, relative humidity and solar radiation were retrieved from 2 weather stations in the Global Weather Database.

Watershed Delineation and HRU Definition

The DEM was used to delineate the Big Haynes Creek watershed and estimate watershed characteristics such as slope, topography and flow direction. A threshold of 2% of the watershed area was used as a threshold to initiate a perennial stream to define the stream network and sub-basins in the watershed. This threshold level has been recommended as satisfactory for modeling applications in SWAT (Arabi et al., 2006; Coffey et al., 2010). The watershed was then delineated using the outlet at the USGS gage station (USGS 02207385 Big Haynes Creek at Lenora Road, Nr Snellville, GA). A total of 32 sub-basins were created with the watershed delineation tool in SWAT. An outlet was placed at the location of our ISCO sampling station so that one of the sub-basins (number 7) corresponded to the nested watershed (Figure 1). The septic system file was merged with the NLCD map to generate a single land use map following the steps outlined by Oliver et al. (2014a). The slope of the watershed was then grouped into 3 classes of 0 – 5%, 6 – 10% and >10%. The new land use map and soils data were then redefined into SWAT land use and soil categories for use in HRU definition. To create a reasonable number of HRUs, thresholds of 0%, 10% and 30% were used to define land use, soils and slope respectively. The soils threshold was less stringent in order to generate all soil groups (Hydrologic A to D soils) to allow assessment of soils prone to septic system failure. Septic land

use was exempted from the HRU definition threshold because of the small percentage of watershed area under septic land use. Overall, a total of 2600 HRU's were created.

Bacteria Source Characterization

The main sources of fecal bacteria in the watershed include manure from grazing beef cattle, confined dairy cows and wildlife, plus runoff from failing septic systems.

Livestock

The amount of manure produced by cattle in the watershed was estimated from the stock density of animals in the watershed. A total of 184 cattle were estimated for the watershed based on a stock density of 0.47 cows ha^{-1} in Gwinnett County as reported by the Department of Agriculture (USDA Census, 2002). Personal communication with Robert L. Brannen of the UGA Cooperative Extension Office in Gwinnett County also confirmed the livestock density in the watershed. The 184 cattle include 122 beef cows and 62 dairy cows based on estimates from the Agricultural Census. Manure production rates by cows were obtained from the American Society of Agricultural Engineers (ASAE) Manure Production and Characteristics database (ASAE, 2005) and the U.S.EPA's Bacterial Indicator Tool (BIT) (U.S. EPA, 2000). Grazing operations were assumed to continue year round for beef cows and between the months of April and November for dairy cows. Manure accumulation from dairy cows during the winter was applied as a fertilizer to pasture/hay land use areas once a month from April to November. Table 5.2 provides details of manure production rates and bacterial numbers in manure for livestock in the study area. The fecal coliform levels in manure were estimated in SWAT model input units of CFU g^{-1} using the average manure fecal coliform levels from the BIT. An average waste flow rate of 0.029 m^3 day^{-1} and fecal bacterial loading of 4×10^8 CFU 100 ml^{-1} were estimated as the in-stream contribution of cattle to fecal bacteria. This was based on the assumption that a third of

all cattle in the watershed have direct access to streams and therefore deposit manure directly into the streams in sub-basins with significant pasture/hay land use (U.S. EPA, 2000). Fencing cattle out of streams is not a common practice in this area. No scenario was reported for in-stream cattle manure deposition in sub-basin 7 as the number of animals did not meet the criteria for direct manure input into streams.

Wildlife

Due to inadequate data on wildlife populations in the watershed, only the effect of white-tailed deer was simulated in this study. A density of 0.14 deer per ha was used in the watershed based on average deer populations in Gwinnett County (GDNR, 2007). Average manure deposition from grazing deer in the watershed was estimated based on population density and manure production characteristics reported by Yagow et al. (2001) (Tables 5.1 and 5.2). An average fecal coliform count of 2.2×10^5 CFU g^{-1} of manure was used based on manure production rate and fecal coliform levels reported in the BIT.

Septic system loading

A total of 4,068 and 416 septic systems were located in the Big Haynes Creek watershed and sub-basin 7 respectively. Because the septic system algorithm in SWAT does not currently quantify bacterial loads from failing septic systems, effluent from failing systems was land applied to septic HRU's or treated as a point source input deposited directly into streams. For land application, septic effluent was added to septic land use areas through continuous fertilization under management operations. We assumed that 30% of septic systems were failing in the study area under the land application scenario. It is estimated that septic systems fail at rates of 5 – 40% (Swann, 2001), whilst the U.S. EPA puts it at 10 – 20% (U.S. EPA, 2002). In this study, a high failure rate of 30% was used based on previous studies that showed poor

SWAT model response to septic effluent applied to land areas (Parajuli et al., 2009b; Coffey et al., 2010). A total of 1,220 septic units were therefore considered failing, producing approximately 31 kg ha^{-1} day^{-1} of septic waste.

Under the scenario where failing septic systems were treated as a point source input, failing systems were identified as the systems that were in close proximity to the nearest stream. These systems have the potential to discharge effluent into streams due to their location. These septic systems were determined using the 'generate near table' option in the proximity analysis toolbox in ArcGIS. The rational for this approach was to allow for septic effluent to be introduced directly into the streams as a point source as suggested by previous studies (Parajuli et al., 2009a; Frey et al., 2013). According to the previous authors, SWAT bacteria model results are most sensitive to septic effluent introduced directly into streams. Four proximity thresholds (<5 m, <10 m, <20m and <30 m from the nearest stream) were used to assess the effect of different failure rates on bacterial loads. A total of 5, 8 and 18 septic units were considered failing and discharging directly into streams when the thresholds were <5 m, <10 m and 20 m respectively at the watershed level. At the sub-basin level, 1, 2 and 3 septic systems were treated as failing at thresholds of <10 m, <20 m and <30 m respectively. Failing septic systems contributed 0.32 m^3 of effluent per day per septic system and fecal coliform concentration of 6.3 x 10^6 CFU 100 ml^{-1} (Parajuli et al., 2009a). Properly functioning septic systems, on the other hand, were simulated using the new septic algorithm to estimate the contribution of septic to daily flow regime. For flow simulation, default septic system parameters were used with the exception of the following parameters: SEP_CAP (number of permanent residents in each house) of 2.8, SEP_STRM_DIST (average distance to the stream from the septic systems) of 0.63 and SEP_DEN (number of septic systems per square kilometer) ranging from 2 – 232 units km^{-2}.

Sensitivity analysis and calibration of SWAT model

The SWAT-Cup SUFI-2 calibration and uncertainty analysis program (Abbaspour, 2007) was used to calibrate and validate the SWAT model. The SUFI-2 algorithm captures uncertainties in model output by propagating uncertainties in parameters. The assumption is that uncertainty in parameters, which are expressed in the parameter ranges, accounts for all sources of uncertainty including uncertainty in variables such as rainfall, conceptual model and measured data (Abbaspour, 2007). The SWAT model was first calibrated for flow using 22 flow parameters identified from the literature and using expert judgment (Table 5.3). During the calibration process, global sensitivity analysis was performed to determine sensitive parameters to focus calibration efforts. The flow model for Big Haynes Creek was calibrated for the period 1998 – 2007 and validated for the period 2008 – 2013 with a warm-up of 4 years. The model was then calibrated and validated for bacteria by adding bacteria parameters to the calibrated flow parameters and running the different source scenarios (Tables 5.4 and 5.5). Due to uncertainty of bacterial source loads, different source scenarios were run in this study to assess the potential contribution of each source to total fecal pollution. The bacteria source analysis was also geared toward evaluating the potential pathways of septic systems' contribution to fecal pollution of streams observed in the MST analysis in the study area. Other studies in the literature have used a similar approach to estimate bacterial sources and analyze the impact of different source scenarios on bacterial output at the watershed level (Coffey et al., 2010; Parajuli et al., 2009b).

The bacteria sub-model was calibrated and validated in years 2003 and 2004 respectively for fecal coliform concentration (CFU 100 ml^{-1}) at the Big Haynes Creek outlet. At the sub-basin level observed flow, *Escherichia coli* (*E. coli*) and enterococci counts collected between 2012 and 2013 were compared to model results. *E. coli* and enterococci were treated as less persistent

and persistent bacteria respectively. This is due to the high concentrations of enterococci observed in streams as compared to *E. coli*. Bacteria parameter values for the default bacterial simulation were obtained from previous SWAT modeling studies (Coffey et al., 2010; Cho et al., 2012; Kim et al., 2010; Parajuli et al., 2009a, b). Fifteen bacteria and sediment parameters were evaluated for sensitivity to bacterial concentrations in the watersheds.

Model evaluation

Model performance was evaluated using the recommendations from Moriasi et al. (2015). A combination of Nash-Sutcliffe efficiency (NSE) coefficient, coefficient of determination (R2) and percent bias (PBIAS) were used to assess model performance. The NSE index indicates how well the plot of observed versus simulated data fits a 1:1 line. Values of NSE ranges between $-\infty$ and 1.0 with NSE = 1 indicative of a perfect fit between predicted and measured values (Moriasi et al. 2007). The equation for estimating NSE is shown below;

$$NSE = 1 - \left[\frac{\sum_{i=1}^{n}\left(Y_i^{obs} - Y_i^{sim}\right)^2}{\sum_{i=1}^{n}\left(Y_i^{obs} - Y^{mean}\right)^2}\right] \quad (2)$$

The R^2 statistic describes the proportion of the measured data variance explained by the model and ranges from 0 to 1 with 1 indicating perfect model fit (Moriasi et al., 2007). Another important performance metric is the PBIAS which measures the tendency of the simulated data to be larger or smaller than the observed values. The NSE, R^2 and PBIAS are widely used in hydrology and water quality modeling with extensive information on reported values for comparison (Moriasi et al., 2007). According to Moriasi et al. (2015), model predictions are considered satisfactory when daily NSE > 0.50, R^2 > 0.60 and PBIAS ≤ ±15% for flow predictions. Similar performance criteria for bacterial modeling have not been described and as

such model accuracy for bacterial predictions in this study was assessed relative to previous studies. An additional model efficiency metric, *p-factor*, was evaluated in this study to estimate uncertainty in model outputs linked to model inputs or structural uncertainty (Abbaspour, 2007). The *p-factor* which varies from 0 to 1, measures the percentage of the observed data that is bracketed by the 95 percent prediction uncertainty (95PPU) of the model predictions resulting from propagating the uncertainties in the selected parameters (Abbaspour et al., 2015). A *p-factor* of > 0.70 was recommended by Abbaspour et al. (2015).

RESULTS AND DISCUSSION

Sensitivity analysis and calibration of flow parameters

The sensitivity analysis results, presented in Table 5.3, showed that parameters such as transmission losses from the main channel (TRNSRCH), effective hydraulic conductivity in main channel (CH_K2), and Manning's "n" value for the main channel (CH_N2) were the dominant processes controlling the flow regime at the watershed scale. This indicates that in-stream processes play a significant role in the flow regime in this watershed. Other flow parameters that were significant included the curve number for moisture condition 2 (CN2), base-flow alpha factor for bank storage (ALPHA_BNK) and the threshold depth of water in the shallow aquifer required for return flow to occur (GWQMN). At the finer scale of Sub-basin 7, flow was influenced by soil parameters such as saturated hydraulic conductivity (SOL_K), available water capacity of the soil layer (SOL_AWC) and moist bulk density (SOL_BD) in addition to parameters affecting flow in the main channel.

In general, flow predictions at the outlet of Big Haynes Creek watershed was satisfactory based on performance criteria recommended by Moriasi et al. (2015). In this study, flow calibration on a daily time-step at the outlet of Big Haynes Creek resulted in NSE, R^2 and PBIAS

of 0.67, 0.68 and 5.9% respectively (Figure 5.2). Model performance was slightly better during the validation period with NSE, R^2 and PBIAS of 0.70, 0.70 and 8.6% (Figure 5.3). The *p-factor* for both calibration and validation time intervals were > 0.70 (Figures 5.2 and 5.3). The flow model performance exceeded results reported by Oliver et al (2014a) who modeled flow in the same watershed using shorter calibration and validation time intervals. At the sub-basin level, the flow model prediction accuracy was lower with NSE and R^2 values of 0.21 and 0.30, and -0.02 and 0.02 for calibration and validation timeframes respectively (Figure 5.4). However, estimates of PBIAS were within the acceptable performance ranges with 6.8% and 3.7% observed for calibration and validation periods respectively. Overall, flow predictions were unsatisfactory at the sub-basin level and this could be attributed to measurement errors in observed data from stage-discharge estimation at the sub-basin outlet. The observed flow was often very low and there were problems with sediment and trash filling in around the water level gauge. This may have caused the step-up and step-down in some parts of the time series in Figure 5.4 in the later part of 2012 and near the end of 2013. Also, poor model accuracy at the sub-basin level may be due to the fact that the NSE statistic is insensitive to low flow periods due to the squared error term in equation (2) (Krause et al., 2005). The calibrated flow model predicted a 7% increase in total water yield as a direct result of the input from septic systems in the Big Haynes Creek watershed. At the sub-basin level, septic systems' contribution to water yield was approximately 13%. The observed contribution of septic systems to water yield in this study compares with previous research in the study area (Oliver et al., 2014a; Landers and Ankcorn, 2008).

Scenario analysis, sensitivity and calibration of bacteria model

Seven bacterial source scenarios were examined to determine the sources of fecal bacteria impacting surface waters (Table 5.5). From the scenario analysis, point source loading of effluent

from failing septic systems in the watersheds was found to best capture variations in bacterial concentrations at the watershed and sub-basin outlets. Typically, point source loads from failing septic systems improved model predictions for both calibration and validation periods. At the watershed level, all three failure rates representing point source loading of septic effluent improved model performance compared to other source scenarios considered in this study. Results of point source loading of septic systems showed NSE values of 0.10 to 0.15 and -0.13 and -0.07 for calibration and validation periods respectively. The scenarios representing point source loading from septic systems also showed the lowest values for PBIAS considering both calibration and validation periods (7.1 to 19.7) and the highest *p-factor* values (0.63 to 0.91) at the watershed level. Estimates of PBIAS close to zero indicate less model bias, with positive values suggesting model under predication bias. The model performance under the failing septic system scenarios was comparable to SWAT bacteria model results reported in the literature (Niazi et al., 2015; Frey et al., 2013). The negative values indicate a poor model fit resulting from the inability of SWAT to simulate some part of the bacterial fate and transport process in the watersheds. One important pathway for bacterial transport to streams that is not currently modeled by SWAT, and which may be contributing to fecal bacterial loads in streams, is the movement of septic effluent through the shallow groundwater. Bacterial source tracking data from the study area suggest the potential for groundwater-associated bacterial transport into streams from the high density of septic systems in the study area and the proximity of these systems to streams (Chapter 4 of Dissertation). Unsatisfactory results for bacteria models can also be attributed to uncertainties in bacterial sources and the paucity of measured data for model calibration (Parajuli et al., 2009b; Coffey et al., 2010).

Sensitivity analysis looked at the effect of changes in model parameters such as BACTKDQ (bacteria partition coefficient in surface runoff) and THBACT (temperature adjustment factor for bacteria die-off/growth) among others (Table 5.4) on bacterial concentrations at the watershed and sub-basin levels. We also examined the impact of input parameters including MANURE_KG (dry weight of manure deposited daily) and FRT_KG (amount of fertilizer applied to HRU). These input parameters directly influence the amount of manure deposited by livestock and wildlife and therefore the amount of fecal bacteria available for runoff into streams. Our results for all source scenarios showed that the model did not respond significantly to changes in the amount of manure deposited in pastures and forest areas by cattle and deer respectively. The results however showed that THBACT and BACTMX (bacteria percolation coefficient) were the most significant bacteria parameters at the watershed level. The most significant parameters at the sub-basin level include THBACT and WDPRCH (die-off factor for less persistent bacteria in streams at 20°C). The fact that in-stream bacterial die-off factors THBACT and WDPRCH were the most significant model factors is not surprising considering that the potential sources of fecal bacteria in the watersheds were found to be direct deposition into streams. Similar studies in the literature found THBACT to be one of the most sensitive parameters that affect bacterial fate and transport at the watershed level (Parajuli et al., 2009a; Niazi et al., 2015; Coffey et al., 2010).

Bacterial source estimation and model predictions

The contribution of animal and human sources to total fecal pollution at the watershed level was estimated for the <10 m failing septic scenario that best represented watershed conditions. Bacteria source contributions were estimated as the ratio of the predicted total fecal coliform load from each source to the predicted total fecal coliform load for the simulation

period. Bacteria from livestock and wildlife manure deposited on land represented a small fraction of the bacterial load observed in streams. We estimated a source load of 1% attributable to livestock and wildlife manure deposited on land with the remaining 99% from direct deposition into streams from failing septic systems in close proximity to streams. It has to be noted that even though the model fit under the direct cattle manure deposition into streams was poor, we cannot discount direct deposition from animals in this watershed due to uncertainties in the number of livestock and wildlife with access to streams. Moreover, streams walks in the study area showed that livestock, especially cattle, had access to streams in the study area (Hoghooghi et al., 2016).

In general, model predictions of bacterial concentrations under the best source scenario followed a similar pattern to the observed bacterial concentrations at the outlet of Big Haynes Creek watershed (Figure 5.5). Predicted bacterial concentrations ranged from 35 – 330 CFU 100 ml^{-1} at the outlet of the watershed and were within the range of observed values. On average, the model under predicted bacterial concentrations by an average of 12%. In contrast, model predictions of bacterial concentrations in Sub-basin 7 were significantly lower by an average of 36% over the calibration and validation periods for the best source scenario (failing septic systems <20 m from streams). Another significant observation is the higher bacterial concentrations at the Sub-basin outlet in comparison to observed values at the outlet of Big Haynes Creek watershed. This is due to the larger number of storm flow samples analyzed at the sub-basin outlet. Runoff during storms can carry fecal bacteria from the land surface into streams (Gonzalez et al., 2012; Baffault and Sadeghi, 2010; Benham et al., 2006) The plot (Figure 5.6) of predicted and observed bacterial concentrations at the sub-basin outlet shows wide variations in observed values compared to the predicted bacterial concentrations. The variation in observed

values suggests that source loadings may vary over time and may also be indicative of the presence of multiple sources impacting bacterial numbers. Our results suggest that at the finer sub-basin scale accurate predictions of bacterial loads will require detailed information on source loadings in order to capture temporal changes in bacterial concentrations.

CONCLUSIONS

This study demonstrated the influence of septic systems on watershed hydrology and microbial water quality. Model simulation of the flow regime in the study area showed that on average septic systems contributed between 7 – 13% of the total water yield. This observation is significant and challenges suggestions that septic systems are 100% consumptive use. Model results also suggest that runoff of fecal bacteria into streams from failing septic systems in close proximity to streams is a significant source of bacterial loads. Analysis of different bacterial source scenarios in the watershed points to the influence of septic systems on microbial water quality when septic systems are <10 m from streams and other water bodies. This result suggests that the current minimum required distance of 15 m from septic drainfields to streams mandated in the state of Georgia may be adequate to protect water resources. However, the results also suggest that there are still a number of septic systems that were installed prior to the current regulatory threshold which could present risks to water quality. Bacteria simulations in this study also highlight the current limitation of the septic system sub-routine in the SWAT model to accurately represent all fate and transport processes pertinent to bacteria in septic effluent. It is our recommendation that future updates to SWAT should include, among others, processes or sub-routines to estimate bacterial output from failing septic systems through effluent ponding on the soil surface and effluent that enters shallow groundwater. This will enable a direct assessment of the effect of septic system condition, that is, whether failing or properly

functioning, on microbial water quality of nearby streams and other water bodies. Finally, the findings of this study provide the tools that can be used at the watershed level to assess septic system critical areas to support septic system management.

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TABLES AND FIGURES

Table 5.1. Land use percentages in Big Haynes Creek and Sub-basin

	Big Haynes Creek		Sub-basin 7	
Land use	Area (ha)	%	Area (ha)	%
Residential	2900.8	64.8	149.2	70.9
Forest	1135.4	25.4	41.9	19.9
Pasture/Hay	389.7	8.7	15.3	7.3
Septic	40.6	0.9	4.2	2.0
Water	11.4	0.3	0.0	0.0

Table 5.2. Manure application rates for animals and failing septic systems

Source	Type of application	Manure input	Fecal coliform load (CFU/g)
Cattle	Grazing (kg dry wt. ha^{-1} day^{-1})	1.74	3.2×10^6
	Fertilizer application (kg ha^{-1})	129	3.2×10^6
Deer	Grazing (kg dry wt. ha^{-1} day^{-1})	0.03	2.2×10^5
Failing septic systems	Continuous fertilization (kg ha^{-1} day^{-1})	31	1.0×10^5

Table 5.3. Sensitivity analysis and calibrated hydrologic parameters

				Big Haynes Creek			Sub-basin 7			
Parameter	Min	Max	Criteria[a]	Fitted value	S[b] (p-value)	Rank	Fitted value	S (p-value)	Rank	Definition
CN2	-0.3	0.2	r	-0.11	0.03	7	-0.28	0.00	3	Curve number for moisture condition 2
ALPHA_BNK	0	1	v	0.46	0.01	5	0.01	0.00	8	Baseflow alpha factor for bank storage
CH_K2	-0	500	v	1.29	0.00	2	71.2	0.00	1	Effective hydraulic conductivity in main channel alluvium
CH_N2	-0	0.3	v	0.20	0.00	3	0.04	0.00	5	Manning's "n" value for the main channel
GW_REVAP	0.02	0.2	v	0.02	0.66	15	0.02	0.18	13	Groundwater "revap" coefficient
RCHRG_DP	0	1	v	0.63	0.34	12	0.7	0.24	14	Deep aquifer percolation fraction
GWQMN	-0.5	2	r	-0.06	0.00	4	0.2	0.31	16	Threshold depth of water in the shallow aquifer required for return flow to occur
GW_DELAY	-0.5	4	r	2.8	0.79	17	0.84	0.10	11	Groundwater delay
DEEPST	-0.8	2	r	-0.11	0.45	13	0.8	0.47	19	Initial depth of water in the deep aquifer
SHALLST	-0.8	2	r	-0.4	0.15	10	0.36	0.11	12	Initial depth of water in the shallow aquifer
ESCO	0	1	v	0.86	0.89	19	0.61	0.3	15	Soil evaporation compensation factor
EPCO	0	1	v	0.53	0.73	16	0.52	0.54	20	Plant uptake compensation factor
SURLAG	1	24	v	16.3	0.07	8	15.5	0.43	18	Surface runoff lag time
TRNSRCH	0	1	v	0.26	0.00	1	0.02	0.00	4	Fraction of transmission losses from main channel that enter deep aquifer
CH_K1	0	300	v	16.7	0.97	22	267	0.36	17	Effective hydraulic conductivity in tributary channel alluvium
CH_S1	-0.5	10	r	7.38	0.91	20	4.95	0.68	21	Average slope of tributary channels
OV_N	-0.5	10	r	15.2	0.82	18	3.85	0.00	7	Manning's "n" value for overland flow
HRU_SLP	-0.5	0.8	r	0.07	0.22	11	-0.18	0.00	6	Average slope steepness
SOL_K	-0.8	0.8	r	0.73	0.03	6	0.65	0.00	2	Saturated hydraulic conductivity
SOL_AWC	-0.5	1	r	0.03	0.09	9	-0.38	0.03	10	Available water capacity of the soil layer
SOL_BD	-0.2	0.5	r	0.01	0.56	14	0.04	0.03	9	Moist bulk density
RES_RR	-0.2	2	r	1.52	0.94	21	-0.07	0.70	22	Reservoir average daily principal spillway release rate

[a] Modification criteria used [b] Global sensitivity measure

Table 5.4. Results of sensitivity analysis and calibration of bacteria parameters for best case source scenarios (<10 septic: Big Haynes, <20 septic: Sub-basin 7)

				Big Haynes Creek			Sub-basin 7			
Parameter	Min	Max	Criteria	Fitted value	S (p-value)	Rank	Fitted value	S (p-value)	Rank	Definition
BACT_SWF	0	1	v	0.77	0.74	13	0.91	0.05	5	Fraction of manure applied to land areas that has active colony forming units
BACTKDQ	100	250	v	204	0.64	12	159	0.01	3	Bacteria soil partition coefficient.
BACTMX	8	19	v	15.5	0.07	2	13.9	0.23	7	Bacteria percolation coefficient in manure
WOF_LP	0	1	v	0.85	0.81	14	0.52	0.36	9	Wash-off fraction for less persistent bacteria
THBACT	0.5	8	v	4.38	0.017	1	0.94	0.00	1	Temperature adjustment factor for bacteria die-off/growth.
WDLPS	0	1	v	0.97	0.43	9	0.02	0.18	6	Die-off factor for less persistent bacteria adsorbed to soil particles.
WDLPRCH	0.5	1	v	0.83	0.18	7	0.94	0.00	2	Die-off factor for less persistent bacteria in streams (moving water) at 20°C
BACTKDDB	0	1	v	0.9	0.2	8	0.88	0.02	4	Bacteria partition coefficient.
BIO_MIN	-0.2	2	r	0.3	0.11	4	-0.01	0.91	14	Minimum plant biomass for grazing
BIO_INIT	-0.2	2	r	1.54	0.54	10	0.12	0.34	8	Initial dry weight biomass (kg/ha)
FRT_KG	-0.5	2	r	1.61	0.12	5	1.5	0.42	10	Amount of fertilizer applied to HRU
MANURE_KG	-0.5	2	r	-0.12	0.92	15	0.90	0.93	15	Dry weight of manure deposited daily
SPEXP	1	1.5	v	1.25	0.16	6	1.10	0.70	12	Exponent parameter for calculating sediment reentrained in channel sediment routing.
ADJ_PKR	0.6	1.8	v	1.52	0.58	11	0.69	0.52	11	Peak rate adjustment factor for sediment routing in the sub-basin (tributary channels)
SPCON	0.0001	0.01	v	0.01	0.1	3	0.00	0.71	13	Linear parameter for calculating the maximum amount of sediment that can be reentrained during channel sediment routing.

Table 5.5. Model performance statistics for bacteria in Big Haynes Creek and Sub-basin 7

		NSE		PBIAS		*p-factor*	
Watershed	Scenario	Calibration	Validation	Calibration	Validation	Calibration	Validation
Big Haynes Creek	Manure application to land from cattle and wildlife (Baseline)	-1.3	-1.16	100	100	0	0
	Land application of septic effluent	-1.26	-1.13	99.4	99.6	0	0
	Septic as point source (<5 m)	0.10	-0.07	30	31.6	0.91	0.75
	Septic point source (<10 m)	0.13	-0.09	16.8	7.1	0.91	0.75
	Septic as point source (<20 m)	0.15	-0.13	8.9	19.7	0.91	0.63
	Instream cattle manure deposition	0.05	-23.27	-4.4	-218	0.55	0.50
	Instream cattle manure deposition plus septic as point source (<5 m)	-119	-2382	-645	-2982	0.27	0.38
Sub-basin 7	Manure application to land from cattle and wildlife (Baseline)	-0.06	-0.74	92.8	100	0.08	0
	Land application of septic effluent	-0.04	-0.74	92.1	99.9	0	0
	Septic point source (<10 m)	-0.25	-0.53	37.6	63	0.38	0.17
	Septic as point source (<20 m)	-0.55	-0.42	19.4	53.4	0.46	0.33
	Septic as point source (<30 m)	-1.05	-0.42	20.9	39.6	0.46	0.5

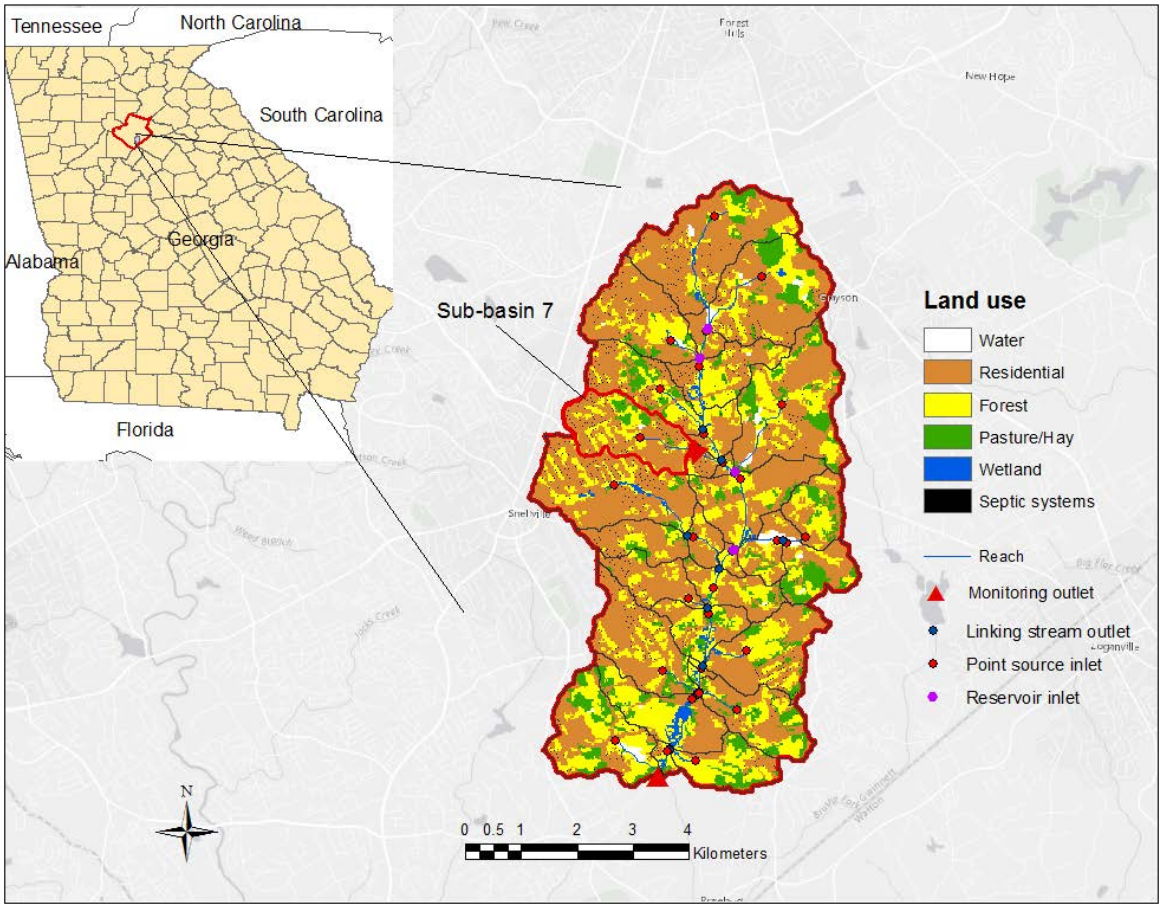

Figure 5.1. Map of study area showing watershed and sub-basin modeled in this study

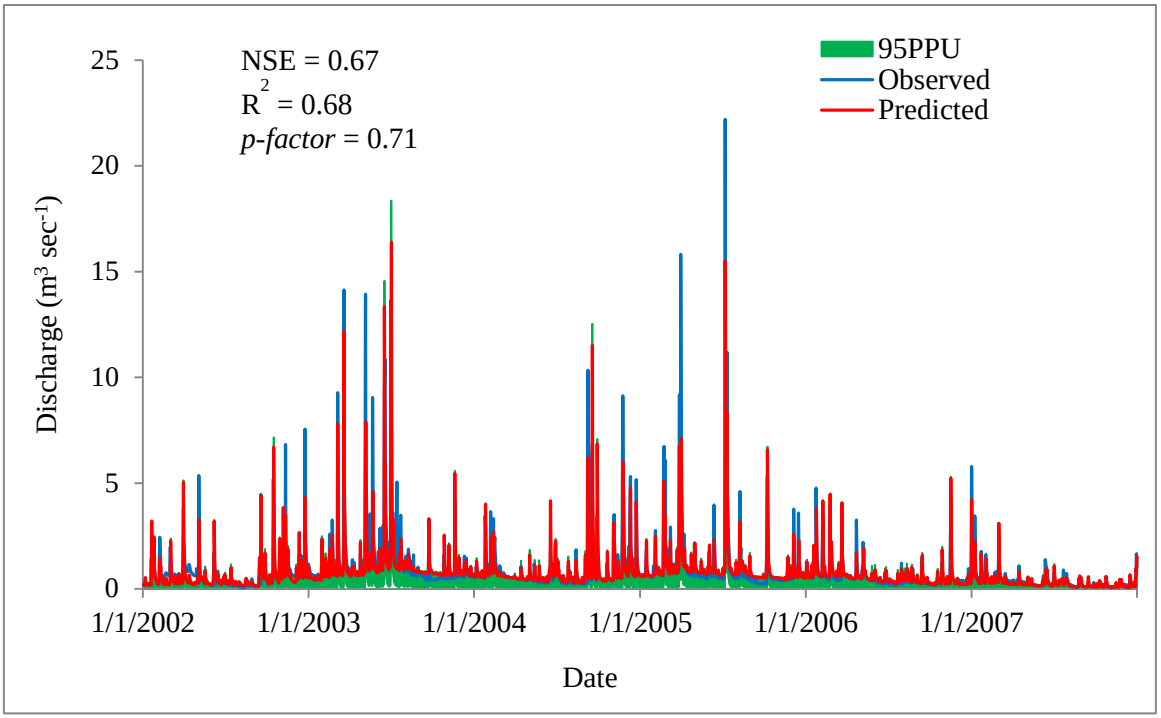

Figure 5.2. Calibrated flow at the outlet of Big Haynes Creek watershed with observed and predicted flow as well as the 95 percent prediction uncertainty band

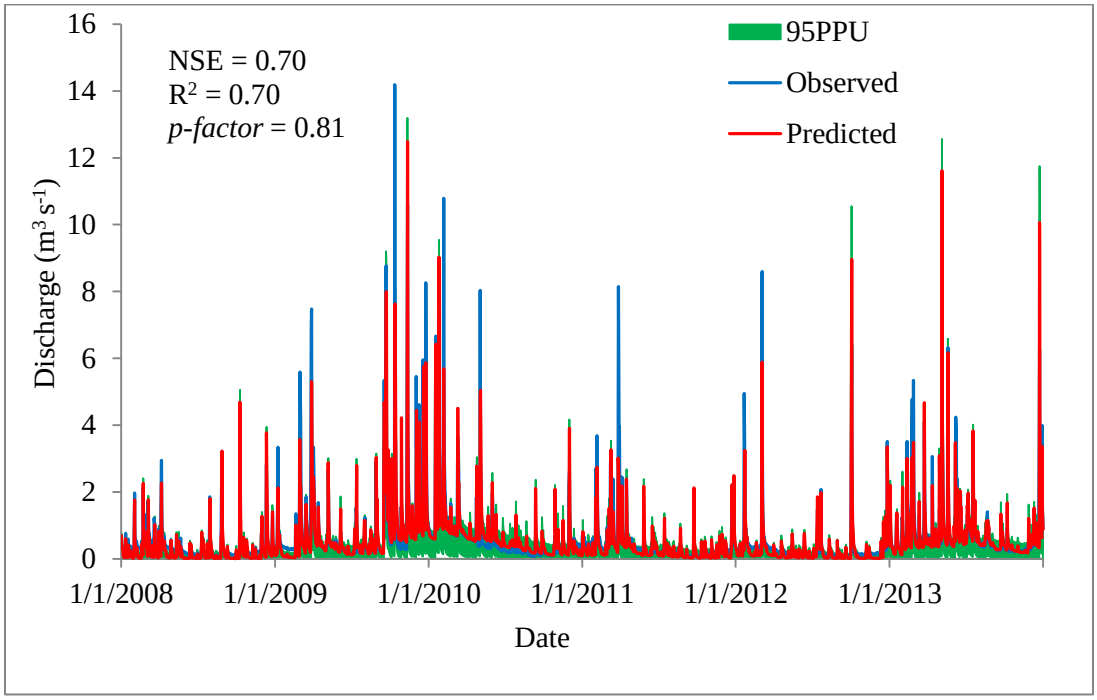

Figure 5.3. Observed and predicted flow at the outlet of Big Haynes Creek watershed for the validation period

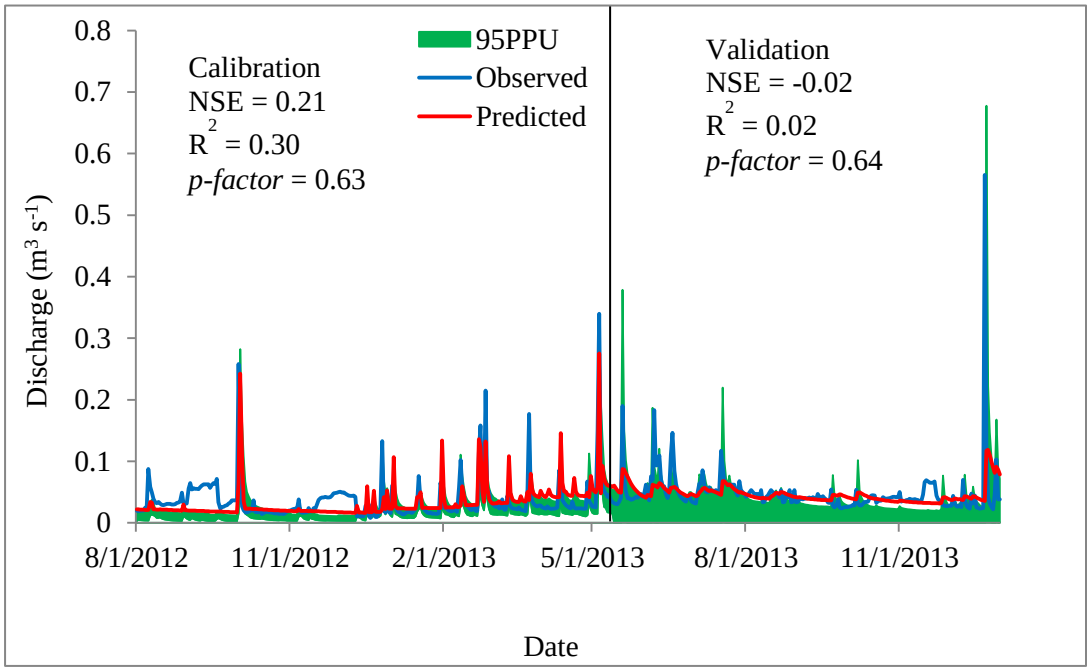

Figure 5.4. Observed and predicted flow at the outlet of sub-basin 7 for the calibration and validation periods

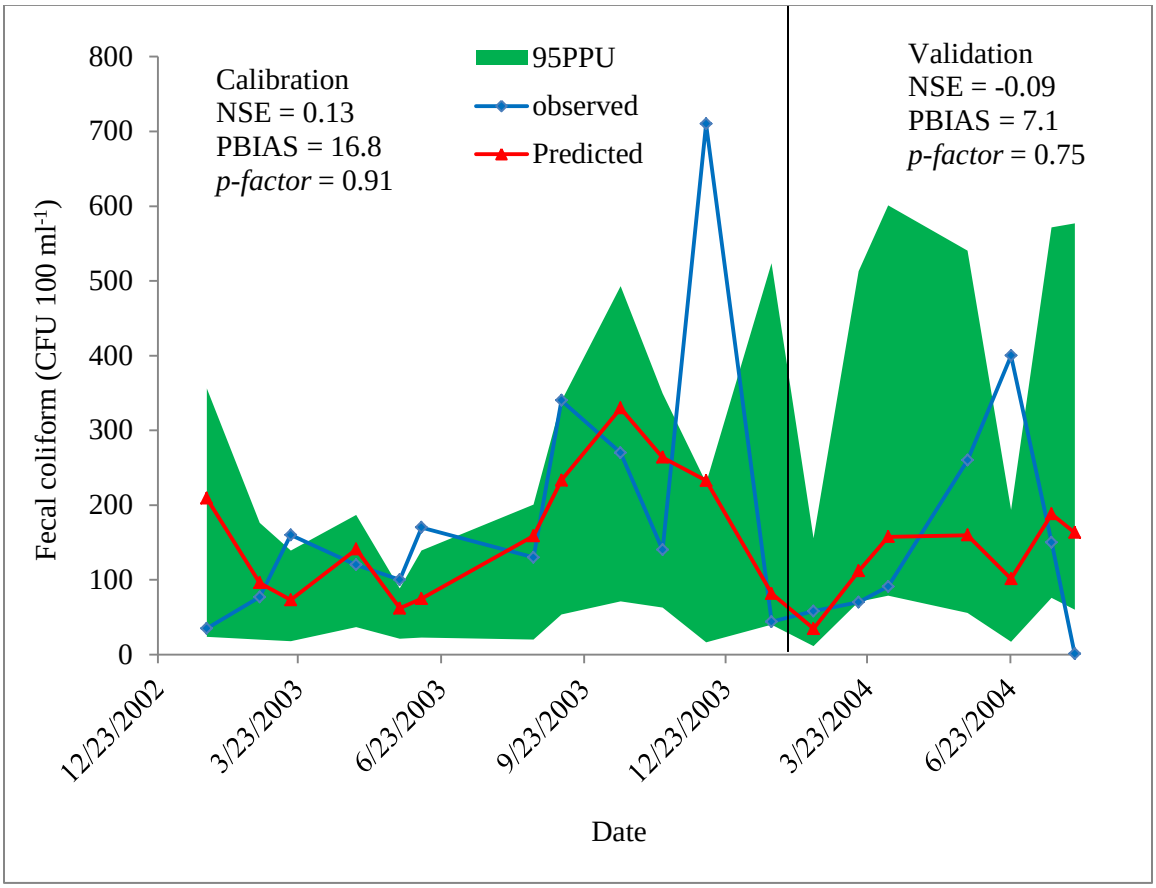

Figure 5.5. Observed and predicted bacterial concentration at the outlet of Big Haynes Creek watershed for the calibration and validation periods

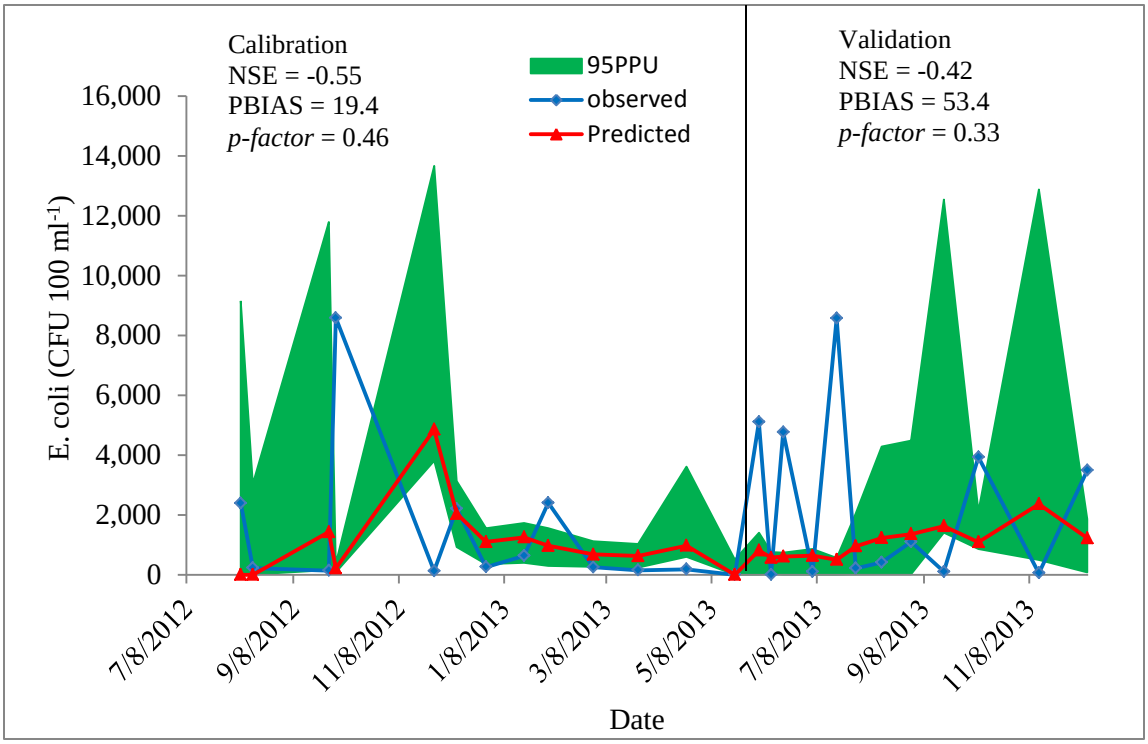

Figure 5.6. Bacterial concentration at the outlet of sub-basin 7 for calibration and validation periods when proximity of septic system to streams is <20 m

CHAPTER 6

SUMMARY AND CONCLUSIONS

This study used multiple approaches including targeted monitoring of FIB, MST markers, watershed characterization and watershed scale modeling to assess the impact of septic systems on microbial water quality in urbanizing watersheds of metropolitan Atlanta. The results suggest widespread fecal pollution of streams in the study area with up to 90% of water samples collected from streams exceeding EPA's single sample recreational water quality criteria for FIB. Analysis of FIB loads indicates seasonal and temporal variations in bacteria levels with significantly higher bacteria loads observed in spring and summer compared to fall. These changes in FIB levels were influenced by watershed level characteristics including the density of septic systems, average distance of septic to streams and forest cover as determined from correlation and multivariable regression analysis. Regression models indicate that ~50% of the variation in FIB levels can be explained by septic system density, average distance of septic systems to streams, per cent developed area, forest cover and water temperature in spring and summer seasons.

Analysis of host-associated *Bacteroidales* markers suggests the influence of septic systems, specifically the density of septic systems and average distance of septic to streams on fecal pollution at the watershed level. Human-associated *Bacteroidales* yield was significantly higher in high density watersheds compared to low density areas and was strongly correlated (r = -0.64) with the average distance of septic systems to streams in spring. The human marker was also positively correlated with the total *Bacteroidales* marker, suggesting that the human source

input was a significant contributor to total fecal pollution in the study area. Multivariable regression analysis indicate that septic systems along with forest cover, impervious area and specific conductance could explain up to 74% of the variation in human fecal pollution in spring. The results suggest septic system impact through contributions to groundwater recharge during baseflow or failing septic system inputs especially in areas with >87 septic units km^{-2}.

The SWAT model developed in this study confirms septic systems' influence on watershed hydrology and microbial water quality. Flow simulation in the study area shows that on average septic systems contribute between 7 – 13% to the total water yield. This observation is very significant and challenges suggestions that septic systems are 100% consumptive use. Model results also suggest that direct deposition of fecal bacteria into streams is the likely source of bacterial loadings. Analysis of different bacterial source scenarios in the watershed points to the influence of septic systems on microbial water quality when septic systems are less than 10 m from streams and other water bodies. This result suggests that the current minimum required distance of 15 m from septic drainfields to streams mandated in the state of Georgia may be adequate to protect water resources. However, the results also suggest that there are still a number of septic systems that were installed prior to the current regulatory threshold which could present risks to water quality.

Lastly, this study supports the use of MST approaches along with traditional FIB monitoring and land use characterization in a tiered approach to isolate the influence of septic systems on water quality in mixed-use watersheds. The findings of this study provide the tools that can be used at the watershed level to assess septic system critical areas to support septic system management. Finally, the results of this study can be used by watershed managers and stakeholders to promote septic system management at the watershed level.