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Fecal indicator bacteria removal by river networks

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Fecal indicator bacteria removal by river networks

Abstract
Fecal bacteria have a significant impact on downstream water quality. Removal of fecal bacteria in river systems potentially attenuates downstream impairments and would represent an important ecosystem service. However, few studies aimed to quantify in-stream removal of fecal bacteria. The goal of this study is to understand the ecosystem service of fecal bacteria removal at the river network scale in both water column and hyporheic zone across hydrologic conditions. I developed a module for routing fecal indicator bacteria (FIB) through river networks in the Framework for Aquatic Modeling of the Earth System (FrAMES) model to understand the fate and transport of FIB. This study focuses on Escherichia coli (E. coli), which is the freshwater indicator for fecal contamination. E. coli loading from land and aquatic removal in both water column and hyporheic zone was simulated for every river grid cell throughout the river network. This study found that the hyporheic zone is important in removing E. coli. The water column and the hyporheic zone removed approximately 10-30% and 30-50% of E. coli input, respectively, during the summer period. Watershed size, land use distribution, and hydrology interact to determine network-scale E. coli removal, but hydrology has the most significant impact. Low-frequency but high-magnitude hydrologic events mobilize a disproportionate amount of E. coli. The attenuation efficiency of river networks decreases as the flow increases, but remains relatively high at higher flows common during critical summer periods. This study found that the ecosystem service of E. coli removal reduces E. coli levels at critical downstream water bodies, such as recreational lakes and estuaries. These results have important implications for managing bacteria contamination.

Keywords
Ecosystem service, Effective discharge, Fecal bacteria, Hyporheic zone, River network, Natural resource management, Environmental studies, Environmental science
FECAL INDICATOR BACTERIA REMOVAL BY RIVER NETWORKS

BY

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THESIS

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ABSTRACT

Fecal Indicator Bacteria Removal by River Networks

by

Tao Huang

University of New Hampshire, December, 2016

Fecal bacteria have a significant impact on downstream water quality. Removal of fecal bacteria in river systems potentially attenuates downstream impairments and would represent an important ecosystem service. However, few studies aimed to quantify in-stream removal of fecal bacteria. The goal of this study is to understand the ecosystem service of fecal bacteria removal at the river network scale in both water column and hyporheic zone across hydrologic conditions. I developed a module for routing fecal indicator bacteria (FIB) through river networks in the Framework for Aquatic Modeling of the Earth System (FrAMES) model to understand the fate and transport of FIB. This study focuses on *Escherichia coli* (*E. coli*), which is the freshwater indicator for fecal contamination. *E. coli* loading from land and aquatic removal in both water column and hyporheic zone was simulated for every river grid cell throughout the river network. This study found that the hyporheic zone is important in removing *E. coli*. The water column and the hyporheic zone removed approximately 10-30% and 30-50% of *E. coli* input, respectively, during the summer period. Watershed size, land use distribution, and hydrology interact to
determine network-scale *E. coli* removal, but hydrology has the most significant impact. Low-frequency but high-magnitude hydrologic events mobilize a disproportionate amount of *E. coli*. The attenuation efficiency of river networks decreases as the flow increases, but remains relatively high at higher flows common during critical summer periods. This study found that the ecosystem service of *E. coli* removal reduces *E. coli* levels at critical downstream water bodies, such as recreational lakes and estuaries. These results have important implications for managing bacteria contamination.
1. Introduction

1.1 Background

Good quality of surface waters is essential for drinking water, irrigation of crops, and recreation. Water-borne pathogen contamination is a major water quality concern throughout the world because harmful microbial contamination causes drinking water degradation and closures of recreational beach and shellfish bed (Rose et al. 2001). Shellfish aquaculture and recreational water use depend on microbiological quality of surface waters in rivers, lakes, oceans and estuaries. Excess water-borne pathogenic bacteria cause many illnesses and contagious diseases, including vomiting and diarrhea. Beach advisories are issued when pathogen indicators exceed some specified limit. The U.S. EPA estimated that pathogens impair more than 480,000 km of rivers and shorelines and 2 million ha of lakes in the U.S. (U.S. Environmental Protection Agency, 2010). Pathogens are one of the top three causes of river and stream impairment in New Hampshire (U.S. Environmental Protection Agency, 2010).

Harmful bacteria are a complex pollutant with diverse sources. Fecal indicator bacteria (FIB) are associated with fecal material from both humans and other warm-blooded animals. Fecal coliform and \textit{E. coli} are considered to be good surrogates for enteric bacterial pathogens and protozoan parasites in freshwater systems (Coffey et al. 2007). The kind and the quantity of fecal contamination sources and associated pathogens existing within watersheds are dependent in part on human activities including dominant land uses. Elevated FIB concentration in rivers is associated with urban runoff, agricultural activity, and wildlife (Servais et al. 2007). Wetlands, forests, and open spaces that are home to a wide variety of wildlife can also contribute FIB
inputs (Byappanahalli et al. 2006). FIB sources can be divided into point sources (e.g., wastewater outfalls) and non-point sources (e.g., non-domestic).

Rivers play an important role in transporting dissolved and particulate materials, including pathogens, from terrestrial environments to the coastal ocean (McKee et al. 2004). Some materials may lose during the transport from sources to sea. They may also play an active role in regulating what proportion of microbial inputs to surface waters reaches critical downstream areas, an important ecosystem service (Liao et al. 2015). However, little is known about the effectiveness of river systems to regulate bacterial contaminant fluxes, and how these vary across flow conditions.

The delivery of pathogens and FIB into receiving waters depends on many environmental factors. Hydrology is an important control of timing and amount of sources (Gburek and Sharpley, 1998), and often concentrations increase during storms, resulting in closure of shellfish beds. For example, FIB concentrations increased significantly during storm-runoff events in both urban and rural settings (Weaver and Fuller, 2007). Peak concentrations of total coliform were broadly linked with diffuse sources and storm events (Whitehead et al. 2016). Reducing FIB in streams is important for many downstream areas, but understanding the natural attenuation capacity of river systems will help to target management actions. A number of physical and biological processes affect the transport and retention of microbes in rivers. Physical (i.e., transient storage) processes control downstream transport of solutes and particles. Biological processes (i.e., predation) influence the survival of FIB. The two processes interact to determine what proportion of FIB loading actually reaches downstream systems.
1.2 Literature review

1.2.1 Fecal indicator bacteria

Fecal indicator bacteria (FIB) are types of bacteria used to detect and estimate the associated potential health risks caused by pathogens. Coliform bacteria usually occur in the intestinal tract of animals and are the most widely accepted indicators of water quality in the United States. Chamberlin (1982) compared pathogen and coliform (combining total coliform, fecal coliform, and E. coli) decay rates measured simultaneously and found they are highly correlated ($r^2=0.73$). The most commonly used microbial contamination indicator is fecal coliform (FC). FC are a group of bacteria found in the feces of warm-blooded animals such as people, livestock, pets, and wildlife. Wastes from warm-blooded animals are a source of bacteria found in waterbodies. The USEPA (2002) uses E. coli, one type of FIB, to indicate the presence of waterborne pathogens in fresh water.

1.2.2 Terrestrial inputs of fecal indicator bacteria

FIB inputs to river systems associated with non-point source runoff have been a concern of government, scientists, and fishermen for decades (Colford Jr et al. 2007). Statistical methods were applied to identify FIB loadings as a function of water runoff. They are useful in producing predictions of FIB presence, concentration, and flux, but they do not allow an in-depth understanding of the processes controlling patterns. Schoonover and Lockaby (2006) used monitoring data in regression models, and they found that impervious surface percentage is the most important factor to predict FIB concentration. Mallin et al. (2001) found strong correlations between mean estuarine FIB counts and watershed population and percent developed area. Oliver et al. (2015) investigated the impact of summer rainfall on E. coli concentration in agricultural headwaters, and they found that temporal variation of E. coli concentrations is highly related to
rainfall. However, limited studies compared the terrestrial input of *E. coli* form headwaters across land use types.

1.2.3 *Fecal indicator bacteria transport*

In the past decade, some researchers developed or modified existing watershed-scale models to predict FIB concentration. These models considered the effect on FIB concentrations due to sources, transport (mostly horizontal transport), decay, and/or sediment-related processes (de Brauwere et al. 2014). Watershed-scale hydrologic and FIB models are used to investigate the effects of climate and land use on water resources (Baffaut et al. 2015). Spatial and temporal variations in FIB sources and sinks can be explicitly represented through watershed modeling. Watershed models can extend field studies over broader temporal and spatial scales and can be used to understand the factors controlling patterns. Models have been used to scale-up plot scale measurements to determine the cumulative pollutant removal by the whole river network scale for many water quality variables, including nitrogen (Stewart et al. 2011). The Hydrological Simulation Program-FORTRAN (HSPF) model was widely applied to predict in-stream FIB concentration (Paul et al. 2004; LaWare and Rifai, 2006). Some modeling studies focused on hydrologic variability and FIB dynamics. Liu et al. (2010) computed the flow and FIB loadings from the watersheds in both low flow period and high flow period, finding greater FIB loading in the wet weather conditions. Cho et al. (2016a) applied the SWAT model to simulate seasonal flow variability and FIB concentration, their results indicated that temperature can be a parameter to reproduce the seasonal variability of FIB. Researchers applied empirical or mechanistic models (e.g., Cha et al. 2016) to calculate microbial fate and transport. However, few of them integrated in-situ measurement and spatial modeling of FIB. The systematic integration of local information enhances the capacity of the model to unravel the site-specific dynamics of FIB. For example, Thériault and Duchesne (2015) applied statistical analysis of the
relationship between rainfall and FIB concentrations in urban rivers, and then simulated hydrology and hydraulics for the identification of the main sources of FIB in urban waters using the Storm Water Management Model (SWMM) hydrological/hydraulic simulation model.

Seasonal and flow-driven dynamics of FIB should be studied to understand the fate and transport of FIB. Hyer and Moyer (2003) found that base-flow FIB concentration showed a seasonal pattern of highest concentrations in the summer and lowest in the winter. Flow also affects the efficiency of pollutant processing. For example, there is little time for biological and chemical filtration to take place under high stream flows if water travels through the sediments too rapidly (Grimaldi and Chaplot, 2000). Hydrology is the key element to determine the efficiency of material transport and removal. Effective discharge analysis (Doyle, 2005) combines the probability of a given flow condition occurring and the loading associated with that flow, to understand when most FIB is transported. It can be expanded to also account for river network scale removal for a given flow condition (Doyle, 2005), to understand when most FIB is removed by the river network. The basic concept behind the effective analysis of FIB is described as follows. Terrestrial FIB inputs enter rivers via surface pathways during high discharge events. These inputs transport rapidly downstream due to increasing flow. The reduction in residence time decreases FIB processing efficiency and exports more FIB from the watershed.

1.2.4 Aquatic removal of fecal indicator bacteria

Knowing the survival rates of water-borne pathogen is important to evaluate the role of river systems in controlling bacterial fluxes. In-stream removal of FIB is controlled by various die-off processes. Main factors controlling the die-off process include residence time, biological processing, attachment to sediments, settling, temperature, and microbial predators. Studies of
the survival of coliform bacteria in waters can be dated back to 1970s. Mancini (1978) established a database consisting of approximately 100 measured coliform mortality rates developed in the laboratory or using in-situ experiments. Temperature and light were found to be the most important factors for coliform survival in freshwater system. Mancini (1978) provided a guide for initial estimates of coliform mortality rates in natural bodies of water. The ability of fecal bacteria to survive in surface water generally increases as the temperature decreases. Brooks and Field (2016) synthesized published decay rate constant estimates for common FIB and found that temperature was a significant variable for all FIB and the best predictor variable for *E. coli* decay rate.

The interface between groundwater and streamwater is critical for stream ecosystem processes. Stream and river ecosystems include the vertical dimension of surface–groundwater linkages via the hyporheic zone (HZ). The sediments of a river channel are a porous interface through which mass exchanges (hyporheic flow) occur. A solute transported in a river can be temporarily trapped in the sediment, follow deep flow paths in the porous medium and return to the surface water after some time (Zaramella et al. 2015). Streams and rivers are heterogeneous systems, with fast surface flow transporting substances quickly in the main channel and slow subsurface flow retaining substances for potentially long periods of time (Aquino et al. 2015). Hydrological exchange between the stream and HZ mediates transport of materials (Boulton et al. 2010). HZ exchange delivers particles into the subsurface, contributing to particle deposition by filtration and settling in pore spaces (Packman et al. 2000). Cooley et al. (2007) indicated that HZ exchange can lead to high rates of suspended particle deposition in sediment beds, even when the suspended particles are very small. HZ is also a sink of DOC and nitrate (Peyrard et al. 2011). Triska et al. (1989) injected chloride and nitrate into the surface waters of a stream to
examine solute retention, and the fate of nitrate, finding that retention of solutes was greater in the HZ than in the channel under summer low-flow condition. The HZ increases solute residence time and solute contact with substrates (Bencala, 2000). HZ acts as a mechanical filter mediated by the sediments and water flows, and has been called the “liver” of river networks due its ability to remove pollutants (Boulton et al. 2010).

Most studies assumed FIB decay in the water column, but not exchange and filtering by HZ. Rivers have the potential to immobilize (i.e. filter, attach, or deposit) fine particles, including FIB within the HZ. Microorganisms and other fine particles can then be trapped within storage areas by filtration within subsurface sediments at the sediment-water interface (Battin et al. 2003; Arnon et al. 2010). Microbes are transferred from surface waters into the sediments by hyporheic exchange. Searcy et al. (2006) used controlled laboratory flume experiments to investigate the deposition of suspended Cryptosporidium parvum oocysts in streambeds. They found that Cryptosporidium parvum oocysts are carried into the subsurface by a combination of advective stream- subsurface exchange and particle settling, leading to extensive oocyst deposition within the streambed and drastically reducing the oocyst concentration in the water column. Few studies focused on FIB retention by HZ, particularly at network scales. Drummond et al. (2014) found that fine particles and bacteria were transported similarly, with both having greater retention than the conservative solute (rhodamine WT). Their study showed that the majority of the particles and E. coli were retained near the sediment-water interface. This study suggested that streambed sediments act as short- and long-term reservoirs for fine organic particles and microbes in streams.
1.3 Knowledge gap

Ecosystems provide ecosystem services including regulation of water flow and quality. Water purification capacity is the ecosystem service that contributes to production of clean water. Many studies showed that in-stream processes are important for the ecosystem service of organic carbon storage (Rosemond et al. 2015) and nitrogen removal (Hill, 1996). River network-scale routing processes delay the timing and reduce the magnitude of various pollutants to the basin mouth (Bergstrom et al. 2016). However, few studies aimed to quantify in-stream removal of FIB. The current FIB removal studies focused on the stormwater and FIB reduction by best management practices (e.g., Mallin et al. 2016). To quantify the ecosystem service of FIB removal taking place after FIB enters the river network, it must be considered at river network scales.

The effects of flow regime on stream ecosystems are often analyzed by the application of the effective discharge concept. Effective discharge analysis has been used to determine the discharge levels that transport most of the sediments or solutes over the annual period (Lenzi et al. 2006), as well as the discharge levels at which most of nutrients are removed from transport over the annual period (Doyle, 2005). The concept has been applied to both solutes and particles, including organic matter (Raymond et al. 2016), sediments, and nutrients (Doyle, 2005), but not to FIB. FIB removal should be quantified for river networks through time and over the range of flow conditions.

Cho et al. (2016b) reviewed current FIB models and stated that most FIB models overlook hyporheic mass exchange, and might underestimate the FIB removed by river networks. Transport of pollutants in streams is controlled by the combination of exchange with HZ or adjacent surface storage areas (e.g., side pools, backwaters), sorption on to particulate matter,
and various biogeochemical reactions. Previously proposed stream transport models, such as the commonly used transient storage model, are successfully used to represent the transport and retention of nitrogen. The ability of streams to remove FIB is key to determining downstream water quality. However, few attempts have been made to understand the removal of FIB by HZ.

1.4 Research questions and hypothesis

(1) Are river networks important regulators of FIB transfer from source areas to critical water bodies?

_Hypothesis: river networks are important regulators of FIB transfer to receiving water bodies._

FIB is not conservative due to retention and die-off processes. Rivers reduce FIB concentration through dilution, temporary retention, and permanent removal. The spatial and temporal pattern of FIB concentrations depends on the distribution of loading, mixing, and removal in the river network. Larger watersheds remove a higher percentage of FIB input than smaller watersheds due to longer traveling time and more removal. Sources nearer the basin mouth will show less removal than those further upstream because of shorter traveling distance, traveling time, and efficiency of removal (Bergstrom et al. 2016). Liu et al. (2010) found that the impact of the lower watershed loading was greater than upper watershed loading on the FIB levels at the outlet.

(2) How does hydrological variability affect FIB removal?

_Hypothesis: FIB removal by river networks is greater at low flow than at high flow conditions._

Aquatic removal of pollutants, including FIB, is important in low stream flow due to longer residence time and greater contact with streambed and exchange with the HZ (Stewart et al. 2011). Storm events result in both increased loading to and reduced FIB removal by river networks. Thus, river networks are important contributors to good water quality under low flow conditions, and provide important ecosystem services under these conditions.
(3) Is HZ important for FIB removal?

**Hypothesis: HZ can remove significant amounts of FIB.**

The HZ exchange process traps sediments and associated FIB within streambed sediments.

There is a high probability of water entering the HZ at some point along its flow path through the river network. Longer residence time provide higher removal efficiency in HZ than in the water column.

**1.5 Objectives**

I used a modeling approach to answer these research questions and test the hypotheses. A FIB module was developed in an existing river network hydrological model (the Framework for Aquatic Modeling of the Earth System, FrAMES; Wollheim et al. 2008b, Stewart et al. 2011) to simulate the mobilization, transport, and fate of FIB. The model was applied with *Escherichia coli* (*E. coli*), a member of the FIB group, to understand the processes of fecal coliform and other pathogen removal by river networks in New England watersheds across flow conditions.

**Specific objectives**

1. Quantify the spatial distribution, the magnitude and timing of terrestrial sources of *E. coli* to the river network as a function of land use and climate.

2. Develop and calibrate the FIB module in FrAMES model (focused on *E. coli*) to route sources through the river network.

3. Quantify river network *E. coli* removal as an ecosystem service under different flow conditions, and identify the distribution of *E. coli* removal in river networks.

4. Evaluate the relative role of water column and HZ removal of *E. coli* in river networks.
2. Methods

**Overarching design**

I quantified *E. coli* removal by river networks using a spatially distributed model to understand the input, transport, removal, and export of *E. coli* at the river network scales. The model accounts for spatially variability in loads as a function of land use, and temporal variability of loads due to terrestrial runoff. It accounts for mixing and transport, and removal of *E. coli* in both surface water (as die-off) and HZ (as permanent filters, where die-off ultimately occurs). This research consisted of four tasks. The tasks were: (1) collecting samples in various headwaters to estimate a terrestrial loading function; (2) development of the module to predict *E. coli* terrestrial loading, removal, and concentrations based on interactions with a hydrological model; (3) comparison of modeling results with observations; and (4) simulation under various model complexities and application to different watersheds to test the hypotheses.

2.1 Study watersheds

Six watersheds were selected to represent various watershed sizes, land use, climate, and source spatial distributions in New England (Figure 1). Watershed size affects dilution, residence time (Kasahara and Wondzell, 2003), and removal. In all watersheds, storm *E. coli* response is a combination of the number, location and magnitude of *E. coli* sources and the transport speed (McKergow and Davies-Colley, 2010). Land use affects amounts of runoff and sources. Source spatial distributions affect traveling distance and the efficiency of pollutant processing. Selected watersheds include small watersheds (Oyster River and Winnicut River, NH), medium watersheds (Lamprey River and Cocheco River, NH), and large watersheds (Merrimack River, NH and Penobscot River, ME). The watershed and land use percentages are
shown in Table 1. I defined small rivers as 4th order or smaller streams, medium rivers as 5th to 7th order, and large rivers as 8th order or larger rivers.

Table 1: Study watershed sizes and land use percentages.

<table>
<thead>
<tr>
<th></th>
<th>Oyster River</th>
<th>Winnicut River</th>
<th>Lamprey River</th>
<th>Cocheco River</th>
<th>Merrimack River</th>
<th>Penobscot River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area (km²)</td>
<td>44</td>
<td>40</td>
<td>551</td>
<td>456</td>
<td>11,996</td>
<td>22,691</td>
</tr>
<tr>
<td>Developed (%)</td>
<td>18</td>
<td>28</td>
<td>14</td>
<td>10</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Forest (%)</td>
<td>55</td>
<td>31</td>
<td>61</td>
<td>65</td>
<td>68</td>
<td>67</td>
</tr>
<tr>
<td>Cultivated (%)</td>
<td>9</td>
<td>10</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Wetland (%)</td>
<td>11</td>
<td>25</td>
<td>9</td>
<td>13</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Other (%)</td>
<td>7</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>7</td>
<td>14</td>
</tr>
</tbody>
</table>

The Oyster, Winnicut, Cocheco, and Lamprey rivers flow through New Hampshire into the Great Bay estuary. The Oyster River (Figure 2) flows for 22 km and drains 44 km². The Winnicut River (Figure 3) is a 15 km long river in the Seacoast region of New Hampshire. Winnicut River drains 40 km² flows north into the Great Bay estuary. The Lamprey River (Figure 4) originates in the Saddleback Mountains in Northwood and drains 551 km². River length is 78 km. The land at the headwaters of the Lamprey River is largely undeveloped and forested. The Cochréco River (Figure 5) flows approximately 56 km in a southeastern direction, through the cities of Rochester and Dover. The river drains 456 km². In the lower Cochréco River Watershed, there is a large urbanized area in Dover and Rochester. Failing septic systems were identified as a source of fecal contamination in this watershed (Truslow, 2006). The levels of
bacteria along certain segments of the Cochecho are considered unacceptable for primary contact recreation (primarily swimming) and in some areas even for secondary contact recreation (boating, fishing). Merrimack River, defined by the head of tide at Lowell, flows approximately 220 km and drains 11,996 km². A large part of the northern Merrimack River Watershed is conserved and protected as part of the White Mountain National Forest (Figure 6). Concord, Manchester, and Nashua are the major cities along the river. Penobscot River (Figure 7) flows approximately 330 km drains 22,691 km². The watershed is characterized by little development (developed area accounts for 2%) and is the largest watershed in this study.

**Figure 1:** Locations of study watersheds.
Figure 2: Oyster River Watershed land use, USGS gauge, and *E. coli* sampling locations.

Figure 3: Winnicut River Watershed land use, USGS gauge, and *E. coli* sampling locations.
Figure 4: Lamprey River Watershed land use, USGS gauge, and *E. coli* sampling locations.

Figure 5: Cocheco River Watershed land use, USGS gauge, and *E. coli* sampling locations.
Figure 6: Merrimack River Watershed land use, USGS gauge, and E. coli sampling locations.

Figure 7: Penobscot River Watershed land use, USGS gauge, and E. coli sampling locations.
2.2 Model description

2.2.1 FrAMES background

The Framework for Aquatic Modeling in the Earth System (FrAMES) simulates the land use and climate impact on aquatic conditions and processes at different spatial scales (Wollheim et al. 2008a; Wollheim et al. 2008b). FrAMES simulates hydrology and water quality at daily time steps, allowing us to account for variability due to storms. The fate and transport of solutes and particles across the full range of hydrologic conditions can be assessed. FrAMES allows analysis of spatially distributed runoff and constituents. Constituents are routed downstream through a gridded river network. FrAMES has been used to simulate nitrogen dynamics (Wollheim et al. 2008a; Wollheim et al. 2008b; Stewart et al. 2011), runoff dynamics (Wisser et al. 2010), river water temperature (Stewart et al. 2013), and dissolved organic carbon (Wollheim et al. 2015). A module describing the dynamics of *E. coli* has been developed and embedded within the FrAMES model. I here describe the new module for understanding *E. coli* dynamics. I used the Simulated Topological Network (STN) of gridded rivers for the Penobscot (3-min resolution), the Merrimack (45-sec resolution), and the rest of the watersheds (15-sec resolution). Precipitation and air temperature data from 2008 to 2014 was extracted from NASA Modern Era-Retrospective Analysis for Research and Applications (MERRA) for the Merrimack River and Penobscot River. The MERRA dataset did not yet include the year 2015, which corresponds to the *E. coli* observations in Oyster River, Winnicut River, Cocheco River, and Lamprey River Watersheds. Daily average air temperature and precipitation data was extracted from NOAA Durham Station for these watersheds. Land use data is from National Land Cover Database 2006.
2.2.2 Terrestrial *E. coli* loading

Terrestrial *E. coli* loading was defined as the number of *E. coli* transported from land to water per unit time. Each spatial unit (grid cell) of a watershed acts as a source of *E. coli* load that is transported to a stream. Modeled runoff was multiplied by *E. coli* concentration using an empirical relationship to estimate *E. coli* loading for each grid cell. The amount of *E. coli* entering water bodies from land is dependent on watershed characteristics and meteorological conditions. Environmental variables were considered as potential correlates with measured *E. coli* concentration in headwater streams. Variables selected in this study include Percentage of forest (Forest%), Percentage of developed land (Developed%), Precipitation, and Air Temperature. To understand the relationship between *E. coli* loading to surface waters and environmental variables, statistical analysis was applied to existing and newly collected data sets. Regression models were applied to link *E. coli* concentrations in headwater streams to watershed land use and hydrologic factors (Verhougstraete et al. 2015). The relationship of *E. coli* concentration and environmental variables was established by Partial Least Squares Regression (PLSR) in this study. PLSR offers a number of advantages over the more traditionally used regression analyses and has been an alternative to ordinary least squares for handling collinearity in variables (Chun et al. 2010). I chose to use PLSR over ordinary least squares regression (OLSR) to model *E. coli* loading for the following reasons: (1) Some of our factors, including Forest % and Developed %, are well-correlated, and violate the assumptions of OLSR (2) PLSR is recommended over OLSR for models using time series as factors (Lin et al. 2003). After conducting the regression, I used the variable importance in the projection (VIP) as a measure of the relative contribution of each of the variables on the response variable (*E. coli* concentration). VIP scores can be used to select predictors in the model according to the magnitude of their values. Variables can be eliminated if their VIPs are below the user-defined threshold.
Headwater, or first-order watersheds are the building blocks of drainage basins (McDonnell and Beven, 2014). They are well suited for terrestrial input estimation due to minimal in-stream losses of the materials (Bormann and Likens et al. 1967). To estimate terrestrial E. coli loading, I measured E. coli concentration in a variety of headwater catchments with different land use to determine whether particular land uses explain E. coli variability. This study assumed there is limited in-stream processing in headwaters. This is a typical assumption for linking headwater stream and terrestrial inputs (e.g., Schade et al. 2016). Sampling locations were selected to include three distinct land uses (forest, agriculture, and urban). The three sites were 1) College Brook, with high impervious surface percentage, 2) Dube Brook, forested site, and 3) Chesley Brook, forested site with agriculture. The locations of each headwater are shown in Figure 8.

This regression-based approach gives a reasonable estimate of the magnitude of E. coli input concentration and their distribution within the basin across flow conditions. The regression model was applied throughout the basin, including higher order rivers. Point sources were assumed to be limited within the study watersheds. Although the dataset size is small, it allows us to test the potential role of the river network in regulating E. coli export to downstream waterbodies, accounting for spatial and temporal variability. As better information becomes available, improved loading models can be incorporated.

Daily rainfall, air temperature, watershed land uses were selected for environmental factors. E. coli concentrations appear to have positive relationships with both temperature and rainfall (Cha et al. 2016). Rainfall is an important factor to quantify the washed-off pollutant concentrations from non-point sources (Maniquiz et al. 2010). Pandey et al. (2012) assessed
linear relationships between in-stream *E. coli* water quality data and rainfall for the Squaw Creek Watershed, IA, USA and found that rainfall is a significant factor. Temperature is a major controlling factor for bacterial growth, representing seasonal variability (Cho et al. 2016a). The variation in *E. coli* level from undeveloped watersheds during dry weather is explained by temperature (Tiefenthaler et al. 2009). Besides hydrologic conditions, land use has significant effects on bacteria indicator concentrations in streams. In general, urban watersheds had higher concentrations of fecal coliforms and *E. coli* than other land covers (Crim et al. 2012).

![Figure 8: Locations of sampling headwaters in the Oyster River watershed, NH.](image)

Table 2 shows the size and land use percentages of small headwaters sampled by this study. The dominant land use of College Brook is developed land, accounting for 69% of the
drainage area. Long-term monitoring work shows that UNH campus has a severe impact on College Brook’s water quality, including high nitrogen concentration. Most developed areas are located near the basin outlet, while most agricultural lands are located near the beginning of the stream. In other words, the measurement at the College Brook mouth can reflect mainly urban with additional agricultural inputs. Dube Brook consists of approximately 60% forest. It is the less human-impacted site in this study. Chesley Brook was selected to represent agricultural site in this study. The main land use types are forest and agriculture. Although the agriculture only accounts for 25% in this watershed, it is still higher than most of watersheds in New England.

Table 2: Sampling headwater characteristics.

<table>
<thead>
<tr>
<th>Site</th>
<th>Watershed size (km²)</th>
<th>Developed (%)</th>
<th>Agriculture (%)</th>
<th>Forest (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>College Brook at Mill Plaza</td>
<td>2.3</td>
<td>68.7</td>
<td>9.8</td>
<td>20.8</td>
</tr>
<tr>
<td>Dube Brook at Cherry Lane</td>
<td>3.3</td>
<td>7.9</td>
<td>15.4</td>
<td>59.4</td>
</tr>
<tr>
<td>Chesley Brook at Packers Falls Rd</td>
<td>4.0</td>
<td>12.9</td>
<td>24.5</td>
<td>48.9</td>
</tr>
</tbody>
</table>

All samples were shipped to the laboratory for analysis within 24 hours of sample collection. Samples were kept on ice and shielded from light until being processed. I used IDEXX Colilert-18, Quanti-Tray/2000 (IDEXX) method to determine E. coli concentration. The method is based upon the use of a chromogenic substrate (Pisciotta et al. 2002). The chromogenic substrate method utilizes enzymes that are specific to particular microbe groups. An enzyme substrate included in Colilert reacts with an enzyme found in E. coli, resulting in fluoresces under UV light (Deepesh et al. 2016). The first step is adding Colilert reagent to the
100 mL sample and pour the sample into Quanti-Tray. The tray separates the sample into 49 large and 48 small wells. Seal the tray in Quanti-Tray Sealer and place in 35°C ± 0.5°C incubator for 24 hours. Following incubation at 35 °C for 24 h, fluorescent wells under UV violet light were reported positive for *E. coli*. A statistical analysis is used to determine the most probable number of bacteria cells present. The number of fluorescing wells were counted and the corresponding most probable number (MPN) was determined using the IDEXX Quanti-Tray® MPN table supplied by IDEXX. 1:10 dilution was conducted with sterile distilled water when sample concentration exceeded the upper limit of detection of 2419 MPN/100 mL. Microbial source tracking was applied to identify the host or environment from which the organisms were derived. This study used human and bovine as target hosts. Microbial DNA sequences were used as markers for detection of human or bovine fecal contamination in water samples.

**2.2.3 E. coli model structure**

The main processes included in the model and the methodology are as follows: (1) the spatial and temporal *E. coli* sources are estimated based on modeled terrestrial runoff and an empirical regression model of *E. coli* concentration versus land use and other environmental factors in headwater streams; (2) the decay rate of *E. coli* in the water column is based on literature values (Mancini, 1978), modified by water temperature; (3) the removal of *E. coli* in the HZ is simulated using a transient storage exchange model (Stewart et al. 2011); and (4) the final *E. coli* concentrations under various removal scenarios are estimated. The conceptual model is shown in Figure 9.

Empirical functions of *E. coli* concentration versus environmental variables were developed from samples and existing data and applied as a terrestrial loading function. New
Hampshire Department of Environmental Services (NH DES) collected samples in the three study headwater sites from 2008-2014. However, most samples were collected at baseflow conditions. The NH DES dataset was integrated with the storm data I collected at the same sampling locations to develop a loading function useful across flow conditions.

In the model, each grid cell has a local *E. coli* load based on its model predicted runoff and the empirical *E. coli* concentration regression (i.e. as a function of land use, precipitation, and temperature) described above. *E. coli* fluxes are then accumulated through the river network as with discharge. *E. coli* removal was simulated within every river grid cell, and the remaining *E. coli* was exported to the next downstream grid cell. A removal model that accounts for transient storage (Mulholland and DeAngelis, 2000; Stewart et al. 2011) was applied to predict *E. coli* removal in each grid cell (river reach). *E. coli* removal in each river reach is partitioned into removal by the main channel (*R_{MC,i}*), which represents decay in the water column, and HZ (*R_{HZ,i}*), which represents filtering by the river bed. Total removal is calculated as

\[ R_i = (1 - T_{HZ,i}) \cdot R_{MC,i} + T_{HZ,i} \cdot R_{HZ,i} \]  

where *R_i* (dimensionless) is the total proportional removal of *E. coli* within grid cell *i*, *R_{MC,i}* (dimensionless) and *R_{HZ,i}* (dimensionless) are the proportional removal of *E. coli* that enters MC and HZ within grid cell *i*, respectively, and *T_{HZ,i}* (dimensionless) is the fraction of *E. coli* entering HZ within grid cell *i*. *R_{HZ,i}* is set as 1 because this study assumes all *E. coli* entering HZ would settle and be stored in the HZ if it enters this zone, so removal by HZ is controlled by hydrologic exchange between the MC and HZ.

To represent *E. coli* removal in the water column, first-order kinetics was applied to characterize the die-off process. Temperature is a known controller of *E. coli* inactivation rates.
The equation for $R_{MC}$ estimates the fraction of *E. coli* removal in the grid cell as function of water travel time, decay rate, and temperature. Generally, the temperature function is represented as a factor multiplied with the reference decay rate at a reference temperature (usually 20 °C) (de Brauwere et al. 2014).

$$R_{MC,i} = 1 - e^{-K_{20} \cdot \text{Tau}_i \cdot A(T_i - 20)}$$  \hspace{1cm} (2)

$$\text{Tau}_i = \frac{RL_i}{V_i} \times 86,400$$  \hspace{1cm} (3)

where $K_{20}$ is first-order die-off rate at 20 °C (day$^{-1}$), $\text{Tau}_i$ (day) is the residence time of water in the MC, $T_i$ is air temperature (°C), $A$ (dimensionless) is temperature adjustment factor, $RL_i$ is the reach length (m), $V_i$ for the mean flow velocity (m/s), and 86,400 is for unit conversion to days. $T_i$ and $V_i$ are predicted by model.

The following equation was modified from Mulholland and DeAngelis (2000) to characterize HZ exchange:

$$TE_{HZ,i} = \alpha_i \cdot A_{cross,i} \cdot RL_i / Q_i$$  \hspace{1cm} (4)

where $TE_{HZ,i}$ is the proportion of water that exchanges with the HZ in each grid cell $i$ (dimensionless), $\alpha_i$ is hyporheic exchange coefficient (1/s), $A_{cross,i}$ is the cross-sectional area (m$^2$), $RL_i$ is reach length (m), and $Q_i$ is discharge (m$^3$/s).

The terrestrial *E. coli* inputs from land is calculated as:

$$\text{Local}_i = \text{Conc}_i \cdot \text{Runoff}_i \cdot 10^4 \cdot 86,400$$  \hspace{1cm} (5)
where Local$_i$ is the terrestrial *E. coli* inputs from land in each grid cell (#/day), Conc$_i$ is *E. coli* concentration estimated from the empirical regression described above (#/100ml), Runoff$_i$ is runoff (m$^3$/s), $10^4$ is for volume unit conversion (100 ml per m$^3$), and 86,400 is for time unit conversion (s/d).

The downstream flux of *E. coli* from grid cell $i$ (Flux$_i$, #/day) is calculated as:

$$\text{Flux}_i = (\text{Upstream}_i + \text{Local}_i)(1.0-R_i)$$

where Upstream$_i$ (#/day) is the sum of *E. coli* input into grid cell $i$ from upstream grid cells, and Local$_i$ (#/day) is the total input generated from land within grid cell $i$.

![Diagram of *E. coli* model structure](image)

**Figure 9: *E. coli* model structure.**

### 2.2.4 Modeling *E. coli* removal under scenarios with various levels of the ecosystem service

I applied the model with three levels of complexity regarding *E. coli* removal at the river network scale. These three scenarios also allow us to test the hypotheses. *E. coli* removal at the river network scale is calculated as dividing the total amount of *E. coli* inputs by the total amount of *E. coli* removed at the river network scale. Three scenarios were simulated in this study:
(1) Mixing scenario: *E. coli* was assumed conservative (non-reactive).

(2) RMC scenario: *E. coli* removal occurs in MC only.

(3) RMC+RHZ scenario: *E. coli* removal occurs in MC and HZ.

The Mixing scenario assumes *E. coli* is non-reactive and all *E. coli* would be transported to the basin mouth, with concentration patterns only affected by mixing and dilution depending on land use in different tributaries and along river corridors. In other words, there is no ecosystem service of *E. coli* removal in this scenario.

The other two scenarios assume there is a certain amount of *E. coli* being removed in rivers. In this study, permanent *E. coli* removal was defined as die-off processes. The RMC scenario simulates permanent *E. coli* removal in the water column only. For each grid cell, FrAMES calculates the amount of *E. coli* removal by multiplying input by removal efficiency, and the remaining *E. coli* exports to the next grid. There is *E. coli* removal ecosystem service provided by MC in this scenario.

The last scenario (RMC+RHZ) adds HZ removal processes. HZ has the ability to retain fine particles and attached organic matter (Harvey et al. 2012). This study assumes that *E. coli* behaves like fine particles and that *E. coli* entering the HZ is also permanently removed and is not re-suspended for further downstream transport. Except for the portion removed by MC or HZ, *E. coli* entering a reach was presumed to travel downstream with the water to the next reach. There is *E. coli* removal ecosystem service provided by MC and HZ in this scenario.

### 2.2.5 Model parameterization

Two parameters define *E. coli* die-off in the water column, including *E. coli* die-off rate ($K_{20}$) (Parajuli, 2007; Whitehead et al. 2016) and the temperature adjustment factor ($A$) (Parajuli, 2007; Niazi et al. 2015; Coffey et al. 2010). Measurements of *E. coli* decay rates specific for this
study area are lacking. The *E. coli* die-off rate was determined from experimental data of previous studies. Mancini (1978) integrated the data found in other studies and came out with the value of $K_{20}$ as 0.8 (d$^{-1}$) and temperature adjustment factor as 1.07. These values have been widely used by watershed scale studies (e.g., Reder et al. 2015; Characklis et al. 2009; Benham et al. 2006; Coffey et al. 2010). The unit, ranges, value chosen by this study, and references of key model parameters are shown in Table 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Value used in this study</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> die-off rate ($K_{20}$)</td>
<td>0.86±0.46</td>
<td>0.8 ($K_{20}$)</td>
<td>1/d</td>
</tr>
<tr>
<td>(Selvakumar et al. 2007)</td>
<td>(Mancini, 1978)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyporheic exchange coefficient ($\alpha$)</td>
<td>0.4-42.8 ($10^{-6}$)</td>
<td>9.5*10$^{-6}$ ($\alpha$)</td>
<td>1/s</td>
</tr>
<tr>
<td>(Briggs et al. 2010)</td>
<td>(Briggs et al. 2010)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature adjustment factor (A)</td>
<td>1.07 ±0.05 (Reddy et al. 1981)</td>
<td>1.07 (A)</td>
<td>Dimensionless</td>
</tr>
<tr>
<td>(Mancini, 1978)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The proportional removal of <em>E. coli</em> that enters HZ ($R_{HZ}$)</td>
<td>0-1</td>
<td>1 ($R_{HZ}$)</td>
<td>Dimensionless</td>
</tr>
<tr>
<td>(Assumption of this study)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HZ hydraulic parameters are based on values reported for six tracer experiments conducted during summer low-flow periods in first- through fifth-order stream segments within the Ipswich and Parker rivers, Massachusetts (Briggs et al. 2010), and previously applied at river network scales to understand nitrogen removal (Stewart et al. 2011). The mean hyporheic exchange coefficient ($\alpha$) is 9.5*10$^{-6}$ s$^{-1}$ (Stewart et al. 2011). No relationship was found between
α and river size, so a constant value was applied throughout the river network. Channel velocity and cross section area, were calculated using power law relationships from measurements of three USGS gauges (Oyster River (USGS 01073000), Lamprey River (USGS 01073500), and Merrimack River (USGS 01100000)) from year 2012 to year 2016.

\[ V = 0.16 \times Q^{0.23} \quad (R^2 = 0.59) \quad (7) \]

\[ A_{\text{cross}} = 4.7 \times Q^{0.61} \quad (R^2 = 0.74) \quad (8) \]

\[ W = 13.7 \times Q^{0.35} \quad (R^2 = 0.84) \quad (9) \]

\[ D = 0.21 \times Q^{0.26} \quad (10) \]

where \( V \) = channel velocity (m/s), \( A_{\text{cross}} \) = cross section area (m\(^2\)), \( W \) = river width (m), \( D \) = river depth (m) and \( Q \) = discharge (m\(^3\)/s). Above relationships were estimated based on particular river cross sections.

### 2.3 Model validation

#### 2.3.1 Discharge validation

Time series of simulated daily discharge for each site were generated for the period beginning in year 2011 through the end of year 2014. For Oyster, Winnicut, Lamprey, and Cocheco Rivers, daily discharges of year 2015 were also generated for *E. coli* modeling and validation with the New England Sustainability Consortium (NEST) data. To validate the
simulated discharge, a daily time series of USGS discharge observations of each watershed was compared with model results.

The coefficient of determination ($R^2$), Nash-Sutcliffe efficiency (NSE), and percent bias (PBIAS) were used to evaluate the model performance of discharge simulation. The coefficient of determination ($R^2$) is defined as the squared value of the coefficient of correlation. Nash-Sutcliffe efficiency (NSE) is defined as one minus the sum of the absolute squared differences between the predicted and observed values normalized by the variance of the observed values during the period under investigation. Percent bias (PBIAS) measures the average tendency of the simulated data to be larger or smaller than their observed counterparts (Moriasi et al. 2007).

### 2.3.2 *E. coli* validation

*E coli* predictions were validated against storm event data collected throughout a single storm at the mouth of the Oyster River (at Mill Pond), against data collected monthly at the outlet of watersheds draining to Great Bay (Oyster, Winnicut, Lamprey, and Cocheco Rivers), and against measurements made by state environment departments in the Merrimack River (NH DES) and Penobscott River (Maine Department of Environmental Protection). Summer storm sampling in Oyster River consisted of a series of water samples collected during a storm event from 6/21/2015 to 6/23/2015. Base flow samples were collected on 20th of June. A sigma autosampler was used to automatically collect samples throughout the storm hydrograph (every two hours once the storm began). Samples were classified into three flow classes for analysis—baseflow, rising limb and falling limb. Baseflow and storm event categorization was carried out by visual inspection of discharge, which was obtained from a stage logger and discharge vs. stage relationships developed for the sites. Two samples were collected on the rising limb, two
samples were collected around the peak, and two samples were collected on the falling limb, distributed over three days. Since the FrAMES-FIB model simulates *E. coli* at daily time step, these measurements were summarized as flow-weighted daily *E. coli* concentration based on instantaneous discharge and *E. coli* concentration. The New England Sustainability Consortium (NEST) monthly samples were collected in the Oyster River, Winnicut River, Lamprey River, and Cocheco River from April, 2015 to Dec, 2015 at the basin outlets by the Experimental Program to Stimulate Competitive Research (EPSCoR) project NEST and analyzed for *E. coli*. *E. coli* data in Merrimack River (Station UMMP-11, below Concord) was extracted from Upper Merrimack Monitoring Program, supported in part by the NH Department of Environmental Services. Sampling period is from 1995 to 2014. This study used the year 2014 data for validation. *E. coli* in the Penobscott River was extracted from Maine Department of Environmental Protection database. *E. coli* data of station 71357 in Penobscot River Watershed is located in Brewer, Maine. Only three samples were found and used in this study.

### 2.3.3 Sensitivity analysis

Sensitivity analyses were performed to evaluate how river network scale *E. coli* removal adjusts with changes in hydraulic characteristics and removal rates in MC and HZ and to understand the interactions of different parameter values at river network scales. I applied the sensitivity analysis for the Oyster River watershed to examine the model responses to adjusting parameters. Model responses were characterized by percent changes of *E. coli* removal at the river network scale. I changed each parameter by a given percentage while leaving all others constant, and quantifying the change of *E. coli* removal at the river network scale. Each parameter was changed by 25% and -25% except for Temperature adjustment factor (A) and $R_{HZ}$ (Table 4). Temperature adjustment factor (A) was adjusted to the maximum and minimum
values from literatures since 25% changes exceeded the reasonable range. $R_{HZ}$ was only tested by -25% because $R_{HZ}$ cannot exceed 1.

Table 4: Parameter ranges for sensitivity analysis.

<table>
<thead>
<tr>
<th>Model component</th>
<th>Parameter</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC</td>
<td>$K_{20}$</td>
<td>-25%</td>
<td>+25%</td>
</tr>
<tr>
<td></td>
<td>Temperature adjustment factor (A)</td>
<td>1.02</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>Velocity</td>
<td>-25%</td>
<td>+25%</td>
</tr>
<tr>
<td>HZ</td>
<td>$\alpha_{HZ}$</td>
<td>-25%</td>
<td>+25%</td>
</tr>
<tr>
<td></td>
<td>$R_{HZ}$</td>
<td>-25%</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>$A_{cross}$</td>
<td>-25%</td>
<td>+25%</td>
</tr>
</tbody>
</table>

2.4 Model application

2.4.1 Validation of model results

Simulated *E. coli* concentration was compared with observed concentration where observations were available. NEST monthly data from April to December is used for evaluating the model’s ability to simulate seasonal variation of *E. coli*. Continuous daily sampling at Oyster River during a single storm is used to validate the model performance for storm responses. In the Merrimack and Penobscot Rivers, I used data available for the time period of model runs. Three scenarios were parameterized independently to represent the different levels of *E. coli* removal.
Each level of model complexity was compared to determine which scenario provides the best fit to observations.

2.4.2 *E. coli* dynamics along the river network

Longitudinal profiles from the headwaters to the river mouth (basin profiles) have been used to illustrate the spatial variability of human impacts on water quality (Meybeck, 2002) as well as cumulative impacts of regulating ecosystem services such as nitrogen removal (Wollheim et al. 2008b). Basin profiles of discharge and *E. coli* concentration for the three scenarios of each of the study watersheds were compared to demonstrate the role of source area distribution and river network removal. Three scenarios (Mixing, RMC, and RMC+RHZ) represent the effects of terrestrial inputs, water column removal, and HZ removal over spatial scales from headwaters to whole river network. The Mixing scenario set removal processes to zero and only the transport processes were considered to assess how this affected the distribution of *E. coli* sources along the longitudinal profiles. For RMC scenario, MC removal was accounted for and HZ removal was set to zero. For RMC+RHZ scenario, both MC removal and HZ removal were accounted. These results are shown using average conditions during a low flow period (June, 2014).

2.4.3 Effective discharge analysis for *E. coli*

The effective discharge concept has been applied to conservative and reactive solutes or particles. When it is applied to conservative materials, only effective discharge of loading is calculated (e.g., Higgins et al. 2015). Effective discharge of loading and removal are both calculated when it is applied to reactive materials, including dissolved organic carbon (Raymond et al. 2016) and nitrogen (Doyle, 2005). In this study, effective discharge for *E. coli* loading and removal are both calculated because *E. coli* does not behave conservatively in surface waters.

*E. coli* dynamics, including both loading and removal are a function of hydrologic conditions, which vary seasonally and during storm events. Seasonal effects on loading and
transport of *E. coli* are often quite strong (Cho et al. 2016a). I conducted effective discharge analysis based on summer data (June-August), instead of annual data from 2011-2014, when bacterial contamination is of greatest concern. Hydrology is described in terms of the flow regime, including magnitude (discharge at any time interval) and frequency (how often a flow at a given interval occurs) (Poff et al. 1997). Flow frequency and magnitude can both affect the amount of *E. coli* loading and removal. The relative importance of extreme events or more frequent events of small magnitude can be measured in terms of the relative amounts of loading or removal of a certain flow category. The goal of the application of effective discharge analysis is to estimate the dominant discharge which carries or removes the most *E. coli* over time. The effective discharge of *E. coli* removal represents the discharge levels at which the most *E. coli* removal occurs (i.e. what flow conditions does the river network do the most work of removal as an ecosystem service). To demonstrate the effective discharge analysis, I focus on the RMC+RHZ scenario because it had the best fit with the observations among all scenarios.

The procedure to determine the effective discharge at the river network scale for each watershed is executed in three steps (Doyle, 2005): (a) construct a flow-frequency distribution for flows at the basin mouth (b) construct a river network scale load vs. flow or river network scale removal percent vs. flow curve, and (c) construct a curve of the product of flow frequency and load/removal per flow level. The peaks in the curve of $f(\text{discharge}) \times f(\text{input})$ and $f(\text{discharge}) \times f(\text{removal})$ are effective discharges of *E. coli* load and removal respectively. Flow was assumed to follow a log-normal distribution of frequencies. The flow data set was divided into 12 logarithmically distributed bins, and then the flow frequency distribution was used to compute the percentage of time the flow was within each discharge bin. Load/removal of a certain flow was estimated based on the results of the FrAMES model. Load at the river network
scale was calculated by summing up the terrestrial load from all grid cells given the flow on each day at the basin mouth. Similarly, removal by all grid cells was calculated by summing predicted removal by all grid cells. Removal efficiency was calculated as dividing total amount of *E. coli* removal by input at the watershed scale. Proportion of total summer input removed was calculated as multiplying the proportion of total summer inputs by removal efficiency at the river network scale. Integration the curve under the proportion of total input removed is equal to the total percent removal.

2.4.4 Skewness index analysis

Watershed *E. coli* source distribution and their associated flow path distances in the river network may affect the potential for transport, water residence time and removal efficiency at river network scales. Mineau et al. (2015) quantified average source area distribution within watersheds in terms of skewness towards or away from the river mouth. The skewness index (SI) characterizes the spatial distribution of source areas (often based on land use) within watersheds in a manner that is related to the average residence time of pollutant inputs in the river network. The skewness index (SI) is:

\[ SI_{LU} = \frac{\text{LU weighted mean flowpath distance}}{\text{Unweighted mean flowpath distance}} \]  

The unweighted mean flow path distance is the average distance travelled by water through the hydrologic network from all land use types. Land use weighted mean flowpath distance is calculated as:

\[ \text{LU weighted mean flowpath distance} = \frac{\sum_{i=1}^{n} \text{LU} \times \text{FD}}{\sum_{i=1}^{n} \text{FD}} \]
where LU is the proportion of each grid cell in the watershed occupied by a given land use type, FD is the flowpath distance from that grid cell along the river network to the watershed mouth, and i is the ith grid cell and n is the total number of grid cells in the river network. A SI value of 1 represents no skewness in the distribution of land use within the watershed while SI < 1 represents skewness of land use and associated *E. coli* sources toward the river mouth, and SI > 1 represents skewness towards the most distant headwaters. This study will test similar-sized watersheds with different SI to identify the impacts of land use distribution on *E. coli* removal. The type of LU quantified is determined based on the *E. coli* loading regression.

**2.4.5 Predictions of *E. coli* exceedance level**

The ecosystem service of *E. coli* removal reduces *E. coli* levels and water quality standard exceedance probability. In New Hampshire, surface waters are required to meet specific standards of water quality. There are two classes for surface waters: Class A is for water potential for public water supply, and Class B is for recreational water use. Class A New Hampshire surface water quality standards for *E. coli* are as follows: any single sample is below 153 (#/100 mL) or a geometric mean calculated from three samples collected within a 60-day period is below 47 (#/100 mL). Class B New Hampshire surface water quality standards for *E. coli* are as follows: any single sample is below 406 (#/100 mL), or a geometric mean calculated from three samples collected within a 60-day period 126 (#/100 mL). Although Maine has different water quality criteria (the instantaneous bacteria standard for a Class B stream is 236 (#/100 mL) of sample while the geometric mean standard is 64 (#/100 mL) of sample), I applied the NH water quality standard in Penobscot River for comparison with other NH watersheds. I demonstrated an example to evaluate the importance of *E. coli* removal as an ecosystem service to prevent water quality exceeding standards. The mixing scenario represents the water quality
without the ecosystem service of \textit{E. coli} removal, while RMC+RHZ scenario represents the water quality with the ecosystem service of \textit{E. coli} removal. Time series of \textit{E. coli} concentrations of the two scenarios were compared with single sample standard of Class A and B standards. The exceedance probability of each watershed was compared to determine the relative importance of the ecosystem service of \textit{E. coli} removal among watersheds for summer period between 2011 and 2014.

3. Results

This section includes two major parts. First, I demonstrate the model performance of the loading function, discharge, and \textit{E. coli} concentration. The aim of validation is to demonstrate that the model results are reasonable and therefore useful for the analysis needed to address the research questions. Second, I use basin profiles, effective discharge analysis, and the skewness index to answer the research questions. The spatial and temporal variations of \textit{E. coli} load and removal of various watersheds across flow conditions are examined.

3.1 \textit{E. coli} loading function

Human DNA was found in the developed site (College Brook) and agricultural site (Chesley Brook), bovine DNA was found in agricultural site (Chesley Brook) only, while human and bovine DNA were both not found in forested site (Dube Brook) (Appendix B). This result supports that land use can be a proxy for various \textit{E. coli} sources. The relative importance of each potential variable (as indicated by the regression output, the Variable Importance in the Projection (VIP)) in the loading function were ranked as follow: Precipitation (mm/d) > Developed\% > Air temperature > Forest\% (Figure 10). Precipitation and Developed\% had VIP larger than 0.8. Most studies eliminate variables with VIP below 0.8 to reduce model complexity (e.g., Thomas et al. 1998). In this study, Air Temperature and Forest\% were not removed
because their VIPs were only slightly below 0.8. Moreover, these two variables have large regression coefficients that are perhaps not negligible, and other studies (e.g., Tiefenthaler et al. 2009, Byappanahalli et al. 2006) showed they are significant variables. The \textit{E. coli} loading function we used in FrAMES is:

\[
\log (E. coli \text{ concentration}) = 0.87 + 0.049 \times \text{Precipitation} + 0.046 \times \text{Air Temperature} + 0.014 \times \text{Developed \%} + 0.0052 \times \text{Forest \%} \quad (R^2=0.60)
\]

As Figure 11 shows, the simulated \textit{E. coli} concentration fitted observed concentration well. \(R^2\) of the loading function was 0.6, indicating the trends of observed and simulated concentrations were similar. The simulated versus observed \textit{E. coli} concentration relationship had a slope similar to 1 (\(R^2=0.60\)). Thus, the loading function provides a reasonable estimate of terrestrial loading.

![Figure 10: VIPs of the loading function, the red line shows the VIP threshold (0.8).](image-url)
3.2 Model performance

3.2.1 Discharge

FrAMES provided a reasonable prediction of daily discharge over the annual period across all study watersheds. Table 5 and Table 6 show the validation results for annual discharge and summer discharge, respectively. Simulated annual discharge at the basin mouth is highly correlated with observed discharge ($R^2$ between 0.46 to 0.65), while model performance as NSE is between 0.20 and 0.55. There is little bias in the predictions (PBIAS between -10.3% to 4.9%). FrAMES also provided reasonable estimation of summer discharge ($R^2$: 0.46 - 0.73; NSE: 0.27 - 0.58; PBIAS: -16.9% - 25.9%).
Table 5: Summary of daily discharge over the annual period.

<table>
<thead>
<tr>
<th>Watershed</th>
<th>R²</th>
<th>NSE</th>
<th>PBIAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oyster</td>
<td>0.46</td>
<td>0.41</td>
<td>-10.3%</td>
</tr>
<tr>
<td>Winnicut</td>
<td>0.49</td>
<td>0.44</td>
<td>+2.3%</td>
</tr>
<tr>
<td>Lamprey</td>
<td>0.48</td>
<td>0.37</td>
<td>+4.9%</td>
</tr>
<tr>
<td>Cochecho</td>
<td>0.44</td>
<td>0.37</td>
<td>-10.2%</td>
</tr>
<tr>
<td>Merrimack</td>
<td>0.59</td>
<td>0.55</td>
<td>-7.5%</td>
</tr>
<tr>
<td>Penobscot</td>
<td>0.65</td>
<td>0.20</td>
<td>+3.8%</td>
</tr>
</tbody>
</table>

Table 6: Summary of daily discharge over the summer period.

<table>
<thead>
<tr>
<th>Watershed</th>
<th>R²</th>
<th>NSE</th>
<th>PBIAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oyster</td>
<td>0.73</td>
<td>0.58</td>
<td>+12.3%</td>
</tr>
<tr>
<td>Winnicut</td>
<td>0.54</td>
<td>0.47</td>
<td>+2.8%</td>
</tr>
<tr>
<td>Lamprey</td>
<td>0.61</td>
<td>0.27</td>
<td>+25.9%</td>
</tr>
<tr>
<td>Cochecho</td>
<td>0.56</td>
<td>0.53</td>
<td>-16.2%</td>
</tr>
<tr>
<td>Merrimack</td>
<td>0.51</td>
<td>0.46</td>
<td>-15.1%</td>
</tr>
<tr>
<td>Penobscot</td>
<td>0.57</td>
<td>0.53</td>
<td>-15.3%</td>
</tr>
</tbody>
</table>

In general, simulated daily flow agreed well with observed values, providing confidence in the hydrological controls of *E. coli* dynamics. Time series of observed mean daily river flows and the corresponding simulated mean daily river flows over a five-year period are shown in Figure 12 to 17. The model tended to underestimate storm peaks. It over-estimated summer base flow slightly in the Oyster River (Figure 12) and Lamprey River (Figure 15), but under-estimated in the Cochecho River (Figure 15), Merrimack River (Figure 16), and Penobscot River (Figure 17).
Figure 12: Observed and simulated flow at Oyster River.

Figure 13: Observed and simulated flow at Winnicut River.
Figure 14: Observed and simulated flow at Lamprey River.

Figure 15: Observed and simulated flow at Cocheco River.
3.2.2 E. coli

In general, the model provided a realistic representation of E. coli concentrations, particularly for the RMC+RHZ scenario (Figure 18). The Mixing scenario had much higher E. coli concentration due to lack of MC and HZ removal. At lower flows, observed values were closer to RMC+RHZ scenario, while the Mixing scenario and RMC scenario over-predicted E. coli concentration. In the Oyster River (Figure 19), FrAMES over-predicted the E. coli concentration at base flow for all scenarios. In the Winnicut River (Figure 20), observations
during high flow period (November and December) were higher than RMC+RHZ scenario and closer to Mixing and RMC scenarios. In the Lamprey River (Figure 21), simulated concentrations compare well to observations during storm in April, June, and November. In the Cocheco River (Figure 22), the September baseflow sample and November post-storm sample were closer to the Mixing scenario. In the Merrimack River (Figure 23), samples were only taken in summer, but simulated and observed concentrations were similar, including in response to storms. The RMC+RHZ scenario was the closest scenario to observations. There are only limited baseflow observation data in the Penobscot River (Figure 24). The RMC+RHZ scenario was closest to observations. I also compared simulated and observed E. coli in a storm event in the Oyster River to examine the model performance in high-flow periods (Figure 25). Observed and simulated concentration show similar patterns. E. coli concentrations increased greatly during the storm. After the concentration reached the peak, it decreased but remained higher than base flow levels. In General, during base flow conditions, RMC+RHZ scenario had a better match with observed data. However, during storm events, the Mixing scenario had a better match with observed data.

Figure 18: Mean Absolute Error for three scenarios of each watershed.
Figure 19: Observed and simulated *E. coli* concentrations at Oyster River (05-OYS). Simulated concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).

Figure 20: Observed and simulated *E. coli* concentrations at Winnicut River (02-WNC). Simulated concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).
Figure 21: Observed and simulated *E. coli* concentrations at Lamprey River (05-LMP). Simulated concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).

Figure 22: Observed and simulated *E. coli* concentrations at Cocheco River (07-CCH). Simulated concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).
Figure 23: Observed and simulated *E. coli* concentrations at Merrimack River (UMMP-11). Simulated concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).

Figure 24: Observed and simulated *E. coli* concentrations at Penobscot River (71357). Simulated concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).
3.3 *E. coli* patterns along the basin profiles

A longitudinal profile of the river (*E. coli* concentration plotted versus downstream direction) shows that cumulative *E. coli* removal increased from upstream to downstream, resulting in lower concentrations further downstream than would be expected from mixing alone. Discharge and *E. coli* concentrations in three scenarios are shown in Figure 26 to Figure 32. Discharge is a function of basin area and there increased from upstream to downstream. Longitudinal patterns in *E. coli* concentration from the Mixing scenario reflected influences of land use characteristics. The separation between conservative mixing (Mixing) and processing (RMC and RMC+RHZ) scenarios increased as drainage area increases due to the loss of *E. coli* during hydrologic transport. In small and medium rivers, removal was dominated by HZ for the entire basin profile. In large rivers, removal was dominated by HZ upstream, but MC became equally or more important in downstream.

Patterns of concentration along basin profile were determined by the location of land use relative to the basin mouth, interacting with removal during transport. In the Oyster River
(Figure 26), there was an increase of \textit{E. coli} concentration near the basin mouth due to urbanized area close to the basin mouth. In the Winnicut River (Figure 27), the major inputs are located towards the headwaters, and therefore a large percentage of \textit{E. coli} was removed during transport. In the Lamprey River (Figure 28), the highest concentration occurred near the middle of the basin profile due to high urban input. In the Cocheco River (Figure 29), \textit{E. coli} concentration increased from headwaters until approximate 10 km from the basin mouth due to the dilution from less-developed Isinglass River. In the Merrimack River (Figure 30), most urban development (Concord and Manchester) occurs in the lower part of the watershed, so \textit{E. coli} concentration increased from upstream to downstream. While the Mixing scenario showed a steady increase from upstream to downstream, the RMC and RMC+RHZ scenarios showed a decreasing trend from upstream to the middle of the basin profile, and then concentration further downstream. Removal was unable to compensate for the greater magnitude of loading in the downstream section of the Merrimack. The basin profile of the Penobscot River is shown in Figure 32. It shows increasing input in the middle section of the river due to increasing developed areas. Concentration in the Mixing scenario is relatively low compared to other scenarios. A large amount of inputs was removed in both RMC and RMC+RHZ scenarios.

Terrestrial \textit{E. coli} input is controlled by developed area. To further understand land use impact on \textit{E. coli} inputs, I chose Merrimack River watershed for a more detailed land use analysis. Figure 31 shows land use change from upstream to downstream at Merrimack River. Compared with \textit{E. coli} concentration of the Mixing scenario in Figure 30, \textit{E. coli} concentration was lower in headwaters (forest area) and higher near the basin mouth (developed area). This analysis shows the significant influence of land use on \textit{E. coli} input.
Figure 26: Basin profile of simulated discharge and *E. coli* concentrations at Oyster River for June, 2014. Simulated *E. coli* concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).

Figure 27: Basin profile of simulated discharge and *E. coli* concentrations at Winnicut River for June, 2014. Simulated *E. coli* concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).
Figure 28: Basin profile of simulated discharge and *E. coli* concentrations at Lamprey River for June, 2014. Simulated *E. coli* concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).

Figure 29: Basin profile of simulated discharge and *E. coli* concentrations at Cocheco River for June, 2014. Simulated *E. coli* concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).
Figure 30: Basin profile of simulated discharge and *E. coli* concentrations at Merrimack River for June, 2014. Simulated *E. coli* concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).

Figure 31: Basin profile analysis of land use percentage at Merrimack River.
Figure 32: Basin profile of simulated discharge and *E. coli* concentrations at Penobscot River for June, 2014. Simulated *E. coli* concentrations are from three scenarios (Mixing, RMC, and RMC+RHZ).

3.4 The relative role of MC and HZ

The HZ removed more *E. coli* than the MC in all watersheds (Figure 33). In small watersheds (Oyster River and Winnicut River, approximately 40 km$^2$), the river networks removed approximate 40% of *E. coli* input during the summer period, with 30% removed by the HZ and 10% removed by the MC. In medium-sized watersheds (Lamprey River and Cocheco River, 456 and 551 km$^2$, respectively), the river networks removed approximate 60% *E. coli* input, with 45% removed by the HZ and 15% removed by the MC. In large watersheds (Merrimack River and Penobscot River, larger than 10,000 km$^2$), the river networks removed approximate 70% of *E. coli* input, with 50% removed by the HZ and 20% removed by the MC. Both HZ and MC removal efficiency increased consistently with watershed size. There is limited amount of *E. coli* removed by the MC in small rivers due to short travel distance and associated short residence times. Although the longer traveling distance enhances the chances of *E. coli* entering HZ, exchange between the MC and HZ becomes relatively less important, resulting in a slight decline in the relative importance of HZ removal. The HZ dominates removal because of
large benthic surface area relative to the overlying water volume of headwaters and other low-order streams. High surface to volume ratios lead to greater contact and exchange of water in the advective main channel with the HZ (Peterson et al. 2001).

The relative importance of MC increased from upstream to downstream. Figure 34 to Figure 39 show the relative importance of MC to HZ from upstream to downstream for each watershed. The x-axis is the accumulated upstream area, while the y-axis is the accumulated upstream \textit{E. coli} removal. As local (grid scale) HZ exchange rates decrease with watershed size (from upstream to downstream), a smaller proportion of \textit{E. coli} entered the HZ and more \textit{E. coli} remained in the water column, where removal would occur. Moreover, the die-off process in MC increased from upstream to downstream because the water temperature was higher in downstream than mountain headwaters.

Figure 33: Percent \textit{E. coli} removal by MC and HZ across watershed size.
Figure 34: The relative role of MC and HZ in *E. coli* removal in Oyster River during a low-flow period of June, 2014.

Figure 35: The relative role of MC and HZ in *E. coli* removal in Winnicut River during a low-flow period of June, 2014.
Figure 36: The relative role of MC and HZ in *E. coli* removal in Lamprey River during a low-flow period of June, 2014.

Figure 37: The relative role of MC and HZ in *E. coli* removal in Cocheco River during a low-flow period of June, 2014.
Figure 38: The relative role of MC and HZ in E. coli removal in Merrimack River during a low-flow period of June, 2014.

Figure 39: The relative role of MC and HZ in E. coli removal in Penobscot River during a low-flow period of June, 2014.

The pattern of increasing E. coli percent removal versus watershed size is the combined effects of hydraulic dimensions, residence time, hyporheic exchange, and temperature (Figure
In our current model, HZ removal variability with river size is controlled by exchange between the advective channel and hyporheic transient storage only, which is a function of alpha and cross sectional area. In contrast, MC removal is controlled by both water residence time and temperature. I did a basin profile analysis of summer discharge and water temperature in the Merrimack River watershed to understand their impact on MC and HZ removal. As Figure 40 shows, both discharge and water temperature increased from upstream to downstream. HZ removal increased slowly because the benthic surface area to water volume ratios (which controls the proportion of water exchanging with the HZ in each reach) declined rapidly and caused HZ exchange efficiency in individual reaches to decrease (Figure 41). Furthermore, from upstream to downstream, MC removal was enhanced by increasing water temperatures (Equation 2). Water temperature increased in the downstream direction during summers due to exchange with atmosphere and additional solar radiation inputs (Stewart et al. 2013). The combined effects make MC removal efficiency steadily more important from upstream to downstream, increasing in importance relative to HZ.

![Figure 40: Basin profile of discharge and water temperature at Merrimack River (2014-06).](image-url)
3.5 Sensitivity analysis

HZ percent *E. coli* removal was higher than MC percent *E. coli* removal for all parameter sets. MC, HZ, and total percent *E. coli* removal, and *E. coli* concentration at the basin mouth for different parameter sets are presented in Table 7. MC percent *E. coli* removal was most sensitive to the temperature adjustment factor. The temperature adjustment factor, which ranged from 1.02-1.12, resulted in MC percent *E. coli* removal ranging from 3%-19%. While all other parameters (plus or minus 25%) caused removal ranged only from 6% to 11%. HZ percent *E. coli* removal was relatively sensitive to all three hyporheic parameters, $a_{HZ}$, $R_{HZ}$ and $A_{cross}$, which resulted in percent removal ranging from 24% to 36%.
Table 7: Percent *E. coli* removal change with adjusting parameters at Oyster River for 2011-2014 summer.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter change</th>
<th>MC percent <em>E. coli</em> removal (%)</th>
<th>HZ percent <em>E. coli</em> removal (%)</th>
<th>Total percent <em>E. coli</em> removal (%)</th>
<th><em>E. coli</em> concentration (#/100ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>8</td>
<td>30</td>
<td>38</td>
<td>395</td>
</tr>
<tr>
<td>$K_{20}$</td>
<td>-25%</td>
<td>6</td>
<td>31</td>
<td>37</td>
<td>406</td>
</tr>
<tr>
<td></td>
<td>+25%</td>
<td>10</td>
<td>30</td>
<td>40</td>
<td>386</td>
</tr>
<tr>
<td>Temperature adjustment factor (A)</td>
<td>1.02</td>
<td>19</td>
<td>28</td>
<td>47</td>
<td>339</td>
</tr>
<tr>
<td></td>
<td>1.12</td>
<td>3</td>
<td>31</td>
<td>35</td>
<td>421</td>
</tr>
<tr>
<td>Velocity</td>
<td>-25%</td>
<td>11</td>
<td>30</td>
<td>41</td>
<td>381</td>
</tr>
<tr>
<td></td>
<td>+25%</td>
<td>7</td>
<td>31</td>
<td>37</td>
<td>403</td>
</tr>
<tr>
<td>$\alpha_{HZ}$</td>
<td>-25%</td>
<td>9</td>
<td>24</td>
<td>33</td>
<td>443</td>
</tr>
<tr>
<td></td>
<td>+25%</td>
<td>7</td>
<td>36</td>
<td>43</td>
<td>356</td>
</tr>
<tr>
<td>$R_{HZ}$</td>
<td>-25%</td>
<td>9</td>
<td>24</td>
<td>33</td>
<td>443</td>
</tr>
<tr>
<td>$A_{cross}$</td>
<td>-25%</td>
<td>9</td>
<td>24</td>
<td>33</td>
<td>442</td>
</tr>
<tr>
<td></td>
<td>+25%</td>
<td>7</td>
<td>36</td>
<td>43</td>
<td>355</td>
</tr>
</tbody>
</table>

3.6 Effective discharge analysis

Over the summer period, most *E. coli* loading occurred during high flow events, even though these events are rare. Runoff frequency distribution was positively skewed for all watersheds. Low flows occurred more frequently than high flows. Storm events increased discharge and runoff *E. coli* concentration. High flows represent a large volume of water, and *E. coli* concentrations increase with higher precipitation and flows. Using the Oyster River and Merrimack River as an example, terrestrial runoff *E. coli* concentration increased with discharge.
during 2011-2014 summer periods (Figure 42). More urbanized Merrimack River increased more greatly than Oyster Ricer. The *E. coli* concentration-to-discharge relationship was compared with the relationships of NO$_3^-$ and SO$_4^{2-}$ for 1997 -2002 at Biscuit Brook from Murdoch and Stanley (2006) (Figure 43). For each pollutant, the concentration was normalized by dividing with the concentration at the lowest flow. *E. coli* shows the greatest increase with flow among all pollutants, and this explains the highest loading at the highest flow level.

![Graph showing concentration of runoff vs. discharge for 2011-2014 summer at Oyster River (05-OYS, blue line) and Merrimack River (UMMP-11, red line).](image)

**Figure 42:** *E. coli* concentration of runoff vs. discharge for 2011-2014 summer at Oyster River (05-OYS, blue line) and Merrimack River (UMMP-11, red line).
Removal efficiency decreased as flow increased, although the rate of change varied with watershed. This supports the hypothesis that removal efficiency decreases with increasing flow. The amount of *E. coli* removal shows the same trend as load, because the variation of load is larger than removal by orders of magnitude. High flow is less frequent than moderate flow, but it can contribute a great proportion of *E. coli* load and removal and should not be ignored.

The effective discharge analysis for each watershed is demonstrated in Figures 44 to 49. The grey line is the proportion of total summer runoff occurring during a given flow interval. The black line is the proportion of total summer *E. coli* inputs occurring during a given flow interval. The integral of the line is 1, representing all input during the interval. The red line is the removal efficiency at the river network scale for a given flow interval. The blue line is the proportion of total summer *E. coli* inputs removed during each flow interval. The integrations of
blue line equals to the total proportion of *E. coli* removed at the river network scale. The peak of the blue line is the effective discharge.

The distribution of *E. coli* input was uneven across flow conditions during the summer period, but in general the effective discharge of *E. coli* load occurred in the highest flow interval. Removal efficiency at river network scale decreased with increasing flow intervals. Nevertheless, effective discharge of *E. coli* removal occurred in the highest flow interval. In the Oyster River (Figure 44), effective discharge of load and removal both occurred in the highest flow category. Compared with the Oyster River, the Winnicut River (Figure 45) had higher removal efficiency at base flow conditions because source areas are located further upstream. The highest flow of the Winnicut River is lower than the Oyster River and removal efficiency at river network scale was slightly higher than the Oyster. Effective discharge of load and removal both occurred at the highest flow. In the Lamprey River (Figure 46), the removal efficiency at river network scale was higher across all flow categories than in the smaller watersheds, and the decline of the removal efficiency at river network scale with increasing flow was less, indicating the greater buffering effect of large watersheds (Mulholland et al. 2008; Wollheim et al. 2008a). Compared to the Lamprey River, the removal efficiency at the river network scale of Cocheco River (Figure 47) was lower than the Lamprey River at both base flow and high flow conditions, possibly because the sources are located closer to the basin mouth, resulting in less total removal. There were two peaks for proportion of total summer inputs. The highest peak occurred at the highest flow category, indicating that most terrestrial inputs entered the river at the highest flow. The secondary peak occurred at just above the moderate flow. The high terrestrial inputs of the secondary peak result from higher frequency than other high flow categories. Most *E. coli* removal occurred at high flow conditions. Although the frequency of the high flow occurrence
was low for the Merrimack (Figure 48), most input occurred at the infrequent high flow. The most *E. coli* was removed at the highest flow. Removal efficiency at river network scale is governed by discharge, but also influenced by water temperature. In Merrimack River, removal efficiency at river network scale generally decreased with flow. The increasing of removal efficiency at river network scale at the highest flow was caused by high water temperature at that flow category.

Figure 44: Effective discharge analysis of *E. coli* removal at Oyster River during summer between 2011 and 2014. The grey line is the proportion of total summer runoff occurring during a given flow interval. The black line is the proportion of total summer *E. coli* inputs occurring during a given flow interval. The red line is the removal efficiency at the river network scale for a given flow interval. The blue line is the proportion of total summer *E. coli* inputs removed during each flow interval.
Figure 45: Effective discharge analysis of *E. coli* removal at Winnicut River during summer between 2011 and 2014. The grey line is the proportion of total summer runoff occurring during a given flow interval. The black line is the proportion of total summer *E. coli* inputs occurring during a given flow interval. The red line is the removal efficiency at the river network scale for a given flow interval. The blue line is the proportion of total summer *E. coli* inputs removed during each flow interval.
Figure 46: Effective discharge analysis of *E. coli* removal at Lamprey River during summer between 2011 and 2014. The grey line is the proportion of total summer runoff occurring during a given flow interval. The black line is the proportion of total summer *E. coli* inputs occurring during a given flow interval. The red line is the removal efficiency at the river network scale for a given flow interval. The blue line is the proportion of total summer *E. coli* inputs removed during each flow interval.
Figure 47: Effective discharge analysis of *E. coli* removal at Cocheco River during summer between 2011 and 2014. The grey line is the proportion of total summer runoff occurring during a given flow interval. The black line is the proportion of total summer *E. coli* inputs occurring during a given flow interval. The red line is the removal efficiency at the river network scale for a given flow interval. The blue line is the proportion of total summer *E. coli* inputs removed during each flow interval.
Figure 48: Effective discharge analysis of *E. coli* removal at Merrimack River during summer between 2011 and 2014. The grey line is the proportion of total summer runoff occurring during a given flow interval. The black line is the proportion of total summer *E. coli* inputs occurring during a given flow interval. The red line is the removal efficiency at the river network scale for a given flow interval. The blue line is the proportion of total summer *E. coli* inputs removed during each flow interval.
Figure 49: Effective discharge analysis of *E. coli* removal at Penobscot River during summer between 2011 and 2014. The grey line is the proportion of total summer runoff occurring during a given flow interval. The black line is the proportion of total summer *E. coli* inputs occurring during a given flow interval. The red line is the removal efficiency at the river network scale for a given flow interval. The blue line is the proportion of total summer *E. coli* inputs removed during each flow interval.

3.7 Watershed size and skewness index analysis
In general, *E. coli* removal was higher in large watersheds and watersheds with high SI (Table 8). In general, large river networks removed more *E. coli* than small river networks. The amount and location of land use within watersheds affected *E. coli* input, removal, and export. When sources of *E. coli* are skewed towards the watershed mouth, *E. coli* sources encounter fewer headwaters and mid-order streams, so overall processing potential declines. The Oyster River and Winnicut River have similar drainage area. However, the Winnicut River has a SI of 1.06 while Oyster River has a SI of 0.77. As a result, the Winnicut River removes more *E. coli* than the Oyster River. The Lamprey River and Cocheco River are medium size river. The Lamprey River has higher SI (0.89) than the Cocheco River (0.72). As a result, the Lamprey
River removed more *E. coli* than the Cochecho River. The Merrimack River has much larger area (11,996 km²) than the Lamprey River (551 km²), but the Merrimack was only slightly more efficient in *E. coli* removal. The Lamprey River has higher SI (0.89) than the Merrimack River (0.64), indicating higher chances of *E. coli* being removed. Although the SI index is low in the Penobscot River, the watershed removes a large amount of *E. coli* due to relative longer traveling length.

Table 8: Watershed characteristics and *E. coli* removal.

<table>
<thead>
<tr>
<th>Watershed</th>
<th>SI</th>
<th>Area (km²)</th>
<th>Percent <em>E. coli</em> removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oyster River</td>
<td>0.77</td>
<td>44</td>
<td>38%</td>
</tr>
<tr>
<td>Winnicut River</td>
<td>1.06</td>
<td>40</td>
<td>44%</td>
</tr>
<tr>
<td>Cochecho River</td>
<td>0.72</td>
<td>456</td>
<td>55%</td>
</tr>
<tr>
<td>Lamprey River</td>
<td>0.89</td>
<td>551</td>
<td>66%</td>
</tr>
<tr>
<td>Merrimack River</td>
<td>0.64</td>
<td>11,996</td>
<td>68%</td>
</tr>
<tr>
<td>Penobscot River</td>
<td>0.56</td>
<td>22,691</td>
<td>79%</td>
</tr>
</tbody>
</table>

Figure 50: Percent *E. coli* removal versus watershed sizes for two SI groups.

In general, watersheds with high SI removed more *E. coli* compared with watersheds with low SI if their sizes are similar. The watersheds were categorized into three groups by sizes. There are two watersheds in each size group, one with higher SI than the other. I extracted
watersheds with high SI from each size group to make a group “High SI, while the remaining watersheds were grouped into “Low SI”. As Figure 50 shows, High SI generally removed more *E. coli* compared with the pair with Low SI. The only exception is Penobscot River. Although Penobscot River and Merrimack River are in the same size group, Penobscot River is twice larger than Merrimack River.

Multiple linear regression was applied to determine the relative importance of SI and watershed area in explaining variability of *E. coli* removal at the river network scale. SI and watershed area were both not statistically significant factors. However, watershed area had much lower p-value than SI had, indicating that watershed area might be more important than SI. It can be explained that large rivers even with low SI, such as Penobscot River, also have the ability to remove great amount *E. coli* because the relatively long travel distance compared with small rivers. Moreover, other factors, including hydrologic condition and temperature, might have more significant impacts on *E. coli* removal. However, with only six points, a multiple regression is not a very powerful test. More watersheds with a range of conditions would be needed.

\[
\text{Percent } E. \text{ coli removal} = 53.8 - 3.8 \times \text{SI} + 0.0013 \times \text{Watershed area (} R^2 = 0.40 \) (14)
\]

<table>
<thead>
<tr>
<th>Variable</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>0.94</td>
</tr>
<tr>
<td>Watershed area</td>
<td>0.24</td>
</tr>
</tbody>
</table>

### 3.8 Prediction of *E. coli* level exceedance probabilities

The ecosystem service of *E. coli* removal reduced *E. coli* concentration and the probability of exceeding water quality thresholds. New Hampshire water quality standards and *E. coli* concentrations without removal (Mixing scenario) and with removal (RMC+RHZ scenario)
of at the sampling locations of each watershed for summer 2014 are shown from Figure 51 to Figure 56. Each watershed shows similar *E. coli* concentrations for the Mixing Scenario except for the lower concentration in the less-developed Penobscot watershed. For the RMC+RHZ Scenario, Oyster River, Winnicut River, and Cocheco River had higher *E. coli* concentration than other watersheds due to less removal. The number of days exceeding Class A or Class B standards was higher in the Mixing Scenario (9-88 days for Class A, 4-24 days for Class B) than the RMC+RHZ Scenario (3-22 days for Class A, 2-13 days for Class B) for the summer period. Large watersheds had lower exceedance probability than small watersheds both in the Mixing and RMC+RHZ scenarios (Table 10). Small and developed watershed will exceed the water quality standard A for almost entire summer if there is no *E. coli* removal. The Penobscot River shows low exceedance probability even without the ecosystem service (Mixing scenario), because the watershed is low-developed and large watershed size leads to long residence time and more HZ exchange for *E. coli* removal. Simulated *E. coli*-impaired rivers agreed with impaired sampling sites from USEPA report (Table 11). Impaired watersheds match the watershed with higher *E. coli* concentration and less *E. coli* removal predicted by this study (Table 11).
Figure 51: Simulated *E. coli* concentrations for Mixing scenario (without *E. coli* removal ecosystem service) and RMC+RHZ scenario (with *E. coli* removal ecosystem service) and water quality standards for 2014 summer at Oyster River (05-OYS).

Figure 52: Simulated *E. coli* concentrations for Mixing scenario (without *E. coli* removal ecosystem service) and RMC+RHZ scenario (with *E. coli* removal ecosystem service) and water quality standards for 2014 summer at Winnicut River (02-WNC).
Figure 53: Simulated *E. coli* concentrations for Mixing scenario (without *E. coli* removal ecosystem service) and RMC+RHZ scenario (with *E. coli* removal ecosystem service) and water quality standards for 2014 summer at Lamprey River (05-LMP).

Figure 54: Simulated *E. coli* concentrations for Mixing scenario (without *E. coli* removal ecosystem service) and RMC+RHZ scenario (with *E. coli* removal ecosystem service) and water quality standards for 2014 summer at Cocheco River (07-CCH).
Figure 55: Simulated *E. coli* concentrations for Mixing scenario (without *E. coli* removal ecosystem service) and RMC+RHZ scenario (with *E. coli* removal ecosystem service) and water quality standards for 2014 summer at Merrimack River (11-UMMP).

Figure 56: Simulated *E. coli* concentrations for Mixing scenario (without *E. coli* removal ecosystem service) and RMC+RHZ scenario (with *E. coli* removal ecosystem service) and water quality standards for 2014 summer at Penobscot River (71357).
Table 10: Summer *E. coli* concentration exceedance probability (percent of days above impairment) for Mixing scenario and RMC+RHZ scenario.

<table>
<thead>
<tr>
<th>Watershed</th>
<th>Exceedance probability of Class A</th>
<th>Exceedance probability of Class B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mixing</td>
<td>RMC+RHZ</td>
</tr>
<tr>
<td>Oyster River</td>
<td>95%</td>
<td>21%</td>
</tr>
<tr>
<td>Winnicut River</td>
<td>95%</td>
<td>18%</td>
</tr>
<tr>
<td>Cocheo River</td>
<td>96%</td>
<td>24%</td>
</tr>
<tr>
<td>Lamprey River</td>
<td>86%</td>
<td>11%</td>
</tr>
<tr>
<td>Merrimack River</td>
<td>85%</td>
<td>7%</td>
</tr>
<tr>
<td>Penobscot River</td>
<td>10%</td>
<td>3%</td>
</tr>
</tbody>
</table>

Table 11: Cause of pathogen impairment of sampling sites at study watershed.

<table>
<thead>
<tr>
<th></th>
<th>Simulated summer <em>E. coli</em> concentration (#/100ml)</th>
<th>Impaired waterbody ID</th>
<th>Cause of pathogen impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oyster River (05-OYS)</td>
<td>395</td>
<td>NHIMP600030902-04</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>Winnicut River (02-WNC)</td>
<td>346</td>
<td>NHIMP600030901-02</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>Cocheo River (07-CCH)</td>
<td>328</td>
<td>NHIMP600030608-04</td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td>Lamprey River (05-LMP)</td>
<td>168</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Merrimack River (11-UMMP)</td>
<td>306</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Penobscot River (71357)</td>
<td>31</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

4. Discussion

4.1 Land use impact on *E. coli* loading

The loading relationship indicates that land use drives loading variability in space, while rainfall drives variability in time. The basin profiles of *E. coli* loads show similar trend with developed lands. It is common for urban and agricultural land to export more *E. coli* as compared to undeveloped land. Previous studies found that *E. coli* is positively correlated to urban and negatively correlated to forest (Pettus et al. 2015; DiDonato et al. 2009). Hennani et al. (2012)
found consistently higher *E. coli* levels across all flow conditions in urban watersheds than other land uses. This study found that developed land has the highest coefficient in the loading model, indicating that it is the most important source contributing to the total *E. coli* load. Other watershed variables that potentially predict bacteria levels include population density and failing septic systems (Verhougstraete et al. 2015). However, population was not an important factor in this study, while we did not have spatial info on septic systems. Land use not only affects *E. coli* loads, but also affects streamflow and the transport of *E. coli*. Urbanization increases stream runoff and discharge variability, which is reflected in the precipitation term in the loading function. The loading function developed in this study provides reasonable estimates of *E. coli* concentration. Moreover, it successfully predicted *E. coli* concentration in the higher ranges. Hence, the loading model adequately accounts for the spatial and temporal distribution of sources, sufficient for exploring the potential factors governing *E. coli* removal by river networks.

### 4.2 *E. coli* removal by MC and HZ

River networks are able to remove a significant proportion of *E. coli* inputs from land. Hydrologic networks do not act as neutral pipes (Cole et al. 2007), but instead are active players in the *E. coli* processing. The *E. coli* concentration under the scenario including MC and HZ removal processes (RMC+RHZ scenario) fitted the observation best, indicating both MC and HZ contributed to *E. coli* removal. All networks removed > 40% of *E. coli* inputs, with much greater removal efficiency at lower flows and in large river networks.

HZ has a considerable control of *E. coli* removal. River hydraulics controlled *E. coli* removal via the extent of HZ exchange. Although the results of this study support the assumption that HZ is the major control of *E. coli*, other factors must be considered to predict *E. coli* removal. The sensitivity analysis shows that HZ removed more *E. coli* than MC with all
parameter sets, indicating that filtering by the HZ contributed a major proportion of *E. coli* removal at river network scale. Hydrologic conditions play a critical role in *E. coli* removal and final concentration because HZ removal efficiency is higher in low flow periods than storm events. The ratio of hyporheic exchange flow to stream discharge decreased from upstream to downstream. This decline is consistent with previous studies. Edwardson et al (2003) found that the ratio of HZ cross-sectional area to the stream cross-sectional area decreased from small to large streams. The ratio of hyporheic exchange flow to stream discharge was large in small stream at low flows, and it is small (near zero) in large river at high flows (Wondzell, 2011). Small streams also generally have greater hyporheic storage relative to the channel water volume than large rivers (Harvey et al. 2003). This study also found that headwaters have greater HZ retention than high-order streams. However, most watersheds in this study have more sources located in downstream, where HZ removal efficiency is lower. During storm events, re-suspension might introduce *E. coli* back to water column (Coffey et al. 2010; Jamieson et al. 2004). During low flow periods, the bed sediment of a stream can act as a transient reservoir that can be released during high-flow events. In this study, re-suspension of *E. coli* was assumed small relative to rate of breakdown of *E. coli* in the sediments.

The hypothesis that HZ removes significant amounts of *E. coli* was also supported by this study, while MC is an important secondary removal component. The relative importance of MC and HZ varied among watersheds, but HZ was always more important. HZ removal is controlled by hydrologic conditions, while MC removal is controlled by both hydrological conditions and temperature. In Merrimack River, HZ removal dominated in mountain headwaters, since the water temperature was low and as a result *E. coli* processing rate in MC was also low. In general, channel hyporheic exchange is more important in headwaters than in larger rivers (Ranalli and
Macalady, 2010).

The MC and HZ function differently for *E. coli* than for reactive nutrients. This study found that MC and HZ removed 20% and 40% *E. coli* inputs in middle size watersheds (Lamprey and Cocheco Rivers) in summer, respectively. Stewart et al. (2011) found that MC and HZ removed 38% and 21% DIN (dissolved inorganic nitrogen) inputs in a middle size watershed (approximately 400 km$^2$) at summer base flow condition, respectively. The major difference between DIN and *E. coli* removal by HZ is the uptake mechanisms. *E. coli* attaches to sediments and is assumed to be removed entirely after entering HZ, while DIN removal follows first-order decay function and the proportion of removal depends on HZ residence time (Stewart et al. 2011), which means some proportion of DIN entering the HZ can return to the water column. As a result, MC removes more DIN than HZ does, while more *E. coli* is removed by HZ than MC. The assumption that all particulate *E. coli* entering the sediments remains there is a major assumption that requires further investigation. However, the sensitivity analysis that reduces HZ removal to 75% of inputs also showed that the HZ dominated removal, suggesting this finding may be robust.

4.3 The effects of flow variability on *E. coli* dynamics

The effective analysis shows that hydrological variability controls dynamics of *E. coli* input and removal. There are substantial differences between *E. coli* loading and removal during high-flow and low-flow periods. Low flow conditions represented a negligible proportion of *E. coli* loads compared with high flow periods. *E. coli* concentration increased by several orders of magnitude during storm events. Greatly elevated concentrations combined with high discharge during storms dominated the transport of *E. coli* to downstream waters, which is consistent with previous studies (Jamwala et al. 2011; Chu et al. 2014; McKergow and Davies-Colley, 2010;
E. coli is shunted rapidly downstream during storm events. Decision makers should focus on the stormwater management practices to reduce E. coli loads during storm events.

High flow reduces water residence time in the MC and the relative importance of HZ exchange fluxes. The removal efficiency of both MC and HZ both decrease during storms. However, total removal integrated through time is highly related to the effective discharge of loading because this is when most E. coli is available for removal. Thus, even the network scale removal declines with increasing flow, on a total numbers basis, high flows are when most removal occurs. The effective discharge analysis suggests that most loading and most river network removal occur during the highest flow events, even though these were infrequent. However, the biggest impact in terms of percentage removal occurred during low flows. Since these flows occur most of the time, and when most recreational activity occurs, this removal is an important ecosystem service.

The effective discharge for E. coli loads differed from those for DIN loads estimated by Wollheim et al. (2008b). Effective discharge for DIN loads occurred at intermediate flows. However, E. coli transport was dominated by the largest discharges. The difference can be explained by concentration-to-discharge relationship. DIN tends to dilute with higher flow (Carey et al. 2014). Effective discharge for DIN occurs at medium flow with high frequency. Effective discharge for E. coli loading and removal occurs at high flow because E. coli concentration increases greatly with flow, while removal does not decline precipitously because exchange with sediments continues to occur. My study shows similar findings with Raymond et al. (2016), who found that even though the percentage of DOM removed during the less frequent larger storm scenario decreases, the total DOM consumed in the drainage network during these
events increases due to the larger transport of terrestrial DOM into the drainage network, and because the network as a whole can compensate for reduced removal in headwater streams. Wollheim et al. (2008b) also suggested that the network as a whole is able to remove a much greater proportion of inputs during higher flow periods.

4.4 The effects of watershed size and land use distribution on $E. \text{coli}$ removal

Watershed size and land use distribution both affected $E. \text{coli}$ removal, but watershed size had a greater effect on $E. \text{coli}$ removal. Model results indicate large rivers removed a greater proportion of total watershed $E. \text{coli}$ input than small streams. Previous studies found that large rivers have a stronger role in regulating pollutants, including nutrients (Hall et al. 2013; Wollheim et al. 2006), due to longer residence time. $E. \text{coli}$ removal depends largely on HZ exchange. More HZ exchange occurs when watershed area is large and with longer travel distance. This increasing removal occurs due to processing in larger rivers (Wollheim et al. 2006, Ensign and Doyle 2005). $E. \text{coli}$ not removed by headwaters eventually pass through large river segments and have probability to be removed, although removal efficiency is less in downstream reaches. Land use heterogeneity also controls $E. \text{coli}$ removal at the river network scale. Land use distribution is an important factor when comparing similar-sized watersheds (Mineau et al. 2015). Developed land distribution in the Winnicut has a SI of 1.09 indicating that developed land is skewed towards the watershed headwaters while other watersheds have SI lower than one indicating developed land is skewed towards the watershed outlet. The SI characterizes the spatial distribution of developed land within watersheds, and is related to the average residence time of runoff from developed land and the proportion of runoff that enters HZ in the river network, which together determine the potential for $E. \text{coli}$ removal. For example, the Oyster and
Winnicut have similar watershed sizes, but developed land in the Winnicut is located more towards headwaters than in the Oyster. As a result, the Winnicut has relatively higher *E. coli* removal, similar to what Mineau et al. (2015) found for nitrogen. Increasing watershed area with development skewed towards the headwater reduces the impacts of development on water quality regarding *E. coli* concentration.

**4.5 The ecosystem service of *E. coli* removal mitigates water quality impairment**

*E. coli* removal by river networks is an important ecosystem service that maintains freshwater quality. In general, the number of days where Class A and B standard were exceeded both decreased due to *E. coli* removal. The effect was greater at low flows and when land use was skewed toward the headwaters (Table 10). Large watersheds (Merrimack River and Penobscot River) had better capacity to regulate *E. coli* levels and maintained water quality. For small watersheds (Oyster River and Winnicut River), there was approximate 20% of probability for *E. coli* concentration to exceed the Class A standard even with the *E. coli* removal. During these exceedance levels, these water bodies are not suitable for drinking water use (required to meet Class A standard). Short travel distance results in less *E. coli* processing in small watersheds. In other words, small watersheds have less self-purification ability to meet water quality standards. Shellfish and beach managers should prioritize mitigation of these small watersheds with sources near the basin mouth, since the removal efficiency of these small watersheds is limited. More actions need to be taken in these small and developed watersheds to protect ecosystem services.

**4.6 Limitation**

Bacteria modeling is challenging since watershed specific data are often inadequate for characterizing sources, resulting in great uncertainties. A sensitivity analysis informs us the relative importance of the uncertain factors in determining the output of FIB concentration or
removal. The role of different sources, including wildlife distribution and densities, and septic systems failing rates are often unknown. Relatively few headwater streams have been sampled during storm events. The loading function in this study was based on limited data from three headwater sites, and uncertainty should also be considered. Nevertheless, the model resulted in reasonable estimates at downstream stations with the simple mixing generally higher than all observation across different New England watersheds. As a result, the loading function is reasonable in this study area.

The model presented in this study is a valuable tool for understanding *E. coli* removal, but it is limited by a number of uncertainties. Other models, including SWAT and HSPF, tended to over-predict *E. coli* concentration at lower measurement values and under-predict *E. coli* concentration at higher measurement values (Iudicello and Chin, 2014). These biases occurred probably because they did not account for exchange with HZ, which relatively important at base flows and ignored input to water column due to *E. coli* re-suspension at high flows (Niazi et al. 2015). Sedimentation is effective in substantially reducing *E. coli* and important in the overall *E. coli* disappearance in river impoundments (Ganno et al. 1983). FrAMES accounts for sedimentation processes through HZ exchange, but as with the above models, does not account for re-suspension. Sediments bring *E. coli* back into water column from the stream bottom under high flow conditions (Cho et al. 2010).

Pond, lake, and reservoir are not considered in this study. Reservoirs can attenuate short-term pulses in bacteria concentration because the large volume of water can dilute bacteria (Town, 2001) and longer residence time result in higher die-off proportions. In this study, FrAMES frequently over-predicted *E. coli* concentration during base flow conditions at Oyster
River. The sampling site (05-OYS) is located at a dam that creates a reservoir, Mill Pond, which has abundant aquatic vegetation. Previous studies (e.g., Chao et al. 2015) show that aquatic vegetation is an important factor influencing the hydraulic conditions of pond systems. Chao et al. (2015) found more sedimentation in the vegetated zone than in the non-vegetated zone due to smaller flow velocity. FrAMES does not account for aquatic vegetation effects on sedimentation and over-predicted \textit{E. coli} concentration in the water column. FrAMES also did not account for the longer residence time of the reservoir, which is would increase under low flow conditions when the greatest mismatch occurs.

There are a number of limitations associated with the assumptions of die-off processes. Other factors potentially affecting the removal of \textit{E. coli} are also not included in this study. Solar radiation can affect the survival of \textit{E. coli} in the water column (Whitehead et al. 2016), a mechanism which was not included in FrAMES. Further, the die-off rate was assumed independent of nutrient availability in this study. \textit{E. coli} can possibly re-grow if it is not nutrient-limited. FrAMES also did not consider point sources, which are important in some watersheds (e.g., the Cocheco, Merrimack). More data regarding these mechanisms is required. Nevertheless, the dynamics of the model compare favorably with observations, providing some measure of confidence in our results regarding the role of river networks in controlling downstream fluxes of pathogens.

The uncertainty is the role of the HZ in \textit{E. coli} removal resulted from limited information on removal rate in HZ (biological reaction kinetics) and variations of HZ coefficient with flow (physical transport kinetics). All \textit{E. coli} entering HZ was assumed to be removed. However, the persistence and survival of \textit{E. coli} in HZ is significantly influenced by a complex array of physical, chemical, and biological factors, including the growth and decay rates and the
concentration of available nutrients (Hipsey et al. 2008). Little is known about the HZ exchange coefficient change with flow both at-a-site and in the downstream direction. HZ exchange coefficient (α) was assumed constant through flow conditions and locations although it might be flow-dependent (Fox et al. 2016).

This study was unable to present the interaction of velocity (V) and cross sectional area (A\textsubscript{cross}). The current V equation and A\textsubscript{cross} equation were independent, and not constrained by continuity with discharge (i.e., Q = W*D*V). In the sensitivity analysis, when V was changed, A\textsubscript{cross} was kept constant, and visa versa. A result, the interpretation of the sensitivity analysis results should be taken with care. The modification of V affects A\textsubscript{cross}. The model did not reflect this constraint. Work is ongoing to properly constrain these variables, as they have been in previous studies (Wollheim et al. 2008a; Stewart et al. 2011, 2013). Qualitatively the results presented in this study will not change, that E. coli removal by the river network is an important consideration, and that the hyporheic zone is an important contributor to this ecosystem service.

5. Conclusion

The ecosystem service of water purification potentially makes an important contribution to human well-being. This study suggests that aquatic systems play a significant role in the ecosystem service of E. coli removal. E. coli removal processes in river systems are important, and are the combination of water column removal and filtering by sediments below flowing water (i.e., hyporheic zone). I have presented a novel model for E. coli fate and transport in river networks that accounts for MC removal and hyporheic zone (HZ) filtration. The goal of this study was to understand the ecosystem service of E. coli removal at the river network scale in MC and HZ across hydrologic conditions. This study found network scale removal is significant, that filtering by sediments removes more E. coli than breakdown in the water column alone.
Watershed size, land use distribution, and hydrology all control *E. coli* removal, but hydrology has the most significant impact. The attenuation efficiency of river networks increases as the flow decreases, but remains relatively high at higher flows common during critical summer periods. Although a certain amount of *E. coli* is removed, most *E. coli* reaches critical water bodies during high flows due to a combination of increased loading and reduced removal. This study found that the ecosystem service of *E. coli* removal reduce *E. coli* levels. The frequency of *E. coli* concentrations over accepted threshold levels increased considerably without the ecosystem service. These results have important implications for managing fecal coliform and other pathogens. Managers should factor the important role of river systems in their management decisions.
List of References


Stewart, R. J. et al., 2013. Horizontal cooling towers: riverine ecosystem services and the fate of thermoelectric heat in the contemporary Northeast US. *Environmental Research Letters*.


### Appendix A

*E. coli* concentration of loading function input

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## Appendix B

### Microbial Source Tracking present/absent results

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