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Modeling In- Stream Escherichia coli Concentrations

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Modeling in- stream *Escherichia coli* concentrations

by

Pramod Kumar Pandey

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Agricultural Engineering

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2012

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DEDICATION

I would like to dedicate this work to my mother, Sunaina Pandey, and father, Radhey Shyam Pandey, for their blessing.

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ABSTRACT

Predicting in-stream pathogen levels has long been known to be a challenging problem due to complex interactions between microorganisms and the natural stream environment, and the spatial heterogeneity involved in stream networks of a watershed. Here we have developed models for predicting *E. coli* (a pathogen indicator) in streams. In *E. coli* estimation, the first modeling approach uses Geographic Information Systems (GIS) based watershed indexes considering the undisturbed land cover, which encompasses the natural land cover area, wetlands, and vegetated stream corridors, and the disturbed land cover extent which includes areas receiving manure from confined animal feeding operations, tile-drained areas, and areas under cropped and urban land cover. The second approach involves developing mathematical models for calculating *E. coli* resuspension, deposition, in-stream routing, and growth in the streams. A hydrological model capable of predicting in-stream *E. coli* concentrations in the streambed sediment as well as in the water column was developed. In order to develop the hydrological model for predicting in-stream *E. coli* concentrations, firstly a model capable of predicting *E. coli* resuspension was formulated. Secondly, formulations for calculating in-stream *E. coli* routing, water temperature depended *E. coli* growth, and the streambed sediment and water column *E. coli* concentrations were developed. Finally, these formulations were programmed in FORTRAN language, and were integrated into the Soil and Water Assessment Tool (SWAT), a watershed scale hydrological model, written in FORTRAN. In addition to the model development, this study also involves monitoring *E. coli* concentrations in the streambed sediment and the water column

extensively starting from May 2009 to December 2011 in the Squaw Creek Watershed, Iowa, USA. The observations were used to verify the model predictions, and results indicated that the models performed well.

The GIS based approach developed here for estimating *E. coli* concentrations in streams can be potentially useful in predicting in-stream waterborne *E. coli* levels using watershed indexes. Approximately 95- 98% of the predictions were within 1 order magnitude of the observed values, when we used hydrologically corrected watershed indexes for *E. coli* estimation. The model skills varied from 0.39 to 0.55.

In *E. coli* resuspension model, approximately 81% of the predicted *E. coli* resuspension rates were within a factor of 2 of the inferred values (i.e., measured *E. coli*). All of the predicted resuspension rates were within a factor of 5 of the inferred values. The model skill value of 0.85 indicated that the model predicts *E. coli* resuspension rates successfully.

The application of the modified SWAT model in the Squaw Creek Watershed, which was developed here, performed well. For example, approximately 62% of the predicted streambed sediment *E. coli*, and 82% of the predicted water column *E. coli* concentrations were within 1 order magnitude of the measured concentrations. The R^2 for monthly average daily flow was 0.99, while for daily flow predictions R^2 was 0.42. The Nash-Sutcliffe's efficiency (*NSE*) for monthly average daily and daily flow predictions were 0.75 and 0.39, respectively.

We also developed a model for calculating in-stream total *E. coli* loads (i.e., contributions from the streambed as well as from free floating) in order to improve Total Maximum Daily Loads (TMDLs) estimation, and understand the potential impacts of streambed sediment *E.*

coli on total in-stream *E. coli* loads. While comparing the total predicted *E. coli* loads with the measured *E. coli* loads, coefficient of determination (R^2) was 0.82, and model skill was 0.78; these results indicate that the model for calculating total in-stream *E. coli* loads performed well, and should help in developing Total Maximum Daily Loads (TMDLs) for stream bacteria.

In addition to in-stream processes and overland flow, weather pattern can potentially impact in-stream *E. coli* concentrations. To understand the impacts of weather pattern on in-stream *E. coli* concentrations, *E. coli* observations in the streambed sediment and the water column (from two locations) were related with climate data (i.e., air temperature, soil temperature, solar radiation, and rainfall). The results show that increase in temperature increases *E. coli* concentrations not only in the water column but also in the streambed sediment. Moreover, *E. coli* in the streambed sediment remained elevated even at relatively lower temperature. These results signify that increase in ambient temperature can potentially increase *E. coli* levels in the water bodies.

The results from monitoring and modeling of in-stream *E. coli* presented here will have significant importance in developing Total Maximum Daily Loads (TMDL) for in-stream pathogens as well as predicting *E. coli* concentrations in the streams at the watershed scale.

CHAPTER 1. GENERAL INTRODUCTION

1. Introduction

In-stream pathogen contamination, which is often assessed by enumerating pathogen indicators such as *Escherichia coli* (*E. coli*) in stream water, is a major water quality concern in the United States of America (USA). For example, approximately 27% of the total rivers and streams (5,653,128 km) in the USA are assessed, and 53% of the assessed are impaired (USEPA, 2012). The leading cause of impairment is elevated pathogen levels in streams. The method by which pathogens proliferate in natural streams has long been understood to be a complex problem. Understanding pathogen transport in streams requires a symbiotic union of computational approaches and field observations. Emphasize on controlling in-stream pathogen contaminations necessitates an improvement of our understanding how the combined impacts of in-stream processes, climatic conditions, hydrology, land cover, and anthropogenic activities at the watershed scale influence stream water. This research integrates field observations and computational modeling approaches to improve our understanding of *E. coli* (a pathogen indicator) transport in the streams.

1.1 Goals and objectives

The overall goal of this research was to improve understanding of *E. coli* transport in natural streams. Here we have developed computational models, and carried out extensive monitoring of *E. coli* concentrations in the streambed sediment and the water column of the Squaw Creek Watershed, Iowa, USA, which will provide much needed information to improve Total Maximum Daily Loads (TMDLs) estimation, and hydrological models for predicting *E. coli* concentrations in the streams. In addition, we have used *E. coli* monitoring

data to understand the impacts of weather pattern on in-stream *E. coli* concentrations (i.e., in the streambed sediment and the water column).

The specific objectives of this study are to:

1. Assess the impacts of watershed indexes and precipitation on spatial in-stream *E. coli* concentrations.

Hypothesis: Waterborne *E. coli* concentrations in a stream can be estimated using the landscape (i.e., land cover) characteristics of the stream watershed.

2. Develop a model for predicting resuspension of *E. coli* from streambed sediments.

Hypothesis: In-stream *E. coli* resuspension can be calculated using the stream flow properties and characteristics of both cohesive and non-cohesive sediments.

3. Improve SWAT for developing TMDLs for bacteria.

Hypothesis: Integrating a pathogen transport model, capable of predicting *E. coli* concentrations in the streambed sediment and in the water column, into Soil and Water Assessment Tool (SWAT), can improve SWAT applicability for predicting in-stream *E. coli* concentrations at the watershed scale.

4. Assess the impacts of streambed sediment on in-stream total *E. coli* loads over a range of flow conditions and the streambed sediment *E. coli* concentrations.

Hypothesis: Current U.S. EPA methodology for assessing stream water pathogen contaminations, which relies solely on analysis of water samples, may underestimate in-stream pathogen loads.

5. Assess the impacts of weather pattern on in-stream *E. coli* concentrations.

Hypothesis: Weather pattern can have a potential impact on *E. coli* concentrations in the streambed sediment and the water column.

In order to improve our understanding of *E. coli* transport in natural streams, here we have developed several approaches (i.e., Geographical Information System (GIS), *E. coli* resuspension model, and hydrological model), and each approach has its own unique purpose. For example, the purpose of the first approach, which is described in chapter 1, was to create watershed indexes using Geographic Information System (GIS) software to understand the relationships between *E. coli* concentrations in the water column and disturbed and undisturbed natural land cover. These watershed indexes were used to calculate *E. coli* concentrations in stream water column. While the first approach does not include in-stream processes such as resuspension, deposition, and growth of *E. coli*, the second approach, which is described in chapter 3, incorporates in-stream processes. The primary purpose of the second approach was to develop a resuspension model capable of predicting *E. coli* release from the streambed to the water column. The predictions of this model were verified at 16 unique locations of the Squaw Creek Watershed. The *E. coli* resuspension model described in chapter 3 was further modified for improving hydrological model capable of predicting in-stream *E. coli* levels at the watershed scale, which is described in chapter 4. The pathogen transport model was formulated and written in FORTRAN language to improve Soil and Water Assessment Tool (SWAT) (hydrological model), which is explained in chapter 4. The predictions of modified SWAT model were verified using the field data collected at Squaw Creek Watershed. The model predicts *E. coli* concentrations in streambed sediment as well as in the water column. In addition, we developed a model to estimate total

pathogen loads in streams (i.e., *E. coli* loads in water column caused by streambed sediment under various flow conditions), which is described in chapter 5. The pathogen load model explains the impacts of streambed sediment and flow on water column *E. coli* concentrations. The model was used to quantify and compare pathogen loads in two different situations: 1) stream water meets existing EPA water quality standards, which do not include the impacts of streambed sediment on water column *E. coli*; and 2) streambed sediment impacts on water column *E. coli* was incorporated.

The research proposed here which includes field studies as well as computer modeling, will provide tools to predict *E. coli* concentrations in streambed sediment as well as in the water column, and will improve bacteria TMDL estimation.

1.1 Thesis organization

The research performed here is divided into five parts. The first part is focused on developing a Geographical Information System (GIS) based model for predicting in-stream waterborne *E. coli* concentrations, which is described in chapter 2, and the chapter has been published in *Ecological Indicator* (Pandey et al., 2012, vol. 23, 641 – 652). In order to calculate *E. coli* concentrations, watershed indexes considering the undisturbed land cover extent which encompasses the natural land cover area, wetlands, and vegetated stream corridors, and the disturbed land cover extent which includes areas receiving manure from confined animal feeding operations (CAFOs), tile-drained areas, and areas in cropped and urban land, were used. For developing and validating the model, waterborne *E. coli* concentrations, which were measured at 46 sampling locations in the Squaw Creek Watershed, Iowa, USA, by Squaw Creek Watershed Coalition, were used.

The second part of the research is focused on developing a model for predicting resuspension of *E. coli* from the streambed, which is described in chapter 3, and the chapter has been published in *Water Research* (Pandey et al., 2012, vol. 46, 115-126). A model for predicting *E. coli* resuspension from the streambed to the water column was developed. Sediment particle attached *E. coli* concentrations and erosion rates estimated from sediment transport theory were used to calculate *E. coli* resuspension rates. We monitored *E. coli* concentrations in the streambed sediment and the water column of the Squaw Creek Watershed at 16 sampling locations, which were used to verify the *E. coli* resuspension rates.

The third part of the research is concentrated on improving an existing watershed scale water quality model to improve Total Maximum Daily Loads (TMDLs) estimation for pathogen impaired waters, which is described in chapter 4. This chapter has been submitted as a final project report to the EPA Regions 7 (contract no. X7-97703701-1) “Improving SWAT for Developing TMDLs for Bacteria”, and has been approved. A manuscript based on this report is ready to be submitted for publication in *Water Research*. In this study, first a watershed-scale *E. coli* transport model, which includes in-stream routing, resuspension, deposition, and *E. coli* growth, and capable of predicting *E. coli* in the streambed sediment and the water column was developed. Second, the model was programmed in FORTRAN, and integrated with the existing Soil and Water Assessment Tool (SWAT), a hydrological model, to predict the *E. coli* concentrations in the streambed sediment as well as in the water column. Finally, the predictions were verified using monitored streambed sediment and water column *E. coli* concentrations of the Squaw Creek Watershed.

The fourth part of the research develops a model for predicting in-stream total *E. coli* loads, which is described in chapter 5, and ready to be submitted. The model was used to calculate in-stream total *E. coli* loads in the Squaw Creek Watershed under a range of flow conditions using particle attached *E. coli* concentrations, and water column *E. coli* concentrations. Here we have shown how in-stream *E. coli* loads are underestimated by ignoring the impacts of streambed sediment *E. coli*. This work emphasizes the need for improving the United States Environmental Protection Agency (USEPA) current water quality testing methodology, which currently relies solely on water borne *E. coli* concentrations to assess stream pathogen levels and identify impaired waters.

The fifth part assesses the impacts of weather pattern on in-stream *E. coli* concentrations, which is described in chapter 6, and a draft is submitted for rapid communication in *Water Resources Research*. In this work, *E. coli* measurements taken in the streambed sediment and the water column of the Squaw Creek Watershed were related to air temperature, soil temperature, solar radiation, and rainfall to investigate the impacts of temperature, solar radiation, and rainfall on in-stream *E. coli* levels. The work provides foreknowledge and evidences for a potential increase in pathogen contamination by increases in ambient temperatures.

2. Literature review

Water borne pathogen contamination in ambient water bodies and related diseases are a major water quality concern in all over the world. To find the current trends and possible future research directions in the field, this literature review provides a historical perspective of pathogen contaminations and elaborates on pathogen contaminations in ambient water

bodies. Besides this, we also discussed the state of art of pathogen survival under various environmental conditions. We present a short synopsis elaborating water resources development and global warming impacts on pathogen contaminations. Finally, we expound on future challenges and recommendations to address the issue of pathogen contaminations of ambient water bodies.

Pathogen contamination is a serious issue for almost all types of ambient water bodies (USEPA, 2012), therefore, understanding waterborne pathogen contamination in a relatively broader sense is necessary. As scientific evidence for warming of Earth climate is unequivocal (IPCC, 2007), it is important to understand how changes in weather patterns can potentially impact *E. coli* levels in ambient water bodies. To meet the future water demand for food, increasing water resource structures are necessary (Word Bank, 2010); however, these new water storage structures can also aggravate the public health risk, which also needs to be addressed.

We found that there is a clear need for studies that brings knowledge from different fields and aspects of pathogen contamination, and put them in a single place to present the problem as a whole. Therefore, our goal in this review is to present pathogen contamination problems at a relatively broad scale. We attempt to summarize the prevalence of potential health risks imposed by various ambient water bodies under different environmental conditions, and to find the current trends and possible future directions in this field. The study is divided into sections: 1) historical perspective of water borne pathogen contaminations; 2) health risks associated with water borne pathogens; 3) pathogen contaminations in various ambient water bodies; 4) environmental factors affecting pathogens survival in ambient water bodies; 5) the

impact of water resources development and climate warming on pathogen contamination; 6) future challenges and recommendations.

Each section has a specific objective. For example, the first provides historical studies elaborating early concern and pathogen related human causalities. The second section emphasizes potential health risks caused by water borne pathogens. The third is to describe pathogen contaminations in various ambient water bodies such as coastal waters, estuaries water, ground waters, stream waters, and lakes and reservoirs. The fourth focuses on the impacts of environmental factors (i.e., solar radiation, temperature, pH, predators, dissolved oxygen, salinity, protienaceous material, and solid attachment) on pathogen survival. The fifth is to render a short synopsis of water resources development and global warming impacts on pathogen contaminations; and the sixth section expound on future challenges and recommendations.

2.1 Historical perspective of water borne pathogen contaminations

Issues of water contamination have a long history and there are descriptions in Sushruta Samshita, a foundation text on ayurvedic medicine (Indian traditional medicine), about water borne diseases resembling cholera from 500 to 400 B.C. in India (Colwell, 1996). The pathogens such as *Vibrio Cholera*, which causes cholera, infects several million people each year (Nelson et al., 2009). There have been several historical cholera pandemics. The first occurred between 1817 and 1823, resulting in the deaths of 10,000 British troops and countless Indian deaths. It later spread across China, Indonesia, and the Caspian Sea totaling more than 100,000 deaths. The second cholera pandemic between 1829 and 1851, which

began in Russia, caused 100,000 deaths in Hungary, and it later spread across the Atlantic to New York on 23 June 1832 (Colwell, 1996).

The classical map of cholera deaths in London in the 1840s, which was produced by Dr. John Snow (Snow, 1854), a physician to Queen Victoria, shows potential health risks caused by contaminated water. In his investigation, Dr. Snow hypothesized that the cause of cholera was drinking water from the well on Broad Street, London. This was the first instance on record of the implementation of an appropriate measure to prevent the transmission of waterborne pathogens (Colwell, 1996; Okun, 1996). Also this was the first work, which used a geographic method to show the spread and epicenter of cholera, which resulted in locating a contaminated water body, responsible for spreading the disease. His tracking of death rates caused by contaminated water, was an important observation in the understanding of the epidemiology of waterborne diseases (Colwell, 1996).

Besides Dr. Snow's study, there are several other noteworthy works. For example, the first study on the longevity of the typhoid bacillus in water (Jordan et al., 1904), which showed the viability of the typhoid bacillus in contaminated water. On the request of the sanitary districts of Chicago, authors examined the life of typhoid bacillus in the waters of Lake Michigan, the Chicago Drainage Canal, and the Illinois River. The purpose of the study was to understand if typhoid bacillus could survive during water transport from the Chicago Drainage Canal to the mouth of the Illinois River. The authors concluded that the typhoid germ loses its vitality over time. Ruediger (1911) reported that typhoid bacilli disappear from polluted river water faster in summer than in winter, when the river is

covered with ice and snow, which indicates that winter seasons prolong survival of pathogens (Phelps, 1914).

The prevalence of infectious diseases which were potentially transmitted by recreational water contact was presented by the Committee on Bathing Places (Simons et al., 1922), who gathered the information on the methods employed at different bathing places for washing and disinfecting suits and towels. The study found that infection was caused by infected water at the bathing places. A similar study by Winslow and Moxon (1928) found that the bathing beach water of New Haven Harbor was highly polluted. This study surveyed the harbor waters of New Haven, Connecticut, and suggested that the average coliform count in bathing water should not exceed 100 counts per 100 ml in order to be considered as safe water. The author's suggestion was used to convince the New Haven authorities to develop a sewage treatment plant to eliminate the discharge of crude sewage water into the Harbor.

A similar study by Rubentschik et al. (1936) for salt lakes (limans) found that a large number of pathogens were absorbed in lake sediment. In the 1920s, the American Public Health Association reviewed the incidences of disease associated with the use of recreation water, and proposed recommendations to control pathogen contaminations. A review study on pathogen contamination by Rudolfs et al. (1950) provided an excellent summary of early work on the occurrence and survival of pathogenic bacteria in soil, water, sewage sludge and vegetation.

Moore (1954) carried out a bacteriological survey of beach pollution in the summer of 1948 and 1950, and discovered that the sewer system was a primary source of coliform organisms in the sea water. Many other notable studies such as the studies on sewage contamination for

coastal bathing waters in England and Wales (The Committee on Bathing Beach Contamination of the Public Health Laboratory, 1959) also described the potential health risks caused by pathogens in the coastal environment. Other relatively recent studies describing pathogen contamination in various water bodies (i.e., streams, reservoirs, lakes) are described in later sections of this study.

2.2 Health risks associated with water borne pathogens

The unsafe levels of pathogens in ambient water bodies are a major cause of water contaminations, which causes public health risks. According to the World Health Organization (WHO), over 2.6 billion people lack access to clean water, which is responsible for about 2.2 million deaths annually, of which 1.4 million are children (WHO, 2010). Improving water quality could reduce about 4% of the global disease burden (WHO, 2010). The WHO estimated that 88% of that burden is attributable to contaminated water.

In order to improve people's livelihood, the United Nations envisioned Millennium Development Goals (MDGs)—eight international development goals—and improving water quality is one of them. The target is to reduce the number of people without access to safe water by 50% by the year 2015 (WHO, 2011). To achieve this target, it is imperative to understand how pathogen contaminations impact ambient water bodies, and what the potential sources of contaminations are. Improving water quality of the ambient water bodies, particularly controlling pathogen levels, is a viable option for achieving MDGs.

Water borne diseases (i.e., diarrhea, gastrointestinal illness) caused by various bacteria, viruses, and protozoa were the reasons for many of the outbreaks (Craun et al., 2006). In

developing countries such as Africa, waterborne diseases infect millions (Fenwick, 2006). Even in the United States, these diseases are a major cause of illnesses. A study by Craun et al. (2006) reported statistics on waterborne outbreaks in the U.S., which shows that at least 1870 outbreaks (23 per year) occurred between 1920 and 2002.

A relatively recent report of the U.S. Environmental Protection Agency (EPA) estimated that pathogens impair 480,000 km of rivers and shorelines and 2 million ha of lakes of the U.S. (USEPA 2010a). Approximately 900,000 illnesses and 900 deaths each year are reported in the U.S. because of exposure to water-borne pathogens (Arnone and Walling, 2007). Besides acute gastroenteritis, a major etiological agent, many others such as *Giardia*, *Cryptosporidium*, *E. coli* O157:H7, *V. cholera*, and *Salmonella* were the grounds for many outbreaks (Craun et al., 2006). In mid and late 18th century diseases such as cholera, infected millions of people all over the world (Colwell, 1996). Studies [for example, Jordan et al. (1904), Ruediger (1911), Simons et al. (1922), and Rudolfs et al. (1950)] provided excellent reviews on incidences during the early 19th century.

Relatively newer studies [for example Diffey (1991), Brookes et al. (2004), Jamieson et al. (2004), Gerba and Smith (2005), Gerba and McLeod (1976), John and Rose (2005), Hipsey et al. (2008), and Pachepsky and Shelton (2011)] have reviewed the current state of art and advancement in this field, particularly, for freshwater and estuarine sediments. However, there is a gap in studies. Besides this, many of the current reviews are on specific water bodies, for instance, John and Rose (2005) focuses on ground water, Brookes (2004) focuses on reservoirs and lakes, and Jamieson et al. (2004) focuses on agriculture watershed. Others, for example, Kay et al. (2007) reviewed on catchment microbial dynamics.

2.3 Pathogen contaminations in ambient water bodies

In the previous section, we provided the review/case studies from the late 18th century and the mid-19th century, concerning water borne pathogen contamination, and related issues. In this section, we describe pathogen contamination problems in various ambient water bodies such as coastal environment, estuaries, groundwater, streams, and reservoirs and lakes. This section will review studies focused on pathogen survival in various water bodies, and potential sources. In Table 1.1, previous studies relevant to pathogen contamination are categorized by the ambient water bodies.

2.3.1 Coastal environment

In the U.S., pathogens are a leading cause of impairments of coastal environments; urban runoff and sewers have been identified as the primary source of coastal water impairments (Arnone and Walling, 2007). The studies elaborating pathogens contamination in coastal environments are summarized in Table 1.1. A study by Rippey (1994) reported about 400 outbreaks and 14,000 cases caused by pathogen contaminated coastal water since the late 1800s in the USA. Impairments in coastal environments have major economic impacts on the U.S. For example, losses caused by bacterial contamination in Massachusetts are more than \$75 million each year (Weiskelet al., 1996; Arnone and Walling, 2007).

The sources of coastal water contamination are: point discharges of treated and untreated sewage from shoreline outfalls, and non-point discharges. The non-point sources such as runoff from naturally vegetated areas discharges pathogens into coastal water. Besides runoff from vegetated areas, the storm water runoff from urban, commercial, and industrial land

also discharges pathogens into coastal water. Other sources such as malfunctioning or poorly-sited septic systems can also introduce significant amounts of pathogens (Sayler et al., 1975; Howe et al., 2002). A study by Weiskel et al. (1996) reported that the direct deposition of waterfowl feces is the considerable source for pathogens. A review paper by Fayer and Trout (2005) summarizes the transport of various pathogens such as *Giardia*, *Toxoplasma*, and *Cryptosporidium*—zoonotic parasites in the coastal environment.

The direct discharge of storm water runoff to coastal waters through storm drain systems could cause pathogen contaminations; even where separate storm and sanitary sewer systems are in place. Weiskel et al. (1996) found that about 16% of the total fecal coliform inputs were caused by storm water entering Buttermilk Bay in Massachusetts. Coastal streams draining largely undeveloped watersheds with extensive riparian wetlands can be the natural sources of fecal bacteria to coastal waters. On-site septic systems contribute significant amounts of fecal bacteria to coastal waters in low-lying, fine-grained geological settings where saturated soils enhance bacteria growth. Weiskel et al. (1996) reported that wrack deposits in shoreline could act as a reservoir of fecal bacteria, and removal of wrack deposits from inter tidal zone can improve the water quality of adjacent coastal waters.

2.3.2 Estuaries

Human activities have impacted estuaries significantly as they are often adjacent to populated areas, and often provide a means of transportation and receive substantial recreational use (Schriewer et al., 2010). The studies describing pathogen sources in estuaries and potential survival are summarized in Table 1.1. Municipal point sources are the primary cause of pathogen contaminations in estuaries. Urban water disposed through combined sewer

outflows is the cause of approximately 12% of estuary impairments in the U.S. (Arnone and Walling, 2007). Pathogens such as *Vibrio vulnificus*, that carries the highest fatality rate of any food-borne pathogen in the U.S., were detected in Gulf of Mexico Estuary (Lipp et al., 2001; Rippey, 1994; Baker-Austin et al., 2009). The presence of other pathogens such as *Bacteroidale* is also reported in the water samples of 10 major rivers and estuaries within the Monterey Bay Region (Schriewer et al., 2010). The most common pathogens previously identified in estuaries by Rhodes and Kator (1988) were *Vibrio cholerae*, *Giardia*, *Cryptosporidium*, *Salmonella*, and *Campylobacter* spp. The researchers compared die-off of various pathogens in estuaries water and determined that die-off of *Salmonella* was lower than *E. coli*, which means that *E. coli* concentrations may not indicate true levels of salmonella in waters.

2.3.3 Ground water

Groundwater is heavily used all over the world as the primary source for domestic drinking water supplies. Nationally, 40% of the U.S. domestic water supply originates from groundwater, and over 40 million people use groundwater as their drinking water via private wells (Alley et al., 1999; John and Rose, 2005). Groundwater pathogen contaminations has led to numerous disease outbreaks in the U.S., for example, at least 46 outbreaks of disease occurred between 1992 and 1999; resulting in 2,739 cases of illness and several deaths (John and Rose, 2005). These are reported cases; however, actual occurrence could be higher.

Table 1.1 summarizes studies relevant to pathogen contaminations in ground water.

Controlling groundwater pathogen contamination has recently been emphasized in many countries. For example, identifying the sources of ground water pathogen contamination has

received significant importance in Australia. Many studies reported that the health risks caused by chlorine-resistant protozoa such as *Cryptosporidium* spp (Ferguson et al., 2003; Kay et al., 2007; Kay et al., 2008) are considerable. Also in the UK, regulators have expressed concern; the main concern was that unlined wetlands might cause pathogen contamination to groundwater (Kay et al., 2007). Similarly, the European Union (EU) has emphasized protecting ground water from pathogen contaminations.

Pathogen contaminated ground water can cause pollution in coastal environments. For example, a study of Buttermilk Bay has shown that groundwater is capable of transporting a large amount of pathogens from surface to coastal waters either by direct discharge or by discharge to streams that flow into the Bay (Moog, 1987; Weiskel et al., 1996). The risk of contaminating groundwater increases particularly in areas where shallow aquifers exist. In these situations it is more likely that contaminated surface water or water from septic tanks could reach to groundwater (Weiskel et al., 1996; John and Rose, 2005).

It has been found that precipitation events increase groundwater pathogen contaminations (John and Rose, 2005), which could be the result of increased recharge of contaminated ground water during rainy seasons. Besides, ground water can also be contaminated by seepage and percolation of contaminated water from the vadose zone (Darnault et al., 2004). The macropores of agriculturally managed soils may play a considerable role in polluting ground water, particularly from the fields where manure is applied (Jamieson et al., 2002).

2.3.4 Streams

Pathogen contamination is a major cause of stream impairments. The sources of impairment, and health risks induced by water borne pathogens are extensively reported (Table 1.1). In the U.S. pathogen contamination is the leading cause of stream water pollutions. The EPA's National Water Quality Inventory Report suggests that about 53% of the assessed streams (USEPA, 2012) are impaired, and majority of them are contaminated by pathogens. The cost to implement total maximum daily load (TMDL), a plan to improve stream water, is estimated as \$0.9 to \$4.3 billion per year (USEPA, 2010b). According to USEPA, the elevated levels of pathogen are the leading cause of impairment in Iowa streams as well as in the streams of the USA.

Pathogen influxes into streams from agriculture land are the main cause of stream impairments (USEPA, 2012). Weak understanding on pathogen transport from agricultural

Table 1.1. Studies describing contamination in ambient water bodies

Type of water	Author (s) year	Organisms	Results	Remarks
Coastal water				
	Gerba and McLeod (1976)	<i>E. coli</i> , fecal coliform	Longer survival in sediment presence	Sediment influence in pathogen survival
	Goyal et al. (1977)	Total coliform, fecal coliform	Sediments of shallow canal can act as reservoir	Pathogens distribution in water and sediment
	Kapuscinski and Mitchell (1983)	<i>E. coli</i> , bacteriophages	<i>E. coli</i> survive longer than bacteriophages	Survival of pathogens in sunlight
	Rao et al. (1984)	Enteroviruses (Polio and Rota)	Abundance viruses were attached with sediment	Viruses distribution in water and sediment
	Gonzalez et al. (1990)	<i>E. coli</i> and <i>E. faecalis</i>	<i>E. faecalis</i> digestion was higher than <i>E. coli</i>	Predators digestion rate in fresh and marine water
	Alkan et al. (1995)	<i>E. coli</i> and enterococci	Light intensity influenced bacterial die-off	Die-off on solar radiation and environmental factors
	Weiskel et al. (1996)	Fecal coliform	Waterfowl major	Pathogen source and

Table 1.1. (continued)

Type of water	Author (s) year	Organisms	Results	Remarks
			source of fecal coliform inputs	transport pathways
	Sinton et al. (1999)	Bacteria and Fecal bacteriophages	Somatic coliphages shown prolonged survival	Sunlight influence in sewage-polluted seawater
	Solo-Gabriele et al. (2000)	<i>E. coli</i>	Riverbanks as the primary pathogen source	Sources of pathogens in subtropical environment
	Griffin et al. (2003)	<i>Adenoviridae</i> , <i>Caliciviridae</i>	Human viruses released by human sewage responsible for viral	Pathogenic human viruses transmitted via the fecal-oral route.
	Nasser et al. (2003)	<i>Cryptosporidium</i> , viruses, <i>E. coli</i>	<i>E. coli</i> die-off was faster than other pathogens	Comparative survival of various pathogens
	Schriewer et al. (2010)	<i>Bacteroidales</i> , fecal indicator, protozoa, bacteria	<i>Bacteroidales</i> have shown higher predictive skill than fecal indicators	<i>Bacteroidales</i> as a predictor of pathogens in coastal water

Table 1.1. (continued)

Type of water	Author (s) year	Organisms	Results	Remarks
Estuaries water				
	Ketchum et al. (1952)	Coliform, zooplankton	Sactericidal and predation caused coliform die-off	Processes responsible for pathogens decrease
	Smith et al. (1978)	Echovirus 1, coxsackieviruses	Sediment prolonged viruses survival	Persistence of pathogen viruses
	Anderson et al. (1983)	<i>E. coli</i>	Survival was related with temperature and eukaryote	Seasonal variation in pathogen survival in diffusion chamber
	Rhodes and Kator (1990)	<i>E. coli</i>	Mortality rises in sunlight	Indigenous microbiota and sunlight influence
	White et al. (1998)	<i>Perkinsus marinus</i>	Use of Kriging analysis for disease prevalence	Use of GIS in pathogen distribution analysis
	Lipp et al. (2001)	<i>Vibrio vulnificus</i>	Salinity controls the distribution of pathogens	Distribution of human pathogens
	Desmarais et al. (2002)	<i>E. coli, C. perfringens</i>	Pathogens re-grown with tides and sterile	Influence of soil on fecal indicator in tidally

Table 1.1. (continued)

Type of water	Author (s) year	Organisms	Results	Remarks
	Frias-Lopez et al. (2002)	<i>Clostridium</i> , <i>Campylobacter</i> , <i>Arobacter</i>	sediment Pathogen partitioning was found in surface and overlying water	influenced environment Bacterial communities partitioning between sea water, dead coral surface
	Chandran and Mohamed (2005)	<i>E. coli</i> , <i>Salmonella</i>	<i>E. coli</i> shows better survival capacity than salmonella	Relative survival in microcosm studies
	Baker-Austin et al. (2009)	<i>Vibrio vulnificus</i>	Prevalence of antibiotic resistance in a human pathogen	Multi-site analysis shows widespread antibiotic resistance in Pathogens
<hr/>				
Ground water	Bitton et al. (1983)	<i>Salmonella</i> , <i>E. coli</i> , <i>S. faecalis</i> , <i>enteroviruses</i>	<i>S. faecalis</i> decay rate was similar to viruses	Survival of pathogens in ground water
	Schijven and Hassanizadeh (2000)	Viruses	Viruses attachment with soil was influenced by pH, and favorable sites	Soil passage impacts in virus removal at field scale

Table 1.1. (continued)

Type of water	Author (s) year	Organisms	Results	Remarks
	Gordon and Toze (2003)	Bacteriophages, <i>E. coli</i> , viruses	<i>E. coli</i> and viruses shown maximum mortality in presence of predators	Ground water characteristics influence on survival of pathogens
	Pang et al. (2004)	<i>E. coli</i> and F-RNA phages	Pathogens were sorbed in aquifer material	Transport of <i>E. coli</i> and F-RNA phages
	Nevecherya et al. (2005)	Salmonella, viruses, <i>E. coli</i> , shigellos	Mathematical model was derived for temperature depends inactivation rate	Pathogenic bacteria and viruses survival in ground water
	Filip and Demnerova (2009)	<i>Bacillus megaterium</i> and <i>Staphylococcus</i>	Pathogens survived 10 to 100 days	Pathogens survival in ground water; FT-IR characterization
	Grisey et al. (2010)	Total coliforms, <i>E. coli</i> , <i>Enterococci</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella</i> and <i>Staphylococcus aureus</i>	Bacterial density monitoring coupled with artificial trace experiments proved useful in locating pathogens sources	Pathogens survival in groundwater and landfill leachate

Table 1.1. (continued)

Type of water	Author (s) year	Organisms	Results	Remarks
Streams				
	Chin (2010)	Fecal coliform	Summer and rainfall raises pathogens	Urban areas impacts in stream pathogens
	Smith et al. (1973)	Salmonela, fecal coliforms, streptococci	Salmonella decline was close to fecal coliform	Usefulness of indicators organisms
	Burton et al. (1987)	<i>Pseudomonas aeruginosa</i> , <i>Salmonella newport</i> , <i>E. coli</i>	Clay in sediments improves <i>E. coli</i> survival	Survival of pathogens in fresh water sediments
	Smith et al. (1987)	<i>E. coli</i> , Fecal bacteria	U.S. rivers shows decline in fecal indicator	Water quality assessment from 1974 to 1981
	Terzieva and McFeters (1991)	<i>E. coli</i> , <i>Campylobacter jejuni</i> , <i>Yersinia enterocolitica</i>	Temperate zone surface water serve as a persistent vehicle in transmission of bacteria between animals and humans	Survival and injury study of bacteria in agricultural surface water
	McFeters and Terzieva (1991)	<i>Yersinia enterocolitica</i> and <i>E. coli</i>	Experimental design and environmental play major role in pathogens survival	Survival in stream water, comparison between field and lab studies
	Fraser et al. (1998)	Fecal coliform	Model predicted pathogens	Modelling non-point source pollution

Table 1.1. (continued)

Type of water	Author (s) year	Organisms	Results	Remarks
Lake & reservoirs				
	Beaver and Crisman (1989)	Ciliates	Grazing habits of ciliates are discussed	Predators roles in fresh water
	Rubentschik et al. (1936)	<i>Serratia marcescens</i>	Adsorption of bacteria could be questionable	Adsorption of bacteria in salt lakes
	Mac Kenzie et al. (1994)	<i>Cryptosporidium</i>	<i>C. oocysts</i> study passes through the filtration system of water supply	Contaminated water from Milwaukee lake caused outbreak
	Wcislo and Chrost (2000)	<i>E. coli</i>	Predators controlled pathogen levels	Survival of pathogens in man-made reservoir
	Kistemann et al. (2002)	<i>E. coli</i> , coliform, fecal streptococcal, and <i>Clostridium perfringens</i>	Most of the pathogens increases during extreme runoff events	Microbial load in drinking water reservoir during rainfall events
	Howe et al. (2002)	<i>Cryptosporidium oocysts</i>	Animal feces was a major source of pathogens	Water supply's oocysts caused outbreak in northern England
	Ishii et al. (2006)	<i>E. coli</i>	<i>E. coli</i> survived longer in soil	Presence and growth of <i>E. coli</i> in Lake superior watershed

land is considered the major challenge in implementing corrective measures to improve stream water quality. For example, it is difficult to identify the origin point of pathogens and the pathway in which they enter the stream. Pathogens may enter into streams from many potential sources including lateral inputs from pastures and riparian zones, the influx of pathogen-contaminated ground water, direct deposit of fecal matter from livestock and wildlife, discharge of contaminated sanitary sewer flows, and wastewater treatment plant effluent.

Controlling pathogen contaminations from livestock is challenging. For example, there is doubt that pathogen contamination can be prevented by fencing off riparian buffers, and if buffers are useful in controlling stream water pathogens, we are not certain about the width they must be (Nagels et al., 2002). There are review studies, which elaborated the stream water pathogen contamination (Jamieson et al., 2004; Pachepsky et al., 2006). Many studies have emphasized using mathematical models to understand pathogen transport in stream water (Kim et al., 2010; Muirhead et al., 2004; Jamieson et al., 2005a; Jamieson et al., 2005b).

2.3.5 Reservoirs & Lakes

Studies, which have shown the threat of pathogen contaminations in lakes and reservoirs, are summarized in Table 1.1. In many countries surface reservoirs serve as the main source of drinking water, and these surface water bodies are often vulnerable to pathogen contamination (Kistemann et al., 2002). In the developed world, although there is increased awareness of water quality and water treatment for pathogen contamination, outbreaks of

water-borne disease via public water supplies continue to be reported (Gibson et al., 1998; Howe et al., 2002; Brookes et al., 2004).

For example, during the spring of 1993 an estimated 403,000 residents of the greater Milwaukee, Wisconsin area, experienced gastrointestinal illness due to infection with the parasite *Cryptosporidium parvum* following contamination of the city's water supply, which was associated with inadequate filtration of contaminated water from Lake Michigan (Mac Kenzie et al., 1994; Cicirello et al., 1997). In the 1990s, *Cryptosporidiosis* became the most common cause of outbreaks associated with public drinking water supplies in the United Kingdom (Howe et al., 2002). In developing countries, it is difficult to estimate the exact morbidity and mortality of diseases caused by water borne pathogens because the surveillance systems are rudimentary, and many cases are not reported; however, diseases such as diarrhea and cholera are the leading cause of morbidity (Nelson et al., 2009). Overall, diarrhea disease associated with drinking water is responsible for 2 to 2.5 million deaths annually (Fenwick, 2006).

In lakes and reservoirs, increased pathogens are often associated with storm events, and the stream inflow is considered to be the major source of pathogens. During rainy seasons, the influx of contaminated water from streams to lakes and reservoirs can increase pathogen levels substantially (Kistemann et al., 2002). The quantity of pathogen influxes from lakes' and reservoirs' tributaries during rainy seasons is of particular importance in determining pathogen transport and distribution (Brookes et al., 2004).

2.4 Factors affecting pathogen survival and transport

Previous sections described the pathogen contaminations in various ambient water bodies and potential sources. Factors affecting pathogen survival and transport in ambient water bodies are of a great interest, which is discussed in this section. The pathogen contamination in water bodies, which are used by public either for drinking water or recreational purposes causes health risks to human health as well as significant economic losses (Scott et al., 2002). Numerous review studies describing environmental factors impacts on pathogen survival are available (Brookes et al., 2004; Fayer and Trout, 2005; Gerba, 2005; Hipsey et al., 2008). In this section, we discussed major environmental factors, which impacts pathogen survival and growth. Modeling approaches for estimating growth and survival are also discussed. Previous studies summarizing potential impacts of environment on waterborne pathogens are categorized by environmental factors in Table 1.2.

Pathogen concentrations in water bodies are influenced by many environmental factors. The effect of these factors may vary with season and the type of ambient water bodies (Van Donsel et al., 1967; Gallagher and Spino, 1968; Niemi, 1976). For example, in stream water, temperature is considered to be the governing factor in *E. coli* survival (McFeters and Stuart, 1972); however, in groundwater and reservoir, the presence of predators controls their survival (Wcislo and Chrost, 2000; Gordon and Toze, 2003; John and Rose, 2005).

2.4.1 Solar radiation

Solar radiation is considered to be the most important factor that influences the survival of pathogens, however, the influence may vary with depth of water, type of water bodies, and

type of pathogen (Sinton et al., 2002). For example, sunlight inactivation rates vary. From greatest to least they are: coliforms > enterococci > F-RNA phages > somatic coliphages (Davies-Colley et al., 1999; Davies-Colley et al., 1994; Davies-Colley et al., 2000). Another study reported the sunlight inactivation rate as: enterococci > fecal coliforms > *E. coli* > somatic coliphages > F-RNA phages (Sinton et al., 2002). The authors found that inactivation rates depend on cumulative solar radiation (insolation), and inactivation rates were more than 10 times higher than the corresponding dark inactivation rates in enclosed (control) chambers.

Table 1.2 summarizes the studies describing the relationship between sunlight and pathogen inactivation. Previous studies have reported that the incoming solar radiation (insolation) is arguably the most crucial in the inactivation of *E. coli* and enterococci in water (Whitman et al., 2004). As solar light has high impact on pathogen survival, the current recommendation for maximum *E. coli* density in freshwater (235 CFU/ 100 ml) could be biased. To protect swimmers effectively from potential waterborne infection, criteria should take into consideration two factors—time of day and amount of insolation—that influence *E. coli* counts in the water (Whitman et al., 2004).

The simplest type of function to estimate the solar radiation impact in pathogen survival, for a given exposure is formulated as (Diffey, 1991):

$$S = S_0 \text{EXP}(-\gamma D) \quad (1)$$

where S_0 is the initial number of cells, D is the ultraviolet dose and γ is factor that characterizes biological sensitivity. To estimate the vertical attenuation of UV light in water,

the Beer's law, which is an exponential function, is often used; this involves the attenuation coefficient and depth of water (Brookes et al., 2004; Tung et al., 2007).

The above simple function (Eq 1) is valid if a single harmful event (hit) is sufficient to inactivate a cell, however, very often inactivation of a single cell requires more than one hit and mathematical treatment of this condition leads to shoulder survival conditions (Harm, 1980; Diffey, 1991). Considering shoulder conditions, a two parameters multi-target kinetic expression is proposed as follows (Sinton et al., 1999; Sinton et al., 2002):

$$P_s = 100 \{1 - [1 - e^{-k_s S}]^{n_s}\} \quad (2)$$

where P_s is survival percentage under shoulder conditions, S is insolation (MJ m^{-2}), k_s is an inactivation coefficient ($\text{m}^2 \text{KJ}^{-1}$), and n_s is a dimensionless parameter quantifying the size of the shoulder. The inactivation coefficient was obtained from the slope of the inactivation curve (Sinton et al., 2002).

2.4.2 Temperature

Temperature influence in the growth and inactivation of various viruses and pathogens is reported extensively (Robertson et al., 1992; Jenkins et al., 1997; Walker and Stedinger, 1999), and considered to be the only well-defined factor with consistent effects on virus survival (Gerba, 2005). We have summarized previous studies, describing temperature impacts on pathogen survival in Table 1.2. Various studies found that inactivation of pathogens increases with high temperature, with greater inactivation rates above 20°C . The possible mechanism for high virus inactivation at high temperatures are reported to be more rapid denaturation of viral capsid proteins or potential degradation by extracellular enzymes

Table 1.2. Influence of environmental factors on pathogen survival and growth

Factors	Author(s) (year)	Organisms	Results	Remarks
Sun light				
	Harm (1969)	<i>E. coli</i> strains B _{s-1} and AB 2480	300 nm wavelength was more sensitive to strain AB 2480 than B _{s-1}	Lab scale; sunlight germicidal activity
	Webb (1978), Webb and Brown (1976), Webb and	<i>E. coli</i>	Radiation of 365 nm and 460 nm inactivated <i>E. coli</i>	Lab scale; sensitivity to UV, near UV and visible radiation
	Brown (1977) Kapuscinski and Mitchell (1983) Diffey (1991)	<i>E. coli</i> , MS2, øx-174, and T7 Pathogens	<i>E. coli</i> died more rapidly than other populations Time, seasons, clouds, reflection, altitude impacts pathogens	Lab scale; sunlight induced mortality. Review; solar ultraviolet radiation effects on pathogens
	Sinton et al. (1999)	Somatic coliphages, bacterio-phages	Somatic coliphages exhibited superior survival	Sunlight inactivation of pathogens in sewage
	Davies-Colley et al. (1999)	Enterococci, F-RNA phages, <i>E. coli</i>	Sunlight was crucial in pathogens disinfections	Compared to DO and pH, sunlight was more influential

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
	Sinton et al. (1994)	Fecal coliforms and enterococci	Solar spectrum between 318 and 340 nm and > 400nm caused most inactivation	Lab scale; inactivation of pathogens from sewage and meat works
	Craik et al. (2001), Craik et al. (2000)	<i>Cryptosporidium parvum</i> oocysts, <i>Giardia muris</i> cysts	Inactivation was found to be very sensitive to UV dose.	Lab scale; Medium pressure ultraviolet radiation impact on survival
	Sinton et al. (2002)	Fecal coliforms, <i>E. coli</i> , somatic coliphages, F-RNA phages	Resistance of Somatic coliphages and F-RNA was superior in sea and fresh water, respectively	Lab scale; sunlight inactivation of pathogens from sewage plant effluent in fresh and saline waters
	Whitman et al. (2004)	<i>E. coli</i>	Solar radiation controls the natural mortality	In-situ microcosms; survival study
	Chandran and Mohamed (2005)	<i>E. coli</i> and Salmonella	<i>E. coli</i> survived better than salmonella	Microcosm studies; relative survival

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
	Maiga et al. (2009)	<i>E. coli</i> and enterococci	Sunlight increases the mortality of both indicators	Microcosm studies; dark and real sunlight
Temperature				
	Van Donsel et al. (1967)	<i>E. coli</i> , Streptococcus	In autumn, streptococcus survive longer than <i>E. coli</i>	Shaded and exposed outdoor soil plots experiment
	Niemi (1976)	<i>E. coli</i> , phage T7	Survival varies with water types and seasons	Survival in different water types
	Gordon and Terzieva (1991)	<i>E. coli</i> , <i>Yersinia enterocolitica</i>	High injuries cause a rapid decrease in <i>E. coli</i>	Lab scale; pathogenic and nonpathogenic bacteria in stream
	Solic and Krstulovic (1992)	Fecal coliform	Environmental influenced mortality of pathogens	Lab scale; pH, salinity, solar radiation and temperature
	Abdul-Raouf et al. (1993)	<i>E. coli</i> 0157:H7	Unchanged population at 5 °C, however, increased between at 21 and 30 °C	Lab scale; pathogen survival in ground and roasted beef

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
	Presser et al. (1998)	<i>E. coli</i>	Change in temperature influenced	Lab scale; survival and modeling
	Salter et al. (2000)	<i>E. coli</i>	Regression growth model predicted the effect of temperature and NaCl	Modeling the combined effect of temperature and NaCl on pathogen growth
	Panagou et al. (2003)	<i>Monascus ruber</i> (a fungus)	Predicted influence of temperature, pH on fungus growth	Experiment; modeling growth of heat resistance fungus
	Ross et al. (2003)	<i>E. coli</i>	Effect of temperature, water activity, pH, and lactic acid on <i>E. coli</i> growth	Square root-type model describing <i>E. coli</i> growth.
	King et al. (2005a)	<i>Cryptosporidium</i> oocysts	Mortality increased at temperatures > 15 °C, and predators reduced oocysts	Lab scale; useful for hydrodynamic modeling in oocysts risk estimation

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
pH	Robertson et al. (1992)	<i>Cryptosporidium parvum</i>	High and low pH have a significant impact on oocyst viability.	Lab scale; survival under various environment
	Solic and Krstulovic(1992)	Faecal coliforms	Pathogens optimal survival was between pH 6 and pH 7	Lab scale; combined effects of environments
	Abdul-Raouf et al. (1993)	<i>E. coli</i> 0157:H7	Citric and lactic acids ineffective in inactivation at pH 4.7, and 5.4	Lab scale; survival in different pH and temperature conditions
	Presser et al. (1998)	<i>E. coli</i>	The inhibitory effect of combinations of water activity and pH varied with temperature	Lab scale; growth as a function of temp, pH, lactic acid, and water activity
	Ross et al. (2003)	<i>E. coli</i>	pH effects was varied with changes in other environmental factors	Growth modeling; temperature, pH, lactic acid

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
	Gerba (2005)	Pathogenic viruses	Enteric viruses are stable at pH of natural water bodies	Review; viruses survival
	John and Rose (2005)	Pathogenic viruses	pH over the range from 6 to 7.8 has effects on mortality	Review; microbial survival
	Hipsey et al. (2008)	Pathogenic bacteria	Gradual increase in mortality beyond pH of 6 to 8, and substantial increase outside the range 4–10	Modeling; generic modeling of microbial population in aquatic systems
<hr/>				
Predators				
	Enzinger and Cooper (1976)	Bacteria and protozoa	Protozoa influenced <i>E. coli</i> survival	Lab scale; predators role in pathogen removal
	McCambridge and McMeekin (1980)	<i>E. coli</i>	Protozoan influence was critical during initial decline	Lab scale; relative effects of bacterial and protozoa in <i>E. coli</i> mortality

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
	Anderson et al. (1983)	Fecal bacteria	<i>E. coli</i> disappearance increases in the presence of natural biota and eucaryote	Membrane diffusion chamber; predators influence in mortality
	Gonzalez et al. (1990)	Protozoa, <i>E. coli</i> and <i>Enterococcus faecalis</i>	<i>Enterococcus faecalis</i> survived longer than <i>E. coli</i>	Field scale; differential digestion rates of bacteria by protozoa in fresh water
	Rhodes and Kator (1990)	<i>E. coli</i>	Autochthonous microbiota enhanced <i>E. coli</i> mortality	Lab scale; effects of sunlight and predators
	Menon et al. (1996)	Heterotrophic flagellate	Ingestion kinetics was consistent with competitive inhibition of enzymatic reactions	Lab scale; kinetics of flagellate grazing in the presence of bacterial prey
	Wcislo and Chrost (2000)	<i>E. coli</i>	Predators played major role in <i>E. coli</i> mortality	Field scale; predator and aquatic environment

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
	Menon et al. (2003)	Fecal bacteria	Predator's grazing was dominant coliform mortality	Field scale; protozoan induced mortality
	John and Rose (2005)	Pathogenic viruses and bacteria	Predatory and competing organisms reduces viruses population in aquifer	Review; various factors including predators influence in pathogens
Dissolved oxygen				
	Webb and Brown (1979)	<i>E. coli</i> k12	Lethality of radiation was oxygen-dependent	Lab scale; oxygen dependent radiation lethality in <i>E. coli</i> DNA repairs capability
	John and Rose (2005)	Pathogenic bacteria and viruses	Does not includes the potential impact of dissolve oxygen	Review; survival of pathogens in ground water
	Mackenzie et al. (1992)	Fecal bacteria	Oxygen is crucial in sunlight inactivation of fecal bacteria	Solar inactivation under aerobic and anaerobic conditions

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
	Reed (1997)	<i>E. coli</i> , <i>enterococcus faecalis</i>	Solar based pathogens inactivation require fully aerobic conditions	Lab scale: critical role of oxygen in sunlight pathogen inactivation
	(Davies-Colley, Donnison et al. 1999)	<i>E. coli</i> , enterococci and F-RNA phages	Inactivation of enterococci, F-RNA phages and <i>E. coli</i> was increased strongly with increased oxygen	Lab scale; interaction of DO, pH, and sunlight in inactivation of indicator organisms
	Gordon and Toze (2003)	<i>E. coli</i> , bacteriophage MS2, viruses	<i>E. coli</i> and viruses displayed maximum decay under aerobic conditions	Field scale; influence of water characteristics in pathogens survival
<hr/>				
Salinity				
	Carlucci and Pramer (1959)	Pathogens	Rapid pathogens decrease in the sea water	Report; factors affecting the survival of bacteria

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
	Elliot and Colwell (1985)	Indicator organisms	Direct detection method for pathogens	Letters; estuarine and marine water
	Cornax et al. (1990)	18 pathogenic strains	Pathogenic organisms showed inactivation in saline environment	Lab scales; survival of various pathogenic strains in seawater
	Alkan et al. (1995)	<i>E. coli</i> and enterococci	High level of light intensity in saline water increases inactivation	Lab scale; survival of pathogens in saline water and environment
	Mallin et al. (2000)	<i>E. coli</i>	Fecal coliform and <i>E. coli</i> abundance was inversely related with salinity	Field scale; effect of human development in coastal watershed
	Bordalo et al. (2002)	Faecal indicator bacteria	Overall survival was higher in low salinities	Dark and light microcosm study; survival study
	Allwood et al. (2003)	<i>Feline calicivirus</i> , <i>E. coli</i> and coliphages	Decay rate changes with salinity	Comparative study on pathogen survival in various water

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
	Griffin et al. (2003)	Pathogenic human viruses	Salinity influenced viability of pathogenic human viruses	Review; pathogenic human viruses in saline waters
Proteinaceous matter				
	Carlucci and Pramer (1959)	Pathogens	Increased nutrients decreased the die-off	Report; environmental factors affecting mortality
	LaLiberte and Grimes (1982)	<i>E. coli</i>	Increased organic content enhanced survival	Lab and field scale; survival of <i>E. coli</i>
	Baker et al. (1983)	<i>Vibrio cholera</i>	Nutrient supplementation increased growth	Lab scale; effects of nutrient deprivation
	Tertera et al. (1989)	<i>B. fragilis</i>	Nutrient and anaerobic conditions enhanced growth	Field scale; human origin pathogens growth
	Davies et al. (1995)	<i>C. perfringens</i> , protozoa, <i>E. coli</i>	Nutrients and sediments improved growth	Lab scale; fecal bacteria sediments

Table 1.2. (continued)

Factors	Author(s) (year)	Organisms	Results	Remarks
	Barcina et al. (1997)	Allochthonous bacteria	Survival study	Biological approach
Solid attachment				
	Bitton and Mitchell (1974)	Bacteriophage T ₇	Addition of inorganic and organic colloids reduced the inactivation	Lab scale; colloid effects in pathogen mortality
	Gerba and Schaiberger (1975)	Viruses	Viruses adsorption to particulate matters prolonged survival	Lab scale; effects of particulate on virus survival
	Goyal et al. (1977)	Coliforms, salmonella	Bacteria in sediment	Field scale; pathogens in water and sediments
	Smith et al. (1978)	Enteroviruses	Adsorption to sediment enhanced survival	Field scale; persistence of pathogen in sediment
	Rao et al. (1984)	Enteroviruses	Solid attached viruses survived longer	Field scale; sediment adsorbed viruses
	Ryan et al. (2002)	Viruses (PRD1 and MS2)	Sand attached viruses shown slow inactivation	Effect of iron oxide-coated sand
	Pang et al. (2004)	<i>E. coli</i>	Solid attachment prolonged survival	Model; reversible and irreversible attachment

(John and Rose 2005). A previous study (Gordon and Toze, 2003) found that inactivation of *E. coli* increases above 20 °C in deionized water-saturated soils; however, no significant differences were observed on *E. coli* inactivation in filtered or raw groundwater between 15 and 28 °C. The most influential factor besides temperature in ground water affecting *E. coli* inactivation is the presence of groundwater microorganisms (predators) (Gordon and Toze, 2003). To estimate the effect of temperature on the survival of pathogens, the first-order decay function is often used. The temperature dependent pathogen inactivation function for *Cryptosporidium* is (Jenkins et al., 1997; Brookes et al., 2004):

$$C(t) = C_0 \cdot \exp(-k_D t) \quad (3)$$

$$k_D = 10^{-2.68} \cdot 10^{0.058T} \quad (4)$$

where t is the time in days, C is the concentration of viable oocysts, k_D is dark inactivation, and T is the temperature in °C. *Cryptosporidium* is a significant cause of water-borne enteric disease throughout the world and represents a challenge to the water industry and a threat to public health (King et al., 2005a). The general expression for the temperature dependence of bacterial growth is formulated (Salter et al., 2000; Ross et al., 2003):

$$k_g = \mu_{max} f^T(T) \quad (5)$$

where k_g is growth rate, μ_{max} is maximum growth rate of bacteria at 20 °C, $f^T(T)$ is a function of temperature (Hipsey et al., 2008).

2.4.3 pH

Pathogens such as *E. coli* O15: H7 have been found to have a high tolerance to low pH. No loss of viability was observed in O157:H7 EHEC at pH levels of 3.0 to 2.5 for at least 5 hours, and it has been proposed that this tolerance to low pH could be the reason that outbreaks of *E. coli* infections caused by certain acidic foods occur (Miller and Kaspar, 1994; Benjamin and Datta, 1995; Presser et al., 1998). Table 1.2 summarizes the studies relating pH to pathogen growth and survival.

In fresh and saline water, pH influence on the survival of coliform have been observed (McFeters and Stuart, 1972; Solic and Krstulovic, 1992; Abdul-Raouf et al., 1993; Presser et al., 1998; Ross et al., 2003; John and Rose, 2005). However, compared to studies on temperature and solar radiation, less emphasis has been given on pH influence on the survival of pathogens in ambient water bodies, and the possible reason could be because many ambient water bodies usually maintain relatively stable pH, close to neutral (≈ 7.0). Research on pathogen survival in acidic food has been done extensively and has shown relationships between pathogen survival and disease outbreaks (Benjamin and Datta, 1995). Hipsey et al. (2008) proposed a typical formulation for evaluating the pH influence in bacteria inactivation.

$$k_d = 1 + C_{pHM} \left[\frac{(pH^*)^{\delta_M}}{K_{pHM}^{\delta_M} + (pH^*)^{\delta_M}} \right] \quad (6)$$

where C_{pHM} is the maximum effect pH toxicity can have on the mortality rate, K_{pHM} and δ_M mediate the sensitivity of mortality to change in pH, and pH^* is the magnitude of the pH departure from natural pH ($pH^* = \text{absolute}(pH - 7)$).

2.4.4 Predators

Numerous studies recognized the impact of predators in pathogen mortality (Enzinger and Cooper, 1976; McCambridge and McMeekin, 1980; McCambridge and McMeekin, 1981; Anderson et al., 2005). Table 1.2 shows the various studies evaluating the impact of predators in pathogen destruction. Naturally occurring microbial predators, i.e., phagotrophic protozoa, bacterivorous protists, and other predator bacteria, graze pathogens. In a lab-scale study, Enzinger and Cooper (Enzinger and Cooper, 1976) have shown that *E. coli* survival depended on the presence of protozoan predators. In other study, McCambridge and McMeekin, (1980) have shown that predators played a major role in *E. coli* survival.

Several studies provide information on the relative impact of predators on various pathogens (Gonzalez et al., 1990), and they found that *Enterococcus faecalis* survives in estuaries water longer than *E. coli*. The impact of predators in ground water pathogens has been reported extensively (John and Rose, 2005). Similarity has been found between the ingestion kinetics of prey and competitive inhibition enzymatic reactions (Menon et al., 1996). Studies on various environmental factors which impact pathogens in an aquatic environment (Wcislo and Chrost, 2000) concluded that a major factor responsible in pathogen destruction could be microflagellate grazing.

Other environmental factors already discussed, such as solar radiation and warmer temperatures, also enhance the impacts of predators, natural biota and eukaryote. Studies in general have shown that *E. coli* disappearance increases in the present of natural biota and eukaryote in warmer temperatures (Anderson et al., 1983). Previous studies also have concluded that the combined effect of solar radiation and predators was higher than when

each of these factors acting independently (McCambridge and McMeekin, 1981). To evaluate the impact of pathogen inactivation in water, Hipsey et al. (2008) proposed estimation of minimum predation rate.

$$k_p(T) = \vartheta_p^{T-20} \left[k_{p20} + \max \left(0, \varepsilon(C - C_{minp}) \right) \right] \quad (7)$$

where k_{p20} is the minimum rate due to predation at 20 °C (d^{-1}), ϑ_p accounts for the sensitivity of predation to temperature and C is the organisms concentration. Hipsey et al. (2008) explanation for second term on the equation's right hand side was that it enhances the base predation rate, k_{p20} , when above threshold C_{minp} .

2.4.5 Dissolved oxygen

The concentrations of dissolved oxygen may not have a significant influence on pathogen concentration, but it increases pathogen inactivity. In John and Rose's ground water pathogen contamination review study, for example, dissolved oxygen was ignored in evaluating pathogen survival (John and Rose, 2005). However, many authors consider it an influential factor. One study of sunlight's impact on pathogen inactivity has shown that the lethality of radiation was oxygen dependent. Anderson et al. (2005) have found that dissolved oxygen concentrations impact the pathogen's DNA repair capability.

Table 1.2 shows the relevant studies, which focus on dissolved oxygen's influence on pathogen survival. Studies concluded that *E. coli*, bacteriophages, MS2, polio virus, and coxsackie virus display maximum decay under aerobic conditions (Davies-Colley et al., 1999). Mackenzie et al. (1992) found that the sunlight inactivation rate is influenced by oxygen concentration in water. The study described the influence of aerobic and anaerobic

conditions in pathogen inactivation, concluding that enterococci, F-RNA phages, and *E. coli* survival was higher in aerobic conditions. Webb and Brown (1979) found that oxygen concentration influenced the UV irradiated *E. coli* inactivation. Similarly, Reed (1997) found that salmonella inactivation using γ - irradiation was influenced by oxygen concentrations.

2.4.6 Salinity

Numerous early twentieth-century studies have emphasized on the health risk caused by pathogens in saline water (Winslow and Moxon, 1928). However, more recent research has reported a rapid decline of pathogens when surface water enters to saline water (Carlucci and Pramer, 1959). Cornax et al. (1990), who studied influence of salinity on eighteen pathogenic strains, found that salinity enhanced inactivation.

Table 1.2 presents the studies that evaluate salinity's impact on pathogen survival. Alkan et al. (1995) reported that increased sunlight was more effective in saline water than freshwater, meaning salinity enhanced sunlight-mediated inactivation. Results in marine water research have shown that fecal coliform and *E. coli* abundance is inversely related to water salinity (Mallin et al., 2000). In a microcosm study intended for evaluating the impact of environmental factors on pathogen survival, Bordalo et al. (2002) found that overall pathogen survival was higher in low salinities.

Many studies such as Griffin et al. (2003) have proposed that pathogen contamination is reduced once freshwater enters into a saline environment. In numerous coastal water bodies, however, high levels of pathogenic human viruses are common (Griffin et al., 2003). This

indicates that saline water may reduce the pathogen levels, but the contaminated saline water can still contain sufficient pathogens to create a potential threat to human health.

2.4.7 Protienaceous material

Pathogen survival is enhanced by the presence of soluble organic matter and nutrients. In a seawater experiment, the mortality of pathogens was decreased when nutrient concentration was increased in (Carlucci and Pramer, 1959). Another study which used lake bottom water found that pathogen survival in that water was more likely near the lakebed than the surface because of higher organic content (LaLiberte and Grimes, 1982). Baker et al. (1983), who studied the influence of nutrient supplementation, found that pathogen growth was triggered when nutrients were provided. Table 1.2 shows the various studies focusing on nutrients' impact on pathogen survival.

Tartera et al. (1989) studied nutrient impacts on pathogen growth in aerobic and anaerobic conditions and reported that nutrient supplementation significantly enhances pathogen growth in anaerobic conditions. Although Barcina et al. (1997) reported that starved bacteria show the tendency to shrink, the starving results in enhanced resistance to heat. John and Rose (2005) reviewed nutrients' influence in pathogen survival in groundwater and concluded that peptone and glucose protect bacteria from enzymatic attack. Gordon and Toze (2003) reported that nutrient addition reduces decay of *E. coli*, *polioviruses*, and *coxsackieviruses*.

2.4.8 Solid attachment

Numerous studies have shown that pathogens survive longer in conditions when they are attached to solid particles (Gordon and Toze, 2003; Gerba, 2005; John and Rose, 2005). Muirhead et al. (2004) reported that the number of pathogens in sediment was several fold (10–1,000 times) higher than in water by itself. During high flow conditions, pathogens, which were attached to sediment particles, were released from the streambed into the water. Anderson et al. (2005) compared the differential survival of fecal bacteria in subtropical waters and sediment, and concluded that the decay rate of fecal coliform attached to sediment was much lower in the sediment than the water column; similar results are reported by other authors (Gerba and McLeod, 1976; Fish and Pettibone, 1995; Craig, Fallowfield et al., 2004).

Table 1.2 shows studies which explain the impact of solid particles on pathogen survival. Bitton and Mitchell (1974) evaluated the impacts of organic and inorganic colloids on pathogen survival and documented that the presence of colloids reduced pathogen inactivation. Viruses which are adsorbed to particulate matters have shown prolonged survival (Gerba and Schaiberger, 1975). Goyal et al. (1977) reported that total coliform, fecal coliform, and salmonella have shown a tendency to attach with sediments in order to survive longer. In studying enteroviruses, Rao et al. (1984) have shown that virus associated with solid particles survived longer in various environmental conditions.

Pang et al. (2004) used modeling to analyze reversible and irreversible attachment of *E. coli* with solid particles and the attachment's impact on survival. Many viruses attach with iron oxide particles, which reduces inactivation rates (Ryan et al., 2002). A study of virus transport through ground water explained that pathogens transport is governed by particle

attachment with mineral surfaces (Loveland et al., 1996). The authors explained that the attachment edge occurred at a pH value that was between 2.5 and 3.5 greater than the mineral surface's pH; particles attached above this edge were found to be reversibly attached. The Loveland study also explained the Derjaguin-Landau-Verwey-Overbeek potential energy, which controls the attachment edge.

2.5 The impact of water resources development and climate warming

Besides pathogen transport modeling and evaluating the impacts of various environmental factors on pathogen survival in water bodies, recently there are many studies which emphasize synthesizing and assessing the impacts of water resource development and climate warming on pathogen contamination. Infectious diseases caused by pathogens are the third leading cause of death in the United States, and the leading cause in the world (Binder et al., 1999). The past two decades have seen the emergence of many new pathogenic infectious diseases (Daszak et al., 2000). Many of these diseases are caused by anthropogenic changes such as water resource development, climate warming, and interaction between human and animals, both, domestic and wild (Krause, 1994; Epstein, 2001; Woolhouse, 2002; Foley et al., 2005; Jablasone et al., 2005; Fenwick, 2006; Normile, 2009; Schriewer, et al., 2010).

2.5.1 Water resources development

Water resources development involves altering the natural flow path of rivers, streams, and lakes, as well as designing irrigation schemes and dams. These activities were responsible for causing new diseases and also exacerbated the existing health risks (Fenwick, 2006; Steinmann et al., 2006).

The influence of water resource development in spreading diseases such as schistosomiasis, a parasitic disease which is ranked second only to malaria with regards to the number of people infected, has been reported extensively; one estimate says that about 103 million out of 779 million infected people live in close proximity to large reservoirs and irrigation schemes (Steinmann et al., 2006).

Designing dams and irrigation schemes in tropical and subtropical climate zones has often resulted in disease outbreaks caused by waterborne pathogens. Take, for example, the Sennar Dam on the Blue Nile River and Sudan's Gezira Scheme, the world's largest irrigation project. Because of the dam's commercial success, the irrigation in the region has doubled in size in the 1940s and 1950s. After 1950s, infections from malaria and schistosomiasis increased significantly, becoming the subject of the first integrated disease-control program, the Blue Nile Health Project, which was implemented from 1978 to 1990. The project failed to make a dent in controlling the prevalence of schistosomiasis (Eltoum et al., 1993; Fenwick, 2006; Steinmann et al., 2006). Another example is the Three Gorges Dam in China completed in 2009, which created a 50,700 km² reservoir and submerged more than 220 counties. According to Hotez et al. (1997) the reservoir would produce environmental changes that could lead to the transmission of schistosomiasis in the area served by the dam.

To meet a growing population's demand for food and energy supply, water resources development is necessary; as a result, a large number of people live in close proximity to water bodies, which will expose them to health risks. Lerer and Scudder (1999) assessed the

impacts of water resources development on health risks and concluded that evaluating strategies to mitigate health risks is crucial before executing a new project.

2.5.2 Global climate warming and pathogen-caused disease risks

Climate changes alter pathogens' mortality rate. For example, changes in temperature, rainfall and humidity influence pathogens' survival in ambient water bodies (Harvell et al., 2002). Typically pathogens grow faster in warm rather than cold environments, thus disease introduction and transmission could increase with elevated temperature. For example, El Niño events, which have been linked to global warming, are annual weak warm ocean currents that run southward along the coast of Peru and Ecuador about December and increase ocean temperatures (Trenberth and Hoar, 1996; Trenberth, 1997). Harvell et al. (2002) reported El Niño's influence on marine and terrestrial pathogens, including cholera vibrio, oyster pathogens, crop pathogens and human cholera. Because of these influences, global warming could lead to a rapid declination of terrestrial and aquatic animal population.

Recent worldwide increases in the emergence of new infectious diseases are significant; many infectious diseases can be devastating to the lives of terrestrial and aquatic animals (Williams and Bunkley - Williams, 1990; Harvell et al., 1999; Mallin et al., 2000). For example, pathogens were responsible for massive frog die-offs in the U.S. and Western Australia (Morell 1999), deaths of Hawaiian forest birds (Daily et al., 1993), the extinction of a species of land snail (Cunningham and Daszak, 1998), and the decline in population of wildlife such as lions, eagles and black-footed ferrets (Woodroffe, 1999). The occurrence of infectious disease increases because of global warming. Studies by Smith and Tirpak (1990) and Martens, Niessen et al. (1995) reported the potential impacts of global warming and

climate change on the emergence and transmission of diseases. In 2001, Epstein reported that climate plays a crucial role in determining human health by controlling the spread of many infectious diseases. Epstein described how extreme weather events accelerate the introduction and transmission of diseases by creating advantageous growth conditions for waterborne pathogens.

Harvell et al. (1999) described that changing environmental conditions alter the virulence of existing diseases and increase the possibility of new diseases. Many pathogenic organisms in estuaries and oceans are dormant, but an increase in temperature could enhance their growth and proliferation. The spread of Cholera in Bangladesh, for example, was found to be caused by an increase in sea water's surface temperature (Colwell, 1996). Nevertheless, a few claim that global warming can have positive impacts on human health; for example, Epstein (2001) states that high temperatures in some regions may reduce snail populations, the intermediate source for schistosomiasis. Generally, however, the increase in the spread of other diseases in the ocean and on the land could be catastrophic to the health of ambient water bodies as well as humans (Martens et al., 1995; Colwell, 1996; Trenberth and Hoar, 1996; Harvell et al., 2002; Patz, 2002; Jablasone et al., 2005).

2.6 Challenges and recommendations

Mitigating the threat of global warming and its impacts on ambient water bodies is a major challenge for the future. Although climate warming has significant impacts on pathogen survival and growth, our currently weak understanding about the fate and transport of pathogens could prevent researchers from identifying increased health risks caused by global warming. Multidisciplinary knowledge about how ambient water bodies, wildlife, domestic

animal, and human populations interacting and impacting each other could be crucial in dealing with future challenges. Generally, domestic animals, wildlife and humans are considered to be major sources of waterborne pathogens. Finding the specific culprit—the primary pathogen source—is challenging, however (Malakoff, 2002). A watershed, for example, can have many pathogen sources such as agricultural land, riparian areas, agricultural feeding operations, livestock, wildlife, and humans.

Typically most studies have relied on *E. coli* to indicate pathogen levels in water. Although widely used in monitoring contamination levels, *E. coli* alone can lead to mercurial and misleading information (Gordon, 2001). Schriewer et al. (2010) suggested that with improved pathogen detection technology (i.e., PCR-based detection) an indicator organism such as *E. coli* can be accurate enough in most cases. Overall, improving technology to identify causative agents more accurately, creating standard epidemiological data for diseased populations, and enhancing the knowledge of disease dynamics can improve researchers' understanding of the risks caused by interactions among various populations (Harvell et al., 1999; Daszak et al., 2000; Harvell et al., 2002).

In the past, a considerable number of studies on pathogen contaminations have been conducted on a scale where the conditions of ambient water bodies were simulated in labs. These studies are helpful in understanding pathogen behavior only up to a point. For enhancing the understanding of pathogen interactions in the environment, more emphasis should be given to field-scale studies.

Various publications are available in developing models for predicting pathogen contaminations in ambient water bodies (i.e., Dorner et al., 2006; Kim et al., 2010; Rehmann

and Soupir, 2009; Droppo et al., 2009; Cho et al., 2010; Droppo et al., 2011). In general, pathogen transport is modeled as function of advection, settling, resuspension, lateral influx. Some for example, Droppo et al. (2009) emphasized on flocculation impacts on pathogen transport. However, the evaluations of existing models' predictions show that there is a need for improvements. Many of these approaches have modeled only temperature induced mortality and growth, and did not include interactions among other environmental factors (e.g., pH, nutrients, DO, solar radiation). Inclusion of these environmental factors can be useful, particularly, when models are used at watershed scale. Developing models that are reliable in predicting pathogen survival and transport at watershed scale can be helpful in implementing/evaluating the strategies for mitigating ambient water body pathogen levels.

3. Conclusions

Mitigating the threat of global warming and its impacts on ambient water bodies can be the major challenge for the future, particularly controlling pathogen contaminations. In this review of literature, we examined studies from various disciplines to understand pathogen contamination in ambient water bodies. The worldwide prevalence of pathogen contaminations, major pathogen sources and their significant impacts on ambient water bodies, crucial environmental factors that potentially impact pathogen survival, modeling approaches that are commonly used for predicting waterborne pathogens, the impacts of water resources and climate warming on pathogen contaminations, and future challenges and recommendations for improving water quality were discussed. We found that a considerable number of studies on pathogen contamination have been conducted on a lab-scale; more emphasis should be given to field-scale studies for enhancing the understanding of pathogen

interactions in the environments. Considering existing model's ability in predicting pathogen contamination, improvement and development of new models is necessary so that pathogen levels can be predicted accurately. We emphasized on improving current models for predicting pathogen levels in water bodies. Integrating knowledge from multiple fields (e.g., hydrology, microbiology, and ecology) could increase understanding on pollution levels and potential cause of pollutions, and it can also help devising strategies to improve water quality.

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CHAPTER 2. ASSESSING THE IMPACTS OF WATERSHED INDEXES AND PRECIPITATION ON SPATIAL IN-STREAM *Escherichia coli* CONCENTRATIONS

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Abstract

Pathogen contamination of waterbodies, which is often identified by the presence of pathogen indicators such as *Escherichia coli*, is a major water quality concern in the United States. Reducing in-stream pathogen contamination requires an understanding of the combined impacts of land cover, climatic conditions, and anthropogenic activities at the watershed scale. In this study these factors are considered by assessing linear relationships between in-stream *E. coli* water quality data, watershed indexes, and rainfall for the Squaw Creek Watershed, IA, USA. The watershed indexes consider the undisturbed land cover which encompasses the natural land cover area, wetlands, and vegetated stream corridors, and the disturbed land cover extent which includes areas receiving manure from confined

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animal feeding operations (CAFOs), tile-drained areas, and areas in cropped and urban land. In addition to disturbed and undisturbed land, we also calculated indexes for barren land and land slope. Bivariate analysis was used to assess the linkage between *E. coli* concentrations, watershed indexes and the cumulative rainfall 15, 30, 45, and 60 days prior to water sample collection. To predict in-stream *E. coli* concentrations, we developed multivariate regression models, and predictions were compared with observed *E. coli* concentrations at 46 sampling locations over four sampling periods in two years. Results show that areas receiving manure, wetlands, drained land, and cropped land all influence in-stream *E. coli* concentrations significantly ($p < 0.001$). The coefficient of determination was higher when indexes were corrected using the cumulative rainfall 30 days prior to the sampling event. Model skill varied from 0.29 to 0.55. More than 95% of the predictions across all spatial locations fall within one order of magnitude of the observed values. This Geographic Information System (GIS) based approach for predicting in-stream *E. coli* concentrations appears to be a useful technique for assessing the impacts of land management on water quality.

1. Introduction

Watershed-scale assessments of point and nonpoint source pollutant loads, and recommendations to reduce loads so that water quality criteria may be met are being developed in the United States. In addition to meeting regulatory requirements, contamination of surface waters is a major environmental and public health concern, and new tools are needed to improve understanding of watershed characteristics impacting contaminant transport. For instance, the National Water Quality Report of the United States Environmental Protection Agency (U.S. EPA, 2011) reveals that out of 44,752 watershed

assessments or Total Maximum Daily Loads (TMDLs) which have been developed, approximately 21% are related to pathogens (assuming indicator organisms are representative of pathogen contamination). Of the total 71,509 reported water quality impairments, 15% are due to elevated pathogen levels. Fifty percent of the total 1,496,334 km of assessed rivers and streams are impaired, and pathogens are the leading cause of these impairments.

A major challenge in improving water quality and estimating the pathogen contamination in stream water is our weak understanding of the combined impacts of bacterial loadings from point and non-point (diffuse) sources and their impact on in-stream pathogen concentrations. Mathematical models which incorporate the influence of watershed characteristics and hydrology can be useful to calculate in-stream pathogen concentrations and derive plans for watershed scale water quality improvements. In this study we have focused on developing models for predicting in-stream pathogen concentrations based on the landscape characteristics and hydrology of the watershed.

A primary source of bacterial pollution in streams is considered to be agricultural activities (EPA, 2012), such as manure applications onto cropped land and effluent discharges, often caused by accidental spills (Armstrong et al., 2009), from confined animal feeding operations (CAFOs). Rainfall events occurring shortly after manure application to cropped land can generate overland flow, which in turn may deliver large quantities of bacteria and potentially pathogens into surface waters (Soupir et al., 2006; Guber et al., 2006; Guber et al., 2007). Some manure application practices such as liquid manure injection in the field rather than surface application can potentially reduce *E. coli* transport from cropped land to the streams from overland transport. However, *E. coli* in injected manure can be transported to streams

through tile drainage systems rather than with overland flow (Dorner et al., 2006). Effluent from CAFOs contains high levels of *E. coli* (Mallin and Cahoon, 2003), which can cause water contamination if discharged directly to the stream. Another risk to water quality is accidental spills from CAFOs, which may increase stream *E. coli* levels due to the influx of animal waste (Armstrong et al., 2009; Centner and Feitshans, 2006; Jagai et al., 2010; Lichtfouse et al., 2010).

According to the U.S. EPA (U.S. EPA, 2001b), the U.S. has approximately 238,000 CAFOs, which generate approximately 317 million gallons of manure annually (Armstrong et al., 2009; USEPA, 1999). The potential for water contamination from CAFOs has led to strict federal regulations in the U.S. to minimize degradation of water resources; CAFOs are required to have National Pollution Discharge Elimination Systems (NPDES) permits before they can discharge effluent into nearby waters. Although regulations are in place in the U.S., reducing the bacterial pollution associated with CAFOs and land application of manure is still a major challenge (Centner, 2004).

Although agricultural activities, such as manure application and effluent discharges from CAFOs, have the potential to play a major role in influencing stream water quality, the characteristics of the watershed (i.e., land cover, soils, geology, topographic features and catchment hydrology) also play a key role in determining stream water quality (Rothwell et al., 2010a;b). While assessing environmental conditions, many previous studies have explored new methods to identify representative input parameters, which can be useful to predict stream water quality (Zhao et al., 2006; Wu and Chau, 2006; Xie et al., 2006; Zhang and Chau, 2009; Chau et al., 2002; Muttill and Chau, 2007). A number of studies have

explored the utility of Geographic Information System (GIS) to understand the relationship between watershed characteristics and nutrient concentrations in streams (e.g. Jarvie et al., 2002; Tiner, 2004, King et al., 2005b; Bach et al., 2010; Shiels and Guebert, 2011; Zhang and Huang, 2011). Land cover indexes have been shown to be particularly useful for explaining and predicting spatial variation in waters impaired by nitrate, pH, orthophosphate, suspended sediments, and metals (Roth et al., 1996; Robson and Neal, 1997; Gergel et al., 2002; Buck et al., 2004; Kearns et al., 2005; Aitkenhead-Peterson et al., 2007; Evans et al., 2006; Helliwell et al., 2007; Meynendonckx et al., 2006; Rothwell et al., 2010a;b).

Recently, Rothwell et al. (2010a;b) applied GIS tools to improve understanding between water chemistry (i.e., pH, sulphate, cations, nutrients, and metals) and watershed characteristics such as land cover, topography, soil hydrology, and geology. The authors found that water chemistry is related to land cover and geology, with good linear relationships being identified between nitrate and arable land cover. The approach was less effective for predicting orthophosphate concentrations, likely due to the strong influence of point sources of pollution. The authors concluded that the approach works best when the pollutant sources in a watershed are dominated by nonpoint sources. Other studies, for example, Crowther et al. (2002), Kay et al. (2008), and Wilkes et al. (2011) reported how land use factors in watersheds impact water quality of the ambient water bodies, particularly, faecal indicator concentrations. The authors found that inputs from diffuse sources, particularly, runoff from farmed land are likely to be a significant source of contamination.

While previous studies have investigated linkages between the landscape and in-stream water chemistry using the watershed characteristics approach (Rothwell et al., 2010a;b; Shiels and

Guebert, 2011), relationships between landuse and water pathogen levels need further examination to understand how landcover (i.e., cropping land, undisturbed land cover, and disturbed land cover) potentially impacts in-stream pathogen concentrations. Therefore, our goal was to build upon previous approaches and evaluate the impacts of watershed characteristics and hydrology on in-stream *E. coli* concentrations. Both landuse and hydrology are important in improving our understanding of the sources, fate, and transport of pathogens as they move from the terrestrial to the stream environment. Our study has three objectives: 1) characterize the watershed based on the extent of disturbed and undisturbed land cover; 2) quantify the relationship between in-stream *E. coli* concentrations, watershed characteristics, and rainfall; and 3) use watershed indexes to develop multivariate regression models for predicting in-stream *E. coli* concentrations.

2. Study Area

This study was conducted in the Squaw Creek Watershed. The study area is shown in Figure 1.1 Squaw Creek passes through four counties (Story, Webster, Hamilton, and Boone) of Iowa, USA, and is a tributary of the South Skunk River. The Squaw Creek watershed, Hydrologic Unit Code (HUC) 10 (ID 0708010503), has a total drainage area of 592.39

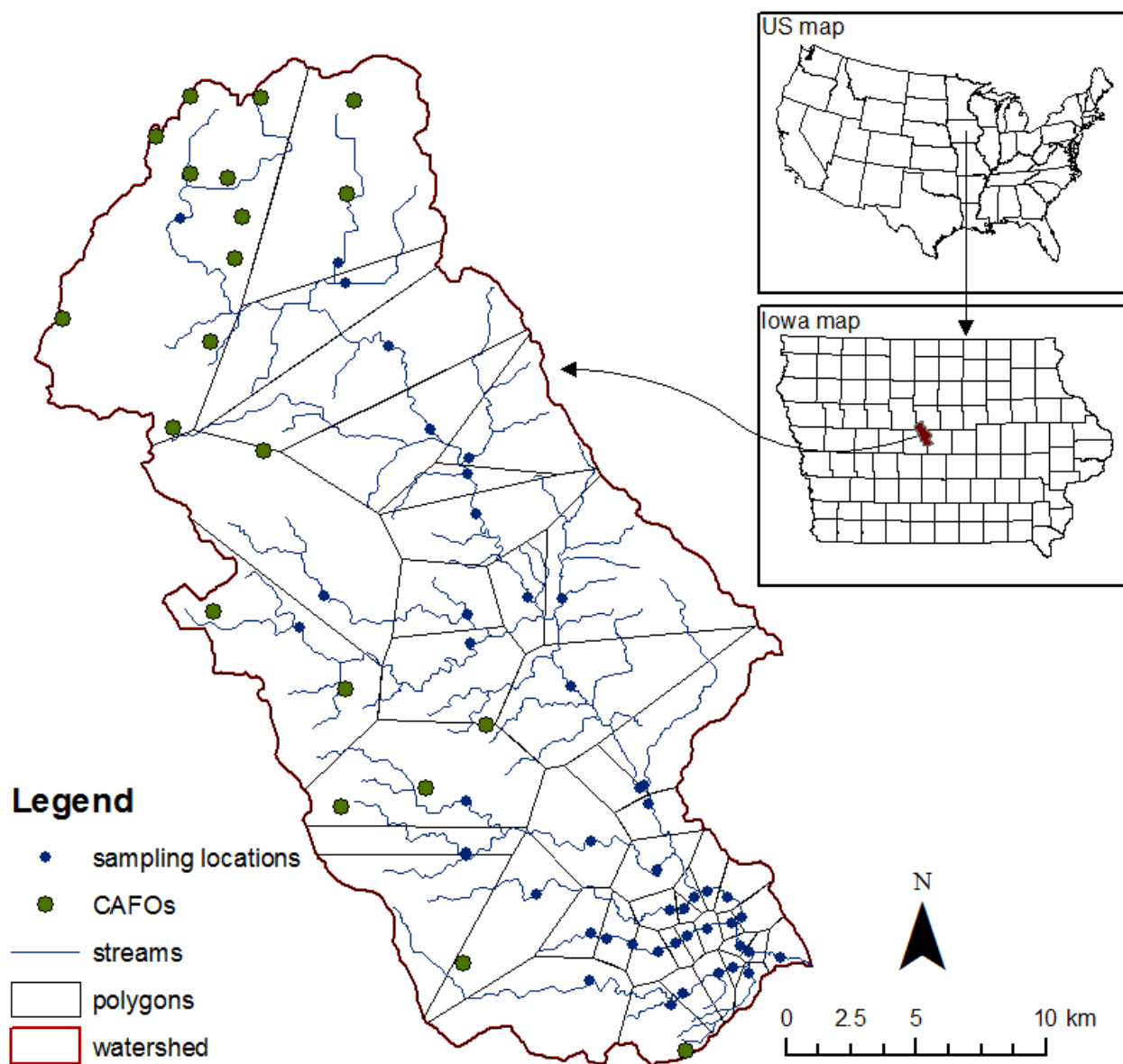


Figure 2.1. The Squaw Creek Watershed in central Iowa, U.S.A. The dark red line shows the watershed boundary, and the thin black line shows the polygon boundaries. The blue line shows streams, green circles indicate locations of CAFOs, and blue circles indicate water sampling locations.

sq km. The basin length and perimeter of the watershed is 43.53 km and 134.02 km, respectively, with an average slope of 2.01%. The basin relief is 111.51 m, the main channel length is 60.46 km and the total stream length within the watershed is 346.72 km. There are 75 first order streams. The 2002 hydrologic Unit Code (HUC 10) watershed land use estimates 0.09%, 0.17% and 0.05% of the watershed land area as water, wetland and wetland forest, respectively. Deciduous forest, ungrazed grass, grazed grass, Conservation Reserve Program (CRP) grassland, and alfalfa were 2.71%, 10.87%, 2.52%, 1.70%, and 1.84%, respectively. Corn and soybeans, and other rowcrops are 41%, 33%, and 0.43%, respectively. Common/industrial, residential, and barren land are 1.67%, 1.27%, and 0.06%, respectively.

3. Methods

3.1. Water quality data

We obtained water quality data from the Squaw Creek Watershed Coalition Program (Iowa Water, 2011). The program started in 2006, and collects stream water at 52 sampling locations in May and October of each year for water quality analysis. For this study we used the water quality data from four sampling periods (October 2008 and 2009, and May 2009 and 2010) from 46 of the 52 sampling locations, as the remaining locations contained missing data. We selected these years solely because of data completeness.

3.2. Spatial datasets

In this study we used seven spatial datasets to characterize the watershed: 1) digital elevation model (DEM); 2) CAFOs; 3) land cover; 4) soils; 5) stream network; 6) wetland; and 7) rainfall. The DEM (30 m resolution) floating point grid was obtained from the Natural

Resources Geographic Information System (NRGIS) library. The library is maintained by GIS section of the Iowa Department of Natural Resources (IDNR). The location map of CAFOs, which are registered with the IDNR, was obtained from NRGIS. Data attributes of the map were updated in January 2011.

The 2002 land cover map (30 m resolution) for the state of Iowa was obtained from NRGIS. The Land cover/Land use classifications used in the map were derived from satellite imagery collected between May 2002 and May 2003. We used a Soil Survey Geographic Database (SSURGO) (2010) hydric soils map of Iowa (30 m grid), which was obtained from NRGIS. This coverage contains a grid representing soil mapping units from the published soil survey reports with a minimum size delineation of 0.80 ha.

The stream network map (2004) of the watershed was obtained from the NRGIS. This coverage contains selected arcs from the 1:100,000 National Hydrography Dataset (NHD), which was developed jointly by the United States Geological Survey (USGS) and EPA. Selected arcs represent the centerlines of wide streams, impoundments, reservoirs, and wetlands. The stream network was produced from a 30 m resolution DEM. A map of the National Wetland Inventory (NWI) (2009) of Iowa was obtained from NRGIS. The NWI digital data files contain wetlands location and classification as developed by the U.S. Fish & Wildlife Service. We used daily rainfall data for Ames, Iowa, which is within the Squaw Creek Watershed, as a representative rainfall for the watershed. The rainfall data was obtained from the Iowa Environmental Mesonet, Agronomy Department, Iowa State University, USA.

3.3. Spatial data analysis

For our analysis, we used GIS (ArcMap™10) and GeoDa 0.9.5-I software. ArcGIS 10 was used for mapping, editing, and map-based analysis. For example, calculations of natural land covers, wetlands, stream corridors, CAFO proximity to streams, CAFO density, drained areas (described in section 4.2.2) , and cropland areas were performed using ArcMap10. GeoDa (0.9.9.12) software was used for creating thiesen polygons of the watershed. A total of 46 thiesen polygons, each for a sampling location were created for the watershed (Figure 1.1). The landscape characteristics of the each polygon were calculated using spatial analysis functionality of ArcMap10, as described in section 4.

4. Watershed indexes

The watershed was divided into 46 thiesen polygons for each of the 46 sampling locations (Figure 1.1). To create the polygons, a point shape file of sampling locations was imported into GeoDa software, and the GeoDa function “change from point files to polygons” was used to produce a thiesen polygon for each sampling location. For each polygon, we calculated both the undisturbed and disturbed land cover extent watershed indexes using ArcMap10.

4.1. Undisturbed land cover extents

To characterize the watershed, we calculated the undisturbed land cover extent and disturbed land cover extent (detailed later). To calculate the undisturbed land cover extent, we estimated three indexes: natural cover (I_{nc}), wetland (I_{wl}), and stream-corridor (I_{sc}). The U.S. Fish and Wildlife Service has used similar approaches for characterizing watersheds, and

evaluating the impacts of changes in environment and natural habitat on water quality (Tiner, 2004). In a relatively recent study, Shiels and Guebert (2011) used indexes of natural land cover, wetland, and stream – corridor integrity for correlating the water quality with natural land cover of the Mississinewa Watershed in east central Indiana.

4.1.1. Natural cover index (I_{nc})

To calculate the natural cover index we first exported all of the natural areas (forest, grassland, shrub and wetland) from the land cover map using a select by attribute functionality. Then the areas of natural cover (A_{nc}) were clipped from the watershed map. The natural cover area calculation method is shown in Figure 1.2. The natural cover index (I_{nc}) was estimated by dividing the total area of natural cover (A_{nc}) in each polygon, by the total polygon area (A_c).

$$I_{nc} = \frac{A_{nc}}{A_c} \quad (1)$$

4.1.2. Wetland index (I_{wl})

Wetland indexes were estimated by dividing the NWI polygon area, by historic wetland extent (HWE) area according to Shiels and Guebert (2011). The HWE area was calculated by merging NWI polygons with the hydric soil layer.

$$I_{wl} = \frac{NWI}{HWE} \quad (2)$$

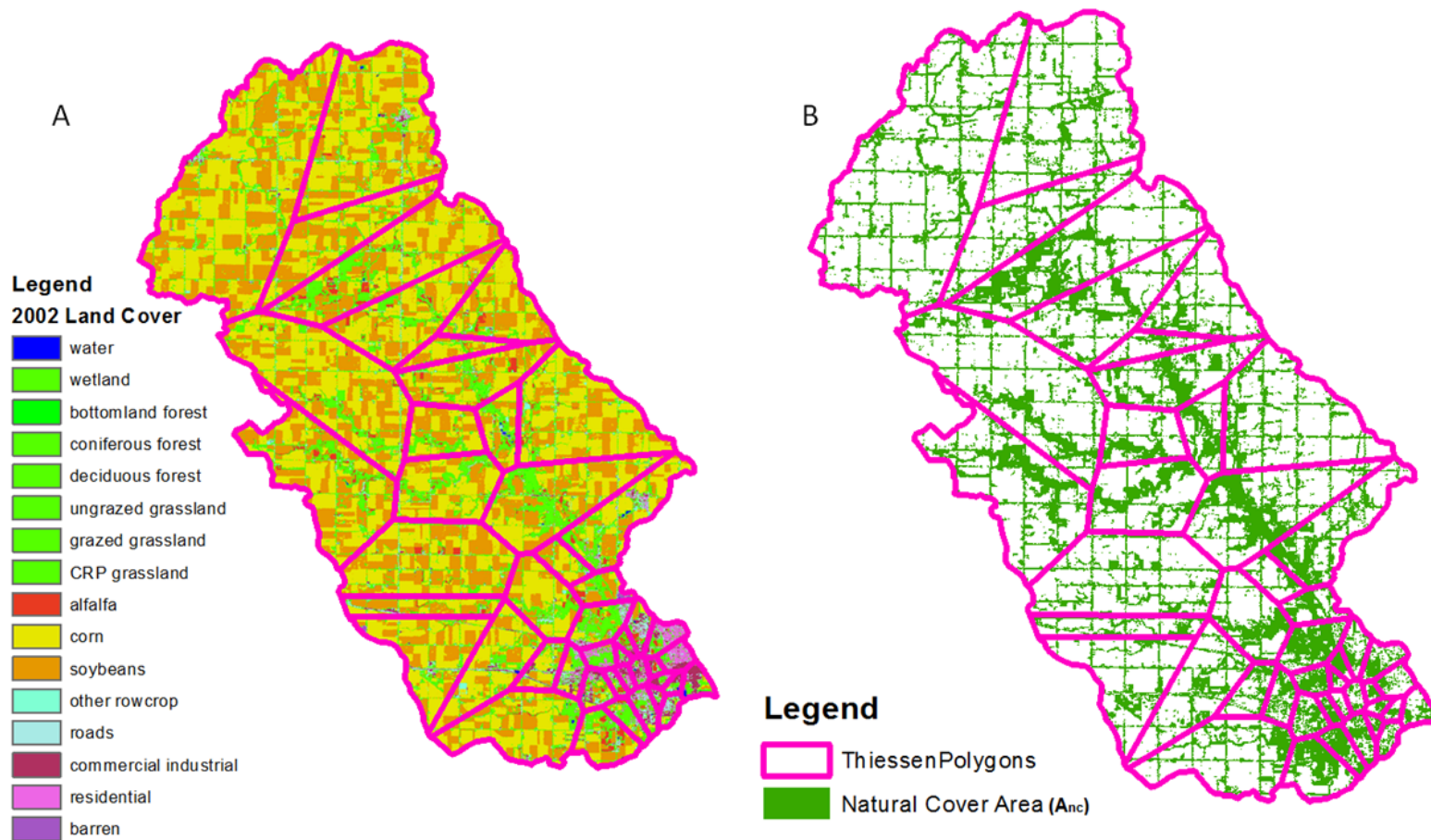


Figure 2.2. The map shows the calculation of the natural cover area index (I_{nc}). The green color indicates natural cover (i.e. wetland, forest, grassland). The map to the right shows where the natural cover area was isolated from watershed landcover map (left).

4.1.3. River – stream corridor integrity index (I_{sc})

To assess the influence of vegetated riparian corridors on stream water quality stream corridor, the integrity index (I_{sc}) was calculated according to Tiner (2004) and Shiels and Guebert (2011):

$$I_{sc} = \frac{A_{vsr}}{A_{tsr}} \quad (3)$$

where A_{vsr} is vegetated stream riparian area and A_{tsr} is total stream riparian area. A buffer of 200 m on each stream bank was used to estimate A_{tsr} . The A_{vsr} was estimated by overlaying the A_{nc} (estimated in section 4.1.1) on A_{tsr} , and clipping the A_{nc} layer with the A_{tsr} layer.

4.1.3. Barren area index (I_{br})

To calculate the barren area index, I_{br} , we extracted the barren areas from 2002 land cover map, and divided it by the total polygon area.

$$I_{br} = \frac{A_{baren}}{A_c} \quad (4)$$

where A_{baren} is area of each polygon classified as barren, and A_c is the total area of the polygon.

4.2. Disturbed land cover extent

The disturbed land cover indexes were calculated to assess the impacts of agricultural activities including CAFOs, tile drainage, and cropped land cover within the watershed.

These indexes are described below:

4.2.1. CAFOs index (I_{cafo})

We calculated I_{cafo} , to assess the impact of manure generated from CAFOs on in-stream *E. coli* concentrations. The 2006 Iowa Department of Natural Resources (DNR) manure application map of the study area, which contains the potential areas receiving manure, assuming a standard application rate of 179.33 N kg/ha prior to corn under cropped land currently in 2-year corn/soybean rotation management, was used to calculate the areas under manure application in each polygon. I_{cafo} was calculated as follows:

$$I_{cafo} = \frac{A_{ma}}{A_c} \quad (5)$$

where A_{ma} is area of each polygon receiving manure application, and A_c is the total area of the polygon.

4.2.2. Drained land index (I_d)

The Squaw Creek Watershed has extensive land area under tile drainage management. To assess the impacts of drained land on stream water quality we estimated I_d the drained land index, using the hydric soil layer and cropped area.

$$I_d = \frac{A_d}{A_c} \quad (6)$$

where A_d is drained area, and A_c is the polygon area. For estimating A_d , areas under agriculture (i.e., corn, soybean, other rowcrops) were extracted from the land cover map, which were intersected by the hydric soil layer as described by Shiels and Guebert (2011).

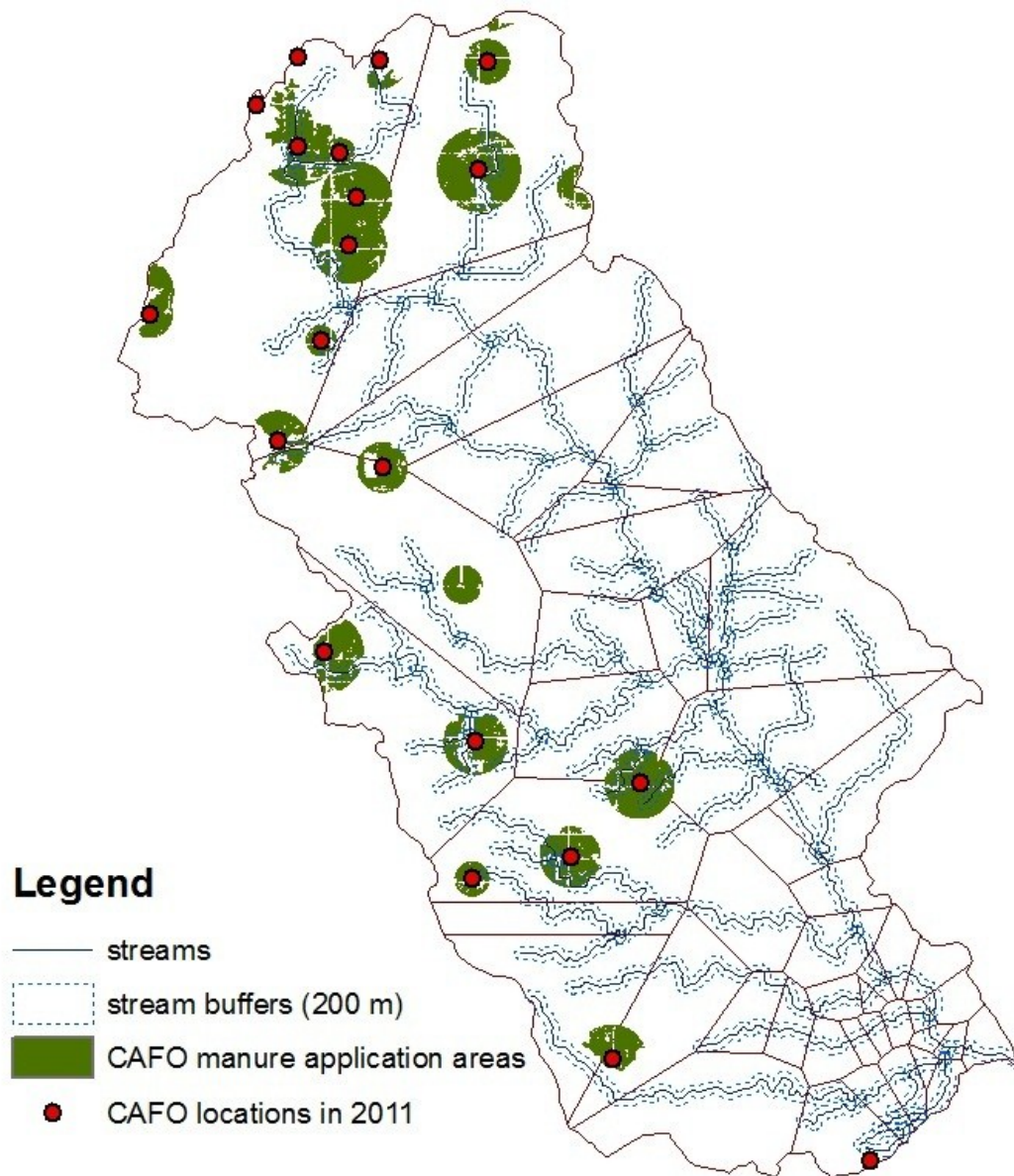


Figure 2.3. The map shows the calculation of the CAFO index (I_{cafo}). Stream buffers of 200 m are shown as dashed lines, streams are shown as blue lines, red dots are CAFO locations, and green circular areas are the regions where manure is potentially applied.

4.2.3. Cropped area index (I_{rcc} and I_{rcs})

The cropped area of the watershed has the potential to impact stream water quality. To calculate the cropped land indexes, we estimated the areas under corn and soybean crop rotation. The cropped land indexes were calculated as:

$$I_{rcc} = \frac{A_{cc}}{A_c} \quad (7)$$

$$I_{rcs} = \frac{A_{cs}}{A_c} \quad (8)$$

where I_{rcc} and I_{rcs} are indexes for corn and soybean crops; A_{cc} and A_{cs} are the total areas under corn and soybean crops; and A_c is the total area of polygon.

4.2.4. Urban area index (I_{urb})

The urban area of the watershed has the potential to impact stream water quality. To calculate urban land indexes, we estimated the areas under residential and commercial/industrial land.

The I_{urb} were calculated as:

$$I_{urb} = \frac{A_{urban}}{A_c} \quad (9)$$

where A_{urban} is land classified as residential, commercial, and industrial area of each polygon, and A_c is the total area of the polygon.

4.2.5. Slope index (I_{slope})

The slope area index (I_{slope}) was calculated as:

$$I_{slope} = \frac{Slp_{avg}}{Slp_{max}} \quad (10)$$

where slp_{avg} is the average slope of each polygon, and slp_{max} is the maximum slope of each polygon.

4.2.6. Hydrological correction

In order to include the impacts of rainfall on in-stream water quality, all indexes were hydrologically corrected. As inclusion of hydrology was crucial in this study, we used four periods of cumulative rainfall (i.e., 15, 30, 45, and 60 days) for hydrological correction of the watershed indexes, which resulted in four different sets of models. To include hydrology, we multiplied all indexes (estimated using equations 1 – 10) with the cumulative rainfall (m) from 15, 30, 45, and 60 days prior to the sampling day. Runoff data were not directly available, and therefore, cumulative rainfall data from the 15, 30, 45, and 60 days prior to sampling for each polygon was used to substitute for runoff, as described previously by Jarvie et al. (2002).

4.3. Statistical analyses and modelling

Bivariate Pearson correlations were used to measure the degree of association between *E. coli* concentrations and the watershed indexes. To develop the model for predicting the impacts of watershed indexes on in-stream *E. coli* concentrations, we performed step wise (forward) multivariate regression analysis using JMP statistical software (JMP^R Pro 9.0.0). In the stepwise regression model, the stopping rule was based on a p-value threshold, and probability to enter and leave were defined as 0.05 and 0.05, respectively, for the 30, 45, and 60 days cumulative rainfall correction. For 15 days cumulative rainfall correction, the probability to enter and leave were defined as 0.10 and 0.10, because no variables were

significant at the 0.05 level. To develop the model, we randomly selected 70% of the data from each year. The remaining 30% of the data were used to validate the model. In the model, four indexes of undisturbed land cover (I_{nc} , I_{wb} , I_{sc} , I_{br}), and six indexes of disturbed land cover (I_{cafo} , I_d , I_{rcc} , I_{rcs} , I_{urb} , I_{slope}) were used as independent variables to predict the dependent variable (*E. coli* concentrations). To evaluate the model efficiencies, suitability of the model, and predictions, we calculated the model skill (Willmott 1981), coefficient of determination (R^2), and Nash-sutcliffe model coefficients (Nash and Sutcliffe, 1970).

$$Skill = 1 - \frac{\sum |P_i - M_i|^2}{\sum (|P_i - \bar{M}| + |M_i - \bar{M}|)^2} \quad (9)$$

$$Nash = 1 - \frac{\sum (M_i - P_i)^2}{\sum (|M_i - \bar{M}|)^2} \quad (10)$$

where skill is the model predictive skill; Nash is the Nash-sutcliffe model efficiency coefficient (NSE); M is the measured value; P is the predicted value, and \bar{M} is the mean of measured values. In addition, we also estimated the coefficient of determination (r^2). The model skill values vary from 0 to 1, and value of 1 is considered a perfect prediction. The NSE values vary from $-\infty$ to 1; the NSE of 1 is considered a perfect prediction. The model skill and NSE values close to 1 are considered as perfect prediction; however, previous studies show that achieving NSE and model skill values close to 1 have not yet been attained due to the complexities involved in bacteria predictions in streams. As described by Dorner et al. (2006), order – of – magnitude estimates are needed for water quality improvement; greater values of NSE, r^2 , and model skill are not expected. Based on the results of previous studies involved in modeling in-stream bacteria predictions, we target achieving the model skill and r^2 values greater than 0.35. Previous studies have reported negative NSE values [a

study by Cho et al., 2010 reported NSE from -0.78 to -0.99 and Parajuli et al., 2009a,b reported NSE values of -0.41], and reported r^2 range from 0.15 – 0.40 (Parajuli et al., 2009a) . We targeted achieving NSE values greater than -0.50; however, while evaluating the model predictions model skill and r^2 values were also taken into consideration.

5. Results

5.1. Variation in *E. coli* concentrations, rainfall and watershed indexes

The descriptive statistics of *E. coli* concentrations and watershed indexes are shown in Table 2.1. In October 2008, the mean of the *E. coli* concentrations of all locations was 927 (\pm 678) Most Probable Number (MPN)/100 ml. The *E. coli* concentration ranged from 100 to 3300 MPN/100 ml. However, in October 2009, the mean *E. coli* concentration was 44% lower (519 ± 1777 MPN/100 ml) than October 2008, and the range was from 10 – 1777 MPN/100 ml. This indicates that the variation in *E. coli* concentrations among the sampling locations as well as seasons was substantial.

Moreover, in May 2009 the mean *E. coli* concentration was 48% higher (1371 ± 1477 MPN/100ml) than in October 2008. The maximum value of *E. coli* in this season was 66% higher than the maximum value in October 2008. Observations from May 2010 show that *E. coli* concentrations were low; they were 30% lower than concentrations in October 2008. In May 2009, *E. coli* concentrations at several locations were considerably higher than other seasons, for example, some of the locations showed *E. coli* concentrations as high as 5500 MPN/100 ml.

To assess the potential impacts of rainfall on *E. coli* concentrations, we calculated the cumulative rainfall for 15, 30, 45, and 60 days prior to the sampling day. For instance, the sampling day in October for both years was the 11th; therefore, we calculated cumulative rainfall 15, 30, 45, and 60 days prior to the 11th of October. Similarly for the May season, we calculated the cumulative rainfall 15, 30, 45, and 60 days prior to the 15th of May (which was the sampling day). In October 2008, the 15, 30, 45, and 60 days cumulative rainfall was 43, 70, 114, and 142 mm, respectively. In October 2009 the weather was relatively dry, the cumulative rainfall values were 1.3, 2, 2, and 5.1 mm respectively. Usually in May central Iowa receives frequent rainfall; the cumulative rainfall in May 2009 was 59, 132, 154, and 189 mm for 15, 30, 45, and 60 days, respectively. In May of 2010, these values were 72.39, 73.66, 73.66, and 73.66, respectively. Except for the May 2009 sampling date, rainfall during sampling was not observed; in May of 2009 a total of 19.60 mm of rainfall was recorded on the sampling day.

The variation in watershed indexes among the sampling locations is shown in Table 2.1. Figures 2.4a and 2.4b show the undisturbed and disturbed land cover indexes and their relationships with *E. coli* concentrations. In these figures, we plotted the watershed indexes versus *E. coli* concentrations of each season at 46 locations. The mean of I_{nc} was $0.25 (\pm 0.10)$ with a range from 0.11 to 0.53. The mean of I_{wl} (0.03 ± 0.03) was 12% of I_{nc} . However, the mean of I_{sc} (0.12 ± 0.08) was 48% of I_{nc} . Since not every polygon contains CAFO operations the values of I_{cafo} were zero for many of the polygons. Polygons showing the CAFO operations and areas receiving manure are shown in Figure 2.3. The mean value for I_{cafo} was $0.0001 (\pm 0.0002)$. Similar to CAFO indexes, minimum values for I_{wl} , I_{br} , I_d , I_{rcc} , I_{rcs} ,

and I_{urb} were also zero as not every polygon contained wetlands, barren land, drained land, cropped land cover, and urban areas. The mean value of I_d was $0.19 (\pm 0.16)$, while these values for I_{rcc} and I_{rcs} were 0.25 ± 0.19 and 0.20 ± 0.16 , respectively.

5.2. Linkages between *E. coli* concentrations, rainfall and watershed indexes

Figures 2.4a and 2.4b show *E. coli* concentrations versus undisturbed land cover indexes and disturbed land cover indexes, respectively. The bivariate correlation matrix showing the linkage between *E. coli*, and watershed indexes is shown in Table 2.2. Analyzing the cumulative rainfall of 15, 30, 45, and 60 days in all seasons shows that mean *E. coli* concentrations of 46 sampling locations was increased when the cumulative rainfall was higher (i.e., higher cumulative rainfall of the season resulted in increased in-stream *E. coli* concentrations). The correlation between disturbed land cover watershed indexes and *E. coli* concentrations were mixed (positive as well as negative). For example, I_d , I_{rcc} , I_{rcs} , I_{cafo} , I_{br} were positively correlated, while I_{nc} , I_{wb} , I_{sc} , I_{slope} , and I_{urb} were negatively correlated (Table 2.2, Figure 2.4a,b).

Table 2.1. Descriptive statistics of in-stream *E. coli* concentrations at 46 sampling location in the Squaw Creek watershed, Iowa and watershed indexes, including natural cover index (I_{nc}), wetland index (I_{wl}), river - stream-corridor index (I_{sc}), barren land index (I_{br}), CAFO index (I_{cafo}), drained land index (I_d), index for corn crop (I_{rcc}), index for soybean crop (I_{rcs}), index for slope (I_{slope}), and index for urban land (I_{urb}).

	Mean	Median	Std. Dev.	Minimum	Maximum
<i>E. coli</i> (MPN/100 ml)					
Oct-08	927	735	678	100	3300
Oct-09	519	360	438	10	1777
May-09	1371	729	1477	80	5500
May-10	651	495	642	50	3900
Watershed indexes					
I_{nc}	0.25	0.23	0.10	0.11	0.53
I_{wl}	0.03	0.02	0.03	0.00	0.14
I_{sc}	0.12	0.10	0.08	0.03	0.31
I_{br}	0.0014	0.00	0.0070	0.00	0.047
I_{cafo}	0.0001	0.00	0.0002	0.00	0.0008
I_d	0.19	0.22	0.16	0.00	0.50
I_{rcc}	0.25	0.34	0.19	0.00	0.53
I_{rcs}	0.20	0.28	0.16	0.00	0.44
I_{slope}	0.42	0.39	0.19	0.18	1.00
I_{urb}	0.17	0.03	0.22	0.00	0.73

5.3. Model development and validation

To develop the models we used stepwise multiple linear regression analyses. The four models for four different types of hydrological corrections (i.e., 15, 30, 45, and 60 days of cumulative rainfall) were developed, and the results are shown in Table 2.3. The coefficient of determination (R^2) for 15, 30, 45, 60 days of cumulative rainfall models were 0.08, 0.36, 0.26, and 0.29, respectively. The best R^2 value (0.36) was achieved using the 30 day cumulative rainfall.

The Nash-Sutcliffe Efficiency (NSE) coefficient value of the developed model for the 30 day cumulative rainfall model was -0.45, and for the 60 day cumulative rainfall model it was -0.59. The model skill for the 30 and 60 day cumulative rainfall models were 0.46 and 0.55, respectively. The model skill and NSE coefficient for 15, and 45 days cumulative rainfall models are shown in Table 2.4. In order to understand differences in predicted and observed *E. coli*, we performed a factor analysis (Table 2.4), and found that about 96% of the predictions are within one order of magnitude of the observed values when applying the 30 day model; similarly approximately 96% are within one order of magnitude of the observed values when applying the 60 day model.

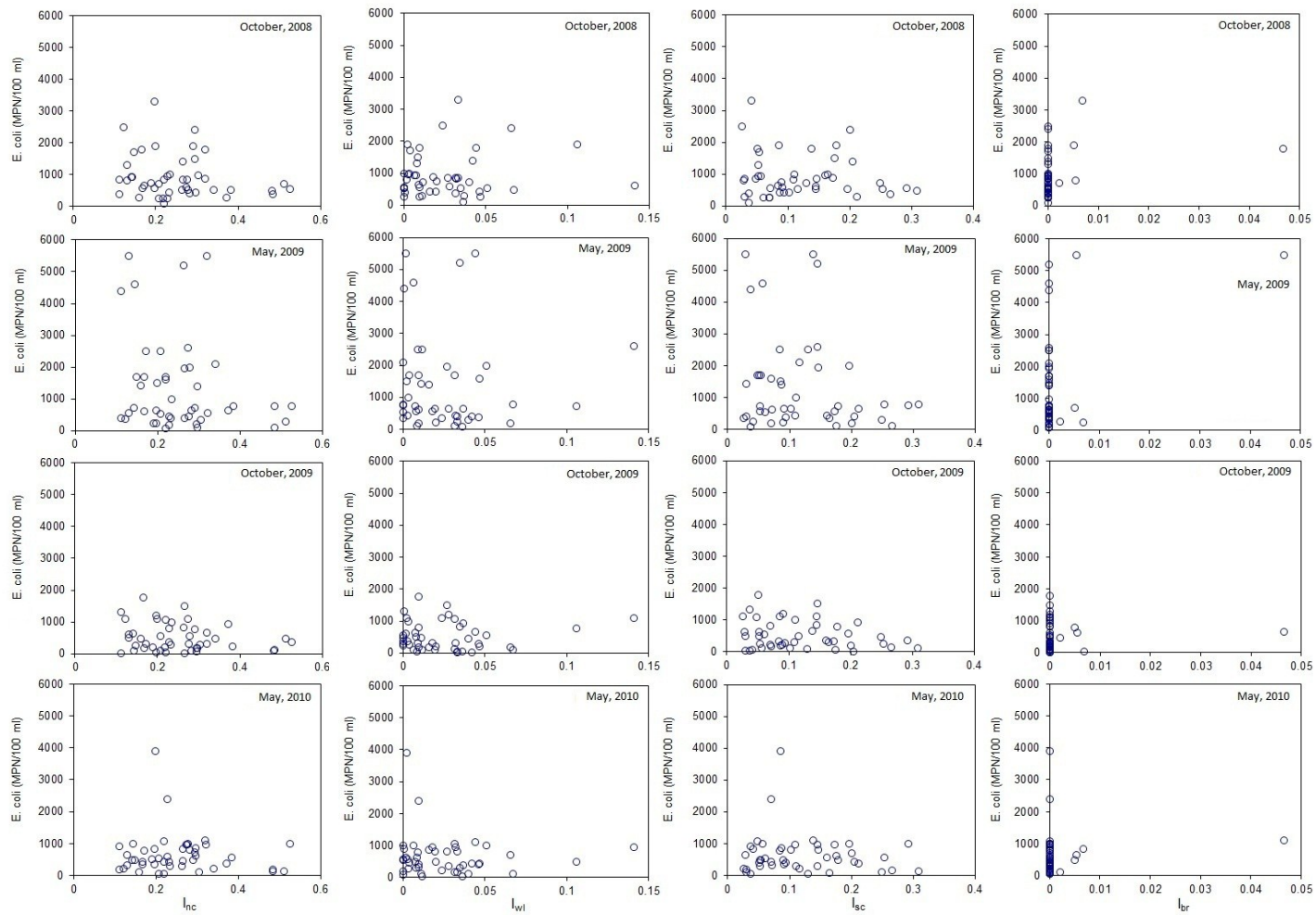


Figure 2.4a. Relationships between *E. coli* (MPN/100 ml) and undisturbed land cover indexes. *E. coli* concentrations at 46 locations for each season (October 2008, May 2009, October 2009, and May 2010) and the corresponding undisturbed land cover (I_{nc} , I_{wl} , I_{sc}) and barren land (I_{br}) indexes are shown in the Figure.

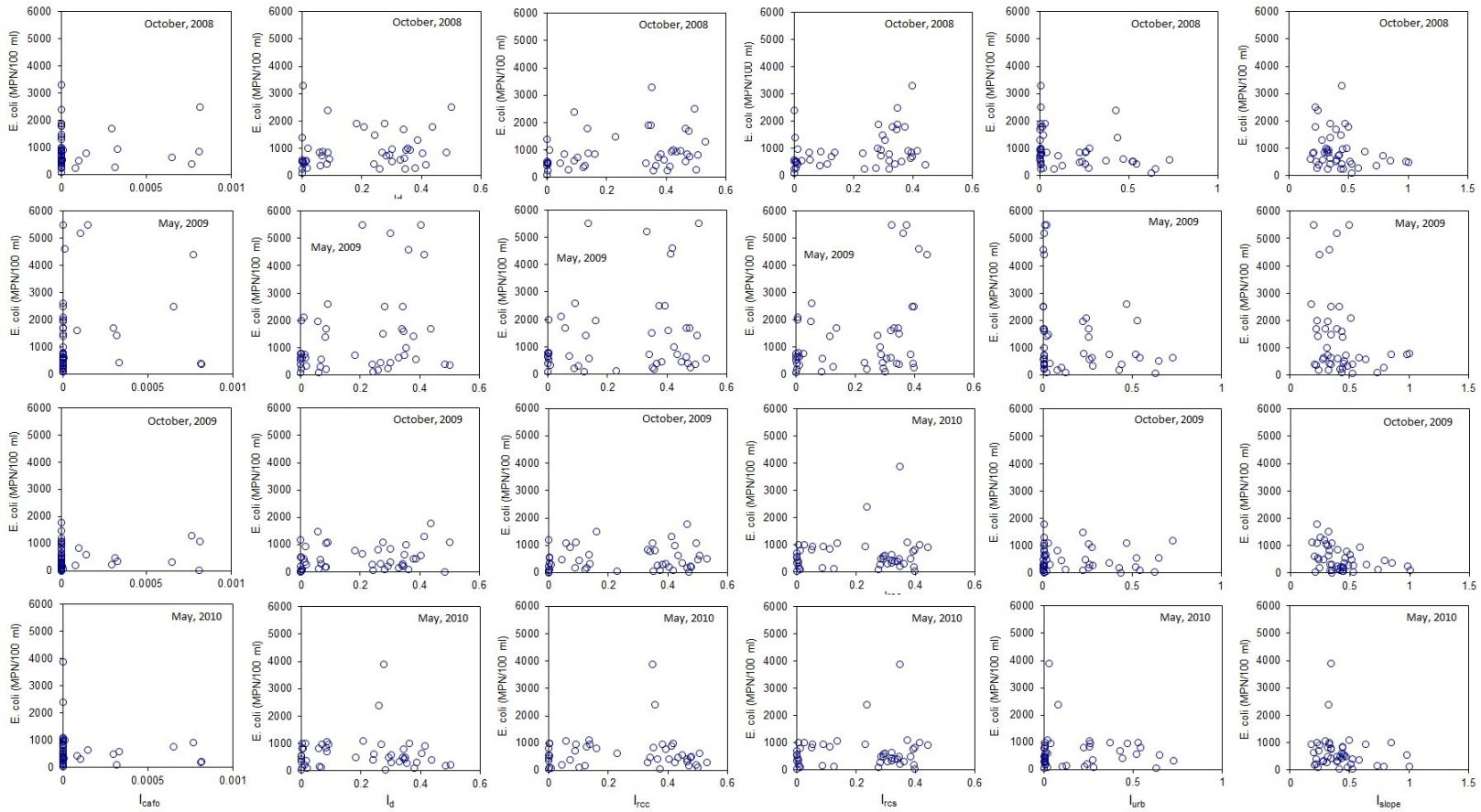


Figure 2.4b. Relationships between *E. coli* (MPN/100 ml) concentrations and disturbed land cover indexes. *E. coli* concentrations at 46 locations for each season (October 2008, May 2009, October 2010, and May 2010) and the corresponding disturbed land cover (I_{cafo} , I_d , I_{rcc} , I_{rcs} , I_{urb}) and slope (I_{slope}) indexes are shown in the Figure.

Table 2.2. Bivariate correlation matrix for in-stream *E. coli* concentration at 46 sampling locations, and watershed indexes, including natural cover index (I_{nc}), wetland index (I_{wl}), river - stream-corridor index (I_{sc}), barren land index (I_{br}), CAFO index (I_{cafo}), drained land index (I_d), index for corn crop (I_{rcc}), index for soybean crop (I_{rcs}), slope index (I_{slope}), and urban land index (I_{urb}).

	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>
	Oct 2008	May 2009	October 2009	May 2010
I_{nc}	-0.21	-0.23	-0.20	-0.08
I_{wl}	0.10	-0.03	0.06	-0.08
I_{sc}	-0.12	-0.21	-0.20	-0.10
I_{br}	0.28	0.44*	0.04	0.10
I_{cafo}	0.08	0.15	0.09	-0.12
I_d	0.16	0.30*	0.19	0.02
I_{rcc}	0.25	0.19	0.10	0.03
I_{rcs}	0.33*	0.37*	0.07	0.12
I_{slope}	-0.23	-0.26	-0.37*	-0.16
I_{urb}	-0.30*	-0.25	-0.03	-0.05

Note: * indicates significant at $\alpha = 0.05$; number of observation = 184.

Figure 2.5 shows the model validation results. The regression equations developed for predicting *E. coli* concentrations are shown in Table 2.3. The result shows that hydrological corrections using 30 days cumulative rainfall produced better results (i.e., r^2 of 0.36). The regression equation of 30 day hydrological correction is marked a by red dotted line in Table 2.3. In table 2.4, we have shown r^2 , NSE , and model skill values for all four regression equations. In developing the equations, we randomly selected 70% of the total data, while 30% of the randomly selected data were reserved for model validation. Figures 2.5a and 2.5c

Table 2.3. Regression equations describing relationships between stream water *E. coli* concentrations and catchment characteristics of Squaw Creek Watershed, including wetland index (I_{wl}), drained land index (I_d), confined animal feeding operation index (I_{cafo}), index for corn crop (I_{rcc}) and index for soybean crop (I_{rcs}). Coefficient of determination (R^2), degree of freedom (DF), p-value, and number of samples (n) are shown in the Table.

Models	Regression equations
15 Day cumulative rainfall	$613.56 - 11387.33 \times I_{cafo} - 24.93 \times I_{rcc} + 45.71 \times I_{rcs}$ $R^2 = 0.07$; DF = 125; n = 128; p = 0.0193
30 Day cumulative rainfall	$432.47 + 75.17 \times I_{wl} + 51.58 \times I_d - 54.34 \times I_{rcc} + 44.55 \times I_{rcs}$ $R^2 = 0.36$; DF = 124; n = 128; p < 0.0001
45 Day cumulative rainfall	$491.60 - 27.04 \times I_{rcc} + 52.79 \times I_{rcs}$ $R^2 = 0.26$; DF = 126; n = 128; p < 0.0001
60 Day cumulative rainfall	$505.12 - 32.97 \times I_{rcc} + 62.44 \times I_{rcs}$ $R^2 = 0.29$; DF = 126; n = 128; p < 0.0001

shows the result of 15 and 45 days of cumulative rainfall regression models, respectively. The results of 30 and 60 day cumulative rainfall models are shown in Figure 2.5b, and 2.5d. In the Figures, the 1:1 line and the one order of magnitude lines are drawn to show the proximity of the predictions to the observed values. As shown in Figure 2.5d (60 day cumulative rainfall model), 95 – 98% of the predictions are within one order of magnitude of the observed values; however, in the 30 day cumulative rainfall model (Fig. 2.5b), more predictions fall along the 1:1 line, and the R^2 value supports this observation as it is higher than the other models. The predictions statistics for all models are shown in Table 2.3.

6. Discussion

In this study we assessed the dependence of in-stream *E. coli* concentrations on watershed indexes. During the four sampling events in 2008, 2009, and 2010, most locations had *E. coli* concentrations higher than the U.S. EPA water quality standard (USEPA, 2001a) which states that the geometric mean of at least five samples during a 30-day period must not exceed 126 MPN/100 ml and that a single sample must not exceed 235 MPN/100 ml. The spatial (variation among the sampling locations) and temporal variability (variation among the sampling seasons) of in-stream *E. coli* concentrations and relationships with rainfall is also shown. The temporal variability (among sampling events) in *E. coli* concentrations was considerably high, with mean values ranging from 519 – 1371 MPN/100 ml (Table 2.1). For example, samples collected during May of 2009 show exceptionally high *E. coli* concentrations compared to the other sampling events. Spatial variability was also large (standard deviation = 1477 MPN/100 ml) during the May 2009 sampling period (Table 2.1). The exceptionally high *E. coli* concentration in May 2009 can be attributed to overland flow

due to rainfall on the day of sampling. Several previous studies have found that the overland flow (i.e., runoff from agriculture land) increases the influx of *E. coli* into stream water (Guber et al., 2010; Soupir et al., 2006; Edwards et al., 2000; Khaleel et al., 1982; Moore et al., 1988).

Due to availability, we used the land cover, and manure data from 2002 and 2006, respectively; however, the observed *E. coli* data used in this study are from 2008 to 2010. For the study area, the land cover map of 2002 is the most comprehensive. In addition to this study, many other recent studies have also relied on these dated land cover maps for watershed scale assessments (Secchi et al., 2011; Burkart and Jha, 2012; Jha et al. 2011). Although the land cover map used in this study is approximately 10 years old, few changes in land cover have occurred over this time period. Iowa, U.S.A. is renowned for intensive agricultural production, and cropped land cover dominates Iowa's watersheds. While estimating watershed indexes, we used the land cover map of 2002; the recent changes in land cover in the watershed were not included. Due to increased demand of corn for biofuel production, a trend of increasing corn acreages in Iowa has been reported. For example, a recent study by Khanal et al. (2012) studied the potential impacts of the expansion of corn ethanol production on cropping pattern and emphasized that corn demand will be met by growing corn more intensively and shifting cropland from cropping systems with lower environmental impacts into continuous corn (CC). The authors predicted that the probability of CC will increase in the vicinity of ethanol plants; however, additional studies are required to better understand the impacts of CC on in-stream pathogen concentrations. We do not anticipate considerable impacts of recent land cover (i.e., increased core acreages) changes

on in-stream *E. coli* concentrations; however the latest land cover map is preferred for calculating watershed indexes over the land cover map of 2002.

Similarly, the manure application area map of 2006 was obtained from Iowa DNR, which based these estimates on animal feeding operation (AFO) locations as well as the amount of manure produced at the AFOs in 2006. It is possible that some changes in the areas which currently receive land application of manure have occurred; however, we expect these changes to be minimal. To assess potential changes, we compared maps locating confined feeding operation in 2011 and 2006 (Fig. 2.3), which indicates that there are some slight changes, for instance, one new CAFO is now located at the south end of the watershed (Fig. 2.3) in the 2011 map that was not present in the 2006 map. Also in this study we used 30 m DEMs, and applying finer resolution (10 m or lower) might result in a more precise estimation of watershed indexes.

For the 15 day cumulative rainfall model, the manure application areas (I_{cafo}) and cropped land cover (I_{rcc} and I_{rcs}) were found to be significantly related with in-stream *E. coli* concentrations ($p = 0.019$); however, the probability for variables to be included in this model were set at a higher level ($p = 0.10$). When we used the 30 day cumulative rainfall, in addition to cropped land, wetland (I_{wl}) and drained land (I_d) indexes were also found to be significant ($p < 0.0001$) indicators (Table 2.3) in *E. coli* predictions. In the 45 and 60 day cumulative rainfall models, only the cropped land indexes (I_{rcc} , and I_{rcs}) were found to be significantly related with in-stream *E. coli* ($p < 0.0001$). This indicates that the amount of rainfall and subsequent runoff, potentially can impact in-stream water quality.

Many studies have also noted the role of wetlands in reducing *E. coli* discharges to surface waters (Ibekwe et al., 2003; Nerella et al., 2000). Landscape such as natural land cover (particularly riparian areas) can act as buffer between the stream and disturbed land cover, reducing the influx of contamination into streams. However, natural land cover also attracts wildlife, and in the past many studies have shown that wildlife in riparian areas often increases stream *E. coli* concentrations (Buckely et al., 1998; Weiskel et al., 1996; Cox et al., 2005).

Our results (Table 2.2, Figs. 2.4a, 2.4b) show that I_{rcs} , I_{rcs} , and I_d , which link the inputs and changes from cropped land to stream water (Hamilton, 2002), are positively correlated with in-stream *E. coli* concentrations. This indicates that water drainage/runoff from cropped land can potentially increase in-stream *E. coli* concentrations. The primary reason for high *E. coli* concentration is likely the cropped land in the watershed, which receives manure as fertilizer. Based on analyses of the manure application map of 2006, approximately 6.8% of the watershed area receives manure as a fertilizer with an application rate of 179.33 N kg/ha. This can potentially elevate the *E. coli* levels in stream water. Previous studies such as Soupir et al. (2006) and Guber et al. (2010) have studied the transport of bacteria from manures applied to pasture land, and found that rainfall events occurring shortly after manure application to cropped land can increase pathogen loads to surface waters. In addition, the tile drains in cropland can also transport bacterial contamination into stream waters (Guber et al., 2006; Ferguson et al., 2007; Gerba and Smith, 2005; Duffy, 2003). The study area is mostly flat cropped land (averaging 2% slope) under tile drainage management. A recent study by VanderZaag et al. (2010) has detected high concentrations of bacteria in tile

drainage effluent. Other studies by Lapen et al. (2008) and Gottschall et al. (2009) tested the impacts of municipal biosolid applications to cropped land, and found that nutrient (N, NH₄-N, Total P, PO₄ – P) and bacteria (*E. coli* and *Clostridium perfringens*) concentrations were significantly higher in tile drain effluent as well as in ground water, when the agriculture land received municipal biosolids.

The cropped land indexes were identified as significant variables in all four models, supporting previous studies that have shown that cropped land potentially increases *E. coli* concentrations in stream water (Guber et al., 2006; Ferguson et al., 2007; VanderZaag et al., 2010; Lapen et al., 2008). Fields under corn-soybean rotation in Iowa will typically receive manure applications in alternate years during the fall prior to corn planting. The land cover under corn crop receives manure as fertilizer. Manure is typically only applied to about half of the land under corn-soybean rotation management in any one year, which could lead to difficulties in linking that land cover to in-stream *E. coli* levels. The CAFO index (I_{cafo}) surprisingly was only significant in the 15 day rainfall model (Table 2.4). In addition to CAFOs, upstream livestock pasture density can also potentially impact stream bacteria levels. About 2.5% of the watershed area is under grazed grassland (based on the land cover map of 2002), and potentially used as pasture. Previous studies have shown that pasture land can also influence in-stream water quality. Additional information including number of animals, size of the animals, grazing schedule, and access to surface waters is needed to estimate the impacts of grazing on in-stream *E. coli* concentrations. Including grazing information in the analysis can potentially improve the predictions, especially in watersheds with a higher percentage of the total land area under grazing management. A recent study by

Wilkes et al. (2011) found that the detections of pathogenic bacteria including *Salmonella enterica*, *Campylobacter spp.*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 was related to livestock pasture density; and pathogen detection was higher in areas where cattle have access to the watercourse. Our bivariate correlation analysis found that I_{cafo} was positively correlated with *E. coli* concentrations during all sampling seasons except May 2010 (Table 2.2, Figs. 2.4a, 2.4b).

As discussed in method section, r^2 and model skill values greater than 0.35 is considered satisfactory for predicting in-stream *E. coli* concentrations. We also targeted NSE values greater than -0.50. The model performance analysis (Table 2.4 & Fig 2.5) did produce reasonable R^2 , model skill, and NSE values for the models, and it is expected that a larger and perhaps continuous dataset would improve the results further. However, when comparing this approach to the performance of other watershed-scale models that are capable of simulating in-stream bacteria concentrations, the results are promising. Cho et al. (2010) and Parajuli et al. (2009a; 2009b) both reported NSE values for the Soil and Water Assessment Tool (SWAT). Cho at al. (2010) reported the NSE range from -0.78 to -0.99 (except one station with a positive value of 0.01), and the study by Parajuli (2009 a;b) reported the NSE value of -0.41 on an uncalibrated model. Parajuli's study reported an r^2 value of 0.26. The R^2 values of the predictions are low; however, when compared to the results of this study (0.08 – 0.36). Moreover, as described by Dorner et al. (2006) order-of-magnitude estimates are needed for

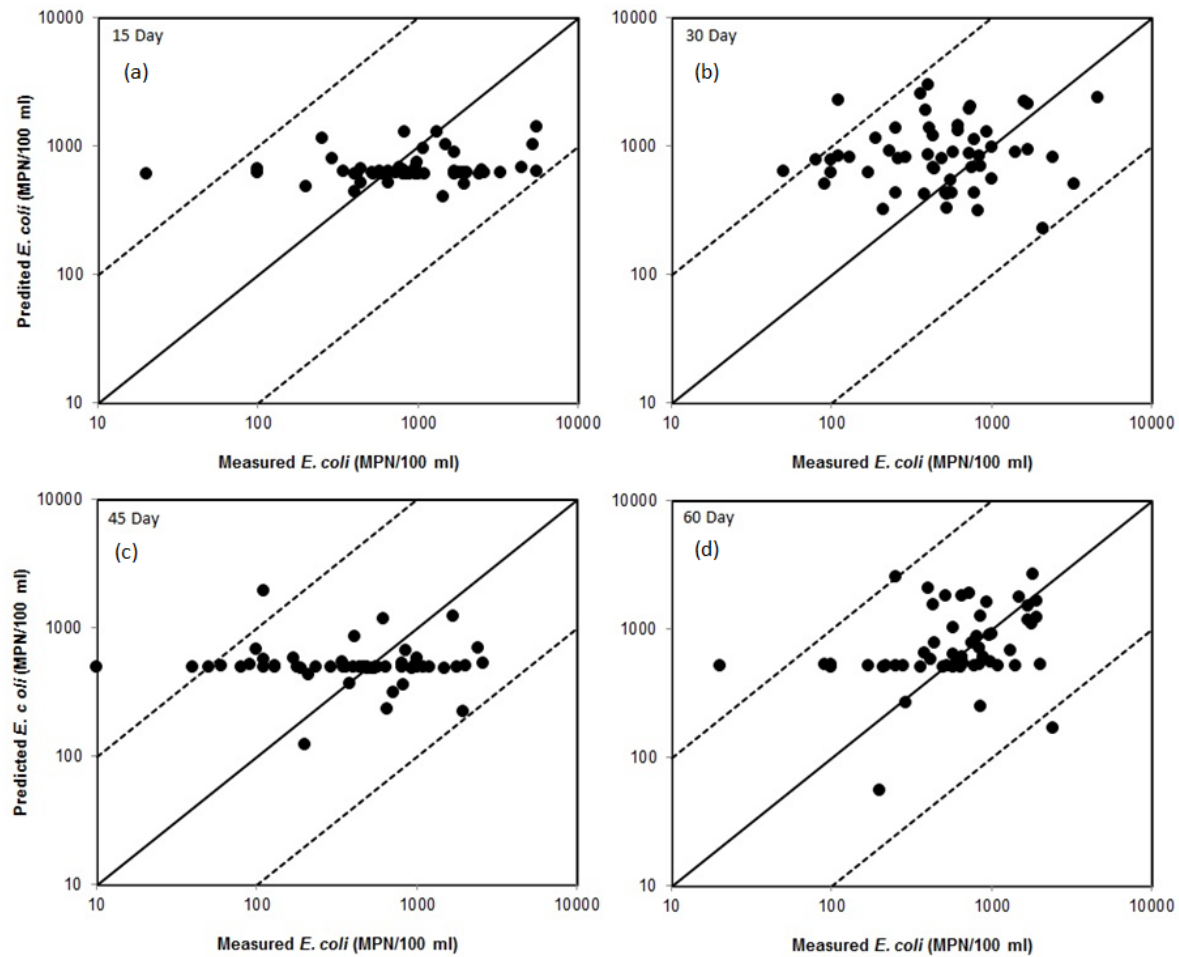


Figure 2.5a,b,c,d. Model validation based on 30% of randomly selected observed data. Comparisons between observed and predicted *E. coli* concentrations are shown for four different models (hydrological corrections of 15, 30, 45, and 60 days of cumulative rainfall). The plots show the 1:1 line (solid line) and the line representing one order of magnitude difference between observed and predicted values (dashed line).

Table 2.4. Model performance statistics for 15 day, 30 day, 45 day, and 60 day cumulative rainfall.

Model performance indicators	cumulative rainfall			
	15 day	30 day	45 day	60 day
Model skill	0.39	0.46	0.29	0.55
NSE coefficient	-0.15	-0.45	-0.20	-0.59
Coefficient of determination (R^2)	0.08	0.36	0.26	0.29
Percentages of predictions within one order magnitude of observed values	98	96	95	96

water quality improvement and greater precision is not necessary or expected for stream water bacteria predictions. Our results show that more than 95% of the predictions are within 1 order of magnitude, which is acceptable for in-stream bacteria predictions at the watershed scale.

A major difference in this study compared to previous work is the number of locations at which the *E. coli* concentrations are being predicted. We examine water quality at forty-six different locations over different seasons, while Parajuli et al. (2009 a;b) assessed model prediction at only six locations and the study by Cho et al. (2010) examined water quality at four stations. In this study, approximately 96% of the predictions fall within one order of magnitude of the observed values (Table 2.4 and Figure 2.5) when applying the 30 day cumulative rainfall model, and 96% fall within one order of magnitude of observed values when applying the 60 day cumulative rainfall model. As expected some extreme values were

not predicted well by the model; however, the percentages of these predictions were low (4 %) for both 30 and 60 day rainfall models. Model performance statistics for 15 and 45 day cumulative rainfall corrections are shown in Table 2.4.

While developing the models for predicting in-stream *E. coli* concentration, several assumptions were made. As previously discussed, the land cover map of 2002 and manure application area map of 2006 were used along with observed *E. coli* data from 4 seasons in 2008 – 2010. Changes in landcover or manure management practices between the development of the GIS layers used in this assessment and the collection of water quality data are expected to be minimal; however, increases in cropped land receiving animal waste could lead the model to underpredict in-stream *E. coli* levels. The hydrological corrections of watershed indexes using cumulative rainfall was used instead of actual runoff data. A hydrological corrections for each polygon based off of runoff data may have improved the results; however, the use of cumulative rainfall in the absence of runoff data has been successfully applied previously (Jarvie et al., 2002). In order to improve the robustness of the study, we considered four sets of hydrological corrections (15, 30, 45, and 60 days) which provided insight into how changes in hydrology influence predictions. In this study, the watershed indexes were estimated using a thiessen polygon approach; other methods such as creating subwatersheds for each sampling location could also potentially improve predictions.

This work demonstrates the usefulness of hydrologically corrected watershed indexes for predicting in-stream *E. coli* concentrations; however, future work is recommended to further explore this approach. Additional information such as wildlife population within the

watershed could also be important because many previous studies have shown that wildlife potentially contribute *E. coli* to stream waters, especially from riparian zones (Cox et al., 2005; Weiskel et al., 1996). We anticipate that precise estimation of indexes denoting manure application rates and timing of application in each polygon might also potentially improve model prediction, as might delineating the sub watershed for each sampling location prior to calculating the watershed indexes. In addition, using flow weighted *E. coli* concentrations and a larger in-stream *E. coli* dataset would be useful when relating mean *E. coli* concentrations with watershed indexes. Beyond the stepwise regression approach proposed in this study, application of non-linear modeling, such as regression trees, could also be a viable alternative.

The GIS based method proposed here can be a useful approach to characterize the watershed, and linking the watershed indexes with in-stream contamination (i.e., *E. coli* levels); however, the approach requires further verification. Implementing the approach to a different watershed and verifying the results can potentially improve the methods (i.e., identifying the indexes which have most significant impacts on in-stream water quality) as well as regression equations proposed in Table 2.3. The calculations provided here are based on a limited dataset e.g., single sampling event from four seasons. Although we used the data from 46 sampling locations, which provided a considerable spatial heterogeneity useful for modeling, more frequent monitoring (i.e., multiple sampling in same season) is required to develop a robust model. Therefore, we do not recommend using the regression equations presented in Table 2.3 for implementing the land cover change plan in order to control in-stream *E. coli* concentrations without verifying the model using the data from the other

watershed. Nevertheless, the method adapted here for calculating the watershed indexes and deriving the relationships between watershed indexes and in-stream *E. coli* concentrations can be a potentially useful tool, which can support decision making and identifying the potential sources of *E. coli* contamination in stream water.

7. Conclusions

This study assessed the impacts of hydrologically corrected watershed indexes on in-stream *E. coli* concentrations at 46 locations over four sampling periods in Squaw Creek Watershed, Iowa, using a GIS based method. The watershed indexes were estimated using disturbed and undisturbed land cover of the Squaw Creek Watershed, and a bivariate analysis was used to assess relationships between watershed indexes, rainfall, and in-stream *E. coli* concentrations. Using watershed indexes, step-wise multivariate regression models were developed to predict in-stream *E. coli* concentrations, and predictions were compared with observed *E. coli* data. The model performance was evaluated using factor analysis as well as statistical indicators such as model skill, NSE coefficient, and coefficient of determination. The results demonstrate that the approach developed in this study has the potential to be a useful tool for predicting in-stream *E. coli* concentrations, and for evaluating the impacts of watershed characteristics on water pathogens in agricultural catchments. This new method of using watershed indexes to predict in-stream *E. coli* concentrations will be useful to planners who are responsible for predicting the impacts of land management decisions on stream water quality, and for regulatory agents responsible for conducting watershed scale assessments and remediation projects.

Acknowledgments

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CHAPTER 3. A MODEL FOR PREDICTING RESUSPENSION OF *Escherichia coli* FROM STREAMBED SEDIMENT

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Abstract

To improve the modeling of water quality in watersheds, a model is developed to predict resuspension of *Escherichia coli* from sediment beds in streams. The resuspension rate is expressed as the product of the concentration of *E. coli* attached to sediment particles and an erosion rate adapted from work on sediment transport. The model uses parameter values mostly taken from previous work, and it accounts for properties of the flow through the bottom shear stress and properties of the sediment through the critical shear stresses for cohesive and non-cohesive sediment. Predictions were compared to resuspension rates inferred from a steady mass balance applied to measurements at sixteen locations in a watershed. The model's predictions matched the inferred rates well, especially when the diameter of particles to which *E. coli* attach was allowed to depend on the bottom shear

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stress. The model's sensitivity to the parameters depends on the contributions of particle packing and binding effects of clay to the critical shear stress. For the current data set, the uncertainty in the predictions is controlled by the concentration of *E. coli* attached to sediment particles and the slope used to estimate the bottom shear stress.

1. Introduction

Pathogens impair 480,000 km of rivers and shorelines and 2 million ha of lakes in the U.S., and the cost to implement total maximum daily load (TMDL) plans is estimated as \$0.9 to \$4.3 billion per year (USEPA, 2010a;b). To predict the risk of bacteria to public health and allocate load reductions fairly, models that include accurate representations of the key processes of fate and transport are required (Fries et al., 2008). For example, the high concentrations of bacteria in suspended sediment and bed sediment suggest that the understanding of interactions between pathogens and sediment must be improved (Droppo et al., 2009). Sediment disturbance can account for the majority of total bacterial contamination (Nagels et al., 2002), and a one-dimensional model applied to the field measurements of Jamieson et al. (2005b) showed that including interactions with the sediment improved the predictions of *E. coli* concentrations in the stream (Rehmann and Soupir, 2009). However, models that U.S. regulatory agencies use to determine pollutant load reductions usually do not include resuspension of bacteria as a source.

Even when resuspension is included in models, how to predict it is uncertain. Many researchers have either specified the resuspension rate (e.g., Petersen et al., 2009) or expressed it mainly as a function of flow (Wilkinson et al., 1995; Tian et al., 2002; Collins and Rutherford 2004). Kim et al. (2010) added a model of resuspension of *E. coli* to the Soil

and Water Assessment Tool (SWAT); resuspension was estimated using a simplified version of Bagnold's stream power equation, which has been criticized for not including the effect of grain size on sediment transport (Ferguson 2005). Hipsey et al. (2008) accounted for properties of the sediment by including a critical shear stress computed from the Shields criterion, but although the Shields criterion holds for non-cohesive sediment, its validity for cohesive sediment is questionable (Mehta and Lee, 1993). Sanders et al. (2005) assumed resuspension to be proportional to the shear stress, while Bai and Lung (2005) expressed resuspension as a nonlinear function of the difference between the shear stress and a critical shear stress. As Rehmann and Soupir (2009) noted, resuspension of microorganisms from a sediment bed depends in general on properties of the flow and sediment (e.g., Lick, 2009, ch. 3), the type of microorganism (Hipsey et al., 2008), and the presence of biofilms (e.g., Droppo et al., 2001).

To predict *E. coli* resuspension reliably, theory for transport of cohesive sediment must be considered because most bacteria attach to cohesive particles (Black et al., 2002). For non-cohesive sediments such as sands, which typically have particle diameters greater than 62 μm , the main forces to consider are the dislodging tendency of the fluid shear stress and the submerged weight of a particle. For cohesive sediment, however, inter-grain forces complicate the predictions. Clay and very fine silt ($< 8 \mu\text{m}$) exhibit strong cohesion, while larger silt particles (8-62 μm) are more weakly cohesive (van Rijn, 2007). Furthermore, because cohesion of deposited flocs renders the critical shear stress for erosion higher than that for deposition, the conditions under which the bed is deposited and the time for consolidation can be expected to affect erosion (Krishnappan, 2007), as well as the properties

of the eroded flocs (Stone et al., 2008). For example, sediment beds formed in a sheared flow eroded at higher shear stresses than those formed in quiescent conditions (Droppo et al., 2001). Also, biofilms increase the critical shear stress by forming a coating that protects the sediment against erosion (Droppo et al., 2001). The coating contains extracellular polymeric substances that increase cohesiveness between particles (Paterson, 1989) and strengthens the surficial sediment (e.g., Sutherland et al., 1998).

To improve predictions of in-stream transport of *E. coli*, we develop a formulation for *E. coli* resuspension that accounts for properties of the flow and properties of both cohesive and non-cohesive sediment. The objectives of this study are 1) to develop a model by assuming that the *E. coli* resuspension rate is proportional to the erosion rate of sediment, 2) to compare the predictions from the model to resuspension rates inferred from mass balances at several locations in a watershed, 3) to evaluate the model's predictive skill, and 4) to assess the sensitivity and uncertainty of the resuspension rate to the input parameters so that measurements and modeling can be improved. The model is developed in section 2, and the measurements and calculations used in our application of the model to predict resuspension in a creek are described in section 3. Objectives 2, 3, and 4 are addressed in section 4, and the main conclusions are listed in section 5.

2. Model

We hypothesize that the rate of resuspension of attached *E. coli* can be estimated as the product of the concentration of attached *E. coli* in the sediment bed and an erosion rate similar to that for sediment. Several researchers have proposed formulas to predict erosion (Partheniades, 1965; Mehta, 1989), but we use the formulation of Lick (2009), in which the

erosion rate E depends on the bottom shear stress τ_b , the critical shear stress τ_c for cohesive sediment, and the critical shear stress τ_{cn} for non-cohesive sediment. For $\tau_b > \tau_{cn}$,

$$E = E_0 \left(\frac{\tau_b - \tau_{cn}}{\tau_c - \tau_{cn}} \right)^{n_s}, \quad (1)$$

where $E_0 = 10^{-6}$ m/s is the erosion rate at the threshold of erosion (Lick 2009). Lick (2009) found the exponent n_s to be approximately equal to 2 for small and intermediate particles, while others expressed the erosion rate as linearly proportional (i.e., $n_s = 1$) to the difference between the bottom stress and a critical stress (Amos et al., 1996). A compilation of data shows that the critical shear stress for non-cohesive sediment depends on the particle diameter d :

$$\tau_{cn} = 414d, \quad (2)$$

where τ_{cn} is in N/m² and d is in m (Lick 2009). For cohesive sediment, the packing of the particles, which is quantified by the bulk density ρ_b , and extra binding forces caused by clay must be considered. Combining the work of Roberts et al. (1998) and Lick et al. (2004), Lick (2009) proposed

$$\tau_c = \tau_{cn} \left(1 + \frac{ae^{b\rho_b}}{d^2} + \frac{c_5}{c_3 d} \right), \quad (3)$$

where a and b are coefficients that Lick (2009) specified as 8.5×10^{-16} m² and 9.07 cm³/g, respectively. The coefficient c_3 is given by

$$c_3 = \frac{\pi}{6} \rho g (s - 1)$$

where ρ is the density of water, s is the specific gravity of the sediment particle, and g is the acceleration of gravity. The coefficient c_3 depends on the clay fraction; for 2% bentonite added to quartz particles, $c_3 = 7 \text{ N/m}^2$ (Lick et al., 2004).

Lick (2009) proposed equation (1) as a uniformly valid formulation for erosion. For large particles and no clay fraction, the bulk density does not affect the critical shear stress (Figure 3.1), and equation (1) follows a form that applies to fine-grained, coarse-grained, cohesive sediments, and non-cohesive sediment (Lick, 2009). As the particle size decreases, effects of cohesion dominate (i.e., $\tau_c \gg \tau_{cn}$), and equation (1) reduces to a form similar to that of Roberts et al. (1998) for cohesive sediment. When the binding effects of clay provide the main resistance to particle motion, the critical shear stress depends only weakly on the particle diameter (Figure 3.1) because both the mobilizing force and the resisting force depend on the surface area of the particle.

The erosion rate in equation (1) can be adapted to predict the rate of resuspension of *E. coli*. Bacteria attach to and bioflocculate around solid particles (Black et al., 2002) and deposit to the bottom sediments; attached fractions for streams ranges from 55% during storms (Characklis et al., 2005; Krometis et al., 2007) to between 80 and 100% (Auer and Niehaus, 1993; Hipsey et al., 2008). When sediment is resuspended, an influx of *E. coli* from the stream bed results (Whitman et al., 2006). Therefore, we predict the *E. coli* resuspension rate R_a ($\text{CFU m}^{-2}\text{s}^{-1}$) by multiplying the erosion rate by the concentration C_a (CFU/m^3) of *E. coli* attached to sediment in the bed:

$$R_a = C_a E_{0a} \left(\frac{\tau_b - \tau_{cn}}{\tau_c - \tau_{cn}} \right)^{n_a} . \quad (5)$$

The coefficient and exponent are changed to E_{0a} (m/s) and n_a to allow for possible differences from E_0 and n_s in equation (1). The resuspension rate is nonzero only when the bottom shear stress exceeds the critical shear stress for non-cohesive sediment. We expect equation (5) to be useful in predicting resuspension rates because it accounts for effects of the flow and sediment, as well as the concentration of *E. coli* in the stream bed.

3. Methods

We applied the model to the Squaw Creek watershed to predict the resuspension of *E. coli* attached to stream bottom sediments into the water column. The model was evaluated using data collected from sixteen sites in the watershed. At each site, flow geometry was measured, and the concentrations of *E. coli* in streambed sediment and the overlying water column were determined. The resuspension rates predicted with equation (5) were compared to values inferred from the one-dimensional model of Rehmann and Soupier (2009). A sensitivity analysis was conducted to assess the influence of certain parameters on model output, and the parameters controlling the uncertainty in the resuspension rate were identified. Table 3.1 lists the parameters used in computing the predicted and inferred resuspension rates, and it indicates whether the parameters were measured, estimated, taken from previous work, or calibrated.

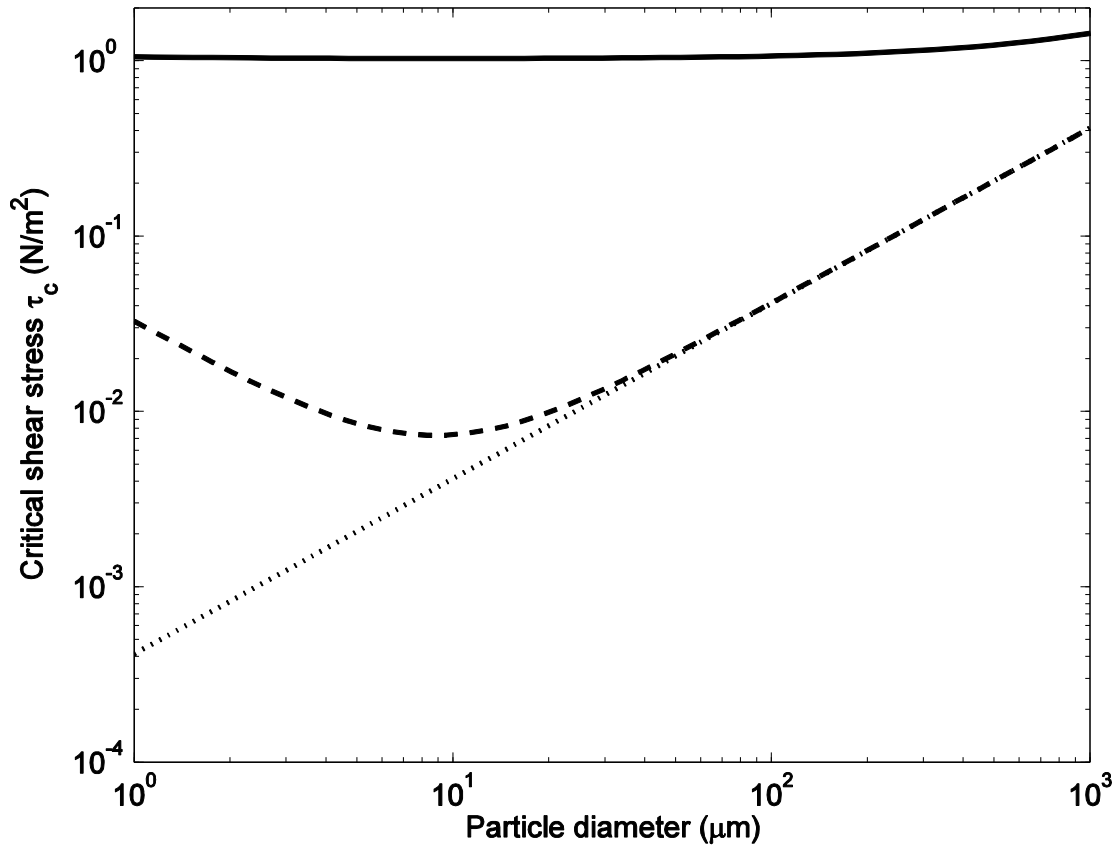


Figure 3.1. Dependence of critical shear stress on particle diameter. The dotted line is the critical stress for non-cohesive sediment. The dashed line is the critical stress for cohesive sediment with a bulk density ρ_b of 1.26 g/cm^3 and no effects of clay ($c_s = 0 \text{ N/m}^2$), and the solid line is the critical stress for cohesive sediment with $\rho_b = 1.26 \text{ g/cm}^3$ and $c_s = 21 \text{ N/m}^2$.

3.1. Study area

Squaw Creek passes through four counties of central Iowa, U.S.A. before discharging into the South Skunk River near Ames (Figure 3.2). The total area of the Squaw Creek watershed, as defined by the 10-digit hydrologic unit code, is 59,327 ha, and the average basin slope is 2%. Soils consist of loamy Wisconsin glacial till and clayey lacustrine deposits, including loam, silty clay, clay loam, and silty clay loam (Iowa NRCS); about 87% of the soil is fine, and another 8% is sandy.

The study area has a humid climate with an average yearly rainfall of 865.4 mm and average annual high and low temperature of 15.6 and 3.3°C, respectively. The stream network of Squaw Creek watershed was generated using 30 m digital elevation maps from the U.S. Geological Survey's Earth Resources Observation and Science Center and the geographic information systems software ArcGIS 9 (ArcMap™ version 9.3.1) to identify the tributaries and main stream.

Land cover was determined with a 2002 map for Iowa obtained from the Natural Resources Geographic Information System library, a repository developed by the Iowa Department of Natural Resources. About 74% of the watershed was under agricultural management (corn 41%, soybean 33%, and row crops 0.4%), 17% of the watershed was under grassland (ungrazed grass 11% , grazed grass 2.5% , CRP grass 1.7%, and alfalfa 1.8%), and 2.7% was deciduous forest. Additionally, 5.4% of the watershed land cover was road, residential, and commercial and 0.3% was water and wetlands. The watershed has 20 listed confined feeding operation units, and hogs are the major livestock.

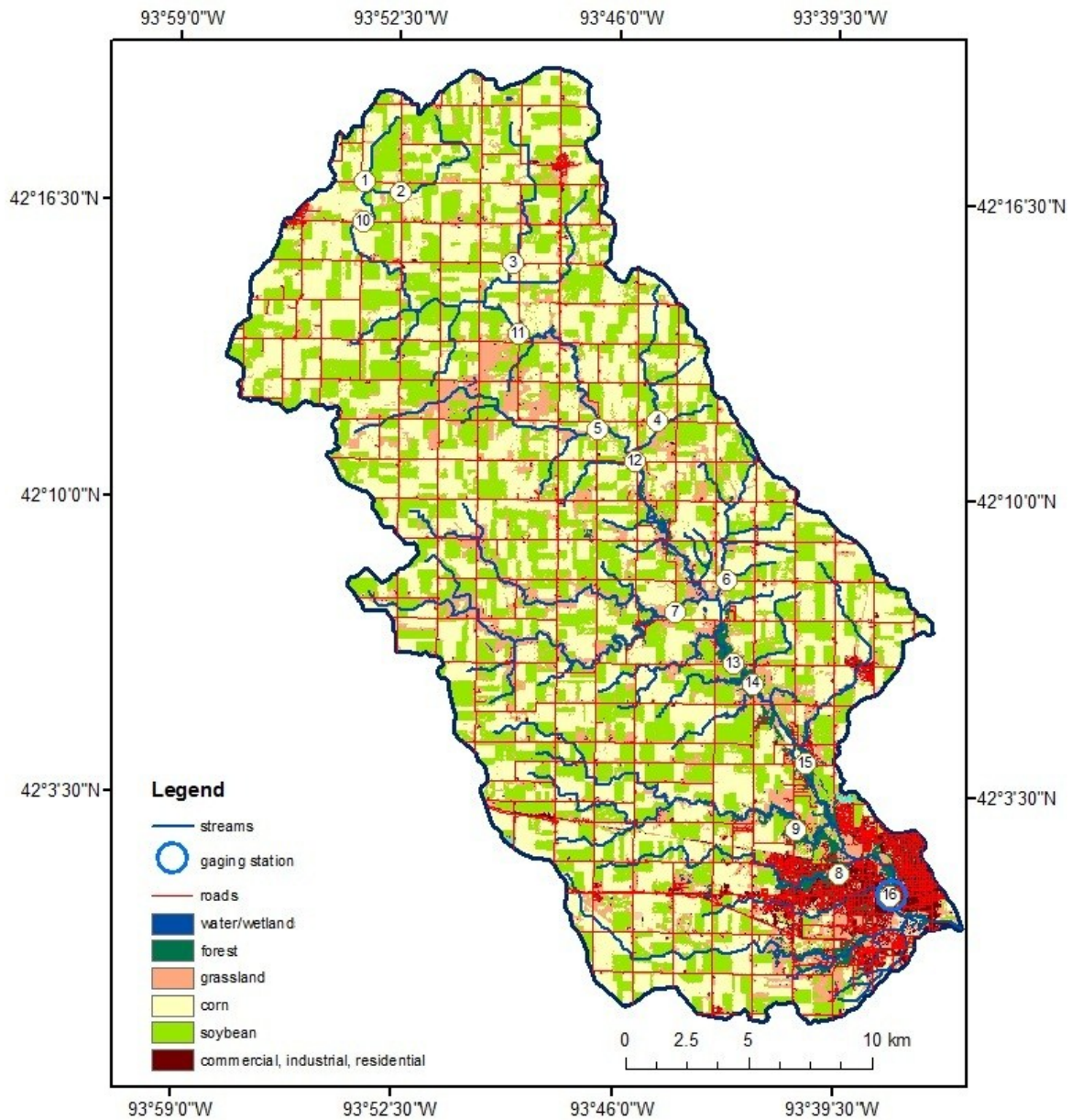


Figure 3.2. Squaw Creek watershed and sampling locations (1-16). Discharge was measured at the U.S. Geological Survey gaging station near station 16. The land cover is also shown.

Table 3.1. Parameters used to compute the predicted and inferred resuspension rates. The second column indicates whether the parameter was used in the predicted rate (P), inferred rate (I), or both (B). The fourth column lists the uncertainty, expressed as a percentage of the parameter's value, assumed in the analysis in section 4.

Parameter	Rates	Value	Uncty (%)	Source
C_1 = conc. of <i>E. coli</i> in water (CFU/100 ml)	I	2.25×10^2 - 5.47×10^3	15	Measured
C_2 = conc. of <i>E. coli</i> in sed. (CFU/m ³)	B	1.85×10^7 - 3.87×10^8	15	Measured
R = hydraulic radius (m)	P	0.10-0.76	10	Measured
A = cross sectional area (m ²)	P	0.5-3.5	10	Measured
ρ_b = bulk density of the sediment (g/cm ³)	P	1.26	5	Obtained through model calibration
T = temperature (°C)	B	17.0-24.6	5	Measured
Q = discharge (m ³ /s)	P	3.6	5	Measured at station 16
E_{0a} = coefficient (m/s)	P	1×10^{-6}	0	Lick (2009)
a = coefficient for bulk density effect (m ²)	P	8.5×10^{-16}	0	Lick (2009)
b = coefficient for bulk density effect (cm ³ /g)	P	9.07	0	Lick (2009)
c_3 = coefficient for clay effect (N/m ³)	P	8.46×10^3	0	Lick (2009), computed with $s = 2.65$
H_2 = depth of sediment containing <i>E. coli</i> (m)	I	0.02	50	Estimated from sediment sampler
n = Manning roughness coefficient	P	0.036	15	Estimated from Chow (1959)
S = slope (m/m)	P	2.5×10^{-4}	20	Estimated with Manning's eq. at station 16
f_a = attached fraction	B	1.0	15	Estimated using range in Hipsey et al. (2008)
n_a = exponent	P	1.0	10	Estimated from Amos et al. (1996)
c_5 = coefficient for clay effect (N/m ²)	P	21	10	Calibrated/estimated from Lick (2009)
d = particle diameter (µm)	B	1.0; 0.5-3.5	50	Calibrated using ranges in Oliver et al. (2007) & by fitting $d = \alpha \tau_b$ with $\alpha = 1.9 \mu\text{m}/\text{Pa}$

3.2. Measurements

Data were collected from the sampling locations to predict *E. coli* resuspension rates. Water temperature and cross section geometry were measured at sixteen locations on 17 July 2009. The mean air temperature during the sampling was 18.4 °C, and although the sky was mostly overcast, precipitation was zero. The mean discharge for the day was reported to be 3.6 m³/s at the U.S. Geological Survey gaging station 05470500 on Squaw Creek in Ames, which is at the same cross section as our sampling location 16 (Figure 3.2); the discharge varied by less than 2% during the sampling. The Manning roughness coefficient n was taken to be 0.036 using information for natural streams in Chow (1959, pp. 112-123). The bulk density of the stream bed sediments, expressed as weight per unit volume, was determined from wet and dry weight (dried in the oven at approximately 75 °C for 2 days) of sediments (Roberts et al., 1998). For estimating the bulk density of streambed sediment of Squaw Creek Watershed, we collected streambed sediment samples at 14 locations (7 sampling locations in main streams and 7 sampling locations in tributaries of the Squaw Creek Watershed). Sampling locations are shown in Appendix I (Fig A4). To collect the streambed sediment samples, we used a soil corer with diameter of 3.175 cm and height of 25.91 cm. We drive a soil corer into the streambed (at center of the stream) and remove the intact core. Immediately after collection, the core samples were stored at 4 °C. The weights of the cores were measured in the lab. We used the volume formula of $\pi r^2 h$ (where r is radius of core, and h is height of the core) to calculate the core volume (205.78 cc). The bulk density of the sediments, expressed as weight per unit volume, was determined from sediment wet and dry weight (dried in the oven at approximately 75°C for 2 days (Roberts et al., 1998). The bulk density data are shown in

Table A of Appendix I. The details of bulk density estimation are also described in Appendix I.

For *E. coli* estimation, water samples were collected from the center of the stream by lowering a Horizontal Polycarbonate Water Bottle Sampler (2.2 L, Forestry Suppliers Inc., Mississippi, U.S) from a bridge into the center of the stream at all the locations. Sediment samples were collected from the top 2-3 cm of the streambed using a Shallow Water Bottom Dredge Sampler (15 cm × 15 cm opening, Forestry Suppliers Inc., Mississippi, U.S) at the same location as water samples. While enumerating *E. coli* in water and sediment samples, three replicates of water and sediment samples were used in microbial analyses. In field, immediately after collection, samples were stored at 4 °C and analyzed in the lab within 24 hours of sample collection. The *E. coli* numbers in water and sediment samples were determined by membrane filtration techniques (APHA, 1999) on modified mTEC agar (EPA, method 1603).

To enumerate *E. coli* in water column, we used 10 ml of stream water sample for filtering through a membrane filter, and then CFU in 10 ml of water sample were converted into CFU/100 ml (by multiplying by 10) and CFU/m³ (by multiplying 10⁵) of water samples. To enumerate the *E. coli* in streambed sediment, sediment attached *E. coli* were detached by stirring the mixture of sediment and purified water (ratio 1:1) for 15 minutes at approximately 200 rpm using a magnetic stir bar. The resulting solution (i.e., mixture) was used for filtration to enumerate *E. coli* in the sediment. We filtered 1 ml of mixture (i.e., solution prepared by stirring 80 g of sediment and 80 ml of water sample) through a membrane filter. Subsequently CFU in 1 ml of sample (i.e., mixture) were converted into

CFU/100 g sediment and CFU/ m³ of sediment, which requires estimation of total mixture volume. The total mixture volume of 143.49 (i.e., 80 of sediment and 80 ml of water) was estimated by adding the volume of 80 g of sediment of 63.49 cc (that was calculated by dividing 80 g of sediment by 1.26 g/cc sediment bulk density) and 80 ml (i.e., cc) of water. The total CFU in 149.49 ml mixture were divided by 80 g of sediment, which yielded CFU in 1 g of sediment. To calculate CFU/m³ of sediment, we multiplied CFU/g of sediment into sediment bulk density (i.e., 1.26×10^6 g/m³). The calculations performed here provided an approximate value of CFU in sediment (either mass or volume basis).

For *E. coli* enumeration in sediment, we assumed that stirring of 80 g of sediment and 80 ml of water samples detached all *E. coli* attached to sediment particles, and were distributed throughout the mixture volume uniformly. Another assumption is that all sediment samples have a unique bulk density of 1.26 g/cc (which was used for calculating mixture volume and estimating CFU per m³ of sediment volume). To understand the potential impacts in predictions caused by uncertainties in bulk density and sediment *E. coli* estimation, we performed sensitivity analysis, which is discussed later in this chapter.

The method adapted here to calculate *E. coli* in sediment has certain limitations caused by assumptions, which were required to estimate the approximate *E. coli* numbers attached to sediment. However, authors are unaware of any established standard method available for enumerating *E. coli* levels in sediment. Developing a method, which can provide precise calculations of *E. coli* in streambed sediment, is a much needed work that can support monitoring of *E. coli* in streambed sediment. Identifying the advanced methods capable of extracting all *E. coli* attached to sediment into water, and then enumerating *E. coli* in water

samples in order to estimate *E. coli* in a certain mass or volume of sediment can potentially improve the results presented in this study.

3.3. Calculation of predicted and inferred resuspension rates

Resuspension rates were predicted with equation (5). All *E. coli* were assumed to be attached to sediment grains; that is, the attached fraction $f_a = 1$ and $C_a = C_2$ (CFU/m³). This choice is consistent with the assumptions and work reviewed in Hipsey et al. (2008), which showed attachment between 80 and 100%. The bottom shear stress was computed from a force balance for steady, uniform flow:

$$\tau_b = \rho g R S, \quad (6)$$

Where ρ is the water density, g is the acceleration of gravity and R is the hydraulic radius.

The slope S was estimated from Manning's equation to be 2.5×10^{-4} . Values of the coefficients a and b from Lick (2009) were used, and the coefficient E_{0a} was assumed to be equal to E_0 given by Lick (2009). The coefficient c_5 was calibrated, and the exponent n_a was taken to be 1, as suggested by Amos et al. (1996), who found the erosion rate to be linearly proportional to the difference between the shear stress and a critical shear stress. The critical shear stresses τ_{cn} and τ_c require an estimate of the diameter d of the particles to which the *E. coli* attach. A constant value of the diameter and a diameter that is linearly proportional to the bottom shear stress were used. The merits of these approaches are discussed in section 4.1.

To evaluate the predictions, resuspension rates were inferred from the mass balance model of Rehmann and Soupir (2009). Considering settling, resuspension, and net growth, they determined that a steady-state mass balance for the sediment yields

$$\frac{C_2}{C_1} = \frac{f_a w_s}{v_r - k_{n2} H_2}, \quad (7)$$

where w_s is the settling velocity, v_r is the resuspension velocity, k_{n2} is the net growth rate in the sediment, and H_2 is the depth of sediment containing *E. coli*, which is estimated to be about 2 cm for our experiments. The settling velocity w_s was estimated with Stokes's law. The net growth rate is the difference between the growth rate and the natural mortality rate, which were computed as functions of water temperature using the relations in Hipsey et al. (2008). The resuspension velocity at each sampling location was computed from equation (7), and the inferred resuspension rate was computed as

$$R_{ai} = v_r C_2 = f_a w_s C_1 + k_{n2} H_2 C_2 = f_a w_s C_1 (1 + \Phi), \quad (8)$$

where $\Phi = k_{n2} H_2 C_2 / f_a w_s C_1$ indicates the relative importance of settling and net growth in the mass balance for *E. coli* in the sediment. For example, when $\Phi \gg 1$, settling is unimportant, and net growth balances resuspension.

Once the exponent n_a was chosen, the parameters to determine or calibrate were the coefficient c_5 and the diameter of the particles to which *E. coli* attach. The optimal values of the parameters for the predictions using equation (5) were chosen by minimizing σ , the sum

of the squares of the differences between the logarithms of the inferred and predicted resuspension rates:

$$\sigma = \sum (\log_{10} R_a - \log_{10} R_{ai})^2. \quad (9)$$

Values of σ were computed with the resuspension rates expressed in CFU/m²s. A quantitative measure of predictive skill (Willmott 1981) was computed to assess the agreement between predicted and inferred *E. coli* resuspension rates:

$$\text{Skill} = 1 - \frac{\sum |R_a - R_{ai}|^2}{\sum (|R_a - \bar{R}_{ai}| + |R_{ai} - \bar{R}_{ai}|)^2}, \quad (10)$$

where the overbar denotes an average over all sampling locations. A skill of 1 indicates perfect agreement between predicted and inferred resuspension rates, while a skill of zero indicates poor performance.

To understand the dependence of the resuspension rate on the parameters and help in applying the model, the sensitivity and uncertainty were computed. The relative sensitivity of the resuspension rate to each parameter y_i was computed (Haan, 2002) with

$$S_{y_i} = \frac{y_i}{R_a} \frac{\partial R_a}{\partial y_i} \quad (11)$$

The relative uncertainty in the resuspension rate was computed by propagating the uncertainties of the individual parameters, which were assumed to be independent, with the formula of Taylor (1997, p. 75):

$$\left(\frac{\Delta R_a}{R_a}\right)^2 = \sum_{i=1}^N \left(\frac{1}{R_a} \frac{\partial R_a}{\partial y_i} \Delta y_i\right)^2 = \sum_{i=1}^N \left(S_{y_i} \frac{\Delta y_i}{y_i}\right)^2, \quad (12)$$

where N is the number of parameters and Δy_i is the uncertainty in y_i .

4. Results and Discussion

4.1. Concentrations, critical stresses, and resuspension rates

E. coli concentrations were large in streambed sediment as well as in the water column. The concentration C_1 of *E. coli* in the water column ranged from 225 to 5467 CFU/100 ml with a mean of 789 CFU/100 ml and standard deviation of 1255 CFU/100 ml (Figure 3.3). All but one of the concentrations exceeded the U.S. water quality standards (USEPA, 2001a), which state that the geometric mean of at least five samples during a 30-day period must not exceed 126 CFU/100 ml and that a single sample must not exceed 235 CFU/100 ml. The concentration C_2 of *E. coli* in the sediment ranged from 1.85×10^7 to 3.87×10^8 CFU/m³ with a mean of 1.54×10^8 CFU/m³ and standard deviation of 1.18×10^8 CFU/m³. Concentrations in the sediment (CFU/m³) were 2-102 times (mean = 34, s.d. = 32) higher than concentrations in the water column (CFU/m³). Previous studies reported the ratio C_2/C_1 to be 10-10,000 (Buckley et al., 1998; Davies and Bavor, 2000; Bai and Lung, 2005).

Resuspension rates inferred using equation (8) ranged from 11-187 CFU/m²s, depending on whether the diameter d of the particles to which *E. coli* attach was set to a constant value (Figure 3.4a) or allowed to vary with hydraulic conditions (Figure 3.4b). The inferred rates are smaller than those of Jamieson et al. (2005b), who measured resuspension rates of 8200-

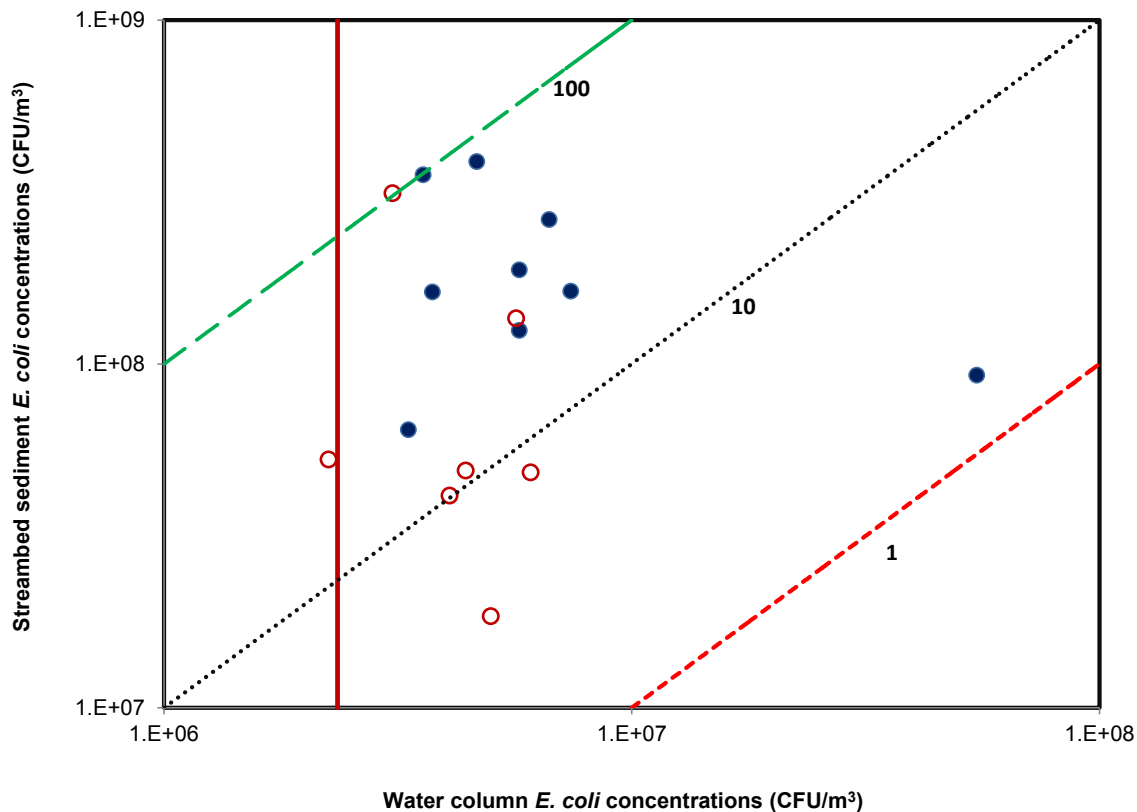


Figure 3.3. Concentrations of *E. coli* in the water column and sediment. Red hollow circles denote measurements from the main channel, and blue filled circles denote measurements from the tributaries. The solid vertical red line is set at the USEPA's water single-sample standard for *E. coli* (235 CFU/100 ml), and the dotted lines are contours of C_2/C_1 (i.e., ratio between sediment *E. coli* (CFU/m³) and water *E. coli* (CFU/m³)). The C_2 were estimated by multiplying the *E. coli* in sediment (CFU/g) into bulk density of sediment (1.26×10^6 g/m³).

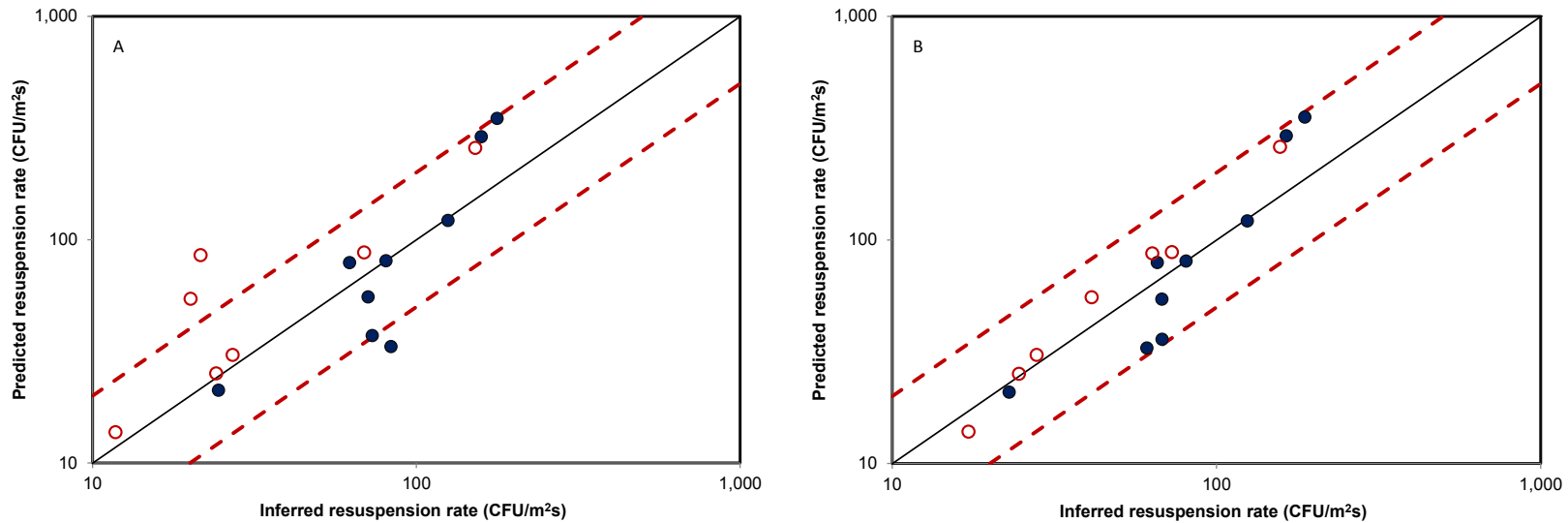


Figure 3.4a,b. Comparison between predicted and inferred resuspension rates. The solid line indicates perfect agreement, and the dashed lines indicate difference by a factor of 2. Red hollow circles denote measurements from the main channel, and blue filled circles denote measurements from the tributaries: (a) Constant value of the particle diameter: $d = 1.0 \mu\text{m}$, $\sigma = 1.04$, and skill = 0.82. (b) Particle diameter linearly related to bottom shear stress: $d = \alpha\tau_b$ with $\alpha = 1.9 \mu\text{m}/\text{Pa}$, $\sigma = 0.40$, and skill = 0.85.

15,000 CFU/m²s in a stream during storms. One cause of the discrepancy is that Jamieson et al. (2005b) artificially seeded the bed with *E. coli* NAR; concentrations of *E. coli* in the sediment corresponding to the three storms highlighted by Jamieson et al. (2005b) were between 1.2×10^5 and 5.5×10^5 CFU/100 ml, or about 3 to 300 times larger than in our experiments. In fact, the resuspension velocities $v_r = R_{crit}/C_2$ in our experiments (3.6×10^{-7} – 1.3×10^{-6} m/s) were only about 2 to 25 times smaller than the values (2×10^{-6} – 1×10^{-5} m/s) Jamieson et al. (2005b) observed. The critical shear stress for cohesive sediment was about 1.1 N/m² at all sampling stations. Because of the effects of clay, the critical stress used for the predictions in Figure 3.4 did not depend strongly on the particle diameter (Figure 3.1). Estimates of critical stress in other cases vary widely because of the characteristics of the sediment, the presence of biofilms, the depositional history of the bed, and the approach used to define the critical stress. For field measurements in a stream in which 32% of the sediment was finer than 75 μm , Jamieson et al. (2005) computed critical shear stresses of 1.5–1.7 N/m² using the Manning's roughness coefficient and the discharge at which *E. coli* NAR first appeared in discrete samples during a storm. El Ganaoui et al. (2004) analyzed sediment samples from a field site and differentiated between the fluff layer, a surface layer of fine sized particles and organics with a mean particle diameter of 10–20 μm and critical shear stresses of 0.025–0.05 N/m², and a layer with coarser particles, which had critical shear stresses that were 10–20 times larger. Droppo et al. (2001) conducted laboratory experiments on kaolinite clay with a mean diameter of 5 μm and contaminated sediment from a field site that had particle sizes less than 63 μm . The critical stress increased from 0.024 N/m² to 0.325 N/m² when a biofilm was allowed to grow, and critical stresses of 0.100–0.135 N/m² for beds

deposited under shear exceeded the stresses of 0.047-0.054 N/m² for beds deposited under quiescent conditions. The critical stress estimated for Squaw Creek had a magnitude representative of natural sediment beds with biofilms and a realistic deposition history.

4.2. Predicting resuspension

Using values of parameters from previous work and a constant value of the particle size (Table 3.1), the model predicted thirteen of the resuspension rates within a factor of 2 and all within a factor of 5 (Figure 3.4a). The model predicted the resuspension rates from the main channel and tributaries about equally well. As noted in section 2, most of the parameters in Table 3.1 were either measured or taken from Lick (2009). The coefficient c_5 was set to 21 N/m²; this value is three times that used by Lick et al. (2004) for quartz particles with 2% bentonite. Because grain size analyses showed that the sediment samples consisted of between 1 and 7% clay (i.e., particle sizes < 8 μm), a larger value of c_5 is reasonable. The diameter d of particles to which *E. coli* attach must be specified to compute both the predicted and inferred resuspension rates. A single value of 1 μm used for all sites yielded $\sigma = 1.04$ and a skill of 0.82. Attachment of *E. coli* to small particles is consistent with previous findings. For example, Oliver et al. (2007) observed that 65% of *E. coli* attached to particles smaller than 2 μm.

Because of the uncertainty in the diameter of particles to which *E. coli* attach, the diameter was allowed to depend on the bottom shear stress as $d = \alpha\tau_b$ where α is a coefficient. The optimal value of α of 1.9 μm/Pa yielded diameters between 0.5 and 3.5 μm, which fall within previously observed ranges (Oliver et al., 2007), and it changed the range of inferred

resuspension rates because of the dependence of the settling velocity on particle diameter. The model predicted all of the resuspension rates within a factor of 2 (Figure 3.4b), and it yielded $\sigma = 0.40$ and a skill of 0.85. Again, the model predicted the resuspension rates from the main channel and tributaries about equally well.

This version of the model involves no more calibration parameters, and the relationship between the diameter and shear stress appeals to physical intuition. Once the exponent n_d and coefficient c_5 were specified using information from Amos et al. (1996) and Lick (2009), the only parameter to adjust in the first application (Figure 3.4a) was the diameter d . The second application (Figure 3.4b) also had only one parameter to adjust, the coefficient α . The assumed relationship $d = \alpha \tau_b$ implies that as the bottom shear stress increases, larger particles can be resuspended. Also, the coefficient α can be related to the Shields parameter, which is used to determine conditions under which non-cohesive sediment will start moving:

$$\psi = \frac{\tau_b}{\rho g (s-1) d} = \frac{\alpha}{\rho g (s-1)}. \quad (13)$$

With $\alpha = 1.9 \mu\text{m}/\text{Pa}$ equation (13) yields a Shields parameter of about 33. This value is much larger than the critical Shields parameter for initiation of motion of $2 \mu\text{m}$ quartz particles (Cao et al., 2006). The larger value we obtained is reasonable because it deals with suspended sediment instead of initiation of motion and because the Shields criterion does not account for the cohesive effects involved in the transport of small particles.

4.3. Sensitivity and uncertainty

Calculating the sensitivity can help in determining the parameters for other situations. The relative sensitivity can be computed analytically (Table 3.2); the parameters $\phi_b = a \exp(b\rho_b)/d^2$ and $\phi_c = c_5/c_3d$ represent the contributions of bulk density and clay content, respectively, to the critical shear stress defined in equation (3). Because the resuspension rate is linearly proportional to both the coefficient E_{0a} and the concentration C_a of attached *E. coli* in the sediment, the relative sensitivity to those parameters is always 1. All other sensitivities depend on the parameter values (Figure 3.5). For the parameter set used in Figure 3.4b, the

Table 3.2. Relative sensitivity of the predicted resuspension rate to the various parameters. As noted in the text, the parameters $\phi_b = a \exp(b\rho_b)/d^2$ and $\phi_c = c_5/c_3d$ represent the contributions of bulk density and clay content, respectively, to the critical shear stress τ_c .

Parameter	Relative sensitivity	Parameter	Relative sensitivity
Hydraulic radius R	$n_a \frac{\tau}{\tau - \tau_{cn}}$	Exponent n_a	$n_a \ln \left(\frac{\tau - \tau_{cn}}{\tau_c - \tau_{cn}} \right)$
Slope S	$n_a \frac{\tau}{\tau - \tau_{cn}}$	Bulk density ρ_b	$-n_a \frac{b\rho_b\phi_b}{\phi_b + \phi_c}$
Concentration C_a	1	Coefficient b	$-n_a \frac{b\rho_b\phi_b}{\phi_b + \phi_c}$
Coefficient E_{0a}	1	Coefficient a	$-n_a \frac{\phi_b}{\phi_b + \phi_c}$
Coefficient c_5	$-n_a \frac{\phi_c}{\phi_b + \phi_c}$	Diameter d	$n_a \left(\frac{\phi_b}{\phi_b + \phi_c} - \frac{\tau_{cn}}{\tau - \tau_{cn}} \right)$

resuspension rate is most sensitive to the slope, hydraulic radius (which for these measurements is approximately equal to the water depth), the concentration of attached *E. coli*, and the coefficients E_{0a} and c_5 . The magnitude of these sensitivities is approximately

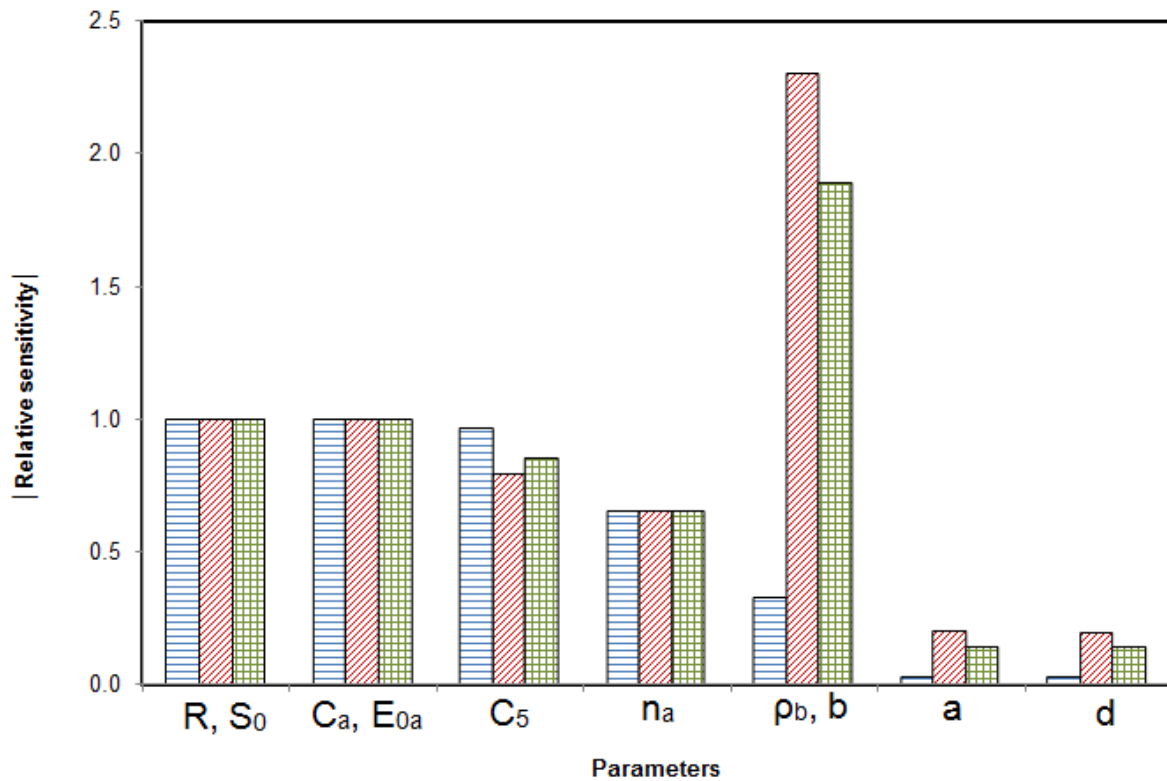


Figure 3.5. Absolute value of the relative sensitivity of the predicted resuspension rate to the parameters listed in Table 3.2. Sensitivity is computed for station 13. Bars with horizontal blue lines are computed for the parameter set used to compute the rates in Figure 3.4b. Bars with diagonal red lines use the base parameter set with $c_5 = 2.5 \text{ N/m}^2$, and bars with green bricks use the base parameter set with $\rho_b = 1.45 \text{ g/cm}^3$.

equal to the exponent n_a , or 1, because the bottom shear stress is much greater than the critical shear stress for non-cohesive sediment ($\tau \gg \tau_{cn}$) and effects of clay control the critical shear stress for cohesive sediment ($\phi_c \gg \phi_b$). For similar reasons, the sensitivity to the bulk density, particle diameter, and coefficients a and b in equation (3) is smaller. When the effects of bulk density outweigh those of the clay content ($\phi_b \gg \phi_c$)—for example, for a soil with greater bulk density or smaller clay content (i.e., reduced c_s), most of the sensitivities change little, but the resuspension rate becomes most sensitive to the bulk density and the coefficient b because of the exponential dependence on both.

Although the predicted resuspension rate is not sensitive to the particle diameter, the inferred resuspension rate can be. With a settling velocity computed with Stokes's law, the inferred rate is always twice as sensitive to the diameter as it is to the concentration of *E. coli* in the water column and the attached fraction (Table 3.3). When settling is more important than net growth in the mass balance for *E. coli* in the sediment ($\Phi < 1$) as at station 14, the inferred resuspension rate is most sensitive to the diameter and less sensitive to the concentration of *E. coli* in the sediment, the depth of sediment containing *E. coli*, and the water temperature T (Figure 3.6). For larger values of Φ , when net growth is more important than settling, the inferred rate is most sensitive to temperature except for temperatures corresponding to the peak in the net growth rate (i.e., $\partial k_{n2}/\partial T = 0$). The growth and decay relations in Hipsey et al. (2008) suggest that sensitivity to temperature will be small around temperatures of 22.6 °C. The differences between these two cases is illustrated by the sensitivities for stations 6 and 11 (Figure 3.6).

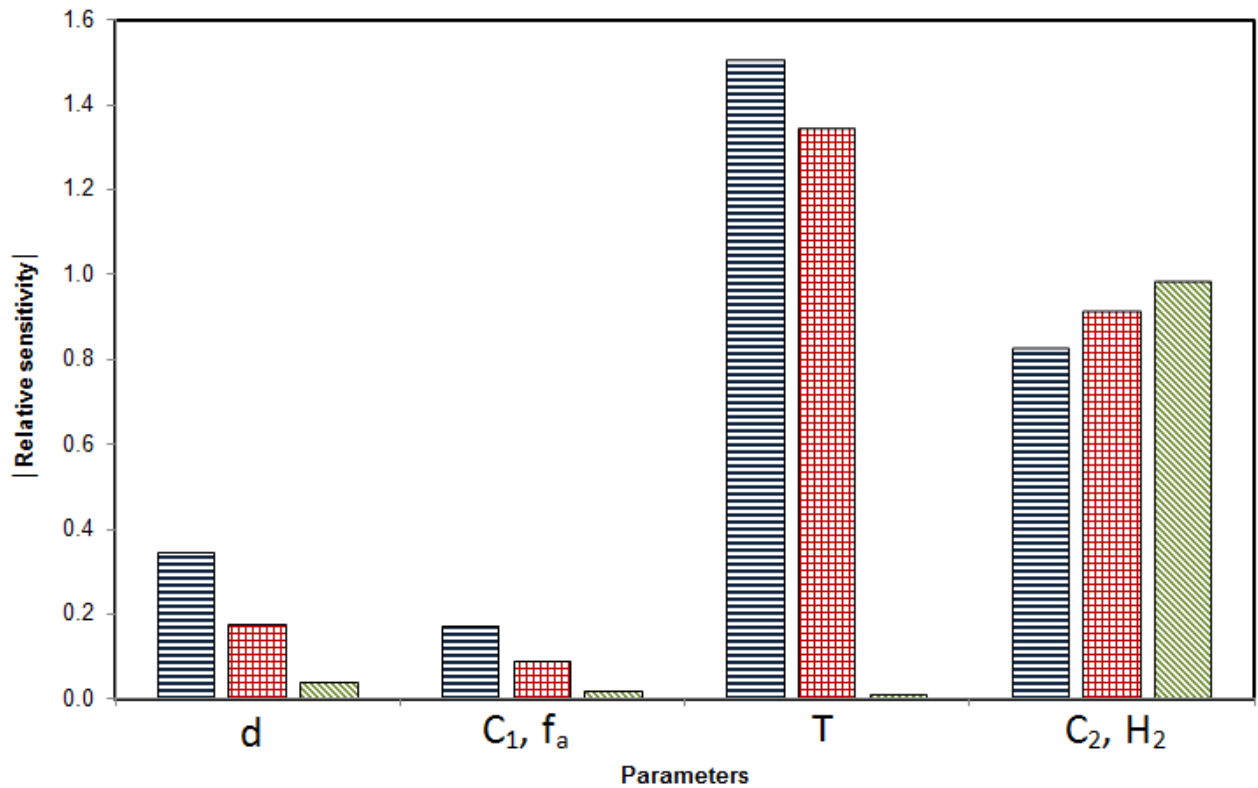


Figure 3.6. Absolute value of the relative sensitivity of the inferred resuspension rate to the parameters listed in Table 3.3: Bars with horizontal lines are computed for station 14 ($\Phi = 0.4, T = 17.7^\circ\text{C}$), bars with red bricks are computed for station 6 ($\Phi = 43, T = 19.0^\circ\text{C}$), and bars with diagonal green lines are computed for station 11 ($\Phi = 16, T = 22.7^\circ\text{C}$).

Uncertainty is 30% in the predicted resuspension rate and 47-75% in the inferred resuspension rate. For the individual uncertainties in the parameters listed in Table 3.1, about 75% of the uncertainty in the predicted rate comes from the slope and the concentration C_a of *E. coli* attached to sediment. Another 20% comes from the coefficient c_5 and the hydraulic radius, which is approximately equal to the water depth in most of our cases, and the remaining uncertainty comes from the exponent n_a . Efforts to reduce uncertainty in the predictions should involve better estimates of C_a and either measuring the slope more accurately or measuring the bottom shear stress with another method, such as one based on velocity measurements at a cross section (e.g., Kim et al., 2000).

Table 3.3. Relative sensitivity of the inferred resuspension rate to the various parameters. As noted in the text, the parameter $\Phi = k_{n_2}H_2C_2 / f_a w_s C_1$ measures the relative importance of settling and net growth in the mass balance for *E. coli* in the sediment. The net growth rate k_{n_2} and its derivative with respect to temperature are taken from Hipsey et al. (2008).

Parameter	Relative sensitivity	Parameter	Relative sensitivity
Diameter d	$\frac{2}{1+\Phi}$	Conc. C_2 in sediment	$\frac{\Phi}{1+\Phi}$
Conc. C_1 in water column	$\frac{1}{1+\Phi}$	Depth H_2 with <i>E. coli</i>	$\frac{\Phi}{1+\Phi}$
Attached fraction f_a	$\frac{1}{1+\Phi}$	Temperature T	$\frac{\Phi}{1+\Phi} \frac{T}{k_{n_2}} \frac{\partial k_{n_2}}{\partial T}$

The main contributions to the uncertainty in the inferred resuspension rate depend on Φ .

When net growth is more important than settling (large Φ), the depth of sediment containing *E. coli* controls the uncertainty, and when settling is more important than net growth (small

Φ), the particle diameter controls the uncertainty. In the former case, the uncertainty can be reduced by measuring *E. coli* concentrations at different depths in the sediment; such measurements would also allow the assumption of uniform concentration to be assessed and revised. In the latter case, the uncertainty occurs because of the dependence on particle diameter through the settling velocity. Reducing the uncertainty in the settling velocity—and thus the resuspension rate—is difficult for several reasons. As Rehmann and Soupir (2009) reviewed in detail, flocculation, which can control the deposition of cohesive sediment (Droppo 2001), can cause Stokes's law to overestimate the settling velocity (Burban et al., 1990). Even without flocculation, settling velocities in a flowing stream fall below those from Stokes's law far from the bed and exceed them near the bed (Cuthbertson and Ervine, 2007). Further uncertainty is introduced by the range of particle sizes present in stream sediment and tendency of *E. coli* to attach to particles of different sizes (Oliver et al., 2007).

4.4 Model assessment

The key advantages of our model are that its parameters are related to observable physical quantities and that it accounts for the properties of the flow, sediment, and organisms. Alternative models for computing resuspension rates include those that assume a constant resuspension velocity v_r (Chapra, 1997) and those that relate resuspension to the discharge Q using a formula of the form

$$R_a = a_1 C_a Q^{b_1}, \quad (14)$$

where a_1 and b_1 are coefficients. Examples of models like (14) include those of Wu et al. (2009), who computed the concentration of resuspended organisms, and Collins and

Rutherford (2004), who computed the number of *E. coli* resuspended per unit time.

Predictions with a constant resuspension velocity of 5.2×10^{-7} m/s and equation (14) with $a_1 = 8 \times 10^{-7}$ and $b_1 = 0.29$ (with discharge expressed in m^3/s) also provide good fits to the inferred resuspension rates in Figure 3.4b (Table 3.4).

Table 3.4. Comparison of methods of predicting resuspension rates. The last two models were evaluated with the dataset computed with variable particle diameter. The last two columns show the number of predictions within factors of 2 and 5 of the measured values.

Model	σ	Skill	Number within a factor of...	
			2	5
Eq. (5), constant d	1.04	0.82	13	16
Eq. (5), constant ψ	0.40	0.85	16	16
Constant v_r	0.45	0.98	14	16
Eq. (14)	0.27	0.95	15	16

However, choosing the parameters in these two models is difficult in situations without measured or inferred resuspension rates to be used for calibration. For example, to specify the resuspension rate in their model, Petersen et al. (2009) used the average of the resuspension rates reported by Jamieson et al. (2005b). As noted in section 4.1, that rate is much higher than the inferred rates from our study. The ranges of resuspension velocity are closer, but even with the smallest value of v_r from Jamieson et al. (2005b)—which is four times larger than the optimal value, the predictions using constant resuspension velocity are

worse than all four cases in Table 3.4. The coefficients a_1 and b_1 in (14) are even harder to specify: Collins and Rutherford (2004) did not report their values of the coefficients. Wu et al. (2009) related the concentration of resuspended organisms to $Q^{4.5}$; this exponent is much larger than b_1 , and the strong dependence on flow was not reflected in our measurements of *E. coli* concentrations in the water column. In contrast, the parameters in equation (5) can be measured, observed, or estimated from previous work; the most challenging parameters to specify are the exponent n_a , which was taken from the work of Amos et al. (1996); the coefficient c_5 , which was estimated from the clay fraction and the results in Lick (2009); and the particle diameter, which was discussed in detail in section 4.1.

The ability of equation (5) to account for sediment properties gives it wider applicability than equation (14). For the data in Figure 3.4b, the bottom shear stress is much larger than the critical shear stress for non-cohesive sediment, and the binding effects of clay make the critical shear stress for cohesive sediment depend only weakly on the particle diameter (Figure 3.1). With τ_c approximately constant, the resuspension rate from (5) is proportional to $C_a \tau_b^{n_a}$, and if the bottom shear stress can be expressed as a function of the discharge raised to some power, then equation (14) should work well. However, in streams with sediment that has a larger bulk density or a smaller clay fraction, the critical shear stress for cohesive sediment will not be constant, and predictions with equation (14) will not be as successful.

The proposed formula (5) for predicting resuspension rates can in principle be applied in unsteady flow. In contrast, a model with specified resuspension velocity would be difficult to apply because the velocity would have to vary in time. The ability to use (5) to predict resuspension in unsteady flows is important because resuspension typically is largest during

the rising limb of storm hydrographs (Jamieson et al., 2005b). To apply equation (5), estimates of the shear stress would need to be obtained by modifying the force balance in equation (6) by considering effects of unsteadiness and nonuniformity or showing that they are negligible, as in Jamieson et al. (2005b).

Our study also demonstrates the challenge of estimating resuspension from field measurements. The inferred resuspension rate from equation (8) was computed from a steady state mass balance in equation (7). An analysis similar to that of Rehmann and Soupir (2009) shows that the flow in Squaw Creek was approximately steady: The time scale of unsteadiness—estimated as $Q/(dQ/dt)$ using discharge measured at the U.S. Geological Survey's gaging station at our station 16—was about 11.5 h. This time scale is about 6 times larger than the time scale for settling (C_2H_2/C_{faw_s}), 20 times larger than the time scale for net growth (k_{n2}^{-1}), and 30 times larger than the time scale for resuspension (H_2/v_r). Therefore, the mass balance in equation (7) should hold approximately. Still, as discussed in section 4.3, resuspension rates inferred with equation (8) are uncertain because they require estimates of the settling velocity and depth of sediment containing *E. coli*, and the various processes contributing to growth and decay of *E. coli* (Hipsey et al., 2008) are difficult to quantify in the field.

Future work involves incorporating the resuspension rate in equation (5) in watershed-scale models such as SWAT. Such models provide discharge and channel geometry, from which shear stresses can be estimated. Spatial variations in quantities such as sediment properties can pose a challenge, especially in cases in which the resuspension rate is sensitive to the bulk density. However, because our model shows that variations in sediment properties

become less important when the binding effects of clay control the critical shear stress τ_c , in those cases—as in the case of the Squaw Creek watershed—the model should be easier to apply. Also, our use of the model for Squaw Creek, as well as future comparisons with the performance of other watershed-scale simulations including resuspension (Collins and Rutherford, 2004; Wu et al., 2009; Kim et al., 2010), will guide users in selecting the model's parameters. The resulting model should help in creating plans to improve water quality in areas affected by *E. coli* contamination.

5. Conclusions

We predicted resuspension of *E. coli* from sediment beds in streams by expressing the resuspension rate as the product of the concentration of *E. coli* attached to sediment particles and an erosion rate adapted from work on sediment transport. The model accounts for properties of the flow through the bottom shear stress and properties of the sediment through the critical shear stresses for cohesive and non-cohesive sediment. To evaluate the model's predictive skill, its predictions were compared to resuspension rates inferred from a steady mass balance applied to measurements at sixteen locations in a watershed. Sensitivity and uncertainty were computed to determine the parameters that affect the predictions most strongly and to identify ways to improve the model. The main conclusions of this study are as follows:

1. The model performed well using parameter values mostly taken from previous work. The coefficient representing the binding effects of clay was increased from a previously reported value because of the higher clay content in the sediment in our study. The application of the model in which the particle diameter was linearly proportional to the bottom shear stress (i.e.,

constant Shields parameter) performed better than an application with constant particle diameter, while maintaining the same number of model coefficients.

2. Although two simpler models also performed well, the proposed model can be applied more easily in situations without measured or inferred resuspension rates because its parameters are related to observable physical quantities and it accounts for properties of the flow, sediment, and organisms. Furthermore, its ability to be applied in unsteady flow is important because resuspension is often largest during the rising limb of a storm hydrograph.
3. When the binding effects of clay control the critical shear stress, the predicted resuspension rate is more sensitive to properties of the flow, and when the bulk density controls the critical shear stress, the predicted resuspension rate is more sensitive to properties of the sediment. For the current data set, the uncertainty in the predictions would be reduced by reducing uncertainty in the concentration of attached *E. coli* and the slope used to compute the bottom shear stress.

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CHAPTER 4. IMPROVING SWAT FOR DEVELOPING TMDLs FOR BACTERIA

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Abstract

Hydrological models capable of predicting streambed sediment pathogen concentrations, which are required for understanding pathogen transport in streams, are lacking, potentially due to the complexities involved in modeling interactions between streambed sediment and water column pathogens. Here we have developed a pathogen transport model which estimates *E. coli* concentrations, a pathogen indicator, in streambed sediment as well as in the water column. Firstly, a new approach, which involves formulations of *E. coli* resuspension from the streambed sediment to the water column, in-stream *E. coli* routing, and *E. coli* growth in the streambed sediment and the water column was developed. Secondly, these formulations were programmed in FORTRAN, and were integrated into the Soil and Water Assessment Tool (SWAT), a watershed scale hydrological model, which calculated *E. coli*

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concentrations in the streambed sediment and the water column. Finally, the modified SWAT was implemented in the Squaw Creek Watershed, Iowa, USA. An extensive *E. coli* monitoring in streambed sediment and the water column was carried out in the Squaw Creek Watershed, and observations were used to verify the modified SWAT predictions. Results show that the modified SWAT is capable of predicting in-stream *E. coli* concentrations (i.e., in the streambed sediment and water column). Majority of the *E. coli* predictions were within 1 order magnitude of the measured values. Approximately 62% of the predicted streambed sediment *E. coli* concentrations and 82% of the predicted water column *E. coli* concentrations were within 1 order magnitude of the measured values. We anticipate that the modified SWAT model, capable of predicting the streambed sediment and the water column *E. coli* concentrations, proposed here should have significant importance in Total Maximum Daily Loads (TMDLs) development and predicting in-stream *E. coli* concentrations at the watershed scale.

1. Introduction

In-stream water pollution is a serious concern in the United States (USA). For example, approximately 27.5% of the total rivers and streams in the USA (total 5,688,460 km) are assessed, and 53% of the assessed streams are contaminated (EPA, 2012). Pathogen contamination is the leading cause of stream water impairment. In the past, many of the outbreak occurrences were found to be related with poor water quality, for instance, more than 400,000 cases of gastroenteritis in Milwaukee, Wisconsin in 1993 were caused by pathogens in city's drinking water supply (Mackenzie et al., 1994).

Previous studies (i.e., Vezzulli et al., 2012; Harvell et al., 2002; Hrudey et al. 2002; Hunter, 2003; Pandey et al. 2012b) have shown linkages between hydrology (i.e., rainfall) and disease outbreaks, indicating a potential linkage between watershed hydrology and disease outbreaks. In a recent study, Pandey et al. (2012b) has used watershed indexes considering disturbed and undisturbed natural land cover of the watershed for finding relationships between in-stream waterborne pathogens and watershed characteristics. Other studies, for example, Dorner et al. (2006) and Kim et al. (2010) have focused on developing pathogen transport models, which were embedded to existing hydrological models for calculating in-stream water borne pathogens.

Dorner's study was focused on embedding pathogen transport model, which includes in-stream routing, overland flow, and subsurface to tile drainage systems, with WATFLOOD; and Kim's study was focused on augmenting pathogen resuspension model with Soil and Water Assessment Tool (SWAT). In Dorner's study resuspension process was not included in the pathogen transport model; however the authors concluded that resuspension of microorganisms from the streambed sediments may be of equal or greater importance than land-based sources of pathogens. In Kim's study bacteria resuspension was included using simplified version of Bagnold's stream power function, which has been criticized for not including the effect of grain size on sediment transport (Ferguson, 2005). Relatively recent studies by Tang et al. (2011) and Coffey et al. (2010) have implemented existing SWAT model, which does not include in-stream processes (i.e., bacteria resuspension and deposition) while predicting *Cryptosporidium* oocysts transport in streams.

Predicting in-stream bacteria concentrations is a difficult task due to the complexities involved in determining bacteria behavior in a natural stream environment. For example, understanding bacteria survival and transport in an environment, where organic matters, sediment characteristics, water levels, and heterogeneous microbial population are changing continuously, can be challenging (Droppo et al., 2011; Droppo et al., 2009; Characklish et al., 2005; Rehmann and Soupir, 2009). Although there has been substantial progress in improving in-stream waterborne bacteria predictions, calculating the impacts of streambed sediment on the water column bacteria remains a major challenge, particularly, calculating resuspension of bacteria from the streambed to the water column at the watershed scale. The resuspension of bacteria from the streambed increases bacteria concentrations in the water column considerably (Jamieson et al., 2005a; Muirhead et al., 2004; Droppo et al., 2009); estimation of *E. coli* resuspension, however, is a challenge.

Predicting bacteria concentrations in the streambed sediment requires understanding of complex interaction between the streambed sediment and the water column. Studies calculating bacteria in natural streambed sediment are not yet reported. Nevertheless, several studies have emphasized the considerable impacts of streambed sediment bacteria on the water column (i.e., Jamieson et al., 2005a; Droppo et al., 2011; Dorner et al., 2006). Here we have developed a watershed scale model for predicting *E. coli*, a pathogen indicator in streambed sediment as well as in the water column, which includes in-stream *E. coli* routing, resuspension and deposition of *E. coli*, overland flow, and *E. coli* growth in the streambed sediment and the water column. The model was written in FORTRAN language. The new subroutines were coded for the existing SWAT (i.e., written in existing SWAT model 2009),

a hydrological model, for predicting in-stream *E. coli* concentrations. The modified SWAT model was built using an Intel (R) Visual FORTRAN Composer XE 2011, and the model has the capability of predicting *E. coli* concentrations in the streambed sediment as well as in the water column. The objectives of this study are: 1) predicting streambed sediment *E. coli* concentrations; 2) predicting stream water column *E. coli* concentrations; 3) verify the model's prediction using extensive *E. coli* data, monitored in the Squaw Creek Watershed, Iowa, USA.

2. Methodology for calculating in-stream *E. coli* concentrations

To improve modeling of in-stream *E. coli* fate and transport processes, we formulated the model for predicting *E. coli* changes in the streambed and the water column. In addition we modeled *E. coli* growth in the streambed as well as in the water column. The conceptual model is shown in Figure 4.1. The streambed *E. coli* prediction model involved calculating *E. coli* changes in the streambed, which was divided into an upper zone and lower zone. The division of streambed into two zones was necessary in order to include the effects of vertical distribution of *E. coli* in streambed with depth.

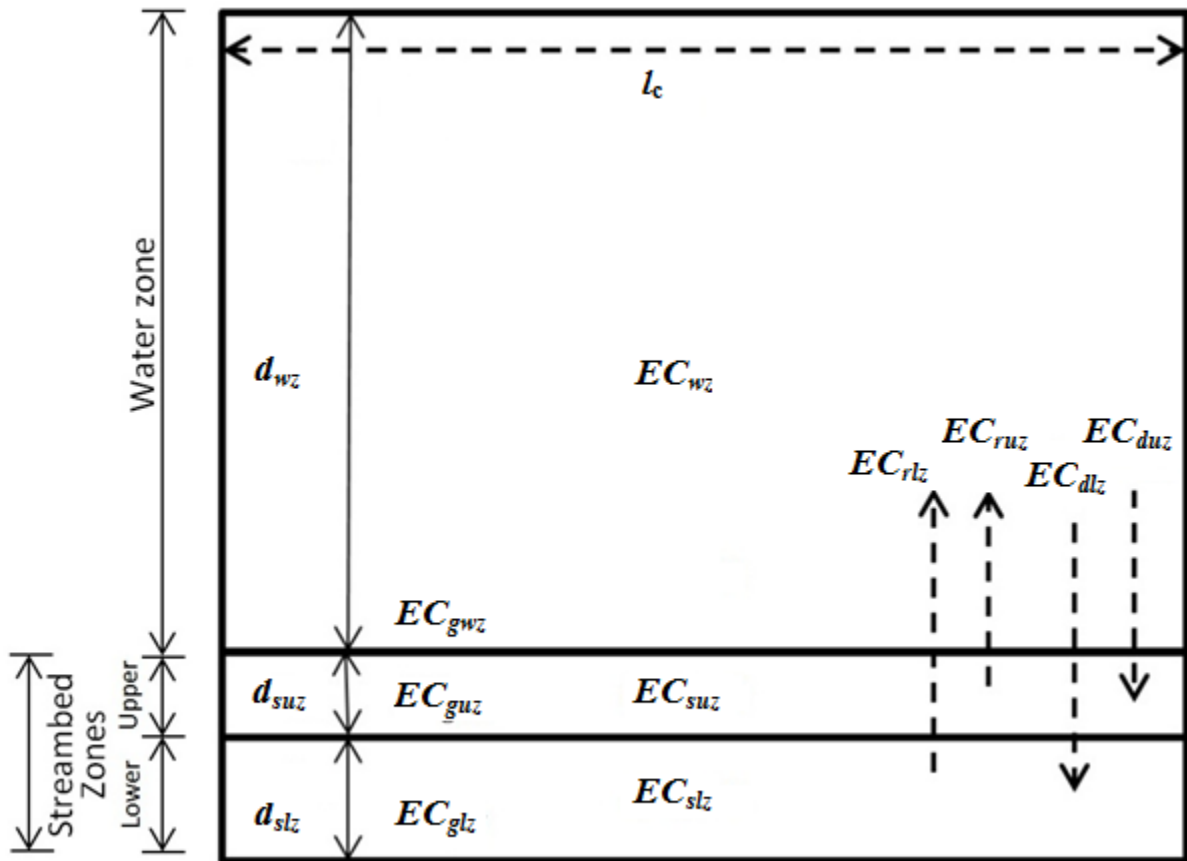


Figure 4.1. Conceptual map of the model. The EC_{wz} denotes change in *E. coli* concentrations of the water column over 1 day; EC_{suz} denotes change in *E. coli* concentrations in upper zone of the streambed over 1 day; and EC_{slz} denotes change in *E. coli* concentrations in the lower zone of streambed (over 1 day). d_{wz} is depth of the water zone (water column), d_{suz} and d_{slz} are the depths of streambed upper and lower zones, respectively. EC_{ruz} and EC_{rlz} are the resuspension from the streambed upper and lower zones to the water zone, respectively, while EC_{duz} and EC_{dlz} are the depositions of *E. coli* from the water zone to the streambed upper and lower zones, respectively. EC_{gwz} indicates *E. coli* growth in the water zone; EC_{guz} , and EC_{glz} indicate *E. coli* growth in the upper and lower zones of the streambed. l_c is the channel length.

$$EC_{suz} = EC_{duz} - EC_{ruz} + EC_{guz} \quad (1)$$

$$EC_{slz} = EC_{dlz} - EC_{rlz} + EC_{glz} \quad (2)$$

where EC_{suz} and EC_{slz} are the changes in *E. coli* (CFU) in the upper and lower zones over 1 day period, respectively. The EC_{duz} (CFU) is the deposited *E. coli* in the upper zone, and EC_{dlz} (CFU) is the deposited *E. coli* in the lower zone. The EC_{ruz} (CFU) and EC_{rlz} (CFU) are the resuspended *E. coli* from the streambed upper and lower zones, respectively. While EC_{guz} (CFU) is *E. coli* growth in the streambed upper zone, EC_{glz} (CFU) is *E. coli* growth in the streambed lower zone. The *E. coli* resuspension from the upper and lower zones was estimated based on the availability of sediment mass in the upper and lower zones. For example, lower zone's *E. coli* and sediment resuspension occurs, when estimated total daily resuspended sediment mass exceeds the available sediment mass of the upper zones. The available sediment mass in the upper and lower zones was determined from the depths of upper and lower zones, and depths (of upper and lower zones) were as input parameters.

To predict the *E. coli* concentration in the water column, the *E. coli* change in the water zone was calculated as follows:

$$EC_{wz} = EC_{ruz} + EC_{rlz} - EC_{duz} - EC_{dlz} + EC_{gwz} \quad (3)$$

where EC_{wz} (CFU) is the change in *E. coli* in the water zone over 1 day period; EC_{ruz} (CFU) is the release of *E. coli* from the streambed upper zone to the water zone; EC_{rlz} (CFU) is the release of *E. coli* from the streambed lower zone to the water zone; EC_{duz} (CFU) and EC_{dlz} (CFU) are the deposition (settling) of *E. coli* from the water zone to the streambed upper and lower zones, respectively; and EC_{gwz} (CFU) is *E. coli* growth in the water zone.

The resuspension of *E. coli* from the streambed to the water column shown in equations 1, 2, and 3 was calculated as follows.

$$EC_{ruz} = EC_{cuz} \times RM_{uz} \quad (4)$$

$$EC_{rlz} = EC_{clz} \times RM_{lz} \quad (5)$$

where EC_{ruz} and EC_{rlz} are the resuspended *E. coli* from the streambed upper and lower zones to the water column (CFU) over 1 day, respectively; EC_{cuz} and EC_{clz} are the *E. coli* concentrations in the streambed upper and lower zones (CFU/g); and RM_{uz} and RM_{lz} are the resuspended sediment mass (g) over 1 day from the streambed upper and lower zones, respectively. The particle attached *E. coli* are resuspended from the streambed (upper and lower zones) to the water column. We assumed 80% of the *E. coli* cells present in the water column are attached with suspended sediment particles (Hipsey et al., 2008). The resuspended mass from the streambed upper (RM_{uz}) and lower zones (RM_{lz}) was calculated by streambed erosion.

$$RM_{uz} = r_{suz} w_p l_c \rho_s dt \quad (6)$$

where RM_{uz} is resuspended sediment mass from the streambed upper zone (g), r_{suz} is the streambed upper surface erosion rate (m/d), w_p is wetted perimeter of the reach (m), l_c is reach length (m), ρ_s is bulk density of sediment particles (g/m³), and dt is time step of 1 day. Similarly we calculated RM_{lz} using the erosion rate from the streambed lower zone (r_{slz}). The total daily erosion rate (m/d), r_{st} , was the sum of r_{suz} and r_{slz} . The r_{st} was estimated using the approach of Lick (2009). First we estimated r_{st} ; however, if r_{st} was greater than the available sediment in the top layer (r_{suz}) (estimated using the depth of the top layer, stream wetted area,

and bulk density) of the streambed then remaining resuspended ($r_{st} - r_{suz}$) sediment is released from the the lower layer of the streambed.

$$r_{st} = E_{oa} \times \left[\frac{\tau_b - \tau_{nc}}{\tau_c - \tau_{nc}} \right]^{n_a} \quad (7)$$

where $E_{oa} = 8.64 \times 10^{-02} m/d$ is erosion coefficient (Lick, 2009), τ_b is streambed shear stress (Pa) caused by stream flow, τ_{nc} is critical shear stress for non-cohesive sediment (Pa), τ_c is critical shear stress for cohesive sediment (Pa), and n_a is an erosion coefficient. The streambed shear stress of equation 7 was estimated (in equation 8) using the hydraulic properties (i.e., stream flow, depth, and water slope) of the stream.

$$\tau_b = \rho_w g R S \quad (8)$$

where ρ_w ($998 kg/m^3$) is density of water; the change in temperature may have slight impacts on water density, however, this is neglected in the model. The g ($= 9.8 m/s^2$) is the acceleration of gravity; R is hydraulic radius (m); and S is streambed slope (i.e., water slope) (m/m). The hydraulic radius of the stream (R) was estimated from the cross-sectional area of stream flow (m^2), and the depth of water (d_{wz} , (m)). The critical shear stress of cohesive and non-cohesive sediment of equation 7 was estimated using the approach of Lick (2009) described in detail by Pandey et al. (2012a).

$$\tau_{nc} = 414 d \quad (9)$$

$$\tau_c = \tau_{nc} \left[1 + \frac{a \cdot \exp(b \cdot \rho_s)}{d^2} + \frac{c_1}{c_2 d} \right] \quad (10)$$

where $a = 8.5 \times 10^{-16} m^2$, $b = 9.07 cm^3/g$, c_1 and c_2 are constants, and d is particle size (m).

The settling of *E. coli* from the water column to the streambed used in equation 1, 2, and 3 was calculated using the suspended *E. coli*, suspended sediment into the water column, and Stokes's law of sediment settling velocity. While calculating deposition, we assumed 80% of *E. coli* of the water column are attached with sediment particles (Hipsey et al., 2008).

$$EC_{duz} = \frac{EC_{wc}}{TSS} \times \frac{g \cdot d^2}{d_w \times 18\mu} (\rho_w - \rho_s) \times SS \times 86400 \quad (11)$$

where EC_{duz} is *E. coli* deposition in the streambed upper zone (CFU); EC_{wc} is *E. coli* concentrations (CFU/ m^3) in the water column; TSS is suspended sediment concentrations in water column (g/m^3); g is acceleration due to gravity (m/s^2); d is effective spherical particle diameter (m); d_w is water depth in stream (m); and μ ($= 8.91 \times 10^{-04}$ Pa s) is the viscosity of water. The SS is suspended sediment mass (g) in the water column, which was estimated by multiplying the volume of water (m^3) in reach by the TSS (g/m^3). The second term of equation 11 shows the settling velocity estimation using Stokes's Law. The value of 86,400 is a multiplication constant to obtain settling over a day. Initially all of the deposited *E. coli* remain in the streambed upper zone; subsequently due to streambed mixing/disturbance caused by stream flow, a fraction (f_{lz}) of total deposited *E. coli* in the streambed upper zone was moved to the streambed lower zone. Due to complexity involved in streambed modeling, it can be extremely difficult to calculate the transfer of *E. coli* from the streambed upper zone to the lower zone. In addition, it can be difficult to verify the predictions. In order to simplify the simulation, we used f_{lz} of 0.15 (15% of the upper zone *E. coli* transferred to the lower zone). The validity of this assumption was tested through calibration (i.e., changing the value of f_{lz} by 15%), and we found that this assumption of 15% can be reasonable. Also we

provided an option for changing the value of f_{lz} (a calibration parameter). The sensitivity of this parameter to *E. coli* predictions is explained later. The transfer of *E. coli* from the upper zone to the lower zone accounted for the effects of streambed mixing, movement, and disturbance. Due to the fact that the streambed sediment particles are always in motion (vertically as well as horizontally) within the streambed, the *E. coli* deposited in the top 2cm layer (upper zone) potentially can enter the streambed lower zones. Using this transferring approach in the model, we simulated the vertical distribution of the *E. coli* over streambed depth.

In addition to the movement of the *E. coli* from the streambed to the water column (or vice versa), the growth of *E. coli* in the streambed and the water column has significant influence on in-stream *E. coli* concentrations. In addition to growth, *E. coli* mortality can also impact the *E. coli* concentration in streams. Both *E. coli* growth and mortality, however, are reported to be controlled by temperature (Hipsey et al., 2008). Previous studies, for example, Kim et al. (2010) excluded the growth function, and the temperature influence was modeled by mortality equations only. Another study, for example, Hipsey et al. (2008), emphasized the use of a growth function. Since both growth and mortality are functions of temperature, the impacts of temperature on *E. coli* growth and decay can be incorporated using one function (either growth or decay). In this study, we used a growth function for calculating the *E. coli* growth in the streambed upper and lower zones, and the water zone, the function described by Hipsey et al. (2008).

$$EC_g = \mu_{max} \times \left[CT_1 \cdot (T_w - T_{min}) \cdot \left(1 - \exp(CT_2 \cdot (T_w - T_{max})) \right) \right]^2 \quad (12)$$

where EC_g is daily *E. coli* growth (d^{-1}); μ_{max} is maximum growth rate constant (d^{-1}); CT_1 and CT_2 are growth constants; T_{min} and T_{max} are the minimum and maximum growth temperatures ($^{\circ}C$); and T_w is water temperature ($^{\circ}C$). Using equation 12, we calculated the *E. coli* growth in the streambed (upper and lower zones) as well as in the water column. The growth constants used in equation 12 are provided in model application section (section 4). The water temperature T_w was estimated from the maximum and minimum daily air temperatures (average) using the method proposed by Stefan and Preud'Homme (1993):

$$T_w = 5.0 + 0.75 \times T_{air} \quad (13)$$

where T_{air} is average air temperature ($^{\circ}C$).

Parameters sensitivities were analysed to estimate the influence of the parameters on sediment *E. coli* and water *E. coli* predictions. Relative sensitivity (S_r) was estimated as:

$$S_r = \left| \left(\frac{X}{Y} \right) \times \frac{(Y_2 - Y_1)}{(X_2 - X_1)} \right| \quad (14)$$

where X is a base value of a parameter and Y is corresponding prediction; X_1 and X_2 are 15% increase and 15% decrease in the parameter values, and Y_1 and Y_2 are the corresponding predictions (James and Burges, 1982; White and Chaubey, 2005).

3. Integrating in-stream *E. coli* transport model into the SWAT

The Soil and Water Assessment Tool (SWAT), a river basin/watershed scale hydrological model, was developed by United State Department of Agriculture (USDA) Agricultural Research Service. In addition to USDA, several other federal agencies including the U.S. Environmental Protection Agency, Natural Resources Conservation Service, National

Oceanic and Atmospheric Administrations, and Bureau of Indian Affairs have contributed to the model. The model has been extensively used in predicting daily/monthly stream flow and water quality (i.e., nutrients, pesticides, sediment) around the world (Gassman et al. 2007). Many of the previous studies, which are reviewed by Gassman et al. (2007) and Moriasi (2007) have shown the applicability of the SWAT in non-point source modeling. The SWAT model simulates the impacts of land use and land management practices on water quantity and quality. In SWAT, a watershed is divided into multiple subbasins, and hydrological response units (HRU), which consist of homogenous land use and management, soil types, and slopes. During model simulations, stream flow and non-point source loads from each HRU are summed, and the resulting loads are routed through streams to the watershed outlets.

In order to integrate the model developed in Section 2, we wrote three new FORTRAN subroutines (modules): 1) `rtbact.f90` (for in-stream *E. coli* resuspension, and routing); 2) `netgrowth_sed.f90` (*E. coli* growth in the streambed); and 3) `netgrowth_wat.f90` (*E. coli* growth in the water column). Programming was done with Intel (R) Visual FORTRAN Composer XE 2011. The model predicts *E. coli* concentrations in the streambed and the water column. The developed modules were included in the version of SWAT released in 2009. Compiling and building of the subroutines and model was done in Parallel Studio XE 2011 with VS 2010. Subroutines for resuspension and settling estimation, *E. coli* growth in sediment, and *E. coli* growth in the water column were imported into Solution Explorer (of Intel (R) Visual FORTRAN), where all of the subroutines (a total of 329) from the current SWAT 2009 model were also imported for compiling and building the modified SWAT

model. Importing all subroutines in Solution Explorer was required as many of the parameters of the SWAT are global, and parameter values estimated in one subroutine are also used in many other subroutines.

In order to predict in-stream *E. coli* concentrations, we also considered *E. coli* transport to streams via over land flow. Overland *E. coli* transport is available in the original SWAT model, and we used the existing equations in the modified SWAT model. To predict overland *E. coli* transport, the current SWAT model includes *E. coli* in surface runoff, *E. coli* attached to sediment in surface runoff, and *E. coli* lag in surface runoff. The details of the overland *E. coli* transports processes and related parameters are discussed elsewhere (Neitsch et al. 2005), as the primary goal of this study was to model in-stream processes.

4. Model Application

4.1 Study area and watershed data

The modified SWAT model, which included the new in-stream *E. coli* transport model was tested in the Squaw Creek Watershed, Iowa, USA. The study area is shown in Figure 4.2. The spatial datasets of the watershed (i.e., land cover, elevation, streams, and manure application) were obtained from the Natural Resources Geographic Information System (NRGIS) library. The library is maintained by the GIS section of the Iowa Department of Natural Resources (IDNR). Soil data (STATSGO) used in this study was developed by the National Cooperative Soil Survey and distributed by the Natural Resources Conservation Services (NRCS) of the U.S. Department of Agriculture (USDA). The DEM and soil maps are shown in Figure 4.3a. The watershed has 20 Confined Animal Feeding Operation Units

(CAFO). The CAFO and land cover map is shown in 4.3b. The total area of the watershed which receives manure is approximately 4000 ha at a rate of 179 N kg/ha. The CAFO locations and manure receiving areas are shown in Figure 4.3b. Due to the lack of detail information, we did not include the impacts of septic system and point-source on streams; however, these may influence stream water quality; incorporation of septic and point source systems in the model may improve the predictions. The Squaw Creek watershed, HUC 10 (ID 0708010503), has a total drainage area of 592.39 sq km. The basin length and perimeter of the watershed is 43.53 km and 134.02 km, respectively, with an average slope of 2.01%. The basin relief is 111.51 m, the main channel length is 60.46 km and the total stream length within the watershed is 346.72 km. The digital elevation model (DEM) map of 30 m resolution (floating point grid) and soil map used in the model is shown in Figure 4.3a. Land cover and areas receiving manure is shown in Figure 4.3b.

Squaw Creek passes through four counties (Story, Webster, Hamilton, and Boone) of Iowa, and is a tributary of the South Skunk River. There are 75 first order streams in the watershed. The 2002 hydrologic Unit Code (HUC 10) watershed land use estimates 0.09%, 0.17% and 0.05% of the watershed land area as water, wetland and wetland forest, respectively. Deciduous forest, ungrazed grass, grazed grass, CRP grassland, and alfalfa were 2.71%, 10.87%, 2.52%, 1.70%, and 1.84%, respectively. Corn and soybeans, and other row crops are 41%, 33%, and 0.43%, respectively (based on landcover map of 2002). Common/industrial, residential, and barren land are 1.67%, 1.27%, and 0.06%, respectively (Fig 4.3b).

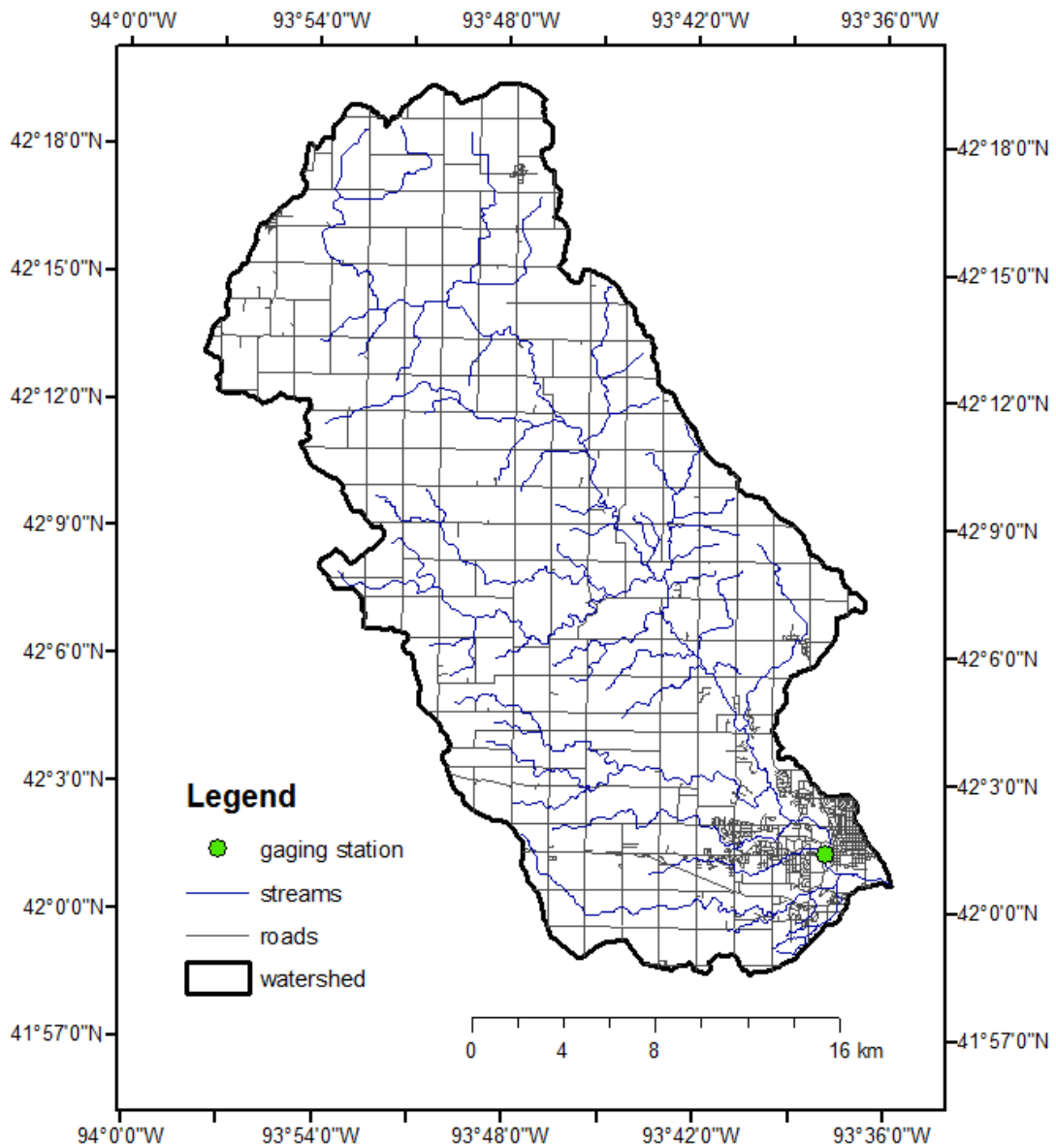


Figure 4.2. Map shows the study area. Green circle indicates gaging station location, streams are shown in blue lines, roads are shown using gray lines, and dark black color line is the watershed boundary line. Latitude and longitude of the study area is shown in the map.

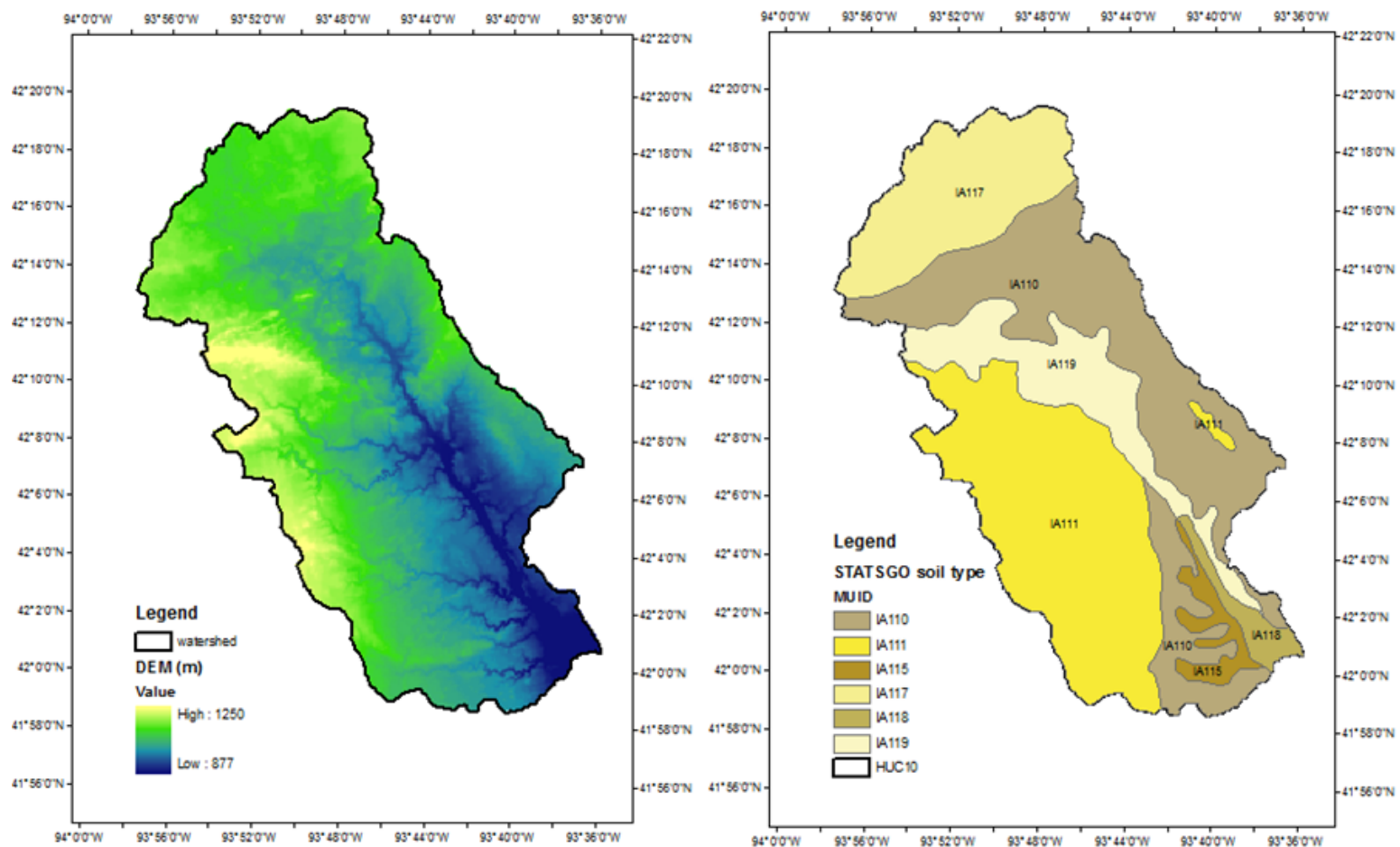


Figure 4.3a. Squaw Creek Watershed: Digital Elevation Model (DEM) (left) and State Soil Geographic database (right).

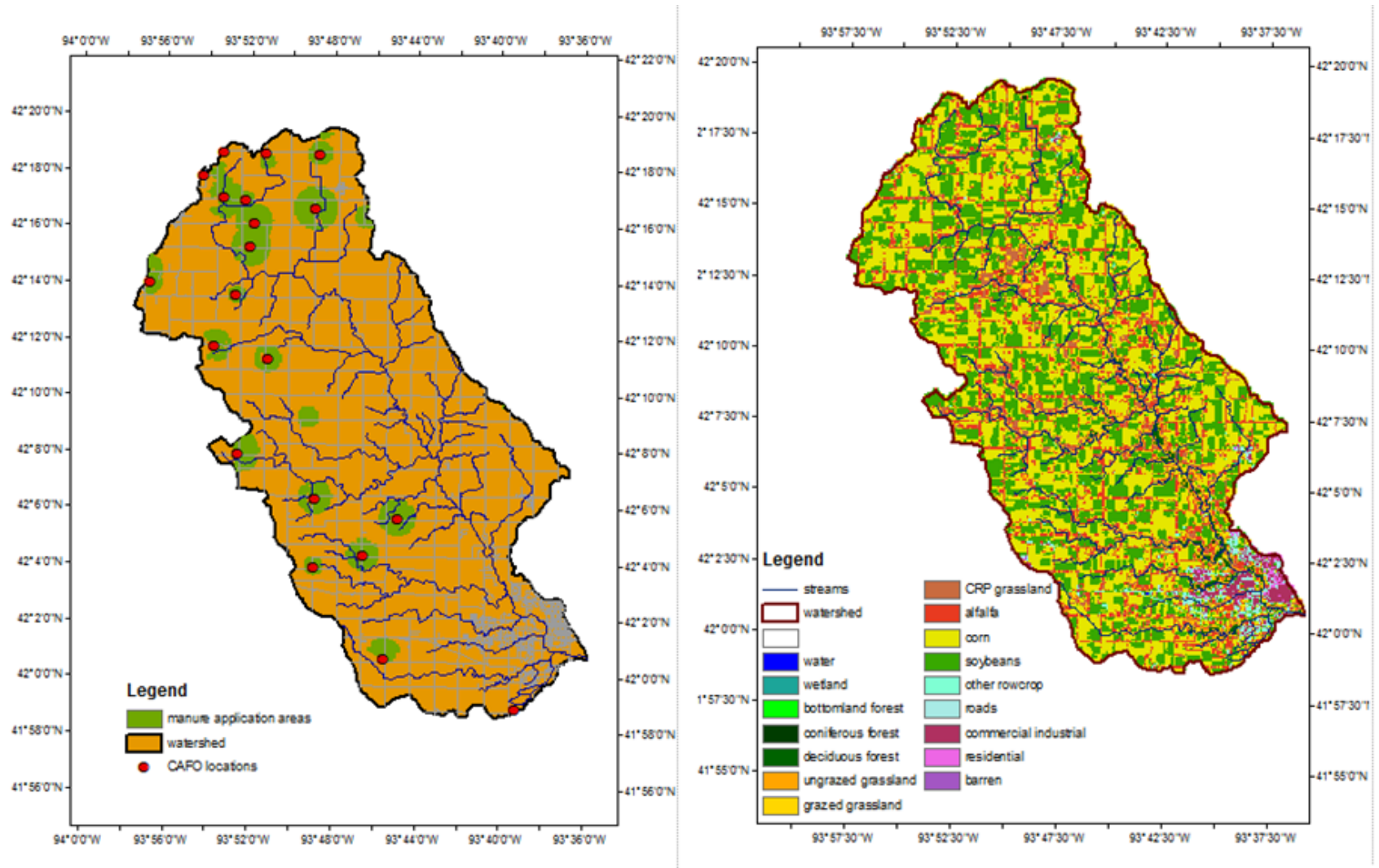


Figure 4.3b. Squaw Creek Watershed land cover: Confined Animal Feeding Operation (CAFO) location (left) and land cover (right).

4.2 *Escherichia coli* data

E. coli concentrations in the streambed and water column were monitored from the summer of 2009 (May) through the fall of 2011 (December). The samples were collected weekly (1 - 2 times per week). The samples were collected at gaging station shown in Figure 4.2. Water samples were collected from the center of the stream using a Horizontal Polycarbonate Water Bottle Sampler (2.2 L, Forestry Suppliers Inc., Mississippi, U.S) by lowering the instrument from a bridge and collecting the sample from the top of the water column at the center of the stream. Sediment samples were collected from the top 2-3 cm of the streambed using a Shallow Water Bottom Dredge Sampler (15 cm × 15 cm opening, Forestry Suppliers Inc., Mississippi, U.S.) at the same location as water samples. Immediately after collection, samples were stored at 4°C and analyzed within 24 hours by membrane filtration techniques (APHA, 1999) on modified mTEC agar (EPA, method 1603). The *E. coli* attached to streambed particles were detached by stirring the mixture of sediment and purified water (ratio 1:1 (weight basis)) for 15 minutes at approximately 150 - 200 rpm using a magnetic stir bar. The resulting solution was used to enumerate *E. coli* in the sediment. The methods used for extracting *E. coli* from sediment, and calculations in enumerating *E. coli* in the sediment and water column are described in Appendix I. The *E. coli* data are shown in Appendix II and III.

4.3 SWAT model application

The SWAT model was calibrated for a set of parameters for the Squaw Creek Watershed to the U.S. Geological Survey gaging station 05470500 (lat 42.02, long 93.63) on Squaw Creek in Ames. The calibrated parameters are discussed later. The stream cross section geometry

was measured at this location. The input data of precipitation and temperature (minimum and maximum) required for the SWAT model were obtained from the Iowa Environmental Mesonet (IEM). We used climate data (precipitation and temperature) from four locations: 1) Ames (lat 42.02, long – 93.77); 2) Boone (lat 42.05, long -93.85); 3) Gilbert (lat 42.11, long – 93.58); and 4) Webster City (lat 42.43, long -93.87) to run the SWAT model. The climate monitoring points and gaging station are shown in Figure 4.4. The delineation of the stream network and subbasins was performed using a DEM with a 30 m resolution (Fig 4.3a), which resulted in a watershed configuration of 31 subbasins. The HRUs were then created by combining 2002 land cover map data with STATSGO soil data. All together a total of 250 HRUs were created (HRU classification of the watershed is described in Appendix IV). Figure 4.4 shows the SWAT model setup. The SWAT model was then run on a daily time step for 2000 to 2011. In the simulation we used the land cover map of 2002 and the STATSGO soil data; however, over the years land cover acreage have changed slightly (i.e., increased corn acreage) in Iowa, which may have slight impacts on the overland *E. coli* transport. This model application, however, does not consider those changes. In the simulation, we consider the first two years as the initialization period because predictions in the initial time period can be erroneous; however, we included these years while calculating the flow prediction statistics to maintain the data integrity. Stream flow predictions discussed in section 5.1 indicate that the modified SWAT model performed well in Squaw Creek Watershed. The predictions of initial years also matched well with observations.

4.4 SWAT model calibration

4.4.1 Flow calibration

The flow data from 2000 to 2005 was used as the calibration period, and the period from 2006 to 2011 was used as the validation period. The model was first calibrated for monthly average daily flow and then for daily flow. In order to calibrate the model, several model parameters, which affect the flow trends, recession, daily peaks, and base flow, were adjusted within the recommended ranges. Table 4.1 shows the calibrated, default, and range of the parameter values, which were subjected to adjustment while calibrating the model. The calibration process adjusted the base flow ratio to the surface runoff, amount of evapotranspiration, and total water yield. While predicting stream flow, we calibrated the curve number (CN2), soil available water capacity (SOL_AWC), groundwater delay coefficient (GW_DELAY), base flow recession coefficient (GW_ALFA), and surface runoff lag coefficient (SURLAG). The calibrated parameter values used in stream flow predictions are in the same range as other published SWAT model applications in Iowa (Jha et al. 2010a). In addition, we estimated the contribution from the tile drains to the stream flow. Since Squaw Creek Watershed is an agricultural watershed ($\approx 75\%$ of total watershed as cropping land), and tile drains are extensively used to reduce the water table in agricultural lands located in the Des Moines Lobe of Iowa, flow contribution from tiles is considerable. In order to estimate the tile flow, we calibrated the value of depth of subsurface drain (DDRAIN), time to drain soil to field capacity (TDRAIN), drain tile lag time (GDRAIN), and depth to impervious layer (DEP_IMP). The value of DDRAIN and TDRAIN were set to 1200 mm and 48 hr, while the value of GDRAIN and DEP_IMP were 24 hr, and 3200 mm, respectively. Tile drainage is known to be an important component to the hydrology of the Des Moines Lobe landscape region; however, precise estimation of the land areas with

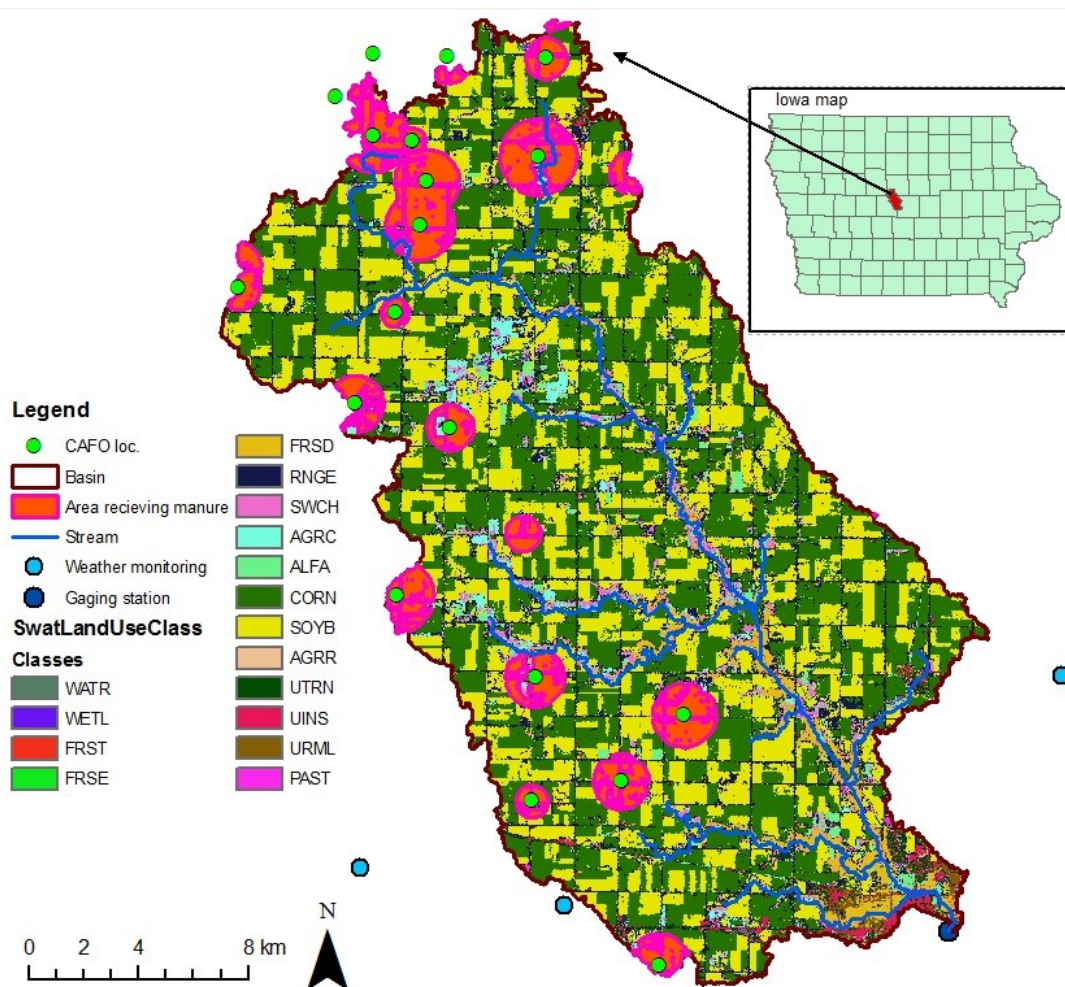


Figure 4.4. The watershed map shown was delineated using Digital Elevation Map (DEM), land cover map of 2002, and STATSGO soil map using SWAT. The subbasins (total 31), HRUs (total 250) and corresponding land use, soils, and slopes are described in Appendix IV. The land cover code names shown in this map are the SWAT code name for the land cover shown in figure 4.3b. The locations of weather stations (light blue circles); gaging station (dark blue circle at the lowest end of the watershed, where we collected samples); stream network (blue lines); manure application areas (red spherical areas); and SWAT land cover (multi colored areas within the watershed boundary).

subsurface tile drained in the watershed can be challenging due to the lack of credible information on tile drains. The method of selecting the tile drain areas based on soil types and slopes are reported elsewhere (Jha et al. 2010b). To simplify simulation, we assumed all subbasins/HRUs in the watershed have tile drains, and the quantity of tile flow was regulated through the calibration parameters. For estimating channel routing and potential evapotranspiration, we used variable storage routing methods and Penman-Monteith method, respectively. The USDA Natural Resources Conservation Service runoff curve number method was used for partitioning daily precipitation between surface runoff and infiltration. The SWAT model selects curve number based on land cover characteristics, which is described elsewhere (Neitsch et al., 2005). To compare the prediction with measured flow values, we used the coefficient of determination (r^2) and Nash-Sutcliffe's coefficient (NSE) (Nash and Surtcliffe, 1970). Both r^2 and NSE are commonly used indicators, particularly in hydrological models, for assessing model performance. The NSE is the proportion of variance in the measured values that is explained by the predicted values. The NSE is considered as a more rigorous fit statistic than the coefficient of determination (USGS Scientific Investigation Report 2010 – 5008). The r^2 indicates the strength of relationship between measured and simulated values (based on differences of predicted and measured values). The r^2 can range from 0 to 1, while NSE ranges from $-\infty$ to 1. A value of one (r^2 and NSE) is considered as an indicator for the perfect match between measured and predicted values. In addition to NSE and r^2 , we also estimated the correlation coefficients between predicted and measured stream flows. Numerous studies are available (Gassman et al., 2007), which report the SWAT model's applicability in predicting stream flow, and results have shown that SWAT performs reasonably well, when it has been applied for predicting

monthly and annual stream flow. However, relatively low r^2 , and NSE values are reported for daily stream flow predictions. For example, Gassman et al. (2007) reviewed several studies, which reported r^2 values ranging from 0.35 to 0.75 for monthly and annual prediction, while

Table 4.1. List of parameter values which were used as calibration parameters. The default parameter values in SWAT model, value range, and calibrated values are all shown. The parameter range is described in Neitsch et al., (2005). The calibrated values used in simulation were obtained by estimating the best r^2 and NSE values for stream flow predictions.

Parameter	Default	Range	Calibrated value
Soil available water capacity (SOL_AWC)	Soil database	± 0.04	Reduced by 0.02
Groundwater delay coefficient (GW_DELAY)	31 days	0 – 100 days	9 days
Surface runoff lag coefficient (SURLAG)	4	0.1 – 10	6
Base flow recession coefficient (GW_Alfa)	0.048	0.1 to 1.0	0.8
Curve number (CN2)	Standard list	$\pm 10\%$	- 20%
Depth of subsurface drain (DDRAIN)	-	0 – 2000 mm	1200 mm
Time to drain soil to field capacity (TDRAIN)	-	0 – 72 hr	48 hr
Drain tile lag time (GDRAIN)	-	0 – 100 hr	24 hr
Depth to impervious layer (DEP_IMP)	-	0 – 6000 mm	3200 mm

r^2 values ranged from 0.24 to 0.50 for daily predictions. Since our target was to predict daily variation in stream *E. coli* concentrations, which requires stream flow in daily time step, we predicted daily stream flow. The daily flow was predicted at the watershed outlet (near the

gaging station at sub-basin of 31 as shown in Fig 4.4). Subsequently we used the daily flow to estimate the monthly average daily flow. Both r^2 and NSE indicators were used during model calibration and validation. 4.4.2 *Overland E. coli transport calibration*

To improve *E. coli* predictions, we compared measured and predicted *E. coli* concentrations i.e., estimated of NSE and r^2 values. The NSE and r^2 values were improved by changing the parameter values (i.e., parameter calibration) [by obtaining the best parameter values within the range described in Neitsch et al., 2005]. In order to calibrate the overland *E. coli* transport process, we adjusted seven parameters, which are shown in Table 4.2.

Table 4.2. Calibrated parameter values linked with overland *E. coli* transport estimation. The parameter ranges, and calibrated values are shown.

Parameters	Range	Calibrated values
<i>E. coli</i> partition coefficient (BACTKDDB)	0 – 1	0.36
Fraction of manure applied to land areas that has active colony forming units (BACTSWF)	0 – 1	0.97
Width of edge-of-field filter strip (m)	0 – 2	1.5 m
Peak rate adjustment factor for sediment routing in the main channel (PRF)	0 – 1	0.61
Linear parameter for calculating the channel sediment routing (SPCON)	$1 \times 10^{-04} - 0.01$	0.0023
Wash-off fraction for <i>E. coli</i> (WOF)	0 – 1	0.5
<i>E. coli</i> soil partitioning coefficient (BACTKDQ)	175	175 m ³ /kg
Temperature adjustment factor (THBACT)	0 – 10	1.07
<i>E. coli</i> percolation coefficient (BACTMX)	0 – 20	10

Other parameters were set at the default values in SWAT. Parameters such as peak rate adjustment factor (PRF) for sediment routing, linear parameter for calculating the channel sediment routing (SPCON), and exponent for calculating the channel sediment routing

(SPEXP) were respectively 0.61, 0.0023, and 1.06. The *E. coli* partition coefficient in surface runoff (BACTKDQ) and the temperature adjustment factor (TBACT) were set to 175 and 1.07, respectively. These parameter values were obtained from previous studies, which have studied overland *E. coli* transport (Parajuli et al., 2009; Kim et al., 2010; Coffey et al., 2010; Neitsch et al., 2005). As in this study our primary focus was to develop model for predicting *E. coli* resuspension, the parameter values governing overland transport were obtained from previous work. The works of Parajuli et al. (2009a;b), Coffey et al. (2010), and Kim et al. (2010) described the range of these parameter values. The bacteria partition coefficient (BACTKDDDB), which partitions deposited bacteria between soil solution and soil solids was set to 0.36 as proposed by Kim et al. (2010), while Parajuli et al. (2009a;b) used BACTKDDDB of 0.9. The default value of percolation coefficient (BACTMIX) of 10, and fraction of manure applied to land areas that has active organisms (BACT_SWF) of 0.97 (Kim et al. 2010) were used.

4.4.3 In-stream *E. coli* transport calibration

The parameter values used in predicting in-stream *E. coli* transport process and their descriptions are shown in Table 4.3. Some of the values were measured at the sampling point, and others were calibrated. The Manning's roughness coefficient n of 0.014 was used, which is a default value of SWAT. We also tested the prediction results at n of 0.036 (the previous value used in Pandey et al., 2012b for the same location), a recommended value for natural streams in Chow (1959, pp. 112 – 123); however, the impact on prediction was negligible. The bulk density of the streambed sediments, expressed as weight per unit volume, was determined from wet and dry weight (dried in the oven at approximately 75 °C

for 2 days) of sediments (Roberts et al. 1998). Based on the previous works of Roberts et al. (1998) and Pandey et al. (2012b), the streambed bulk density was set to 1.26 g/cm^3 for *E. coli* resuspension prediction estimates. The bulk density, however, may change from one sample to the other (as shown in Appendix I (Table A)), which can impact predictions. Particle size distributions in streambed sediment samples (samples collected at 16 unique locations of the Squaw Creek Watershed) were estimated, which is given in Appendix I (Table B). The bulk density of the streambed sediments were also analyzed at 14 unique locations of the Squaw Creek Watershed, and results are provided in Appendix I (Table A). To estimate the potential impacts of bulk density on predictions, we performed a sensitivity analyses (using equation 14) of the bulk density to the predicted *E. coli* concentrations (i.e., streambed and water), which is described in section 5.2. To identify a range of potential bulk density values, we sampled Squaw Creek streambed sediment at 14 locations and calculated the bulk density. The data are described in Appendix I. The results show that the average of the bulk density was 1.46 g/cm^3 with standard deviation of 0.29 g/cm^3 among 14 locations. The bulk density varied from 1.05 to 2.09 g/cm^3 . The analysis based on these 14 samples indicates that the average of bulk densities at 14 locations is higher (15%) than the bulk density used in the simulation (1.26 g/cm^3) on the day that the samples were collected. For the resuspension simulation, the bulk densities of both the zones (upper and lower zones) were assumed to be the same (1.26 g/cm^3).

The parameter values used in predicting *E. coli* concentrations are shown in Table 4.3. The parameter values were set the same for all the subbasins, HRUs and reaches. To simulate in-stream *E. coli* concentrations, the model was initialized by assigning initial *E. coli*

concentrations in the streambed upper and lower zones. The initial *E. coli* concentrations in the streambed upper (EC_{ul}) and lower zones (EC_{bl}) are shown in the Table 4.3. The model predicts *E. coli* concentrations in water column in unit of CFU/100 ml, and in sediment in unit of CFU/100 g.

We assumed that 80% of the *E. coli* in the water column are attached with suspended sediments, which is within the range (80 – 100%) proposed by Hipsey et al. (2008). Pandey et al. (2012b) considered 100% of the stream water column *E. coli* as attached; the simulation performed in the study by Pandey et al. (2012b) was for a single sampling event performed at 16 locations; however, here we have performed simulation for 10 years (on daily time step), which involves in-stream routing and *E. coli* predictions under different flow conditions. Since it is difficult to estimate precise percentages of attached *E. coli* in the water column under different flow conditions (percentages may change in different flow conditions), the selection of unique value was required to simplify the simulations. The slope of the stream bed was estimated from Mannings equation to be 2.5×10^{-04} as described previously by Pandey et al. (2012b). Values of the coefficients a and b were $8.5 \times 10^{-16} m^2$ and $9.07 cm^3/g$, respectively, which were obtained from Lick (2009) as stated in section 2. In order to predict the resuspension of sediment particles from the streambed to the water column, the critical shear stress of cohesive and non-cohesive sediments was calculated. These estimations required knowledge of the particle size to which the *E. coli* were attached. The particles size of $1.5 \mu m$ was used in the model simulation, as a result of calibration. We tested the prediction for the range from 0.5 to $10 \mu m$ particle sizes. The coefficient c_1 (coefficient for clay effects) of 23 and n_a (exponent) of 2.0 were obtained through calibration. These values

Table 4.3. In-stream *E. coli* transport parameters. The parameter values (i.e. calibrated, assumption, and reference) are shown. These calibrated parameter values were used in the final simulation for predicting *E. coli* concentrations in the streambed sediment and the water column. To obtain these calibrated values and verify the predictions, first we ran the modified SWAT model with initially assumed values. Next, the model was rerun multiple times for calibrating the parameter values (i.e., obtaining the parameter values, which improved the predictions). Finally, the calibrated parameter values were used to verify the *E. coli* predictions in streambed sediment and the water column at sampling location (Fig. 4.4).

Parameter	Value	Source
EC _{tl} = initial <i>E. coli</i> conc. in streambed top layer (CFU/100 g)	700	Calibrated
EC _{bl} = initial <i>E. coli</i> conc. in streambed bottom layer (CFU/100 g)	500	Calibrated
EC _{bl} = initial <i>E. coli</i> conc. in – stream water column (CFU/100 ml)	200	Calibrated
d _{suz} = depth of streambed top layer (m)	0.03	Calibrated
d _{slz} = depth of streambed top layer (m)	0.06	Calibrated
f _{uz} = fraction of settled <i>E. coli</i> in streambed top layer	0.85	Calibrated
f _{lz} = fraction of settled <i>E. coli</i> in streambed bottom layer	0.15	Calibrated
a = coefficient for bulk density effect (m ²)	8.5 × 10 ⁻¹⁶	Lick (2009)
b = coefficient for bulk density effect (cm ³ /g)	9.07	Lick (2009)
c ₁ = coefficient for clay effect (N/m ³)	8.46 × 10 ³	Lick (2009), computed with specific gravity (sg = 2.65)
c ₂ = coefficient for clay effect (N/m ²)	23	calibrated/estimated from Lick (2009)
E _{oa} = coefficient (m/s)	1 × 10 ⁻⁶	Lick (2009)
S = slope (m/m)	2.5 × 10 ⁻⁴	Estimated using Manning's equation at sampling location
n _a = erosion coefficient	2.0	Calibrated
d = particle diameter (μm)	1.5	Calibrated
n = Manning's roughness coefficient	0.014	Estimated from Chow (1959)
μ = water viscosity (Pa s)	8.91 × 10 ⁻⁴	Pandey et al. (2012)
sg = specific gravity of sediment	2.65	Pandey et al. (2012)
ρ _s = bulk density of sediment (g/cm ³)	1.26	Calibrated
μ _{max} = maximum growth rate constant (d ⁻¹)	2.4	Hipsey et al. (2008)
CT1 _s = growth constants for streambed <i>E. coli</i>	0.003	Calibrated
CT1 _w = growth constants for water <i>E. coli</i>	0.055	Calibrated
CT2 _s = growth constants for streambed <i>E. coli</i>	0.13	Calibrated
CT2 _s = growth constants for streambed <i>E. coli</i>	0.10	Hipsey et al. (2008)
T _{min} = minimum temperature for growth in streambed and water (°C)	4	Hipsey et al. (2008)
T _{max} = maximum temperature for growth in streambed and water (°C)	35	Hipsey et al. (2008)

differ from Pandey et al. (2012b), which used a different dataset for resuspension predictions. The c_2 value was calculated as proposed in Pandey et al. (2012b).

To evaluate the model prediction, we used the coefficient of determination (r^2) and Nash-Sutcliffe's efficiency coefficient (NSE) to compare predicted and measured stream flow. We targeted an r^2 value greater than 0.40 for daily stream flow prediction and r^2 of greater than 0.75 for monthly average daily stream flow prediction. The target for NSE values for daily stream flow prediction was set to greater than 0.35 and for monthly average daily flow prediction it was set to greater than 0.70. While comparing predicted and measured *E. coli* concentrations in the streambed sediment and the water column, we calculated the percentages of *E. coli* predictions falling within 1 order of magnitude. Our target was to achieve 60% of the predictions within 1 order of magnitude of the observed *E. coli* values. As described by Dorner et al. (2006), *E. coli* predictions in order – of – magnitude are needed for water quality improvement. While predicting *E. coli* concentrations at watershed scale, high precision is not expected.

5. Results and Discussion

5.1 Stream flow and water balance

The results of the annual water balance for the Squaw Creek Watershed, estimated from the SWAT run (2000 to 2011), show average annual precipitation of 780 mm, annual surface runoff of 60.65 mm, and lateral soil flow of 17.48 mm. The contribution from tile flow and ground water was 54.33 and 106.51 mm, respectively. The total annual stream flow (surface runoff, lateral flow, tile flow, and ground water flow) was 238.97 mm, which is 21% of the

total annual precipitation. Tile flow contribution to the total stream flow was approximately 23%, and ground water influx contributed approximately 45%. About 68% of the total stream flow was as base flow (tile and ground water). The water balance of this study is close to the values reported by Jha et al. (2010b). Annual evapotranspiration (608 mm) was 78% of the total annual precipitation. These results are similar to the results of other published studies on watershed in the same region. For example, Jha et al. (2010a) reported 29% of total annual precipitation as stream flow, and 71% of total annual precipitation as evapotranspiration. Another study by Jha et al. (2010b) estimated slightly lower base flow contribution (60% of the stream flow).

Figure 4.5 shows a comparison between predicted and measured daily and monthly average daily stream flow. The r^2 and NSE values for monthly average daily flow were 0.99 and 0.75, respectively. As expected, the r^2 and NSE values for daily stream flow were lower ($r^2 = 0.42$, $NSE = 0.39$). The correlation coefficient (r) between predicted and measured daily flow was 0.65. The r value for monthly average daily flow was 0.99. These results are considered satisfactory. The average monthly daily flow prediction is strong as per the suggested criteria (NSE of 0.75 or greater very good for monthly flow prediction) (Moriasi et al., 2007). As shown in Figure 4.5, the predicted stream flow trends coincide well with measured flow.

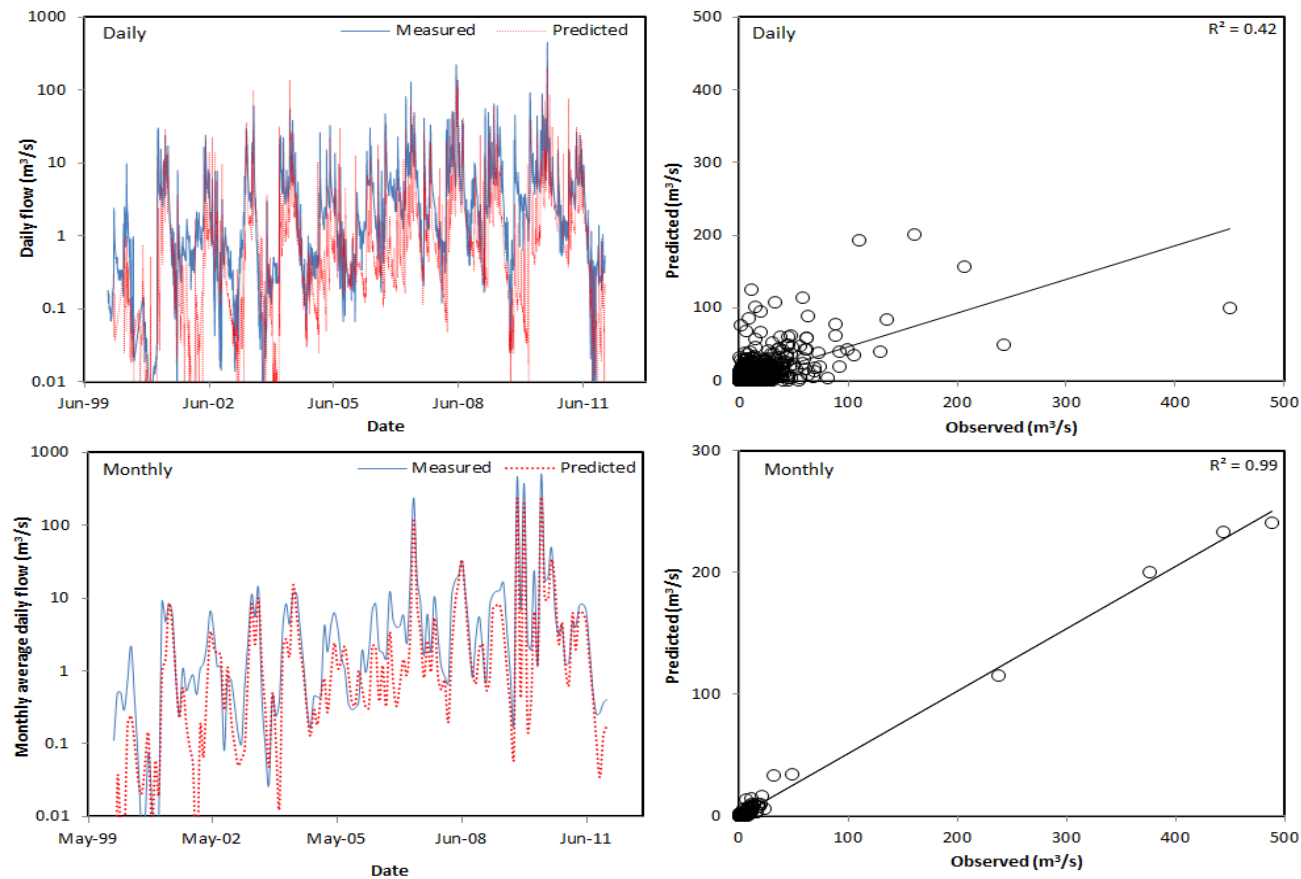


Figure 4.5. Predicted and measured flow in the Squaw Creek Watershed. The top left figure shows the daily stream flow prediction at subbasin 32 (gaging station)(Squaw Creek gaging station), and top right shows comparison and r^2 values between measured and predicted daily flow. The bottom left shows the measured and predicted monthly average daily flows, and bottom right shows a comparison and the r^2 values between observed and predicted monthly average daily stream flow. The calibrated parameter values (i.e., SOL_AWC, GW_DELAY, SURLAG, GW_Alf, CN2, DDRAIN, TDRAIN, GDRAIN, and DEP_IMP) used in stream flow predictions are described in Table 4.1. The location of stream flow observation is shown in Figures 4.2 and 4.4.

5.2 Streambed and water column *E. coli* predictions

Figures 4.6a and 4.6b show the predicted in-stream *E. coli* concentrations. The daily *E. coli* predictions in the streambed and the water column are shown from 2001 to 2011. Plotting *E. coli* concentrations over a 10 year periods shows the oscillatory nature of *E. coli* variation in the stream. For example, during the summer when flow and temperature were high, *E. coli* concentrations peaked. The measured data of Kim et al. (2010) also demonstrated a cyclic behavior of *E. coli* in streams. Figure 4.6a show variations in the streambed *E. coli* concentrations. The secondary vertical axis shows flow variation, while the primary vertical axis shows *E. coli* concentrations (*CFU/100 g*) in the upper zone of the streambed.

Figure 4.6b shows variation in the water column *E. coli* concentrations. These results show that *E. coli* concentration in the streambed as well as in the water increase with high flow in the stream. During high flow events, large amounts of *E. coli* potentially can be released from the streambed to the water column along with resuspended sediment. This increases the level of *E. coli* in the water column. Another potential cause of high levels of *E. coli* during high flow is *E. coli* transport with overland flow. Previous studies, for example, Soupier et al. (2006) reported that runoff from the cropped land which receives manure application can lead to high levels of pathogens in stream water, particularly, during strong rainfall events. High precipitation can cause intense runoff, which can carry large amount of *E. coli* attached to soil particles from the cropped land into streams. While predicting in-stream water pathogen concentrations, a study by Dorner et al. (2006) has modified WATFLOOD hydrological model to include a pathogen transport model. In the study, however, the processes involved were only overland flow, subsurface flow, and in-stream routing. The

conclusions by Dorner et al. (2006) were that the resuspension of microorganisms from stream sediments may be of greater importance than land-based sources of pathogens, and the authors recommended further study of the resuspension process. Other notable studies such as Muirhead et al. (2004), Bai and Lung (2005), and Jamieson et al. (2005a) have also emphasized the importance of streambed *E. coli*, and the resuspension process. For example, Muirhead et al. (2004) created artificial floods in streams during dry weather (in the absence of overland flow) and found that resuspension increased *E. coli* concentrations in stream water by several orders of magnitude. Bai and Lung (2005) studied the impact of sediment on the transport of fecal *E. coli*, and found that the resuspension of sediment and *E. coli* was identical. Similarly, Jamieson et al. (2005a) studied resuspension of sediment-associated *E. coli* in natural streams, and found that high flow increased suspended sediment and *E. coli* in stream water; however, the author proposed that the streambed has a finite supply of sediment associated *E. coli*.

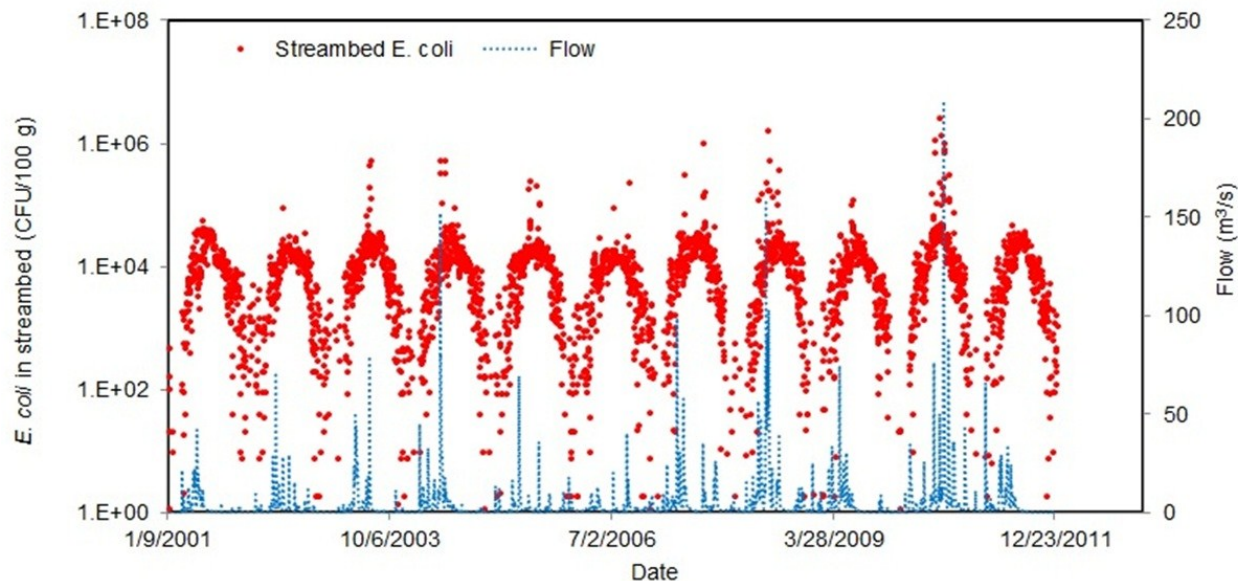


Figure 4.6a. Predicted in-stream *E. coli* concentrations in Squaw Creek and stream flow at the gaging station are shown. The figure shows the *E. coli* concentration (CFU/100 g) in the streambed upper zone (red dots), while the blue dotted line shows stream flow. A parameter list used in predicting streambed sediment *E. coli* concentrations are provided in Table 4.3. For streambed sediment *E. coli* predictions, the values of bulk density and particle sizes were set to 1.26 g/cc 0.15 μm , respectively. The parameter sensitivities is shown in Figure 4.6c. The location of sediment *E. coli* and stream flow prediction is shown in Figures 4.2 and 4.4.

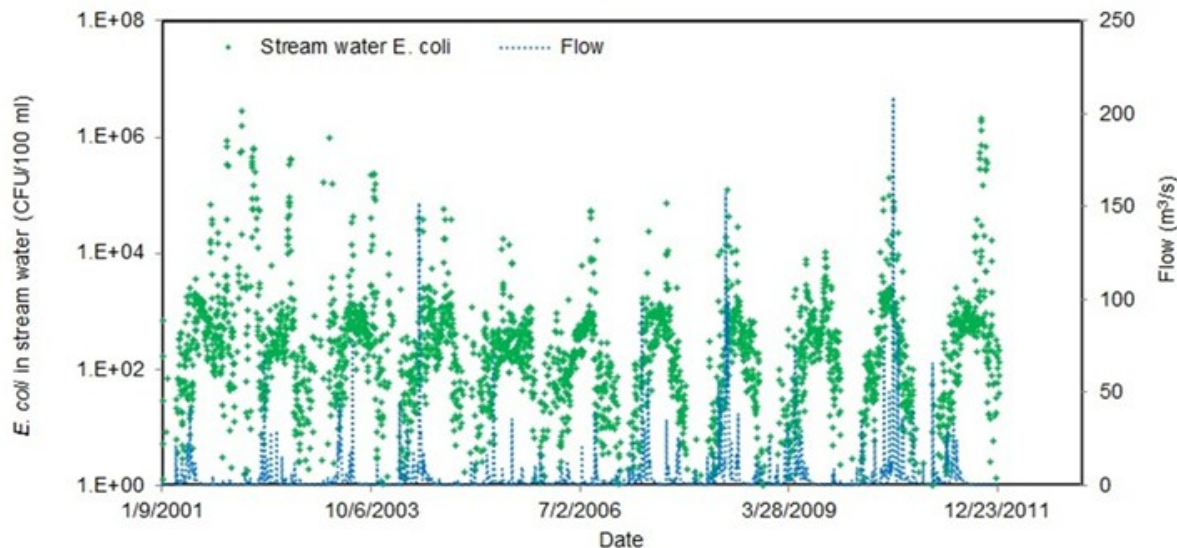


Figure 4.6b. Predicted in-stream *E. coli* concentrations in Squaw Creek at the gaging station and stream flow are shown. Figure shows *E. coli* concentration in the water column (CFU/100 ml). Green dots are *E. coli* concentrations. Blue dotted line indicates stream flow. The parameters used in the water column *E. coli* predictions are described in 4.3. Parameters related to overland flow are described in Table 4.2. The sensitivities of the parameters (i.e., bulk densities, erosion coefficient, and particle size) are shown in Figure 4.6c. The location of water column *E. coli* and stream flow prediction is shown in Figures 4.2 and 4.4.

Sensitivity of parameters important in predicting *E. coli* resuspension (i.e., sediment bulk density (ρ_s), particle size (d), erosion exponent (n_a), fraction of streambed sediment *E. coli* in the top layer i.e., upper zone (f_{uz}), and streambed top depth (d_{suz})) to streambed sediment and water column *E. coli* predictions were evaluated. To find the most sensitive parameter, parameter values were changed $\pm 15\%$ from the base values given in Table 4.3, and corresponding predictions were used to estimate relative sensitivity (S_r) (equation 14).

Figure 4.7 shows the relative sensitivities of the parameters to the streambed sediment *E. coli* and the water column *E. coli*. In both predictions (i.e., streambed sediment *E. coli* and the water column *E. coli*), the S_r values were greater for ρ_s , and S_r values were relatively small for f_{uz} . The ranking of the parameter sensitivities were (highest to lowest) ρ_s , n_a , d , d_{suz} , and f_{uz} .

In addition, we also estimated changes in *E. coli* concentrations of sediment and water column corresponding to a parameter range (i.e., 15 – 80% increase and decrease from the base parameter values). We assessed the impacts of three most sensitive parameters, e.g., particle size, bulk density, and erosion exponent on streambed sediment and water column *E. coli* predictions. Table 4.4 shows the changes in sediment and water column *E. coli* predictions corresponding to the changes in input parameters. The input parameters were changed from 15 – 80% (i.e., increase and decrease), and corresponding changes in sediment and water column *E. coli* were estimated by implementing the modified SWAT model at the Squaw Creek Watershed. When d was increased by 15 to 80%, *E. coli* in sediment was increased by 1×10^2 to 3×10^2 %, and *E. coli* in water was also increased by 1×10^2 to 3×10^2 %, respectively. However, when d value was decreased by 15 to 80%, sediment *E. coli* was decreased by 9×10^1 to 8×10^1 %, and water *E. coli* was decreased by 7×10^2 to 6 %, respectively.

respectively. The impact of bulk density (i.e., ρ_s) on predictions was relatively large (i.e., large relative sensitivity index). For example, when ρ_s was increased by 15 to 80%, sediment *E. coli* decreased by 7×10^1 to 4×10^1 %, and water *E. coli* decreased by 5 to 2%;

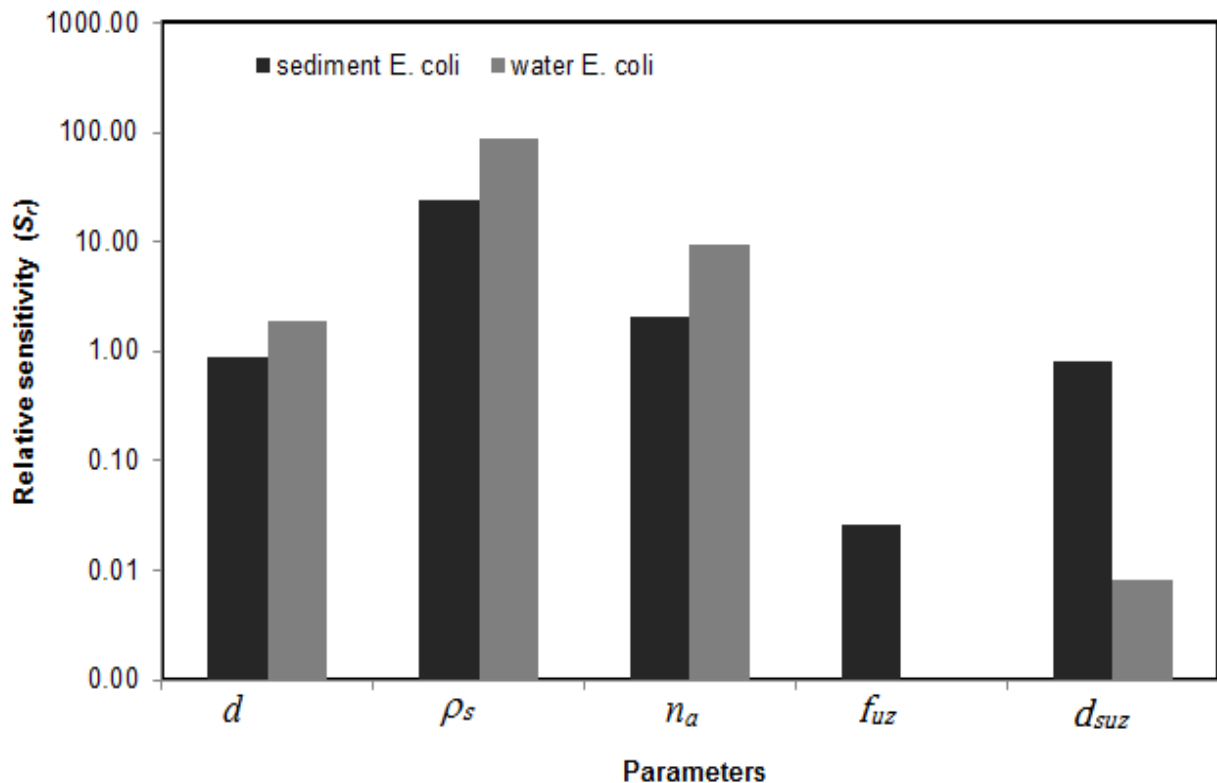


Figure 4.6c. Parameter sensitivities to the streambed sediment and water column *E. coli* predictions. Dark black bars show sensitivities to streambed sediment *E. coli* and light gray bars indicate sensitivities to the water column *E. coli* predictions. In the sensitivity analysis the base values of the parameters i.e., ρ_s , n_a , d , d_{suz} , and f_{uz} were set to 1.26 g/cc, 1.5 μm , 2, 0.85, and 0.030, respectively. Other parameters used in simulation are described in Tables 4.1, 4.2, and 4.3.

however, when ρ_s value was decreased by 15 to 80%, the sediment *E. coli* was increased by 8×10^2 to 2×10^9 %, and water *E. coli* was increased by 3×10^3 to 2×10^9 %, respectively. The impacts of n_a (i.e., erosion exponent) is also shown in Table 4.4. When n_a was increased by

15 to 80%, the sediment *E. coli* was increased by 1×10^2 to 1×10^4 %, and water *E. coli* was increased by 3×10^2 to 5×10^4 %, respectively. Decreasing n_a value by 15 to 80%, resulted in reduced *E. coli* in sediment by 85 to 78%, and in water by 33 to 2%, respectively (Table 4.4).

Table 4.4. Sensitivities of input parameters to streambed sediment and water column *E. coli* predictions

Parameter change	Parameter increase (+) decrease (-)	Parameters			Parameters		
		<i>d</i>	ρ_s	n_a	<i>d</i>	ρ_s	n_a
		Changes in sediment <i>E. coli</i>			Changes in water <i>E. coli</i>		
		%			%		
15%	+	1.E+02	7.E+01	1.E+02	1.E+02	5.E+00	3.E+02
30%	+	1.E+02	6.E+01	3.E+02	2.E+02	2.E+00	1.E+03
45%	+	2.E+02	5.E+01	8.E+02	2.E+02	2.E+00	3.E+03
60%	+	2.E+02	5.E+01	2.E+03	2.E+02	2.E+00	1.E+04
75%	+	3.E+02	5.E+01	5.E+03	3.E+02	2.E+00	2.E+04
80%	+	3.E+02	4.E+01	1.E+04	3.E+02	2.E+00	5.E+04
15%	-	9.E+01	8.E+02	8.E+01	7.E+01	3.E+03	3.E+01
30%	-	8.E+01	2.E+04	8.E+01	5.E+01	7.E+04	1.E+01
45%	-	8.E+01	7.E+05	8.E+01	3.E+01	2.E+06	5.E+00
60%	-	8.E+01	2.E+07	8.E+01	2.E+01	4.E+07	3.E+00
75%	-	8.E+01	2.E+08	8.E+01	1.E+01	3.E+08	2.E+00
80%	-	8.E+01	2.E+09	8.E+01	6.E+00	2.E+09	2.E+00

Note: Table shows changes in streambed sediment *E. coli* concentrations and water column *E. coli* concentrations predictions corresponding to changes in input parameters (i.e., particle size (*d*), buld density (ρ_s), and erosion exponent (n_a)). To calculate the percentage changes in predictions (i.e., *E. coli* concentrations in sediment and water column), simulations were performed for a parameter range (15 – 80% of the base values). Base values of parameters are shown in Table 4.3.

5.3 Model validation

The model predictions were verified by comparing the predicted and measured *E. coli* concentrations in the streambed sediment and the water column in the Squaw Creek Watershed at gaging station shown in Figures 4.4 and 4.2. Figure 4.7 shows the comparison between measured (i.e., estimated from field samples) and predicted *E. coli* concentrations in the upper zone of the streambed sediment. Figure 4.7 (top) shows streambed measured and predicted *E. coli* concentrations (CFU/100g) variation in relation to flow, while Figure 4.7 (bottom) compares predicted streambed *E. coli* concentrations with measured streambed *E. coli* concentrations (CFU/100g). To maintain the data integrity, we used all of the measured data between 3/2010 and 11/2011 (total sample numbers 55), while comparing with measured values. The predictions for water column *E. coli* are better than streambed sediment *E. coli* concentrations. Also predicted streambed sediment *E. coli* are slightly higher in spring 2010 than the observed values. The potential reasons for low observed *E. coli* concentrations in spring 2010 are not known. As shown in the Figure, most of the predicted values of *E. coli* in the streambed are within one order of magnitude of the observed values. Analysis shows that approximately 62% of the predictions are within an order of magnitude, and 36% of the predictions are within 2 orders of magnitude. Only 2% of the predictions fall beyond 2 orders of magnitude. Of course, some predictions are beyond one order of magnitude; however, compared to results of previous studies these results are much improved over the work of others. The data used for model validation are shown in Appendix III. As mentioned by Dorner et al. (2006), order-of-magnitude estimates are what

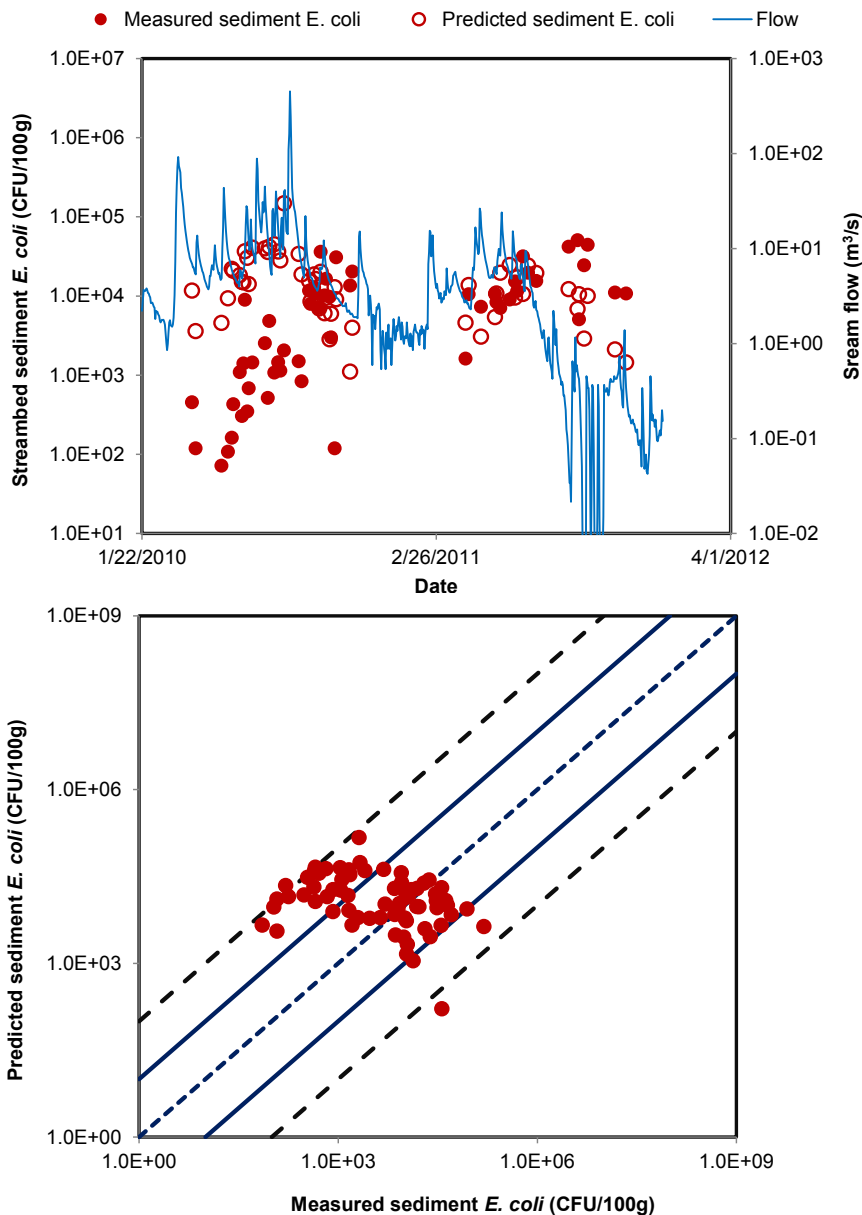


Figure 4.7. Predicted and measured *E. coli* concentrations in the streambed upper zone are shown. The top figure shows predicted (hollow red circles) and measured (filled red circles) *E. coli* concentrations with measured stream flow. The bottom figure shows comparison between predicted and measured *E. coli* concentrations. The small dashed blue line shows the 1:1 line, the solid lines show 1 order of magnitude, and the long dashed lines show 2 orders of magnitude. The parameter values used in predictions are described in Table 4.3 and method used for *E. coli* observation is described in Appendix I. The location of streambed sediment *E. coli* measurement is shown in Figures 4.2 and 4.4.

are required for water quality improvement, and therefore greater precision is not necessary, nor expected in stream water *E. coli* predictions. In a study by Dorner et al. (2006) *E. coli* prediction varied from 1 to 4 orders of magnitude of the observed values, while in a study by Kim et al. (2010), predictions varied from 1 to 3 orders of magnitude of the observed values. Both of these studies, however, focused on predicting stream water column *E. coli* concentrations only. Also in Dorner's study, resuspension process was not included in the model. And Kim's study used a simplified Bagnold's stream power function for resuspension estimation. Figure 4.8 shows the *E. coli* predictions in the water column of the stream. Figure 4.8 (top) shows the measured *E. coli* concentrations from 3/2010 to 12/2011 (total sample numbers 80) and predicted *E. coli* concentrations in relation to stream flow. As shown in the figure, the increased flow elevated the *E. coli* concentrations (*CFU/100 ml*) in the water column similar to the streambed *E. coli* concentrations. The trend of in-stream water column *E. coli* concentrations corroborate the previous results (Kim et al., 2010; Muirhead et al., 2005; Bai and Lung, 2005; Jamieson et al., 2005a;b). Except for the study by Kim et al. (2010), other studies did not involve in-stream routing. Kim et al. (2010) included resuspension of bacteria from the streambed to the water column; however, the resuspension estimation is based on SWAT's default sediment routing, which uses simplified Bagnold's stream power function. This approach has been criticized for not including the impacts of cohesive and non-cohesive properties of sediment as well as the impact of particle sizes in estimating resuspension (Ferguson, 2005).

In our work, we included the impacts of cohesive and non-cohesive sediments properties (i.e., critical shear stresses of cohesive and non-cohesive sediments) and particles sizes on

critical shear stress estimation, which governed the resuspension of *E. coli* from the streambed to the water column. Figure 4.8 (bottom) shows the comparison between measured and predicted values. The analysis shows that 82% of the predictions are within one order of magnitude of the predicted values. Another 15% of the predictions are within 2 orders of magnitudes, and only 3% of the predictions are beyond 2 orders of magnitude. We also predicted *E. coli* concentrations (the predictions deviated greatly from the observed values, therefore the outputs are not listed here) using the original SWAT model (without change), which does not include *E. coli* resuspension. While the original SWAT does not simulate *E. coli* concentrations in the streambed sediment (it predicts *E. coli* concentrations only in the water column without considering the impacts of streambed sediment), the modified SWAT simulates *E. coli* concentrations in the streambed sediment and also includes the impacts of resuspension in predicting *E. coli* concentrations in the water column. In comparison to results reported in previous studies (Dorner et al., 2006; Kim et al., 2010; Parajuli et al., 2006), the work proposed here and the results are a significant improvement, and we expect that the model developed in this study may have significant importance for in-stream *E. coli* prediction and watershed planning. In addition to improving *E. coli* prediction in the water column, we also provided a method to predict *E. coli* concentrations in the streambed, which has never been done.

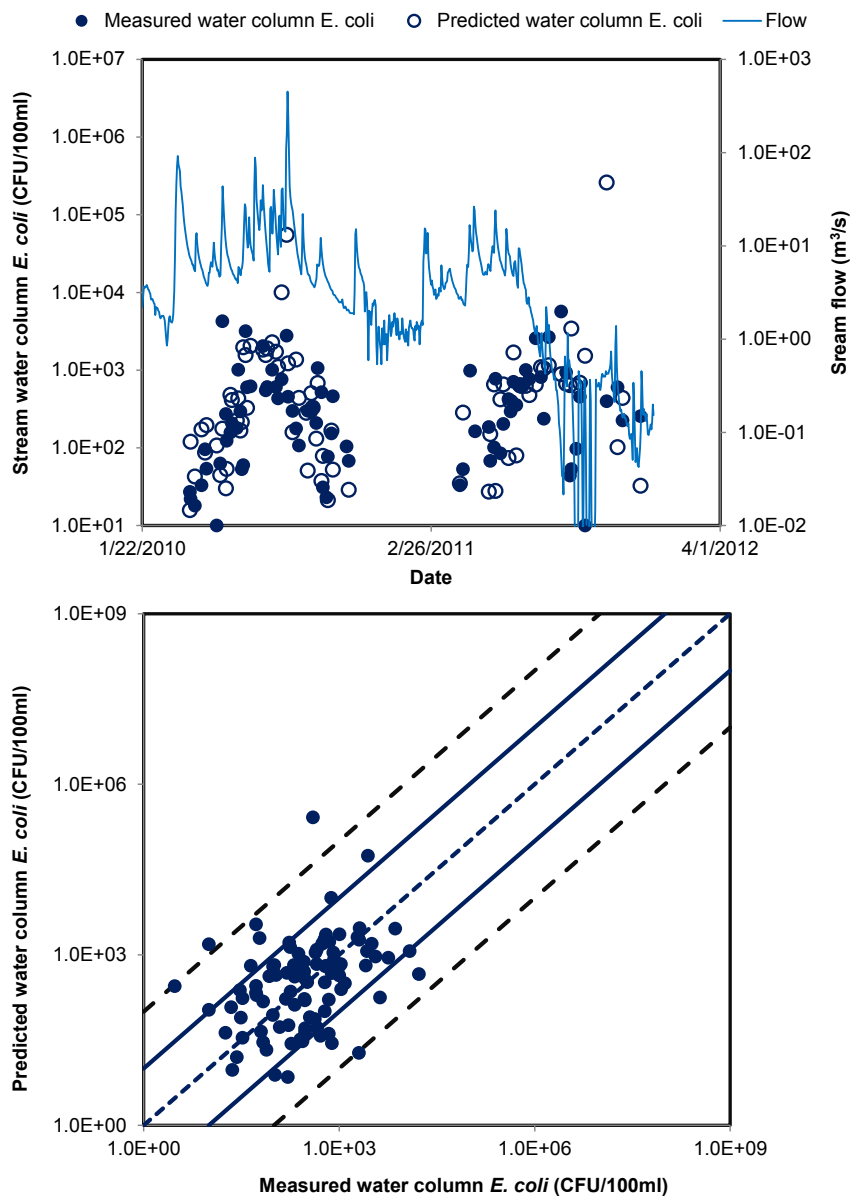


Figure 4.8. Predicted and measured *E. coli* concentrations in the water column are shown. The measurements were taken in the Squaw Creek at gaging station location shown in Figure 4.2 and 4.4. The *E. coli* concentrations were predicted for the same location. The top figure shows predicted (hollow blue circles), measured (filled blue circles) *E. coli* concentrations with flow and the bottom figure shows a comparison between predicted and measured *E. coli* concentrations in the water column. The blue dashed lines show the 1:1 line, the solid line shows 1 order of magnitude, and the small dashed lines show 2 orders of magnitude. The parameter values used in predictions are described in Table 4.3 and method used for *E. coli* observation is described in Appendix I. The location of water column *E. coli* measurement is shown in Figures 4.2 and 4.4.

6. Conclusions

In this study we developed a model for in-stream *E. coli* predictions. *E. coli* in the streambed and the water column were predicted. The developed model was augmented with the existing SWAT model, a hydrological watershed scale model, to improve SWAT bacteria prediction. The modified SWAT model was tested in Squaw Creek Watershed to predict stream flow and *E. coli* concentrations in Squaw Creek. The results of the modified SWAT model show that *E. coli* prediction by SWAT was improved. The results were verified by comparing the prediction results with measured data. For example, the r^2 of flow for monthly average daily prediction was 0.99, and for daily prediction it was 0.42. The *NSE* values for monthly average daily and daily predictions were 0.75 and 0.39, respectively. In the streambed, approximately 62% of the predicted *E. coli* are within 1 order of measured values. In the water column, 82% of the predicted values are within 1 order of magnitude. Only 1 and 3% of the predicted *E. coli* are beyond 2 orders of magnitude of measured values in streambed and water column, respectively.

Considering the results and recommendations of previous studies, the work completed here has significant importance. We predicted *E. coli* concentrations in the streambed of the stream, which has not yet been published in the peer-reviewed literature. Several studies have proposed the importance of streambed *E. coli* concentrations, and we found it as critically important for understanding of stream *E. coli* concentrations; however, a model to predict streambed *E. coli* concentrations is lacking, primarily due to the complexity involved in streambed *E. coli* estimation. Another worthwhile point is that previous work on in-stream *E. coli* prediction has suffered from a lack of observed data. Compared to water *E. coli* data,

the availability of data on sediment *E. coli* concentrations are very scarce. To verify the model results, however, measured *E. coli* data from natural streams is critically important in order to improve the in-stream *E. coli* transport modeling. Our observed *E. coli* data in the streambed and in the water column at same location and same time for multiple years and seasons, was crucial to improve the model predictions. Another significant improvement in this study was that we estimated the resuspension and settling of *E. coli* as a function of flow, which provided more realistic resuspension estimation at various flow conditions.

The SWAT model has rarely been used for TMDLs development when waters are impaired due to elevated pathogen levels. This modified version of SWAT will have significant importance in improving the understanding of in-stream *E. coli* fate and transport, and will be useful for application of SWAT for TMDLs. Additionally, the modified version of SWAT will be useful for comparison of Best Management Practices (BMP) scenarios necessary for EPA-approved watershed management plans.

Acknowledgements

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CHAPTER 5. ASSESSING THE IMPACTS OF STREAMBED SEDIMENT ON TOTAL *Escherichia coli* LOADS OVER A RANGE OF FLOW CONDITIONS

This paper to be submitted
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Abstract

Understanding sediment pathogen levels and their contribution to the water column during resuspension is critical for predicting in-stream pathogen levels and the risk to human health. The U.S. EPA's current water quality testing strategies, however, rely on water borne *E. coli* concentrations to assess stream pathogen levels and identify impaired waters. In this work, we investigated impacts of streambed sediment on in-stream total *E. coli* loads using a range of flows, sediment/water bacteria fractions, and particle sizes to which bacteria attach to assess the impact of *E. coli* in streambed sediments on water column *E. coli* levels. We used a simple sediment transport theory to calculate the potential total bacteria concentrations in a stream with and without the resuspension process. Results clearly indicate that inclusion of resuspending sediment attached bacteria is necessary for watershed assessments and data on sediment bacteria concentrations is much needed. When neglecting the streambed sediment *E. coli* concentrations, the model predicted average *E. coli* loads of 10^7 (cfu/s); however,

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when streambed sediment *E. coli* concentrations were included in the model, the predictions ranged from 10^{10} to 10^{14} (cfu/s). To verify the predictions, *E. coli* data in the streambed sediment and the water column were monitored in Squaw Creek, Iowa, USA. Comparisons between measured and predicted pathogen loads yielded an R^2 -value of 0.85.

1. Introduction

Elevated pathogen levels are the leading cause of stream water quality impairments in the United States. The U.S. Environmental Protection Agency (U.S. EPA) estimates that at least 748,072 kilometers of streams are contaminated with pathogens, potentially posing a risk to human health (USEPA, 2011; Pandey et al., 2012a,b). These estimates, however, could be low because current water quality assessment techniques are based upon environmental sampling methods which assume that indicator bacteria are entirely present in the freely suspended state (Droppo et al., 2011; Bai and Ling, 2005). This approach excludes the pathogens entrained in the stream sediment compartment, potentially underestimating the human health risk during certain flow regimes (Cabelli, 1983; Droppo et al., 2011).

The exclusion of bottom sediment bacteria from water quality monitoring programs has led to insufficient data to include the resuspension process in watershed scale models typically used to set load restrictions and develop watershed management plans. Implications of this are great as currently the burden for water quality improvement is entirely transferred to the stakeholders in the watershed. For example, *E. coli* concentrations in water samples collected for health risk assessment may not represent recent pathogenic contamination but may reflect historically deposited *E. coli* transported from upstream resuspension during high flows (Droppo et al., 2011). While there is limited knowledge available on the pathogenesis of

persistent organisms surviving in stream sediments (Ashbolt et al., 2010), these “background” sources should be acknowledged in watershed assessments.

There is, however, much debate regarding current indicator organisms and their ability to represent the potential presence of pathogens. Current exposure limits have been established to protect human health: the EPA defines acceptable recreational limits as those that will result in eight or fewer swimming-related gastrointestinal (GI) illnesses out of every 1,000 swimmers (USEPA, 1986). The current U.S. EPA fresh water quality criteria for *E. coli* is a geometric mean not exceeding 126 cfu/100 ml or no samples exceeding a single sample maximum of 235 cfu/100 ml (USEPA, 2001a). Criteria were developed based on U.S. EPA measurements of total and highly credible gastrointestinal illnesses (HCGI) which correlated with *E. coli* ($p=0.804$) in fresh recreational waters (Dufour, 1984). Others have also identified trends between indicator organisms in water and gastrointestinal (GI) illness in humans, including vomiting, diarrhea, and fever (Cabelli, 1983; Wade et al., 2006). For example, a study by Wade et al (2006) observed significant trends between increased GI illness and indicator organisms at the Lake Michigan beach, and a positive trend with indicators such as *E. coli* at the Lake Erie beach. Recent work by Edge et al. (2010) detected waterborne pathogens in 80% of water samples with *E. coli* counts of less than 100 cfu/100ml.

Multiple studies have identified high levels of indicator organisms in streambed sediments, ranging from 10 to 10,000 times higher than concentrations in the overlying water column (Davies et al., 1995; Bai and Lung, 2005), and field experiments have confirmed that bacteria associated with the stream sediments resuspend during high flows and contribute an

additional bacteria load to the stream water column (Jamieson et al., 2005a;b ; Nagels et al., 2002). For example, a study by Wu et al (2009) found that a large number of downstream samples were associated with upstream sediment sources of *E. coli*. Muirhead et al. (2004) examined bacteria concentrations during a series of artificial floods in a stream and found strong evidence that the stream bed is an important source of *E. coli*, with high flows mobilizing sediment-associated *E. coli* from the stream bed to the water column. Jamieson et al. (2005b) used mathematical models to understand the processes which control *E. coli* transport in natural streams, and concluded that bottom sediment *E. coli* is a primary source. Similarly, Wilkinson et al. (1995) concluded that resuspension of organisms from storage within the stream bed are critically important to understanding pathogen dynamics in streams and rivers.

There are limited studies, however, focused on understanding the interactions between sediment and water column bacteria (Rehmann and Soupir, 2009; Droppo et al., 2011). Some previous studies have proposed using the critical bed shear stress for erosion for calculating bacteria resuspension (Wu et al., 2009; Rehmann and Soupir, 2009; Droppo et al., 2011; Jamieson et al., 2005b). A study by Cho et al. (2010) emphasized the importance of particle sizes, while estimating the in-stream bacteria resuspension. Studies by Bai and Lung (2005) and Jamieson et al. (2005b) proposed partitioning coefficients to estimate the ratio of attached to freely-suspended bacteria in irreversible and reversible linear adsorption processes; however, verification of these coefficients is difficult (Droppo et al., 2011). Recent work by Pandey et al. (2012a) modified in-stream sediment transport equations derived from the work of Lick (2009) to predict *E. coli* resuspension, and validated the

approach for a natural stream. One of the advantages of this approach is that it is based on simple empirical equations, and the parameter values can be verified from field data.

Currently an assessment differentiating the predicted in-stream pathogen levels with and without the resuspension process is lacking. Here, we developed a model for estimating total stream *E. coli* loads, and performed a scenario analysis to estimate the potential impact of in-stream *E. coli* levels due to resuspending bottom sediments using a range of flows, sediment/water bacteria fractions, and particle sizes to which bacteria attach. The predictions were verified using the observations of the Squaw Creek Watershed, Iowa. The objectives of this work are to: 1) to calculate *E. coli* loads, when *E. coli* levels in the streambed sediment is higher than the water column, 2) verify the pathogen load predictions using measured data of *E. coli* concentrations in the streambed sediment and the water column, 3) and evaluate the impacts of streambed sediment characteristics on *E. coli* load predictions. This exercise provides an insightful quantification of the impact sediment pathogens have on stream water quality.

2. Methods

This work uses the recent bacteria resuspension equations proposed by Pandey et al. (2012a) to estimate the total pathogen discharge through a defined surface area of unit stream length per unit time, L_T (CFU/s). The L_T is the sum of two components: 1) water *E. coli* discharge, L_w (CFU/s), and 2) sediment *E. coli* discharge L_s (CFU/s) as shown in Eq. 1.

$$L_T = L_w + L_s \quad (1)$$

The L_w was estimated by multiplying the *E. coli* concentration in water, C_w (CFU/m³), with water discharge, Q (m³/s) as shown in Eq. 2.

$$L_w = C_w \times Q \quad (2)$$

To determine L_s , we calculated resuspended *E. coli*, R_p (CFU/m²s), and multiplied it by the stream's wetted surface area, W_A (m²) as shown in Eq. 3, ($W_A = W_p \times L$); where W_p is the wetted perimeter (m) and L (=1 m) is a unit meter of stream length.

$$L_s = R_p \times W_A \quad (3)$$

The R_p was estimated by multiplying the sediment *E. coli* concentration, C_s (CFU/m³), which potentially could resuspend, with the sediment erosion rates obtained from sediment transport theory proposed by Lick (2009), as described previously by Pandey et al. (2012a).

$$R_p = C_s \times E_0 \left(\frac{\tau_b - \tau_{cn}}{\tau_c - \tau_{cn}} \right)^{n_a} \quad (4)$$

where, E_0 is erosion rate of 10⁻⁴ cm/s; τ_b is the bottom shear stress caused by water flow (Pa); τ_{cn} and τ_c are the critical shear stresses (N/m²) of non-cohesive and cohesive sediments, respectively. The n_a (= 2.0) is an exponent proposed by Lick (2009) for particle size $d < 432$ μ m. The τ_b is estimated from the specific gravity of water, γ (N/m³), hydraulic radius, R (m), and water surface slope, S , (m/m) ($\tau_b = \gamma \cdot R \cdot S$); τ_{cn} was estimated as a function of particle size, d (m), ($\tau_{cn} = d \cdot 4.14 \times 10^{-3}$), and τ_c was estimated using Lick's approach

(equation 3.17 of the chapter 3) (Lick, 2009) and as described in detail by Pandey et al. (2012a).

$$\tau_c = \tau_{cn} \left(1 + \frac{ae^{bc\rho_b}}{d^2} \right) \quad (5)$$

where a of 8.5×10^{-16} , and b_c of $9.07 \text{ cm}^3/\text{g}$ are constants. In equation 5, Pandey et al. (2012a) has added an additional term to include the impacts of clay content, which is excluded in this simple analysis for simplifying the simulation. The simplification was made due to the fact that critical shear stress of cohesive sediment is primarily governed by bulk density and particle size. As shown in chapter 3 (figs 3.5 and 3.6) and chapter 4 (figure 4.7), particle size and bulk density are the primary parameters (i.e., with greater sensitivities) controlling critical shear stress of cohesive sediment. The slightly simplified equation (eqn 5) also includes governing parameters (i.e., bulk density and particle size). To verify the changes we tested the both type of equations (with and without clay factors), equation 5 of this chapter and equation 3 of chapter 3; however, predictions were not much changed. The R was estimated as W_{sA}/W_p , where W_{sA} (m^2) is water surface area for trapezoidal stream ($W_{sA} = b \cdot d_w + z \cdot d_w^2$), and W_p (m) is wetted perimeter ($W_p = b + 2 d_w \cdot \text{sqrt}(1+z^2)$); the b (m) is stream bottom width, z is side slope (hor : ver) and d_w (m) is stream water depth (m). The S was estimated using Manning's equation ($S^{0.5} = n \cdot Q/R^{2/3} \cdot W_{sA}$), where n ($= 0.036$) is Manning's constant for a natural stream (USDA, 1947). The exponent (n_a) and particle size (d) were used as calibration parameters, while verifying the model predictions.

Since the total *E. coli* load (L_T) was estimated using daily discharge data (average daily rates, m^3/s), the temporal frequency of the model results (L_T) is daily. To obtain *E. coli*

concentrations in water samples (C_w), cfu/100 ml was converted to cfu/m³; and to obtain *E. coli* concentrations in sediments (C_s) (cfu/m³), the *E. coli* concentration in sediment samples (cfu/g) were multiplied by the bulk density of sediment (1.26×10^6 g/m³). The calculation of L_T requires load contributions from both sediment attached *E. coli* and free floating *E. coli*. In the water column, a large number of *E. coli* cells are considered to be attached with particles. For example, a study by Hipsey et al. (2008) suggested that 80 – 90% of the totals *E. coli* in the water column are attached with particles.

We assumed a particle size of $d = 10$ μm , which is within the range of cohesive particles; Black et al. (2002) reported that most pathogens are attached to cohesive particles. The measured stream data (i.e., d_w (stage height) and Q (discharge)), for the monitoring period of 2009 – 2010, used in this study were obtained from the USGS gauging station (05470500) (Lat 42°01'23", long 93°37'49") Squaw Creek, Ames, IA. Stream data, Q and d_w , used in this study are approved by USGS. To calculate b , we measured stream geometry at the location of the gauging station. In W_A and W_P calculations, we used a z value of 2.0 m; the measured z near the gauging stations varied between 2 and 2.5 m during the monitoring period. *E. coli* concentrations were measured weekly (1 -2 times) at Squaw Creek near the USGS gaging station from May 2009 to December 2011. To test *E. coli* levels in the water column, we collected water samples from the center of the stream by lowering a Horizontal Polycarbonate Water Bottle Sampler (2.2 L, Forestry Suppliers Inc., Mississippi, U.S) from a bridge into the center of the stream. Streambed sediment samples were collected using a Shallow Water Bottom Dredge Sampler (15 cm \times 15 cm opening, Forestry Suppliers Inc., Mississippi, U.S.) at the same location as where the water samples were collected. Water and

sediment samples were stored at 4°C, and analyzed in triplicate within 24 hours for *E. coli* concentrations. The *E. coli* attached to stream sediment particles were detached by stirring the mixture of sediment and deionized water ratio 1:1 (weight basis) for 15 minutes at approximately 200 rpm using a magnetic stir bar. The resulting solution was used to enumerate *E. coli* in the sediment. The *E. coli* numbers in the water and the sediment were enumerated by membrane filtration techniques (APHA, 1999) on modified mTEC agar (EPA, method 1603). In Appendix I, we described *E. coli* measurement methods in the sediment and water column.

Previous studies have reported that the pathogen concentrations in the sediment could be up to 10,000 times greater than that in the water column (Kim et al., 2000; Bai and Lung, 2005). For this study, we considered *E. coli* ratios (P_{sw}) between the sediment and the water column of 1, 100, and 10,000. The *E. coli* concentration in the sediment (CFU/100 g) and water column (CFU/100 ml) were both converted into CFU/m³ for consistent units in the ratios (using the sediment bulk density of 1.26 g/cc). The procedure adapted in calculating *E. coli* in sediment is described in Appendix I (section 4.2 in lines 5341 – 5381). The method is also explained in chapter 3 (lines 2438 – 2468). These ratios were used to estimate the potential discharge of sediment attached *E. coli* (CFU/s) and water column *E. coli* (CFU/s) from an upstream unit cross section on downstream water column *E. coli* levels.

The potential *E. coli* discharges were estimated at the USGS gauging station for the range of P_{sw} using “if” and “then” scenarios. For example, a P_{sw} of 1 was used when C_w is equal to C_s . We used a C_w value of 235 CFU/100 ml assuming that stream water quality is set at the EPA water quality single sample maximum. The changes in the *E. coli* discharges at different flow

conditions were estimated first for P_{sw} of 1, then for a range of P_{sw} values (i.e., P_{sw} of 100 and 10,000); all assuming sediment concentrations exceed *E. coli* concentrations in the overlying waters. This approach demonstrates the impacts of sediment *E. coli* on the total *E. coli* discharge, L_T , under the condition when the in-stream (water column) *E. coli* levels are at the EPA limit ($C_w = 235$ CFU/100 ml). While predicting total pathogen load, L_T , the parameter values used in the model were obtained from the studies by Lick (2009) and Pandey et al. (2012a). To calculate the model accuracy, we estimated the model's predictive skill (m_{skill}) (Willmott, 1981) and Nash - Sutcliffe model efficiency (NSE) coefficient (Nash and Sutcliffe, 1970).

$$m_{skill} = 1 - \frac{\sum_i^n (p_i - o_i)^2}{\sum_i^n ((p_i - \bar{o}_i) + (o_i - \bar{o}_i))^2} \quad (6)$$

$$NSE = 1 - \frac{\sum_i^n (o_i - p_i)^2}{\sum_i^n (o_i - \bar{o}_i)^2} \quad (7)$$

Predicted and observed *E. coli* loads were used in calculating model skill and NSE. The o_i and p_i are observed and predicted *E. coli* loads, while o_i overbar indicates average of the observed values. While comparing the predicted total *E. coli* loads and measured *E. coli* loads, we estimated coefficient of determination (r^2), *NSE*, and model skill for verifying the predictions. Here we have targeted achieving r^2 values greater than 0.65, *NSE* values greater than 0.50, and model skill greater than 0.50 in order to consider predictions as satisfactory.

3. Results and Discussion

Our results confirm that sediment-associated *E. coli* are a major fraction of the in-stream pathogen concentrations. Figure 5.1 shows the total *E. coli* load (L_T), which is the sum of the

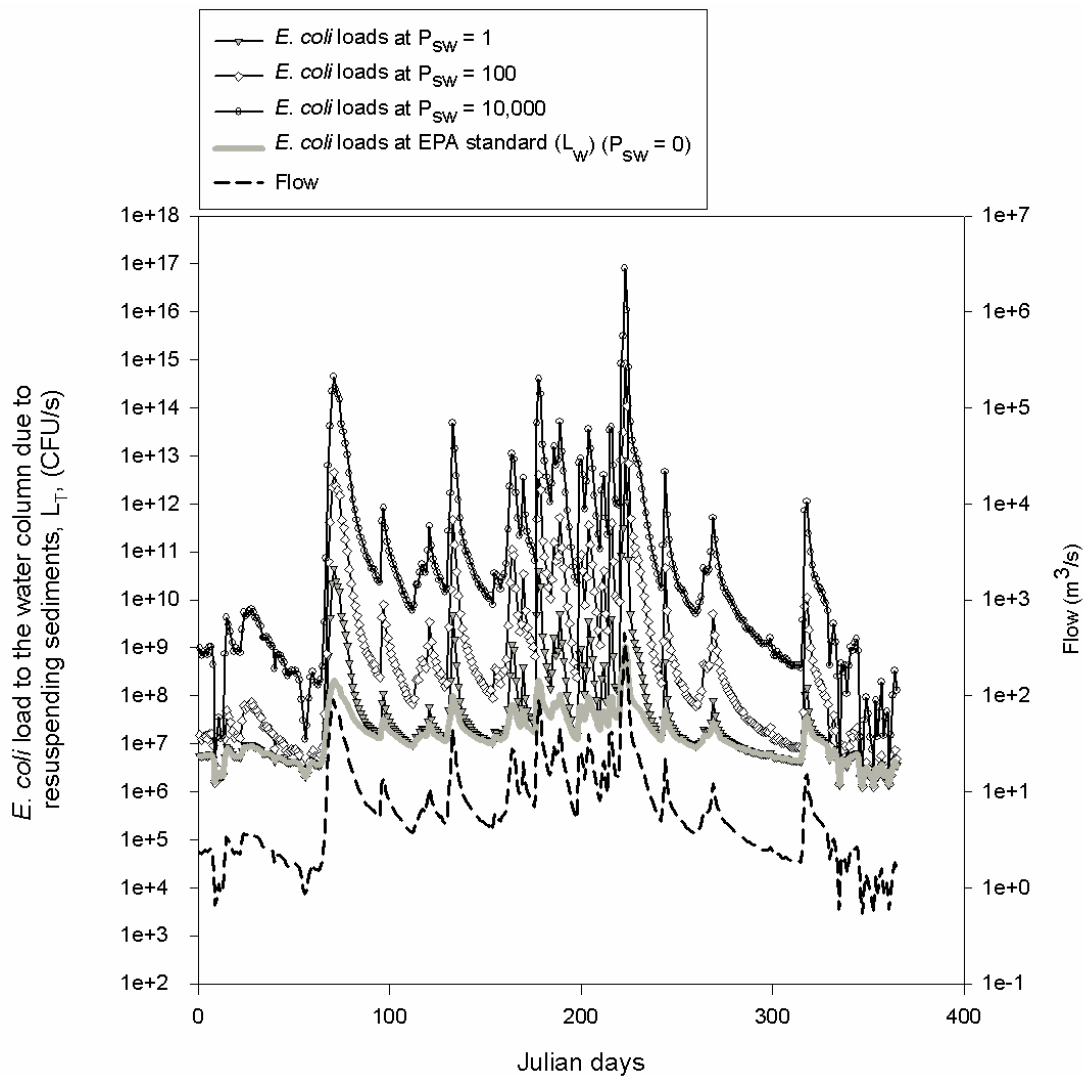


Figure 5.1. Predicted stream total *E. coli* load (L_T , CFU/s) and water flow (m^3/s). The dashed line shows flow, the thick solid gray line indicates total *E. coli* load based on the current EPA standard when sediment impacts are excluded. The solid line with triangles indicates the *E. coli* load when the sediment and water column *E. coli* concentration is equal ($P_{sw} = 1$), the line with diamonds indicates the load when the stream bed sediment *E. coli* concentration is 100 times higher than that of water column ($P_{sw} = 100$), and the line with circles indicates the load when the stream bed sediment *E. coli* concentration is 10,000 times higher than that of the water column ($P_{sw} = 10,000$).

water column *E. coli* load (L_w) and the resuspending *E. coli* load (L_s), along with stream flow over a period of a year. In the Figure, the EPA standard represents the *E. coli* discharge at the current single sample maximum criteria, reflecting current sampling protocols which only consider bacteria in the freely suspended state. The changes in the total *E. coli* load with the addition of resuspending organisms over the range of flows is included for sediment-water ratios (P_{sw}) of 1, 100, and 10,000. As shown in the Figure, the total load when *E. coli* resuspension was included was considerably higher over most of the flow conditions, but particularly for higher flows. For instance at sediment-water *E. coli* ratios of 1 and 100, when the flow was $0.54 \text{ m}^3/\text{s}$, the water column *E. coli* load was equal to the total *E. coli* load, indicating that at low flow the contribution from stream bed as resuspension was negligible, particularly, when sediment – associated *E. coli* concentrations were low. However, as flow increases the total *E. coli* loads were considerably higher than the water column *E. coli* load. For a sediment-water ratio of 1, when the flow was between 0.54 and $9.8 \text{ m}^3/\text{s}$, the water *E. coli* load fraction of the total *E. coli* load varied between 50 and 100%. For a sediment-water ratio of 100, the water *E. coli* load was less than 50% of the total *E. coli* load, even at a relatively low flow of $1.93 \text{ m}^3/\text{s}$. At high sediment-water ratios ($P_{sw} = 10,000$), the water *E. coli* load was less than 50% of total *E. coli* load at very low flows (less than $0.65 \text{ m}^3/\text{s}$).

At a flow of $9.97 \text{ m}^3/\text{s}$, the resuspending *E. coli* load was greater than the water *E. coli* load by a factor of 1, but when flow was increased to $450 \text{ m}^3/\text{s}$, the resuspending *E. coli* load became greater than the water *E. coli* load by three orders of magnitude (at $P_{sw} = 1$).

Moreover, at a sediment-water ratio of 100, the resuspending *E. coli* load became greater than the water *E. coli* load at a relatively low flow ($2.04 \text{ m}^3/\text{s}$); under high flow ($450 \text{ m}^3/\text{s}$)

conditions, the resuspending *E. coli* load was greater than the water *E. coli* load by more than five orders of magnitude (7.4×10^5 factors). Similarly, for sediment-water ratios of 10,000, the resuspending *E. coli* load was greater than the water *E. coli* load during all flow conditions, except when flow was less than $0.65 \text{ m}^3/\text{s}$. These results are supported by the findings of others and clearly indicate that flow and stream sediment *E. coli* concentrations have a considerable impact on in-stream pathogen levels. For example, Wilcock et al. (1999) found that fecal contamination in the stream water column increased 406 times when flow was increased from $0.001 \text{ m}^3/\text{s}$ to $5.72 \text{ m}^3/\text{s}$, with the majority of the increased bacteria being associated with particles.

Other research, for instance, Mahler et al. (2000) studied sediment associated bacteria in the stream bed, and found that during rainy seasons approximately 5 – 100% of the total fecal bacteria in streams were associated with sediments. Wilkenson et al. (1995) reported 2 – 25 times higher fecal bacteria, and Muirhead et al. (2004) found approximately 50 times greater bacteria in the water column due to resuspension from the stream bed. Nagels et al. (2002) found that approximately 30% of the total bacteria were resuspended from the stream bed to the water column.

In another scenario, we consider the condition when the sediment *E. coli* concentration is equal to the concentration in the water ($C_s = 235 \text{ CFU}/\text{cm}^3$, $P_{sw} = 1$). Here, the average sediment *E. coli* discharge (i.e., load) was 2.66×10^{10} (ranging from $4.5 \times 10^1 - 7.9 \times 10^{12}$) cfu/s and the average water *E. coli* discharge was 2.88×10^7 (ranging from $1.3 \times 10^6 - 1.1 \times 10^9$) cfu/s. This shows that the average sediment *E. coli* discharge could be three orders of magnitude greater than the water *E. coli* discharge, even under the condition when the stream

water meets the EPA single sample maximum standard and sediment *E. coli* concentrations do not exceed the water column *E. coli* concentration. In the scenario when the sediment pathogen concentration is 100 times greater than that of water, the average sediment pathogen discharge was 2.66×10^{12} (ranging from $4.5 \times 10^3 - 7.9 \times 10^{14}$) cfu/s; the average sediment pathogen discharge was five orders of magnitude greater than the average water column pathogen discharge. Similarly, at sediment-water ratios of 10,000, the average sediment pathogen discharge was 2.66×10^{14} (with range of $4.5 \times 10^5 - 7.9 \times 10^{16}$) cfu/s, seven orders of magnitude greater than the average water pathogen discharge. Clearly, contaminated upstream sediments have considerable impacts on downstream *E. coli* levels. In an interesting case study, Bai and Lung (2005) created a series of artificial high flow events in a stream by releasing reservoir water (which eliminated the possibility of bacteria contributions from overland flow i.e. runoff from the watershed). They found that *E. coli* concentrations during peak flow ($4.5 \text{ m}^3/\text{s}$) were 14,000 to 16,000 times higher, or four orders of magnitude than the baseflow concentrations. These field results align well with the findings of our scenario analysis and also emphasize the importance of considering the quality of sediments (pathogen concentrations in sediments) when assessing pathogen impaired streams.

We also estimated the total *E. coli* load, and the fraction of the of total *E. coli* load due to the resuspending sediment *E. coli*. Figure 5.2 shows the sediment *E. coli* fraction (L_s/L_T) for corresponding total *E. coli* load and flows, over a range of sediment *E. coli* concentrations. The sediment *E. coli* fraction increases with flow: at high flows resuspending *E. coli* dominates the total *E. coli* load. As sediment-water ratios increase, the resuspending *E. coli*

load contribution exceeds the water *E. coli* load at relatively low flows. In addition to flow, the sediment characteristics (i.e., grain size, density, and mineralogy) (Partheniades, 1990)

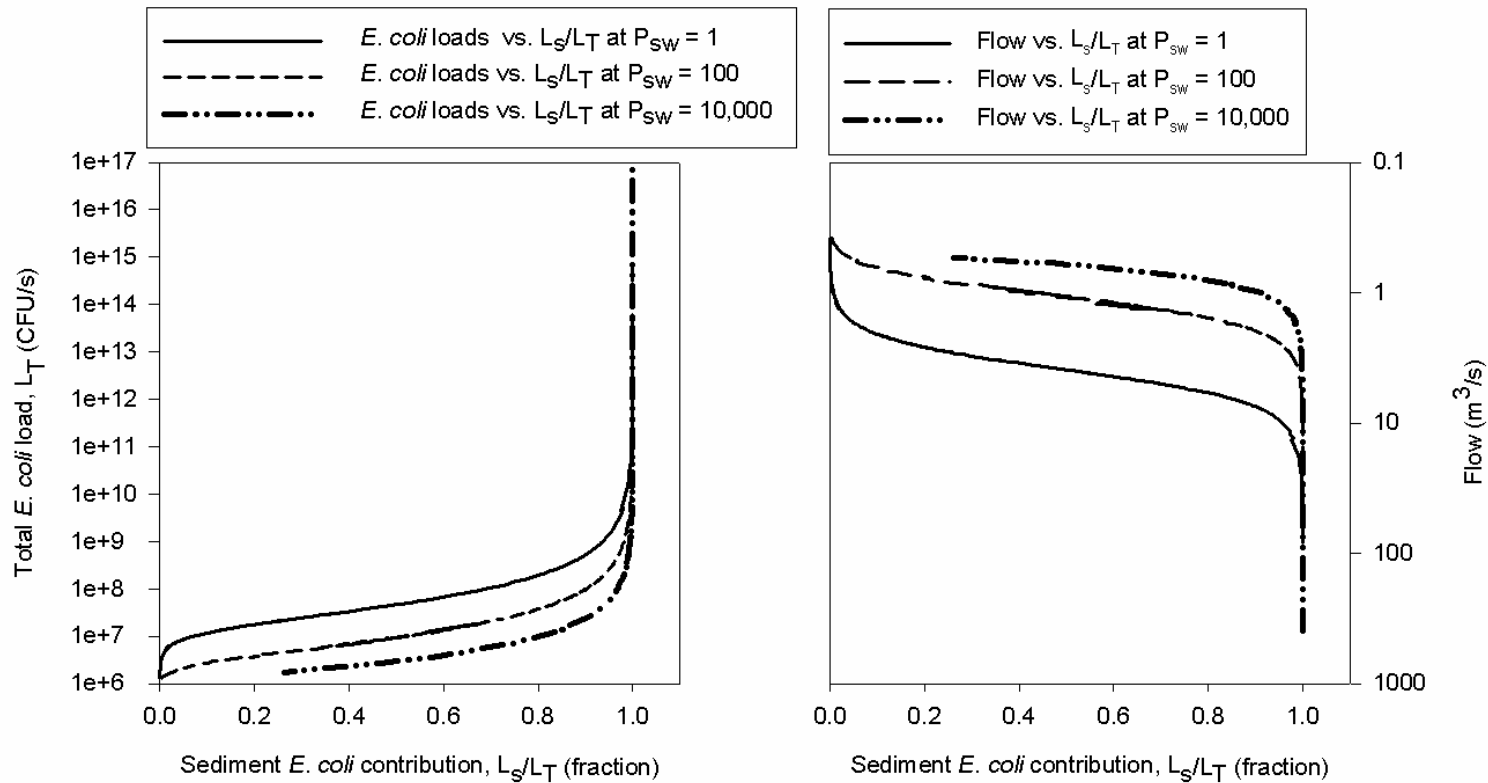


Figure 5.2. The left plot shows the total *E. coli* load (L_T) and sediment *E. coli* contribution (L_S), and the right plot shows the stream flow and sediment *E. coli* contribution. The solid line shows when the *E. coli* concentration in the stream bed sediment and that of the water column are equal ($P_{sw} = 1$), the short dash line shows when the *E. coli* concentrations in the stream bed sediments are 100 times higher than that of the water column ($P_{sw} = 100$), and the dash line with dots represents when the *E. coli* in the stream bed sediments are 10,000 times higher than that of the water column ($P_{sw} = 10,000$).

play a crucial role in resuspending sediment associated *E. coli* to the water column. Previous studies, for example Cho et al. (2010) and Atwill et al. (2007), have emphasized the need to understand the stream bed characteristics while calculating the resuspension of *E. coli* in streams. The total *E. coli* discharge and resuspending sediment *E. coli* load shown in Figures 5.1 and 5.2 were calculated based on the assumption that the *E. coli* are attached to particles 10 μm in size; however, streambeds is a representative of a wide range of different particle sizes which will have different resuspension rates. The potential impacts of particle size on total *E. coli* discharge are demonstrated in Figure 5.3. In the Figure, we show the total *E. coli* discharge for *E. coli* attached to average particle sizes of 2, 8, and 20 μm for a single sediment-water ratio (i.e., P_{sw}) of 100.

For the different particle sizes, the critical bed shear stresses are also shown in Figure 5.3. Critical stress is a minimum value required to initiate erosion, which depends on the particle size (Mehta and Rao, 1985; Shields, 1936) and controls the stream bed particle movement. The bed shear stress (τ_b), which is a function of stream flow is also shown in the Figure. Stream bed shear stress (i.e., τ_b) is a force per unit area exerted by the overlying (Stone et al., 2011; Mehta and Rao, 1985; Partheniades, 1990). As shown in the figure, the total *E. coli* discharge for small particles (i.e., 2 μm) was lower than for large particles. For instance, for a particle size of 2 μm , the mean total *E. coli* discharge was within 11 orders of magnitude (ranging from 1.27×10^6 - 3.15×10^{13}); however, when the particle size was increased to 20 μm , the average total *E. coli* discharge increased by 2 orders of magnitude, and total *E. coli* discharge ranged from 1.27×10^6 to 3.15×10^{15} (cfu/s). The *E. coli* resuspension from larger

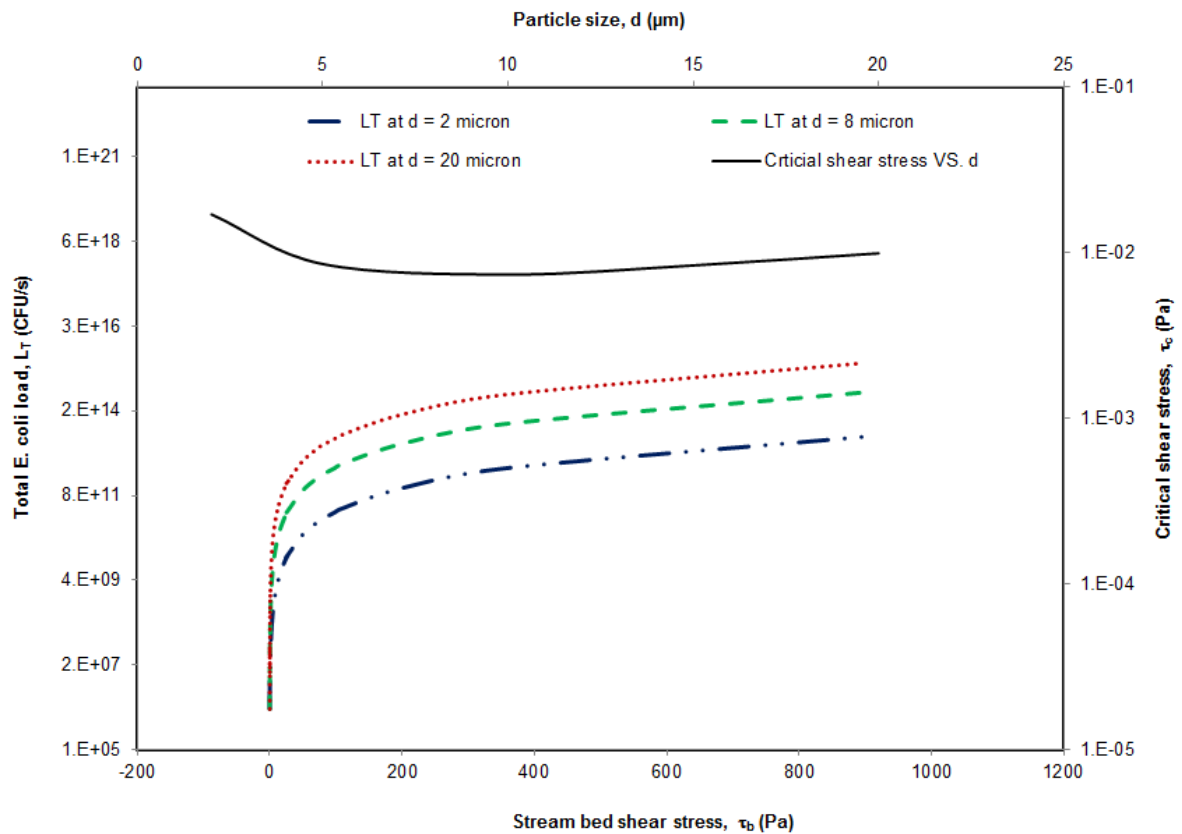


Figure 5.3. The plot shows the impact of particle sizes on *E. coli* loads (L_T). The total load is estimated for the scenario when streambed sediment *E. coli* concentrations are 100 times higher than that of water column ($P_{sw} = 100$). The solid line indicates the critical shear stress (secondary y – axis) for different particle sizes shown in the secondary x – axis. The long dash line shows *E. coli* loads when resuspension was estimated for a particle size (d) of 20 μm , the medium dash line with single dots indicates loads when the particle size considered is 8 μm , and the medium dash line with double dots indicates loads for particle sizes of 2 μm .

particle sizes is greater because smaller particles exhibit cohesive behavior with increased binding forces (Lick, 2009), and thus lower resuspension. Cho et al. (2010) identified variation in sediment properties as a factor leading to large differences in quantifying the release of *E. coli* from the stream bed to the water column. For instance, considerable differences were reported in the critical bed shear stress (τ_c) values, when bed shear stress for erosion is surpassed, which defines the initiation of *E. coli* resuspension. The study by Streets and Holden (2003) estimated τ_c of 0.02 – 0.1 Pa, while Bai and Lung (2005) and Jamieson et al. (2005b) reported τ_c values 3 and 16 times greater. We also estimated the potential impact of particle size on τ_c , which is shown in Figure 5.3. The critical shear stress for particle sizes less than 5 μm , is considerably higher than that for particle sizes $> 5\mu\text{m}$. The τ_c at 2 μm was 99% greater than that at 5 μm . This increased critical shear stress for smaller particles sizes can potentially lower the resuspension of *E. coli*. Very fine silt ($< 8 \mu\text{m}$) exhibit strong cohesion, while larger silt particles (8-62 μm) are more weakly associated (van Rijn, 2007). *E. coli* are generally thought to be associated with fine sediment particles in the aquatic environment (Schillinger and Gannon, 1985; Gannon et al., 1983; Auer and Niehaus, 1993) and the work of Atwill et al. (2007) found a significant direct relationship between fine particles and *E. coli* concentrations in stream bed sediments. Therefore, application of the τ_c values of fine particles is a reasonable approach for quantifying *E. coli* resuspension (Pandey et al., 2012a). While the model clearly reflects the observations of other researchers, the results for scenarios representative of field conditions in the Squaw Creek Watershed were compared to measured *E. coli* loads. The measurements of *E. coli* in the streambed sediment and the water column are shown in Figure 5.4 as the ratio between *E. coli* concentrations in the sediment and the water column. Approximately 87% of the water samples exceeded the

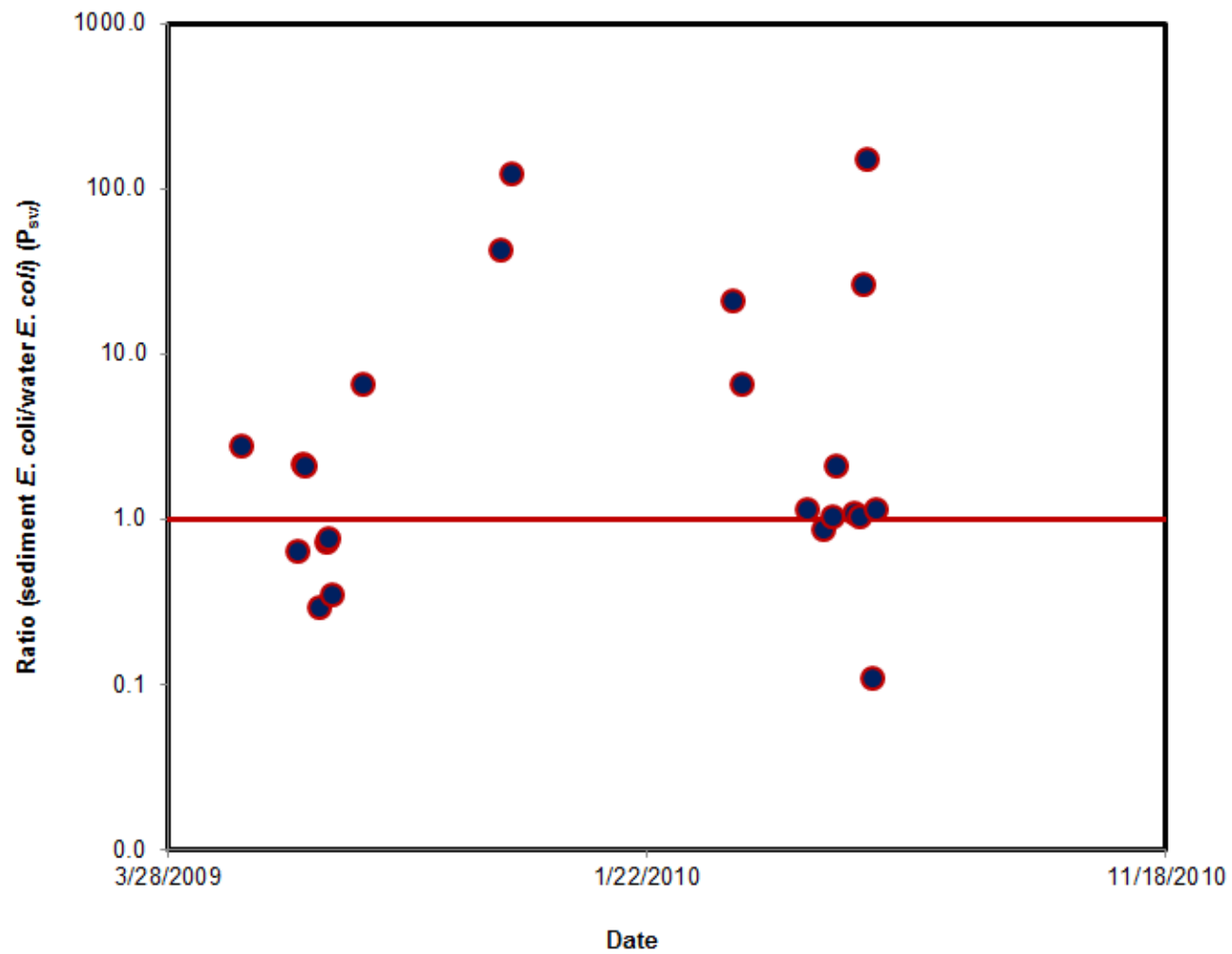


Figure 5.4. Figure shows ratio between measured *E. coli* concentration in sediment (C_s) and the water column (C_w). The horizontal red line indicates 1:1 line, and blue circles are ratio of *E. coli* concentrations between sediment and water column.

single maximum *E. coli* criteria, and 96% of the samples exceed the geometric mean *E. coli* criteria. In 83% of the collected samples, the sediment *E. coli* concentrations exceeded the *E. coli* concentrations in the water samples. The ratio between sediment and water samples *E. coli* concentrations varied from 0.11 to 149. The average ratio for the samples was 17 with standard deviation of 39. Streambed sediment *E. coli* concentrations varied from 9.0×10^5 to 1.0×10^9 (cfu/m³), while the water column *E. coli* concentrations varied from 1.8×10^5 to 7.3×10^7 (cfu/m³) (i.e., 18 – 7267 cfu/100 ml). The average *E. coli* concentration in the streambed sediment was 9.4×10^7 ($\pm 2.4 \times 10^8$) (cfu/m³), while the average *E. coli* concentration in the water column was 1.7×10^7 ($\pm 1.7 \times 10^7$) (cfu/m³).

To validate the model we compared the predictions with the observed *E. coli* loads, which were measured from May 2009 to June 2010 (a total of 23 samples). The data used for model validation are shown in Appendix III. The comparison between observed and predicted values is shown in Figure 5.5. The model skill (m_{skill}) and Nash-Sutcliffe model efficiency coefficient (*NSE*) values were 0.78 and 0.55, respectively. The coefficient of determination (R^2) value was 0.85. Pandey et al. (2012a) previously demonstrated that the resuspension model for predicting the resuspension rate performed well. Previously the model was tested for predicting the resuspension rates of *E. coli* at 16 unique locations in the Squaw Creek watershed. Here, the modified model was applied to a unique location for calculating total in-stream *E. coli* loads.

The models proposed in chapter 3 and chapters 4 are different than the model proposed for total *E. coli* load estimation. Although the fundamental of *E. coli* resuspension estimation (estimated based on critical shear stresses of cohesive and non-cohesive sediment) remained

the same, the model proposed here has different applications and required a different formulation. While the work proposed in chapter 3 primarily focuses on developing and validating *E. coli* resuspension equations, here we have modified formulations for predicting total *E. coli* loads in stream water column, and for understanding the potential impacts of streambed sediment on the water column. We also estimated the potential impacts of stream flow on total *E. coli* loads and transport. In addition, here we have focused on understanding the weaknesses of current EPA method used for assessing in-stream pathogen levels; the current EPA method does not include streambed sediment *E. coli* levels while assessing the bacterial impairment in streams. In summary, this simple scenario analysis provides insightful understanding of the potential magnitude of *E. coli* contamination in a stream due to sediment resuspension. Sediment *E. coli* resuspension is a significant source of *E. coli* contamination in stream waters, and ignoring the impact of this source may lead to unfair allocation of pollutant loads to stakeholders during watershed assessments. Incorporation of *E. coli* resuspension in water quality models used to develop watershed management plans is imperative for correct identification of point and nonpoint sources of pathogens. Current models which do not include sediment pathogen resuspension while predicting in-stream pathogen levels should be applied with great caution.

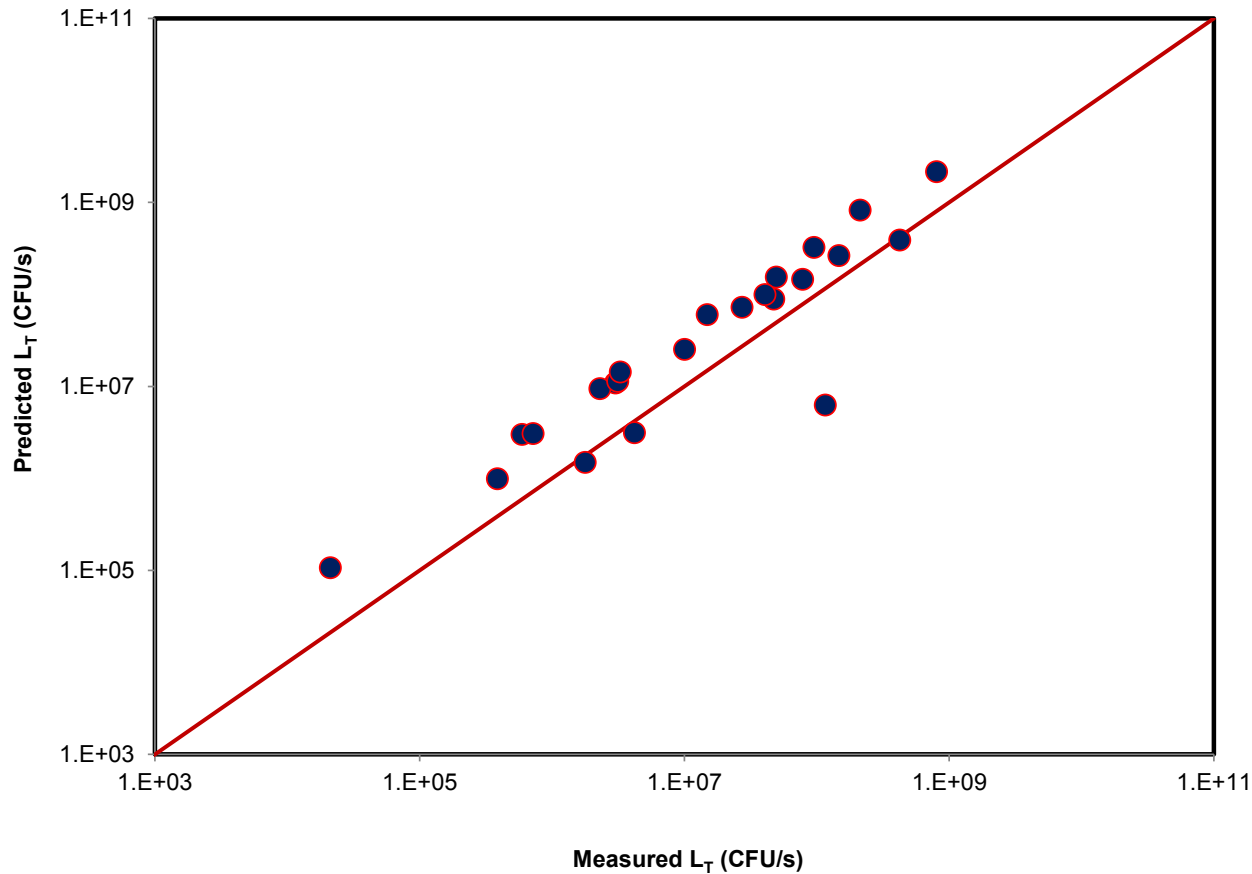


Figure 5.5. The figure shows the comparison between measured and predicted total pathogen loads (L_T) with the red line indicating the 1:1 line. Blue circles are *E. coli* concentrations. Predicted L_T is based on particle diameter (d) of 2 μm and bulk density of 1.26 g/cc. The L_T was estimated as the sum of sediment *E. coli* load (L_s) and water *E. coli* load (L_w). The L_s is *E. coli* load in sediment and the L_w is free floating unattached *E. coli* load. Eighty percent of the *E. coli* in the water column were assumed to be attached to sediment particles in the water column (Hipsey et al., 2008). The coefficient of determination (R^2) value of 0.85 indicates that predictions are well matched with measured values.

Monitoring bacteria levels in stream sediments will provide much needed data for modeling the resuspension process. Current U.S. EPA guidelines for assessing stream water pathogen contamination rely solely on analysis of water samples; no effort is made to assess the *E. coli* concentration of stream sediments. However, this work demonstrates the importance of quantifying sediment *E. coli* concentrations to identify circumstances when a potential risk to human health is present. Monitoring sediment quality is further important for identifying potentially impaired streams since sediment impacts may be negligible at low flows, but dominate the total load during high flow conditions. Further work is recommended to assess the role of persistent *E. coli* in stream bottom sediments. These bacteria are not representative of fresh fecal inputs, but their relation with true pathogens is unknown as they may or may not indicate a risk to human health.

4. Conclusions

In this study, we demonstrated the impacts of streambed on total in-stream *E. coli* loads. The model was developed to calculate the total in-stream *E. coli* load. The inclusion of streambed sediment *E. coli* resulted in the increased levels of pathogen load. For example, when neglecting the streambed sediment *E. coli* concentrations, the average *E. coli* loads was 10^7 (cfu/s); however, when streambed sediment *E. coli* concentrations were included in the model, the predictions ranged from 10^{10} to 10^{14} (cfu/s). The model predictions are verified using field studies. The model skill (m_{skill}) and Nash-Sutcliffe model efficiency coefficient (NSE) values were 0.78 and 0.55, respectively. Results of this study suggest that monitoring streambed sediment *E. coli* concentrations is required in order to improve the assessment of in-stream *E. coli*.

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CHAPTER 6. ASSESSING THE IMPACTS OF WEATHER PATTERN ON IN-STREAM *Escherichia coli* CONCENTRATIONS

This paper is submitted for rapid communication to *Water Resources Research*

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Abstract

Our current weak understanding of in-stream pathogen fate and transport often hinders attempts to improve water quality; and moreover, future challenges associated with climate warming, may exacerbate water quality conditions. Here we examined variability of in-stream *E. coli* concentrations (a pathogen indicator) in the streambed sediment and water zones extensively over three years. The *E. coli* measurements taken in the streambed sediment and the water column of the Squaw Creek Watershed were related to air temperature, soil temperature, solar radiation, and rainfall to investigate the impacts of temperature, solar radiation, and rainfall on in-stream *E. coli* levels. The results show that increase in temperature increases *E. coli* not only in the water column but also in the streambed sediment. Moreover, *E. coli* in the streambed sediment remained elevated even at relatively lower temperature. These results signify that increase in ambient temperature can potentially increase *E. coli* levels in the water bodies, which may results in an increased risk to public health. These findings substantiate previous findings that future increases in temperature may increase the risks to human health via exposure to water borne pathogens.

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1. Introduction

Water borne pathogens are the leading cause of water quality impairments in ambient water bodies (USEPA, 2012), and poses serious health risks to human. Understanding how climate warming can potentially impact persistence of water borne pathogens, therefore, is critical for protection of human health. Many studies have shown linkages between pathogenic disease outbreaks (i.e., for human, wild life, terrestrial, and marine biota) and climate warming (Vezzulli et al., 2012; Harvell et al., 2002; Daszak et al., 2000; Epstein, 1999). For example, data from a study by Harvell et al. (2002), which compiled the World's major outbreaks from 1938 to 1997, has shown that more than 50% outbreaks are correlated with high temperature.

Previous studies have shown the relationships between temperature in East Africa between 1950 and 1998 and outbreaks of Rift Valley fever (Epstein, 1999). The spread of cholera in Bangladesh was found to be related with ocean surface water temperature (Colwell, 1996).

The El Niño Southern Oscillation (ENSO) has been linked with many disease outbreaks including malaria, dengue, and Rift Valley fever (Harvell et al., 2002, Linthicum et al., 1999). Despite the known sensitivity of pathogens to climate factors such as temperature and rainfall, it is challenging to associate climate warming with pathogen contamination, and potential health risks (Harvell et al., 2002; Daszak et al., 2000; Epstein, 1999).

Significant efforts have been made to review the past outbreaks and corresponding climatic conditions to improve our understanding how climate variability is potentially related to the risk to human health (Vezzulli et al., 2012; Harvell et al., 1999). Studies based on intensive measurements of pathogen levels in ambient water bodies for extended periods of time, and relating these data to variability in climate (i.e., temperature, rainfall, solar radiation) and

pathogen levels (i.e., in the streambed sediment and the water column), however, are lacking. Nevertheless, monitoring studies are critical to develop reliable models for predicting future changes in waterborne pathogen levels caused by climate warming.

We addressed this issue by monitoring *E. coli* (a pathogen indicator) in a natural stream for over three years (2009 – 2011) in a field study. The *E. coli* concentrations in the water column and the streambed were measured. The objective of this study is to understand the impacts of climate variability (e.g., changes in temperature, solar radiation, and precipitation) on the streambed sediment and the water column *E. coli* concentrations.

2. Methods and Field Study

2.1. Study Area

Streams, and sampling locations in Squaw Creek Watershed, Iowa, USA, are shown in Figure 6.1. The two locations (L1, and L2 of Figure 6.1) indicates sampling points. Samples of streambed sediment and the water were collected weekly (1 – 2 times) between May 2009 and December 2011. In this study we have used *E. coli* data from the two locations (L1 and L2), while in chapter 4 and 5, we have used the data from a single location (L1). We selected a single location (i.e., L1) in chapter 4 and 5 because the model requires stream flow data, and the gaging station is located at location L1 only.

The sampling procedure is described in Section 2.2. Figure 6.2 shows the land cover, and watershed characteristics. The watershed data were obtained from Natural Resources Geographic Information System (NRGIS) library. The Squaw Creek watershed, HUC 10 (ID

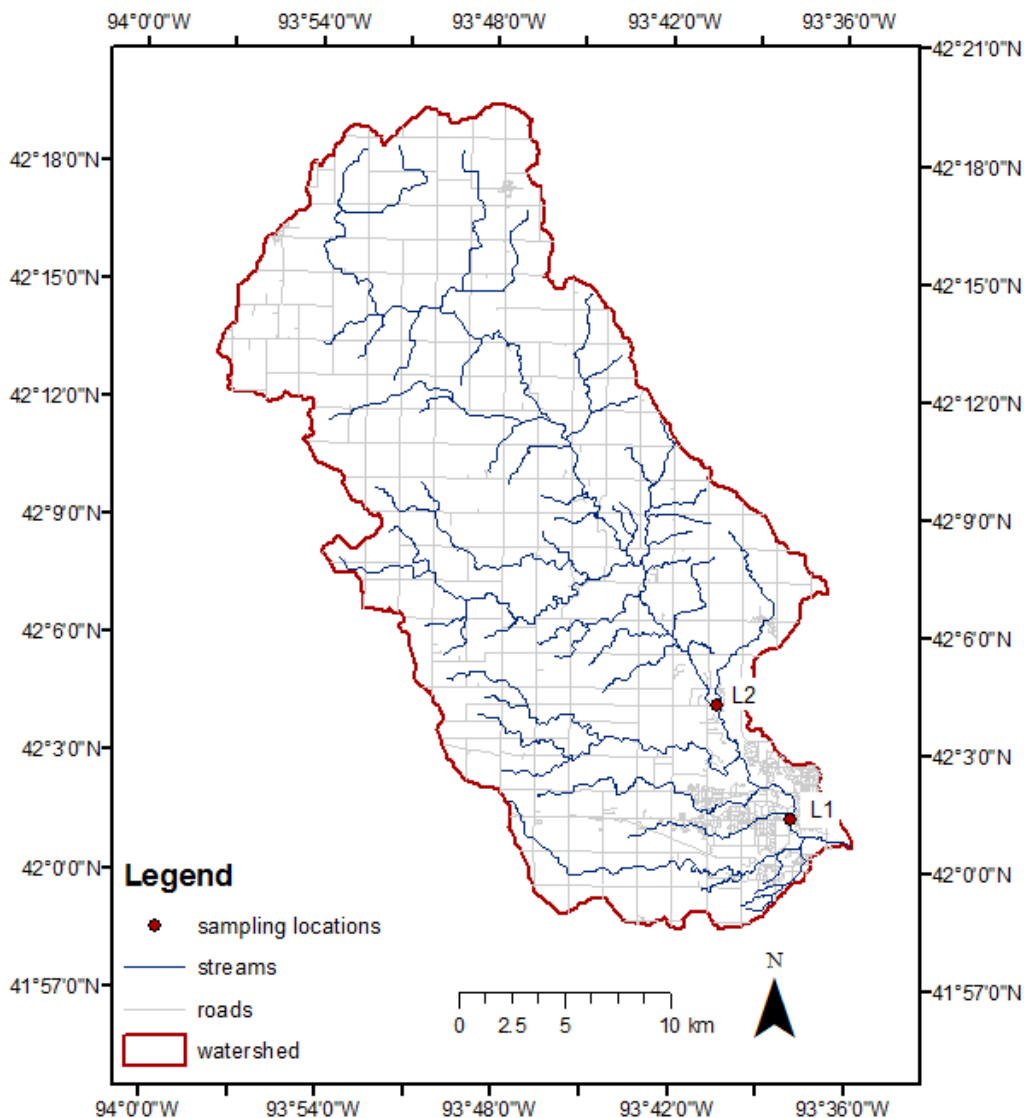


Figure 6.1. Study area map showing Squaw Creek Watershed, streams, and sampling locations. Yellow circles (L1 and L2) indicate two sampling locations. Red color boundary indicates watershed area, blue lines indicate stream lines, and light gray color lines indicate roads. The sampling locations are shown as red circles.

0708010503), has a total drainage area of 592.39 sq km. The basin length and perimeter of the watershed is 43.53 km and 134.02 km, respectively, with an average slope of 2.01%. The basin relief is 111.51 m, the main channel length is 60.46 km and the total stream length within the watershed is 346.72 km. There are 75 first order streams in the watershed. Squaw Creek passes through four counties (Story, Webster, Hamilton, and Boone) of Iowa, and is a tributary of the South Skunk River. Approximately 0.09%, 0.17% and 0.05% of the watershed land area is water, wetland and wetland forest, respectively. Deciduous forest, ungrazed grass, grazed grass, CRP grassland, and alfalfa are 2.71%, 10.87%, 2.52%, 1.70%, and 1.84%, respectively. Cropping land dominates the watershed, 74% of the watershed is under cropping land. Two major crops and corn and soybean rotation, occupies 41%, and 33% of the land of the watershed, respectively. Other row crops are grown in 0.43% of the watershed. Common/industrial, residential, and barren land are 1.67%, 1.27%, and 0.06%, respectively. About 87% of the soil in Iowa is fine, and another 8% is sandy. The soils in the Squaw Creek Watershed consist of loamy Wisconsin glacial till and clayey lacustrine deposits including loam, silty clay, clay loam, and silty clay loam (INRCS, 2011).

2.2. Sample collection and E. coli enumeration

Sediment samples from the streambed sediments and the water sample from the center of the stream were collected at Locations L1, and L2. The point L1 (at intersection of Squaw Creek and Lincoln Way) and L2 (at the intersection of Squaw Creek and Cameron School Road) are shown in Figure 6.1. The L1 receives storm water discharge from the City of Ames urban areas; however, the L2 receives the water from the upstream watershed, primary agricultural

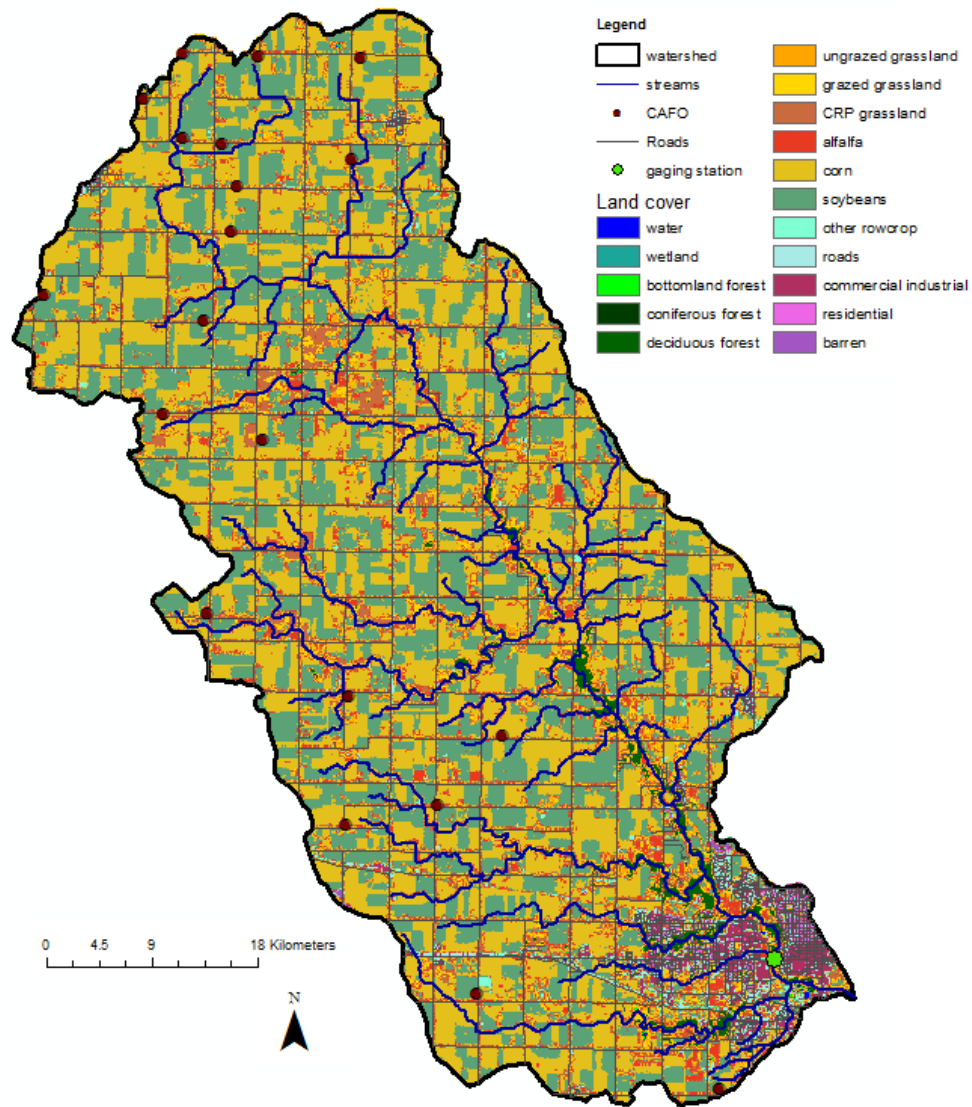


Figure 6.2. Land cover map of Squaw Creek Watershed. Dark red circles indicate location of confined animal feeding operations; light green circle indicate gaging location, blue lines indicate streams, light black lines indicate roads, and dark black line indicate watershed boundary. Land covers are shown in multiple colors.

lands. The population of Ames in 2010 was 58,965 (US Census, 2010). There are no known point pollution sources preceding locations L1 and L2.

The water samples were collected by lowering a Horizontal Polycarbonate Water Bottle Sampler (2.2 L, Forestry Suppliers Inc., Mississippi, U.S) from a bridge into the center of the stream. To collect the streambed sediment samples, we used a Shallow Water Bottom Dredge Sampler (15 cm × 15 cm opening, Forestry Suppliers Inc., Mississippi, U.S). The dredge sampler was lowered to the streambed at the same location as water samples. The water samples were collected prior to sediment samples in order to avoid streambed sediment disturbance, which can potentially cause resuspension of *E. coli* associated with streambed sediment. All samples were analyzed in triplicate. Immediately after collection, samples were stored at 4°C and analyzed within 24 hours. To assess the concentration of *E. coli* in sediment, the *E. coli* attached to particles were detached by stirring the mixture of sediment and purified water (ratio 1:1) for 15 minutes at approximately 150-200 rpm using a magnetic stir bar. The resulting solution was used to enumerate *E. coli* in the sediment. The *E. coli* concentrations in the water and the sediment were enumerated by membrane filtration techniques (APHA, 1999) on modified mTEC agar (EPA, method 1603). Appendix 1 provides *E. coli* enumeration methods.

2.3. Climate data

We used weather station in Ames, Iowa (lat 42° 01'48", long 93° 04'48") for obtaining the precipitation, air temperature, and soil temperature data of Ames. The data were retrieved from Iowa Environmental Mesonet (IEM), Agronomy Department, Iowa State University, USA. To obtain daily solar radiation, a LI-COR pyrometer, model LI200X, was used.

Temperature data was obtained using a Campbell HMP 45, mounted within a radiation grill at 2 m height (IEM, 2012). The sampling locations have a humid climate with an average yearly rainfall of 865.4 mm (from 2000 to 2011). The annual air temperature varies from -30 to 32°C (from 2000 to 2011).

3. Results and Discussion

Figure 6.3a and 6.3b show *E. coli* concentrations in the water column and the streambed sediment column at two locations. To understand how annual variability in air temperatures are related with *E. coli* in the stream water column and streambed sediment, we graphed *E. coli* concentrations and air temperatures from 2009 to 2011 versus Julian Days (Fig. 6.3c, 6.3d). As shown in the Figures, the variability in *E. coli* concentrations in the water column follows the changes in air temperature (Fig. 6.3c). Similarly, *E. coli* concentrations in the streambed sediment also increased during high air temperatures (Fig 6.3d).

While *E. coli* concentrations in the water column decreased with lower temperatures, *E. coli* in the sediment remained elevated, which indicates that contaminated streambed sediment can harbor *E. coli* even at low temperatures (Fig. 6.3d). The precipitation and stream flow are shown in Figure 6.3e, while variability in air and soil temperatures and solar radiation is shown in Figure 6.3f. Spikes in temperature and solar radiation resulted in increased *E. coli* levels in the stream water column as well as in the streambed sediments. Our observed *E. coli* data in the stream water column and the streambed sediment suggests that climate warming could potentially increase pathogen contamination in the sediment and the water column of ambient water bodies, posing an increased risk to public health.

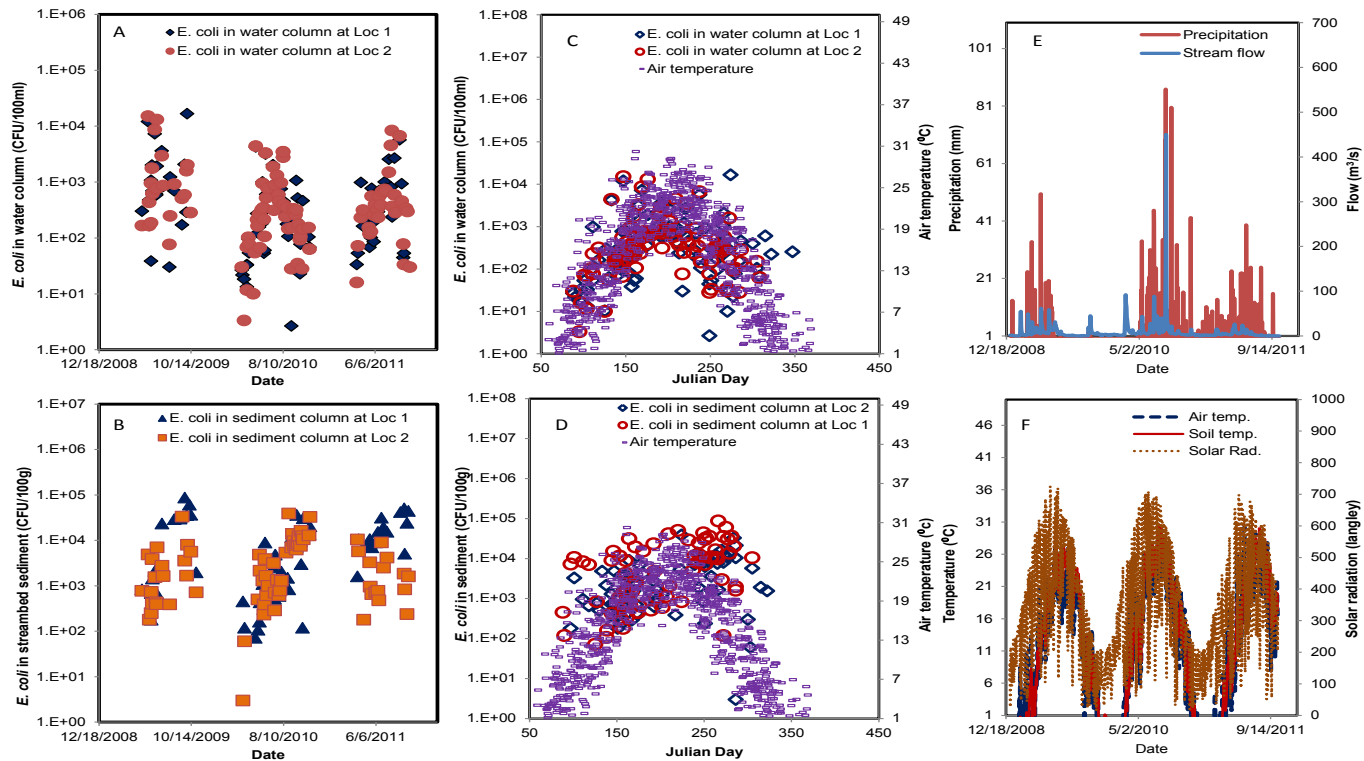


Figure 6.3. *E. coli* concentrations in stream water column, streambed sediment, air and soil temperatures, solar radiation, rainfall, stream flow, and annual changes in *E. coli* concentrations: A) red circles and blue diagonals indicate *E. coli* concentrations in water column at locations 1 and 2, respectively; B) orange squares and blue triangles indicate *E. coli* concentrations in the streambed sediment; C) blue diagonals and red circles indicate annual changes (over the three years) in the water column *E. coli* at locations 1 and 2, respectively, and purple line markers indicate air temperature; D) red circles and blue diagonals indicate annual changes in *E. coli* in the streambed sediment at Locations 1 and 2, respectively, and purple line markers indicate air temperature; E) blue line and dotted red line indicate stream flow and precipitation, respectively; F) dotted blue line indicates air temperature, red line indicates soil temperature, and orange dotted line indicates solar radiation.

Moreover the intensive monitoring of *E. coli* in both the streambed sediment and water column presented here are rare, but much needed to develop and validate models for predicting the impacts of climate warming on human health risks associated with exposure to water borne pathogens.

4. Conclusions

Here we have shown the impacts of weather pattern on in-stream pathogen contaminations. Results show that increase in ambient temperature could potentially result in elevated pathogen concentrations in streams as well as in other ambient water bodies (i.e., lakes, reservoirs, and even oceans). The data shows that increase in temperature increases pathogens not only in the water column but also in the streambed sediment. Moreover, pathogens in the contaminated streambed sediment remained elevated even at relatively lower temperature, while water pathogens were decreasing. This indicates that bed sediment can prolong the contamination time. These results signify that increase in ambient temperature may results in an increased risk to public health. The field study and observations of *E. coli* in the streambed sediment and the water column presented here will be useful to understand potential impacts of climate warming on pathogen contaminations in ambient water bodies

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CHAPTER 7. GENERAL CONCLUSIONS

The goal of this study was to improve understanding of in-stream *E. coli* transport. Here we have developed models for predicting in-stream *E. coli* concentrations, which have long been considered a challenging task due to complex interactions between stream sediment and water columns, and impacts of watershed landscape. The models proposed here predict resuspended *E. coli* from the streambed sediment to the water column, in-stream total *E. coli* loads, and *E. coli* concentrations in the streambed sediment as well as in the water column. We have developed new approaches, which use Geographical Information Systems (GIS) and Soil and Water Assessment Tool (SWAT), for predicting in-stream *E. coli* concentrations. In addition, we have carried out an extensive in-stream *E. coli* monitoring program, which is very rare, for determining *E. coli* concentrations in the streambed sediment and the water column for extended period of time. These *E. coli* measurements were used to verify the predictions. Monitored in-stream *E. coli* data were also used to understand the potential relationships between in-stream *E. coli* concentrations and weather pattern. The following sections summarize the methods and significant findings from each of the five study objectives.

7.1. Objective 1: Assess the impacts of watershed indexes and precipitation on spatial in-stream *E. coli* concentrations

Develop a Geographic Information System (GIS) based model to predict waterborne *E. coli* concentrations in stream water column.

Hypothesis: Waterborne E. coli concentrations in a stream can be estimated using the landscape characteristics of the same stream watershed.

Reducing in-stream pathogen contamination requires an understanding of the combined impacts of land cover, climatic conditions, and anthropogenic activities at the watershed scale. While previous studies have investigated linkages between the landscape and water chemistry using the watershed characteristics approach, relationships between watershed landscape characteristics and waterborne *E. coli* levels need further examination to understand how watershed indexes potentially impacts in-stream pathogen concentrations.

Here we have assessed linear relationships between in-stream *E. coli* water quality data, watershed indexes, and rainfall for the Squaw Creek Watershed, IA, USA. The watershed indexes consider the undisturbed land cover which encompasses the natural land cover area, wetlands, and vegetated stream corridors, and the disturbed land cover extent which includes areas receiving manure from confined animal feeding operations (CAFOs), tile-drained areas, and areas in cropped and urban land. In addition to disturbed and undisturbed land, we also calculated indexes for barren land and slope. Bivariate analysis was used to assess the linkage between waterborne *E. coli* concentrations, watershed indexes and the cumulative rainfall 15, 30, 45, and 60 days prior to water sample collection.

To predict in-stream waterborne *E. coli* concentrations, we developed multivariate regression models, and predictions were compared with the measured *E. coli* concentrations at 46 sampling locations over four sampling periods in two years. Results show that areas receiving manure, wetlands, drained land, and cropped land all influence in-stream waterborne *E. coli* concentrations significantly ($p < 0.001$). The coefficient of determination was higher when indexes were corrected using the cumulative rainfall 30 days prior to the sampling event. Model skill varied from 0.29 to 0.55. More than 95% of the predictions

across all spatial locations fall within one order of magnitude of the observed *E. coli* concentrations. This Geographic Information System (GIS) based approach for predicting in-stream waterborne *E. coli* concentrations appears to be a useful technique for assessing the impacts of land management on water quality.

7.2. Objective 2: Develop a model for predicting resuspension of *E. coli* from streambed sediments

Develop a model to predict *E. coli* resuspension rates from the streambed sediment to the water column.

Hypothesis: In-stream E. coli resuspension rates can be calculated using the stream flow properties and characteristics of both cohesive and non-cohesive sediment.

Predicting in-stream *E. coli* transport requires understanding of resuspension of *E. coli* from the streambed sediment to the water column of a stream. To improve predictions of in-stream *E. coli* transport, here we have developed a formulation for calculating *E. coli* resuspension rates that accounts for properties of the stream flow and properties of cohesive and non-cohesive sediment. In *E. coli* resuspension model, the resuspension rates were expressed as the product of the concentrations of *E. coli* attached to sediment particles and erosion rates, which were estimated using sediment transport theory. Model calculates streambed shear stress, and critical shear stresses of cohesive and non-cohesive sediments for estimating erosion rates. To verify predicted *E. coli* resuspension rates, an *E. coli* monitoring program was carried out at 16 locations of the Squaw Creek Watershed, Iowa; the *E. coli* concentrations in the streambed sediment and the water column were measured. Comparisons between predicted and inferred (i.e., observed) *E. coli* resuspension rates show that model

performed well. Approximately 81% of the predicted *E. coli* resuspension rates were within a factor of 2 of the inferred values, while all predicted values were within a factor of 5 of the inferred values. A relatively higher model skill value of 0.85 indicates that the model predicts *E. coli* resuspension rates successfully, which should help in developing a hydrological model for predicting *E. coli* transport in streams.

7.3 Objective 3: Improve SWAT for developing TMDLs for bacteria

Develop a pathogen transport model for improving SWAT for predicting streambed sediment and water column *E. coli* concentrations.

Hypothesis: Integrating a pathogen transport model, capable of predicting E. coli in the streambed sediment as well as in the water column, into Soil and Water Assessment Tool (SWAT), can improve in-stream E. coli predictions at the watershed scale.

Hydrological models capable of predicting streambed sediment *E. coli* concentrations are lacking, potentially due to the complexities involved in modeling interactions between streambed sediment and water column *E. coli*. Here the primary task was to develop a hydrological model capable of predicting *E. coli* concentrations in the streambed sediment and the water column. To complete this task, a new approach for predicting *E. coli* concentrations in the streambed sediment and the water column was developed. Firstly, a model capable of predicting *E. coli* resuspension was formulated. Secondly, formulations for calculating in-stream *E. coli* routing, water temperature depended *E. coli* growth, and streambed sediment as well as water column *E. coli* concentrations were developed. Finally, these formulations were programmed in FORTRAN, and were integrated into the Soil and

Water Assessment Tool (SWAT), a watershed scale hydrological model, which calculated *E. coli* concentrations in the streambed sediment and the water column.

The modified SWAT model was applied in the Squaw Creek Watershed. Predictions of streambed sediment *E. coli*, water column *E. coli* concentrations, and stream flow were verified using monitored values. Results show that the modified SWAT is capable of predicting in-stream *E. coli* concentrations (i.e., in streambed sediment and water column). Majority of the *E. coli* predictions was within 1 order magnitude of the observed values. For example, approximately 62% of the predicted streambed sediment *E. coli* concentrations, and 82% of the predicted water column *E. coli* concentrations were within 1 order magnitude of the measured concentrations. The coefficient of determination (R^2) for monthly average daily flow was 0.99, while for daily flow predictions R^2 was 0.42. The Nash-Sutcliffe's efficiency (*NSE*) for monthly average daily and daily flow predictions were 0.75 and 0.39, respectively. We anticipate that the new approach developed here, and modified SWAT model, capable of predicting the streambed sediment and the water column *E. coli* concentrations should have significant importance in TMDLs development and predicting in-stream *E. coli* concentrations at the watershed scale.

7.4. Objective 4: Assess the impacts of streambed sediment on in-stream total *E. coli* loads over a range of flow conditions

Develop a model for predicting in-stream total *E. coli* loads.

Hypothesis: Current U.S. EPA methodology for assessing stream water pathogen contaminations, which relies solely on analysis of water samples, may underestimate in-stream pathogen loads.

Currently methods, which include both streambed sediment *E. coli* and water column *E. coli*, to estimate total *E. coli* loads in streams do not exist. Here we have developed a formulation to predict total *E. coli* loads in streams, which involves both sediment and water column *E. coli*. To calculate total *E. coli* loads, we have estimated the potential impact of in-stream *E. coli* levels due to resuspending bottom sediments using a range of flow conditions, sediment/water bacteria fractions, and particle sizes to which bacteria attach. The predictions were verified using *E. coli* data collected in the Squaw Creek Watershed. The comparisons between predicted and observed *E. coli* loads show that model performed very well. The coefficient of determination (R^2) value was 0.85. The model skill (m_{skill}) and Nash-Sutcliffe efficiency (NSE) values were 0.78 and 0.55, respectively. This work provided insightful understanding of the potential magnitude of *E. coli* contamination in a stream caused by sediment resuspension. Here we have shown how in-stream *E. coli* loads are underestimated by ignoring the impacts of streambed sediment *E. coli*. Results suggest that monitoring streambed sediment *E. coli* concentrations is required in order to improve the assessment of in-stream *E. coli*. This work emphasizes the need to improving the United States Environmental Protection Agency (USEPA) current water quality testing methodology, which currently relies solely on water borne *E. coli* concentrations to assess stream pathogen levels and identify impaired waters.

7.5 Objective 5: Assess the impacts of weather pattern on in-stream *E. coli* concentrations

Assess the weather pattern impacts on in-stream *E. coli* concentrations.

Hypothesis: Weather pattern can impact E. coli concentrations in the streambed sediment and the water column.

Previous studies have shown potential linkages between climate and disease outbreaks. However, very few information on how in-stream *E. coli* concentrations in the streambed sediment and the water column changes with weather pattern exists. We addressed this issue by monitoring *E. coli* over extended period of time in the streambed sediment and the water column of the Squaw Creek Watershed, and linking these observations with the weather pattern. The *E. coli* concentrations were linked with air temperature, soil temperature, precipitation, solar radiation, and stream flow. The observations show that increase in temperature resulted in higher level of *E. coli* not only in the water column but also in the streambed sediment. Moreover, *E. coli* levels in the streambed sediment remained elevated even at relatively lower temperatures. These findings signify that increase in ambient temperature can potentially increase *E. coli* levels in the water bodies, which may results in an increased risk to public health. These results can be a foundation for propagating future research to understand how climate warming can potentially impact pathogen levels in our ambient water bodies.

7.6. Implications of the study

Here we have developed models, which improve understanding of in-stream *E. coli* contaminations. The *E. coli* data, which were monitored during this study, are very rare, and are much needed data either for validating the models or understanding the impacts of weather pattern. The GIS based method developed here can be potentially useful in understanding how landscape characteristics of the watershed influence in-stream *E. coli* concentrations.

Many of the existing water quality models do not include *E. coli* resuspension, while predicting waterborne *E. coli* concentrations. We proposed a model for calculating *E. coli* resuspension from the streambed sediment to the water column, which can be potentially useful to improve the water quality models. We modified the SWAT model, which has been used extensively in the USA for predicting stream flow and nutrients concentrations in streams. However, it has rarely been used for TMDLs development when waters are impaired due to elevated pathogen levels. This modified version of SWAT will have significant importance in improving the understanding of in-stream *E. coli* fate and transport, and will be useful for application of SWAT for TMDLs. Additionally, the modified version of SWAT will be useful for comparison of Best Management Practices (BMP) scenarios necessary for EPA-approved watershed management plans.

Total *E. coli* load estimation presented in this study shows that monitoring bacteria levels in streambed sediments are required in order to estimate in-stream total *E. coli* loads and assess stream impairment correctly. Current U.S. EPA methodologies for assessing stream water pathogen contamination, however, rely solely on analysis of water samples; no effort is made to assess the *E. coli* concentration of stream sediments. However, this work demonstrates the importance of quantifying sediment *E. coli* concentrations to identify circumstances when a potential risk to human health is present. We anticipate that the results of this study will be useful for both estimating total *E. coli* loads in streams and embarking monitoring of *E. coli* in the streambed sediment to assess impairment levels.

An interesting understanding of weather pattern impacts on in-stream *E. coli* levels were obtained here. We anticipate these findings will be a foundation for investigating the impacts of climate change on pathogen contaminations in the ambient water bodies.

7.7. Limitations of the study and future research recommendations

The models proposed here are tested in a watershed, where agriculture is dominant land cover; approximately 74% of the watershed is under cropping land. Although the theories used in the models are sound, we anticipate that the models developed here will require further verifications and calibrations, when implementing in the other watersheds. Therefore, we recommend verifying the predictions before making a decision on the land management plans pertaining to controlling in-stream *E. coli* concentrations. For example, study presented in chapter 2, uses data of single sampling event from four seasons (i.e., data from single day sampling in each season). Although the data has considerable spatial heterogeneity (i.e., 46 sampling locations), we suggest for verifying the predictions using another datasets. The dataset, which includes relatively more frequent observations, can be useful for validating the approach. We do not recommend using the regression equations presented in chapter 2 for implementing the land cover change plan in order to control in-stream *E. coli* concentrations without verification using data from the other watershed. Nevertheless, the approach used for calculating the watershed indexes and deriving the relationships between in-stream *E. coli* and watershed indexes can be a potentially useful tool to support decision making and understanding the relationships between watershed indexes and in-stream *E. coli* concentrations.

The data used in Chapter 3, 4, 5, and 6 were obtained by extensive field sampling during this study in the Squaw Creek Watershed. The extensive monitored *E. coli* data used here are rare; however, measurements are from single watershed. The models developed here were implemented in only one watershed (i.e., Squaw Creek Watershed), further verifications are required to evaluate the predictions at the watershed scale. Using the data from another watershed will also help in improving the predictions.

In addition, we used measured data to assess the relationships between weather pattern and in-stream *E. coli* concentrations, which indicated that increase in ambient temperatures, can potentially elevate in-stream *E. coli* levels. Understanding the impacts of climate change on in-stream *E. coli* concentrations, however, will demand relatively large datasets from multiple streams before making any strong conclusions. Therefore, we recommend extending *E. coli* monitoring in the streambed sediment and the water column of the Squaw Creek Watershed. Many agencies such as EPA and USGS are carrying out waterborne *E. coli* monitoring in the ambient water bodies of the USA; however, the data are very sporadic, and understanding the impacts of climate change on pathogen contaminations in ambient water bodies will require consistent and more frequent *E. coli* monitoring in the bed sediment as well as in the water column.

NOMENCLATURE

I_{nc}	natural cover index [-]
A_{nc}	Area of natural cover [m ²]
A_c	Area of polygon [m ²]
I_{wt}	wetland index [-]
NWI	national wetland inventory polygon
HWE	historic wetland index [-]
I_{sc}	river-stream corridor integrity index [-]
A_{vsr}	vegetated stream riparian area [m ²]
A_{tsr}	total stream riparian area [m ²]
I_{br}	barren area index [-]
A_{barren}	area of barren land [m ²]
$CAFO$	confined animal feeding operations
I_{cafo}	CAFO index [-]
A_{ma}	area receiving manure [m ²]
I_d	drained land index [-]
A_d	drained area [m ²]
I_{rcc}	corn crop index [-]
I_{res}	soybean crop index [-]
A_{cc}	area under corn crop [m ²]
A_{cs}	area under soybean crop [m ²]
I_{urb}	urban area index [-]

A_{urban}	area under urban land cover [m^2]
I_{slope}	slope index [-]
slp_{avg}	average slope (%)
slp_{max}	maximum slope (%)
GIS	Geographic Information System
a	coefficient for the effects of particle packing on the critical shear stress τ_c [L^2]
a_1	coefficient in the alternative model of the resuspension rate (eq. 14) [$L^{1-3b_1} T^{b_1-1}$]
b	coefficient for the effects of particle packing on the critical shear stress τ_c [$M^{-1} L^3$]
b_1	exponent in the alternative model of the resuspension rate (equation 14) [-]
C_1	concentration of <i>E. coli</i> in the water column [CFU/ m^3]
C_2	concentration of <i>E. coli</i> in the sediment [CFU/ m^3]
c_3	$\pi\rho g(s-1)/6$, coefficient for the effect of clay on the critical stress τ_c [$M L^{-2} T^{-2}$]
c_5	coefficient for the effect of clay on the critical stress τ_c [$M L^{-1} T^{-2}$]
C_a	concentration of <i>E. coli</i> attached to sediment in the bed [CFU/ m^3]
d	diameter of sediment particles to which <i>E. coli</i> attach [L]
E	erosion rate for sediment [$L T^{-1}$]
E_0	erosion rate at the threshold of erosion [$L T^{-1}$]
E_{0a}	coefficient in the predicted resuspension rate [$L T^{-1}$]
f_a	fraction of <i>E. coli</i> in the water column that are attached to sediment [-]
g	acceleration of gravity [$L T^{-2}$]

H_2	depth of the sediment containing <i>E. coli</i> [L]
k_{n2}	net growth rate in the sediment [T^{-1}]
N	number of parameters [-]
n	Manning roughness coefficient [-]
n_a	exponent in the predicted resuspension rate [-]
n_s	exponent in the erosion rate for sediment [-]
Q	discharge [$L^3 T^{-1}$]
R	hydraulic radius [L]
R_a	predicted resuspension rate [$CFU L^{-2} T^{-1}$]
R_{ai}	inferred resuspension rate [$CFU L^{-2} T^{-1}$]
\bar{R}_{ai}	average inferred resuspension rate [$CFU L^{-2} T^{-1}$]
S	slope [-]
S_{y_i}	relative sensitivity to y_i [-]
s	specific gravity of sediment particles [-]
t	time [T]
T	temperature [Θ]
v_r	resuspension velocity [$L T^{-1}$]
w_s	settling velocity [$L T^{-1}$]
y_i	generic parameter
α	coefficient relating particle diameter to shear stress [$M^{-1} L^2 T^2$]
Δy_i	uncertainty in y_i
ρ	water density [$M L^{-3}$]
ρ_b	bulk density of the sediment [$M L^{-3}$]

σ	sum of the squares of the differences between the logarithms of resuspension rates
τ_b	bottom shear stress [$M L^{-1} T^{-2}$]
τ_c	critical shear stress for cohesive sediment [$M L^{-1} T^{-2}$]
τ_{cn}	critical shear stress for non-cohesive sediment [$M L^{-1} T^{-2}$]
Φ	$k_{n2}H_2C_2/f_aW_sC_1$, parameter measuring the importance of settling and net growth [-]
ϕ_b	$a \exp(b\rho_b)/d^2$, contribution of bulk density to the critical shear stress τ_c [-]
ϕ_c	c_5/c_3d , contribution of binding effects of clay to the critical shear stress τ_c [-]
ψ	Shields parameter [-]
EC_{wz}	change in <i>E. coli</i> concentrations in the water zone [CFU/d]
EC_{suz}	change in <i>E. coli</i> concentrations in upper zone of the streambed [CFU/d]
EC_{slz}	change in <i>E. coli</i> concentrations in the lower zone of streambed [CFU/d]
d_{suz}	depths of streambed upper zone [m]
d_{slz}	depths of streambed lower zone [m]
d_{wz}	depth of the water zone [m]
EC_{ruz}	resuspension from the streambed upper zone [CFU/d]
EC_{rlz}	resuspension from the streambed lower zone [CFU/d]
EC_{duz}	depositions of <i>E. coli</i> from the water zone to the streambed upper zone [CFU/d]
EC_{dlz}	depositions of <i>E. coli</i> from the water zone to the streambed lower zone [CFU/d]
EC_{gwz}	<i>E. coli</i> growth in the water zone [CFU/d]
EC_{guz}	<i>E. coli</i> growth in the streambed upper zone [CFU/d]
EC_{glz}	<i>E. coli</i> growth in the streambed lower zone [CFU/d]
r_{suz}	streambed upper surface erosion rate [m/d]

TSS	total suspended solid concentrations [g/m ³]
T_w	water temperature [°C]
T_{air}	air temperature [°C]
<i>SOL_AWC</i>	soil available water capacity [-]
<i>GW_DELAY</i>	groundwater delay coefficient [-]
<i>SURLAG</i>	surface runoff lag coefficient [-]
<i>GW_Alfα</i>	base flow recession coefficient [-]
<i>CN2</i>	curve number [-]
<i>DDRAIN</i>	depth of subsurface drain [mm]
<i>TDRAIN</i>	time to drain soil to field capacity [hr]
<i>GDRAIN</i>	drain tile lag time [hr]
<i>DEP_IMP</i>	depth to impervious layer [mm]
<i>BACTKDDB</i>	<i>E. coli</i> partition coefficient [-]
<i>BACTSWF</i>	fraction of manure applied to land areas that has active colony forming units [-]
<i>PRF</i>	peak rate adjustment factor for sediment routing in the main channel [-]
<i>SPCON</i>	linear parameter for calculating the channel sediment routing [-]
<i>WOF</i>	wash-off fraction for <i>E. coli</i> [-]
<i>BACTKDQ</i>	<i>E. coli</i> soil partitioning coefficient [m ³ /kg]
<i>THBACT</i>	temperature adjustment factor [-]
<i>BACTMX</i>	<i>E. coli</i> percolation coefficient [-]
<i>TMDL</i>	total maximum daily loads
<i>SWAT</i>	soil and water assessment tool
L_T	total <i>E. coli</i> discharge [CFU/s]

L_w	water <i>E. coli</i> load [CFU/s]
L_s	resuspending <i>E. coli</i> load [CFU/s]
Q	water discharge [m^3/s]
R_p	resuspended pathogens [CFU / m^2s]
C_w	<i>E. coli</i> concentration in water [CFU/ m^3]
C_s	<i>E. coli</i> concentration in sediment [CFU/ m^3]
W_A	wetted surface area [m^2]
P_{ws}	<i>E. coli</i> ratio between sediment and water column [CFU / m^3] / [CFU / m^3]

APPENDIX I: Procedures adapted for *E. coli* enumeration, sample collection, and bulk density estimation

1. Procedure for E. coli analysis

Summary

The modified mTEC agar method (i.e., standard EPA method 1603) provides *E. coli* count in water by membrane filtration using modified Membrane-Thermotolerant *E. coli* agar (modified mTEC). The method gives a direct count of *E. coli* in ambient water based on the development of colonies, which grow on the surface of a membrane filter. A water sample is filtered through the membrane, which retains the *E. coli*. After filtration, the membrane containing the *E. coli* is placed on a selective and differential agents (i.e., medium), modified mTEC Agar. The membrane filter with medium in petri dish is incubated at 35 ± 0.5 °C for 2 h to resuscitate the injured or stressed bacteria, and then incubated at 44.5 ± 0.2 °C for 22 h. The target colonies on modified mTEC agar are red or magenta in color after the incubation period (EPA, 2006).

Materials

- Magnetic stirrer
- Stirrer bar
- Weighing scale
- Pipettes
- Graduated cylinders

- Disposable pipette tips
- Petri dishes, sterile, prepared with Modified mTEC agar
- Filtration units (filter base and funnel)
- Filter flask
- Sterile, white gridded 0.45 μm membrane filters
- Sterile forceps
- Ethanol (for flame-sterilizing forceps)
- Bunsen burner
- Thermometer
- Incubator maintained at $35\pm 0.5^\circ\text{C}$
- Water bath maintained at $44.5\pm 0.2^\circ\text{C}$

Precautions

Always wear gloves when handling samples containing *E. coli*.

Mouth pipetting is prohibited

General Procedures for Membrane filtration

1. First sterile filtration units at the beginning of each filtration series to prevent accidental contamination. A filtration series is considered to be interrupted when an interval of 30 minutes or longer elapses between sample filtrations.
2. A sample volume with expected bacterial density will be passed through the membrane filter.

3. The suggested sample volumes for stream water samples range 5 - 20 ml.
4. For enumerating *E. coli* in streambed sediment, prepare a mixture of streambed sediment and water (1:1) weight basis in a beaker size of 500 – 1000 ml.
5. The suggested weight of sediment range from 50 – 100 g.
6. Mix the sediment and water for 15 minutes using magnetic stirrer with 150 – 200 rpm of stirrer bar speed.
7. The suggested sample volumes for mixture of streambed sediment and water for filtration is 0.5 – 2 ml.
8. Using sterile forceps, place a sterile membrane filter over porous plate of receptacle.
9. Carefully place matched funnel unit over receptacle and lock it in place.
10. Filter samples (i.e., water sample, and mixture of sediment and water) under partial vacuum.
11. Rinse the interior surface of the funnel by filtering three 20 to 30 mL of sterile dilution water.
12. Immediately remove membrane filter with sterile forceps and place on culture dishes prepared with modified mTEC agar.
13. Close the dish, invert, and incubate for 2 hours at $35 \pm 0.5^{\circ}\text{C}$.
14. Next incubate for 22 hours at $44.5 \pm 0.2^{\circ}\text{C}$.

2. Sample collection in Squaw Creek Watershed

The water samples were collected by lowering a Horizontal Polycarbonate Water Bottle Sampler (2.2 L, Forestry Suppliers Inc., Mississippi, U.S) (Figure A1) from a bridge into the



Figure A1. Sample collection devices: stream water samples were collected using horizontal polycarbonate water bottle (left), and streambed sediment samples were collected using Shallow Water Bottom Dredge Sampler (right).

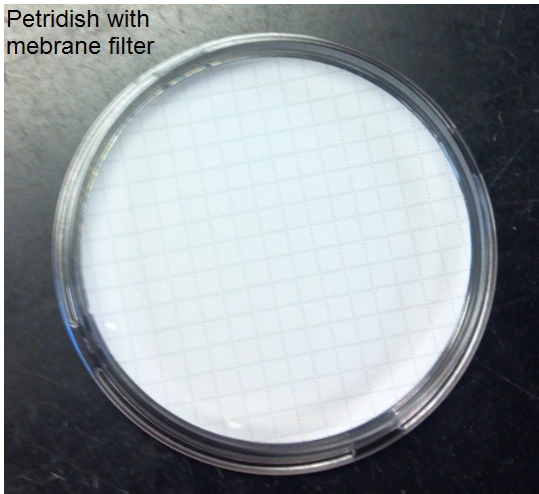
center of the stream. To collect the streambed sediment samples, we used a Shallow Water Bottom Dredge Sampler (15 cm × 15 cm opening, Forestry Suppliers Inc., Mississippi, U.S) (Figure A1). The dredge sampler was lowered to the streambed at the same location as water samples. The water samples were collected prior to sediment samples in order to avoid streambed sediment disturbance, which can potentially cause resuspension of *E. coli* associated with streambed sediment. All samples were analyzed in triplicate. Immediately after collection, samples were stored at 4°C and analyzed within 24 hours.

3. Membrane filtration for *E. coli* enumeration in Squaw Creek Watershed samples

E. coli in stream water column and streambed sediment column were analyzed. *E. coli* were enumerated by membrane filtration apparatus and equipment (Figure A2) using modified mTEC agar (Difco™, Modified mTEC agar, Becton, Dickinson and Company, Sparks, MD, USA). For analyzing *E. coli* cells in water column, a water sample volume of 10 ml was passed through a membrane filter (Millipore, 0.45 µm sterile grided 47 mm, HAG047S6) (Figure A2). For enumerating *E. coli* in sediment samples, we passed 1 ml volume of mixture (sediment and water) through a membrane filter. The procedure for preparing mixture of sediment and water is described in section 1 and 4. The membrane filter was then placed on the petri dish with modified mTEC agar. Subsequently, petri dish with membrane filter was incubated in a water bath (Thermo Scientific, Model 2872, SN 206155-393) (Figure B2). The red or magenta colonies on modified mTEC agar were (Figure A3) counted using a colony counter (Scienceware, Bel-Art Product, F37862-0000) (Figure B3).



Petridish with
mebrane filter



Petridish with modified
mTEC agar

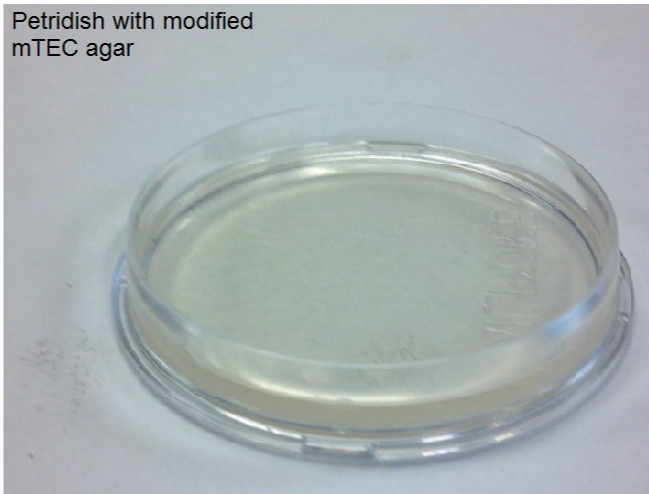
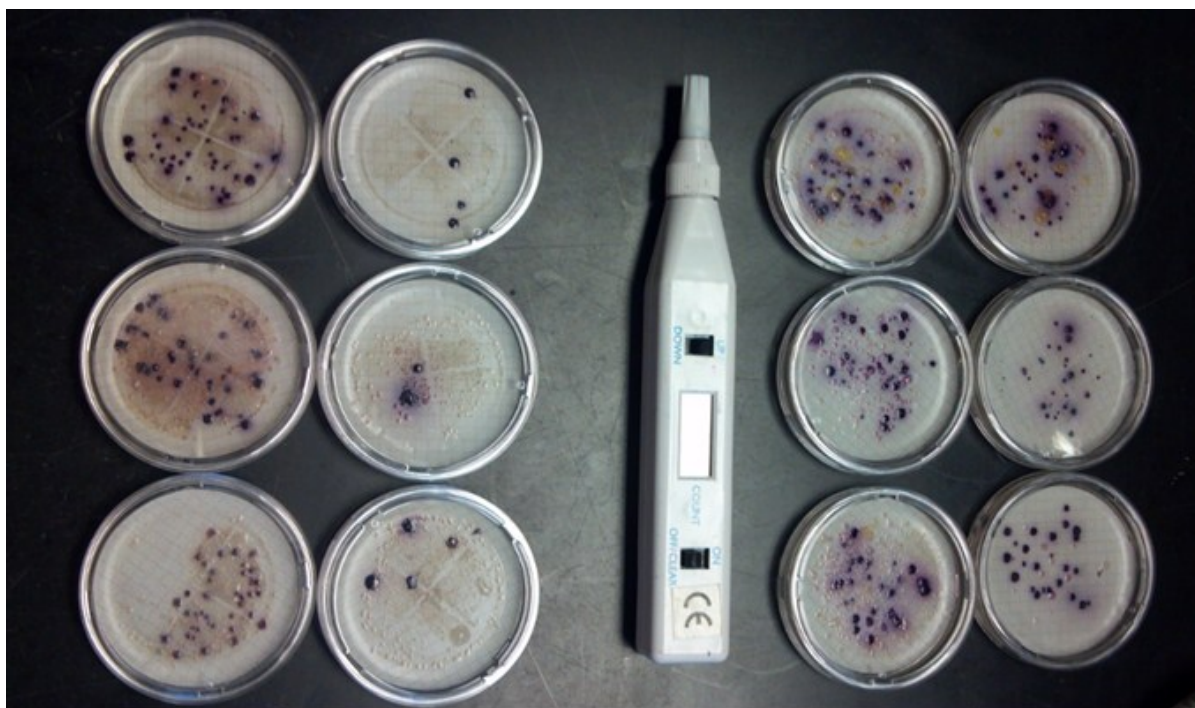


Figure A2. Membrane filtrations for *E. coli* enumeration: upper figure shows membrane filtration apparatus and equipments; bottom figure shows petri dish with membrane filter (left) and petri dish with modified mTEC agar (right).

Sediment *E. coli*

Colony counter

Water *E. coli*

Figure A3. Membrane filters incubation and *E. coli* cells: upper figure shows water bath used for membrane filter incubation and bottom figure shows *E. coli* in sediment sample (left), *E. coli* in water samples (right), and colony counter (middle).

4. *E. coli* calculation in water and sediment samples

4.1. *E. coli* calculation in a unit volume of water samples

In order to enumerate *E. coli* in water sample, we filtered 10 ml water sample through membrane filter using membrane filtration device discussed in section 1 and 3. After filtration, the membrane filter was placed on a petri dish with modified mTEC agar. After 22 hours of incubation of the filter and modified mTEC agar dish at 44.5 ± 0.3 °C, *E. coli* colonies grown in membrane filters were counted. Subsequently, we used equation 1 (shown below) to obtain *E. coli* colonies in 100 ml (or 100 g) of stream water sample.

$$\frac{E.coli (CFU)}{100 \text{ ml of water sample}} = \frac{CFU}{10 \text{ ml water samples}} \times 100 \dots\dots\dots (1)$$

To calculate the *E. coli* in per unit m³ of water sample, we converted *E. coli* in 100 ml of water samples into *E. coli* in m³ of water samples using equation 2.

$$\frac{E.coli (CFU)}{m^3 \text{ of water sample}} = \frac{CFU}{100 \text{ ml water samples}} \times 10^6 \text{ ml/m}^3 \dots\dots\dots (2)$$

4.2. *E. coli* calculation in a unit volume of sediment samples

To enumerate *E. coli* in streambed sediment sample, we mixed 80 g of sediment and 80 g (or 80 ml) of water in a beaker and stirred the mixture at 150 - 200 rpm using a magnetic stirrer (Corning PC -620D) for 15 minutes. Then 1 ml of mixture was passed through a membrane filter, and immediately after filtration the filter was placed on a petri dish with modified mTEC agar. Subsequently the filter with modified mTEC agar dish was incubated in a water bath (Figure A3). The *E. coli* colonies grown in the membrane filter were counted (Figure

A3). To obtain *E. coli* colonies in per 100 g of streambed sediment sample, we used equation 3 for calculations.

$$\frac{CFU}{100 \text{ g sediment}} = \frac{CFU}{1 \text{ ml of mixture}} \times \frac{\text{total mixture volume}}{80 \text{ g sediment}} \times 100 \dots\dots\dots(3)$$

where total mixture volume (i.e., 80 g of sediment and 80 ml of water was calculated using equation 4.

$$\text{mixture volume} = \frac{80 \text{ g of sediment}}{\text{bulk density of sediment (i.e., } 1.26 \frac{\text{g}}{\text{cc}})} + 80 \text{ ml of water} \dots\dots\dots(4)$$

E. coli concentrations in per m³ of sediment volume was calculated using equation 5.

$$\frac{CFU}{\text{m}^3 \text{ of sediment}} = \frac{CFU}{100 \text{ g sediment}} \times \text{sediment bulk density} \left(\frac{1.26 \text{ g}}{\text{cc}} \right) \times \frac{10^6 \text{ CC}}{\text{m}^3} \dots\dots\dots(5)$$

The method adapted here for calculating *E. coli* in sediment does provide an approximate *E. coli* concentration in sediment. Here our assumption is that after stirring sediment of 80 g and water of 80 ml, all of the attached *E. coli* to sediment particles are detached from the particles and are uniformly distributed throughout the mixture. This assumption may lead to a degree of uncertainty in calculation of sediment *E. coli*. For example, there is a possibility that a 15 minutes stirring may not release all of the *E. coli* attached to sediment into mixture. The other source of uncertainty could be caused by 1 ml sample used in filtrations. For instance, we used 1 ml of mixture for filtering through membrane filters. There is a possibility that the particles of large sizes in mixture (i.e., 80 of sediment and 80 ml of water) were not a part of that 1 ml solutions used for filtrations. In calculating *E. coli* in a unit gram of unit volume of sediment, we used a bulk density of 1.26 g/cc. Our measurement shows that bulk density of

streambed sediment (Appendix I, Table A) varies from one sample to the other. To understand the potential uncertainties in predictions, we have calculated impacts of changes in bulk density on sediment and water column *E. coli* concentrations, which is described in chapter 4 (Table 4.4), and readers are encouraged to understand the potential uncertainties involved in *E. coli* measurement and *E. coli* predictions, while using the models proposed in this research.

5. Bulk density estimation

For predicting *E. coli* resuspension from the streambed sediment to the water column, we used a calibrated value of bulk density of 1.26 g/cm³; the calibration was performed while developing resuspension model described in chapter 3. To understand the variability in streambed sediment bulk density from one sample to the other, we calculated the potential changes in the bulk density sediment samples. For estimating a range for bulk density of streambed sediment of Squaw Creek Watershed, we collected streambed sediment samples at 14 locations (7 sampling locations in main streams and 7 sampling locations in tributaries of the Squaw Creek Watershed). Sampling locations are shown in Figure A4. The streambed sediment samples were collected using a soil corer diameter (3.175 cm) and height (25.91 cm). We drive a soil corer into the streambed and remove the intact core; subsequently the sediment core weight and volume were measured. The core volume (205.78 cc) was estimated using the volume formula ($\pi r^2 h$). The bulk density of the sediments, expressed as weight per unit volume, was determined from sediment wet and dry weight (dried in the oven at approximately 75°C for 2 days (Roberts et al., 1998)). The data are shown in Table A.

$$\text{bulk density (g/cc)} = \frac{\text{sample wet weight(g)} - \text{water loss(g)}}{\text{core volume (cc)}} \dots\dots\dots(3)$$

Results show that the bulk density varies from 1.05 – 2.08 g/cc. The average of the bulk density was found to be 1.45 g/cc with standard deviation of 0.29 g/cc. Considering the greater variation (i.e., 1.05 – 2.08 g/cc) in bulk density among 14 location shown in Figure A3, we expect that the bulk density used in this analyses (1.26 g/cc) may require adjustment (or calibration) while predicting in-stream *E. coli* concentrations in other study areas.

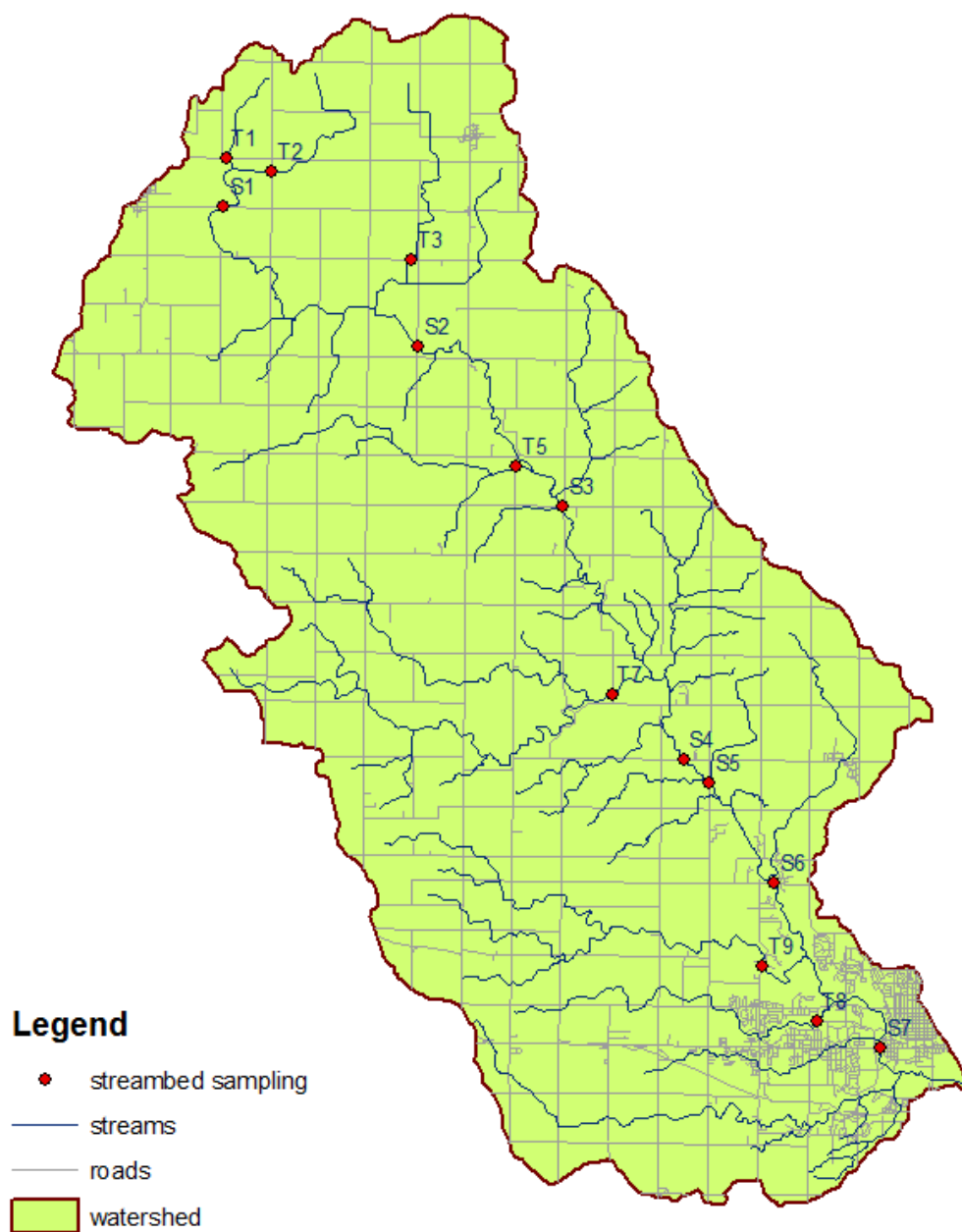


Figure A4. Streambed sediment sampling locations: red dots indicate 14 sampling locations: T1, T2, T3, T5, T7, T8, and T9 are locations in tributaries; and S1, S2, S3, S4, S5, S6, and S7 are locations in main streams. Blue lines indicate streams; light gray lines indicate roads; and dark red lined indicates watershed boundary.

APPENDIX I: Table A. Bulk density values among 14 locations shown in Figure A

Location ID	Dish Weight (g)	Wet Sample Weight (g)	Volume (cm ³)	Dry Sample + Dish Weight (g)	Dry Sample Weight (g)	Dry Bulk Density (g/cm ³)
T1	15.80	352.20	205.78	286.60	270.80	1.32
T2	16.10	315.10	205.78	232.40	216.30	1.05
T3	15.70	335.70	205.78	297.20	281.50	1.37
T5	15.60	577.60	205.78	443.60	428.00	2.08
T7	16.10	485.70	205.78	403.00	386.90	1.88
T8	16.10	386.80	205.78	301.10	285.00	1.38
T9	15.90	401.40	205.78	332.50	316.60	1.54
S1	14.10	400.40	205.78	326.40	312.30	1.52
S2	15.70	393.40	205.78	314.60	298.90	1.45
S3	16.00	327.30	205.78	273.10	257.10	1.25
S4	16.10	294.60	205.78	235.40	219.30	1.07
S5	15.70	321.50	205.78	267.70	252.00	1.22
S6	15.70	394.80	205.78	325.70	310.00	1.51
S7	16.10	418.20	205.78	360.70	344.60	1.67
					AVERAGE	1.45
					STDEV	0.29

Reference

Roberts, J., Jepsen, R., Gotthard, D. and Lick, W. (1998) Effects of particle size and bulk density on erosion of quartz particles. *Journal of Hydraulic Engineering* 124(12), 1261-1267.

U.S. Environmental Protection Agency (2006) Method 1603—*Escherichia coli* in water by membrane filtration using modified membrane-thermotolerant *Escherichia coli* agar: Washington, D.C., EPA 821-R-06-011, 42 p.

APPENDIX I: Table B. Particle Size Distribution of streambed sediment at 16 locations (T1 – T9 samples were from tributaries, and S1 – S7 samples were collected at main streams (Figure A4).

Type	Date	Cobbles %	Gravel %		Sand %			Fines %		mm						Coefficient	
			CRS	FINE	CRS	MEDIUM	FINE	SILT	CLAY	D(85)	D(60)	D(50)	D(30)	D(15)	D(10)	C(U)	C-C
1	10/16/2010	0	0	1.5	33.8	25.1	21.4	11.3	6.9	3.33	1.78	1.32	0.223	0.0525	0.0132	134.5	2.12
2	10/16/2010	0	0	1.3	5.1	47.3	26.8	9.7	9.8	1.15	0.557	0.457	0.291	0.017	0.0059	94.8	25.85
3	10/16/2010	0	22.9	36	10.6	14.2	13.1	3.2		24.1	11.3	7.69	1.89	0.391	0.276	40.85	1.15
4	10/16/2010	0	0	5.8	16.9	26.1	42.5	6	2.7	2.96	0.566	0.41	0.242	0.147	0.103	5.52	1.01
5	10/16/2010	0	0	0	0	1.9	59.9	32.2	6	0.224	0.124	0.0989	0.0602	0.0252	0.0116	10.62	2.52
6	10/17/2010	0	5.4	20.3	24.3	40.2	8.5	1.3		7.58	2.81	2	1	0.547	0.43	6.54	0.83
7	10/17/2010	0	5.1	15	20.4	39.1	17	3.4		7.07	2.04	1.47	0.701	0.304	0.218	9.33	1.1
8	10/17/2010	0	0	0	0	13.5	82.2	3.5	0.8	0.41	0.272	0.241	0.19	0.151	0.118	2.3	1.12
9	10/17/2010	0	0	0	1.3	50	46.2	1.7	0.8	0.831	0.502	0.433	0.319	0.23	0.193	2.6	1.05
10	10/16/2010	0	0	17.2	15.5	30.1	23.5	9.8	3.9	6.11	1.28	0.707	0.325	0.105	0.0366	34.79	2.27
11	10/16/2010	0	16.3	21	21.2	29.2	11.2		1.1	20.6	4.23	2.81	1.2	0.488	0.8	11.14	0.89
12	10/16/2010	0	0	0	2.8	38.1	54.8	3.7	0.6	0.609	0.43	0.382	0.295	0.219	0.185	2.32	1.09
13	10/17/2010	0	0	0.5	7.8	48	41.7	1.4	0.6	1.3	0.553	0.468	0.342	0.255	0.221	2.51	0.96
14	10/17/2010	0	0	11.2	21.5	49.5	17.3	0.5		3.96	1.56	1.11	0.582	0.394	0.34	4.59	0.64
15	10/17/2010	0	0	0	2.3	62.4	34.4	0.3	0.6	0.988	0.599	0.519	0.395	0.309	0.275	2.18	0.95
16	10/17/2010	0	4.4	20.7	4.5	50.9	18.7		0.8	11.9	0.956	0.756	0.518	0.388	0.345	2.77	0.81

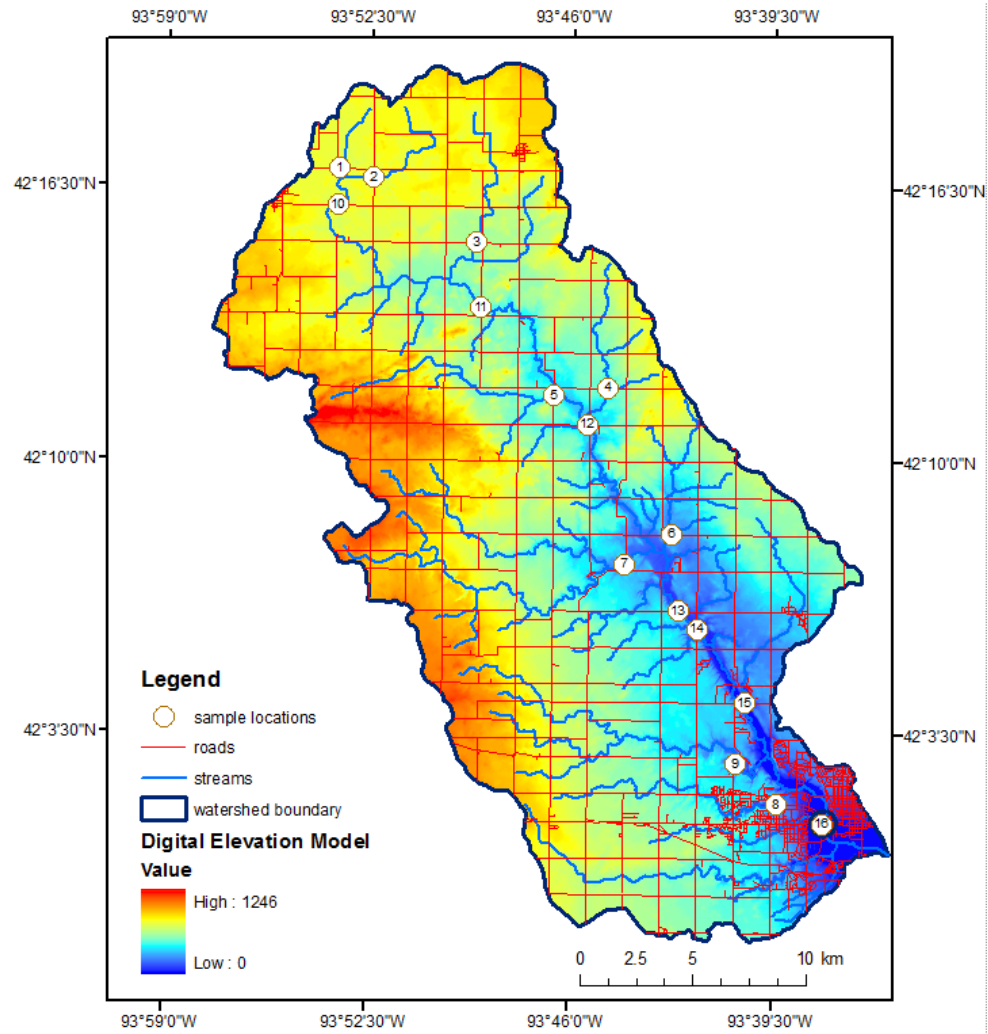


Figure A5. Sampling streambed sediment for distribution analysis.

locations of particle size

APPENDIX II: Table A. Streambed sediment and water column *E. coli* concentrations in Squaw Creek Watershed at 16

locations. The data of *E. coli* presented in this table were used in developing resuspension model described in chapter 3.

Location ID	Date	Sediment	Water
		(CFU/m ³)	(CFU/m ³)
1	7/17/2009	1.251E+08	5.750E+06
2	7/17/2009	2.629E+08	6.667E+06
3	7/17/2009	1.620E+08	3.750E+06
4	7/17/2009	3.548E+08	3.583E+06
5	7/17/2009	3.872E+08	4.667E+06
6	7/17/2009	1.627E+08	7.417E+06
7	7/17/2009	9.266E+07	5.467E+07
8	7/17/2009	6.441E+07	3.333E+06
9	7/17/2009	1.876E+08	5.750E+06
10	7/17/2009	1.356E+08	5.667E+06
11	7/17/2009	3.134E+08	3.083E+06
12	7/17/2009	5.273E+07	2.250E+06
13	7/17/2009	4.840E+07	6.083E+06
14	7/17/2009	4.897E+07	4.417E+06
15	7/17/2009	1.846E+07	5.000E+06
16	7/17/2009	4.143E+07	4.083E+06

APPENDIX II: Table B. Streambed sediment *E. coli* concentrations in Squaw Creek Watershed. The data shown in this table was used to verify the predictions of the modified SWAT model and the model for predicting total *E. coli* loads described in chapter 4 and 5, respectively. Also this data set was used in chapter 6 for understanding the potential impacts of weather pattern in in-stream *E. coli* concentrations.

Streambed sediment <i>E. coli</i> concentrations							
Date	(CFU/100g)	Date	(CFU/100g)	Date	(CFU/100g)	Date	(CFU/100g)
5/7/2009	837	4/5/2010	119	8/3/2010	2057	4/11/2011	10523
6/6/2009	179	5/10/2010	72	8/23/2010	1495	4/28/2011	7294
6/9/2009	4305	5/19/2010	108	8/27/2010	837	5/17/2011	10643
6/10/2009	1447	5/24/2010	161	9/6/2010	11659	5/19/2011	8371
6/18/2009	2128	5/26/2010	431	9/8/2010	7953	5/24/2011	7055
6/22/2009	455	6/4/2010	1088	9/13/2010	12676	6/6/2011	9029
6/23/2009	442	6/7/2010	305	9/15/2010	10882	6/13/2011	15247
6/25/2009	657	6/9/2010	1405	9/20/2010	7115	6/16/2011	11540
7/11/2009	23379	6/11/2010	8921	9/22/2010	36174	6/24/2011	31630
8/20/2009	29357	6/14/2010	347	9/27/2010	10164	7/1/2011	19671
9/8/2009	30016	6/16/2010	682	9/29/2010	16503	7/12/2011	15426
9/9/2009	32886	6/21/2010	1447	10/4/2010	9746	8/25/2011	41854
9/23/2009	87176	7/8/2010	2523	10/6/2010	2989	9/6/2011	50464
9/29/2009	35756	7/12/2010	514	10/11/2010	119	9/8/2011	5083
10/8/2009	156534	7/14/2010	4831	10/13/2010	30792	9/15/2011	24395
10/13/2009	36473	7/21/2010	1076	11/1/2010	13513	9/20/2011	44246
10/30/2009	1973	7/26/2010	1458	11/4/2010	20329	10/27/2011	11061
3/31/2010	455	7/29/2010	1136	4/7/2011	1614	11/11/2011	10763

APPENDIX II: Table C. stream water column *E. coli* concentration used in chapter 4, 5 and 6.

Date	(CFU/100ml)	Date	(CFU/100ml)	Date	(CFU/100ml)	Date	(CFU/100ml)
5/7/2009	300	10/1/2009	16700	6/11/2010	60	9/13/2010	291
5/27/2009	12067	3/29/2010	27	6/14/2010	3160	9/15/2010	323
5/31/2009	167	3/30/2010	22	6/16/2010	600	9/20/2010	208
6/6/2009	1077	4/5/2010	18	6/21/2010	618	9/22/2010	1064
6/9/2009	2013	4/14/2010	33	7/8/2010	2033	9/27/2010	519
6/10/2009	693	4/19/2010	96	7/12/2010	547	9/29/2010	31
6/18/2009	7267	4/21/2010	54	7/14/2010	607	10/4/2010	23
6/22/2009	627	5/5/2010	10	7/21/2010	1013	10/6/2010	77
6/23/2009	587	5/10/2010	63	7/26/2010	607	10/11/2010	153
6/25/2009	1913	5/13/2010	4240	7/29/2010	433	10/13/2010	461
7/11/2009	3620	5/18/2010	273	8/3/2010	757	11/1/2010	104
7/15/2009	833	5/19/2010	123	8/10/2010	2773	11/4/2010	68
8/5/2009	30	5/24/2010	157	8/12/2010	453	4/7/2011	33
8/7/2009	1227	5/26/2010	207	8/18/2010	297	4/11/2011	53
8/21/2009	695	6/2/2010	180	8/23/2010	177	4/21/2011	984
9/15/2009	171	6/4/2010	1007	8/27/2010	107	4/28/2011	163
9/23/2009	2065	6/7/2010	293	9/6/2010	3	5/17/2011	185
9/29/2009	288	6/9/2010	53	9/8/2010	300	5/19/2011	68

APPENDIX II: Table C (continued)

E. coli concentrations

Date	(CFU/100ml)	Date	(CFU/100ml)	Date	(CFU/100ml)
5/24/2011	101	7/1/2011	613	9/6/2011	44
5/26/2011	773	7/7/2011	1003	9/8/2011	53
6/2/2011	85	7/12/2011	770	9/15/2011	97
6/6/2011	203	7/21/2011	2560	9/20/2011	453
6/13/2011	423	7/28/2011	813	9/27/2011	10
6/16/2011	293	8/1/2011	237	10/27/2011	397
6/20/2011	707	8/8/2011	2667	11/11/2011	603
6/24/2011	356	8/25/2011	5667	11/18/2011	225
6/29/2011	640	9/1/2011	933	12/13/2011	255

```

!! ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
!! ii |none |counter
!! jrch |none |reach number
!! pi |3.15159 |pi value
!! c3 |N/m^3 |coefficient for the effect of clay on critial shear stress, tauc
!! c5 |N/m^2 |coefficient for the effect of clay on critial shear stress, tauc
!! dp |m |sediment particle diameter
!! a |m^2 |coefficient for the effects of particle packing on tauc
!! b |m^3/g |coefficient for the effects of particle packing on tauc
!! gr |m/s^2 |acceleration of gravity
!! sr |- |specific gravity of sediment particle
!! rows |g/m^3 |bulk density of the sediment
!! roww |g/m^3 |water density
!! Eoa |m/s |Coefficient in predicted resuspension rate
!! na |unit less |exponenet in predicted resuspension rate
!! muw |g/(m.s) |water viscosity
!! chn |none |Manning's "n" value for the reach
!! chs1 |m/m |stream bed slope of reach
!! dtl |m |depth of top streambed layer
!! dbl |m |depth of bottom streambed layer
!! hydr |m |hydraulic radis
!! wetp |m |wetted perimeter
!! chz |- |change in horizontal distance per unit
!! | | |change in vertical distance on channel side
!! taub |g/(m.s^2) |stream bed shear stress
!! tauc |g/(m.s^2) |critical shear stress for cohesive sediment
!! taunc |g/(m.s^2) |critical shear stress for noncohesive sediment
!! resvel |m/s |resuspension velocity
!! setvel |m/s |settling velocity
!! ressedmass |g |resuspended sediment mass
!! setsedmass |g |settled sediment mass
!! resecoli |10^3 cfu |resuspended E. coli numbers
!! setecoli |10^3 cfu |settled E. coli numbers
!! bedsedvol |m^3 |stream bed sediment volume in reach
!! bedsedmass |g |stream bed sediment mass in reach
!! bedsedmass_t1 |g |stream bed sediment mass in streambed top layer
!! bedsedmass_b1 |g |stream bed sediment mass in streambed bottom layer
!! watvol |m^3 H2O |water volume in reach
!! ressedmass_t1 |g |resuspended sediment mass from top layer

```



```

real :: totwatecoli, atcwatecoli, atcwatecolicon uatcwatecoli
real :: sussedmass, bedsedmass, bedsedvol, acbedsedvol
real :: acbedsedmass, taub, tauc, taunc, Eoa, na, resvel, setvel
real :: ressedmass, setsedmass, resecoli, setecoli, fa, fua
real :: bedsedecoli, bedseddep, acbedseddep
real :: wetp, chz, chn, chs1, c3, c5, gr, sr, a, b, dp, rows
real :: roww, mu, wtmp, hydr, pi, kp, sedin, qdin, sussedcon
real :: bedsedecolicon, totbactp, totbactlp, ressedcon
real :: initotwatlp, initotwatp, inibedsedlp, inibedsedp
real :: atcwatlp, atcwatp, uatcwatlp, uatcwatp
real :: uatcwatlpcon, uatcwatpcon
real :: reslp, resp, setlp, setp
real :: bedsedlpcon, bedsedpcon, bedsedlp, bedsedp
real :: totwatlp, totwatp, atcwatecolicon
real :: atcwatlpcon, atcwatpcon, uatcwatecoli, reachwatr
real :: wtrin, totbactecoli
real :: insussedmass, isedlp_conc, watvol, insussedcon
real :: inwatlp_conc, watlp_conc, insedlp_conc
real :: sussedmass_bf
real :: dtl, dbl, bedsedmass_tl, bedsedmass_bl
real :: bsen_tl, bsen_bl, bsec_tl, bsec_bl
real :: res_f, nres_f, res_ecn_tl, res_ecn_bl
real :: ressedmass_tl, ressedmass_bl
real :: ibsen_tl, ibsen_bl, ibsec_tl, ibsec_bl
real :: ibsec, ibsen, bsen, bsec
real :: wen, wec, in_bedseden, bedseden
real :: in_bedsedec, bedsedec, sussedcon_bs
real :: sussedcon_as, outfract, fen_tl, fen_bl
real :: watlp_end, bsec_tl_end, watlp_conc1
real :: sedin1, sussedmass_bs, totwatlp1
real :: inwatlp_conc1, sussedcon_bfs, sussedcon_afs
real :: totressedmass, sussedcon_t1, sussedcon_ar
real :: ibsedtl, ibsedbl, iwatb, intss
real :: unat_ecoli, at_ecoli, at_ecolicon
real :: sussedcon_avr, totwatlpcon_ar

```

```

jrch = 0
jrch = inum1

```

```

!! all initial values starts
pi = 3.14159
c3 = 8452.      !!pi * roww * gr * (sr - 1.)/6.
c5 = 23.
dp = 1.5E-06    !! m
bedseddep = 0.10 !! m
acbedseddep = 0.02 !!m
a = 8.5E-16
b = 9.07
gr = 9.81
sr = 2.65
rows = 1.26 !g/cm3
roww = 998.
Eoa = 1E-06
mu = 8.91E-04
na = 2.
kp = 0.01
chn = 0.036
chs1 = 2.8E-04
dtl = 0.03
dbl = 0.06
fen_t1 = 0.80
fen_b1 = 1 - fen_t1
res_f = 0.75

hydr = 0.
chz = 2.
wetp = 0.
!! hydraulic radius calculations
if(rchdep > 0.0001) then !!change3/14/2002
wetp = phi(6,jrch) + 2. * rchdep * Sqrt (1. + chz * chz)    !m
hydr = rcharea / wetp      !!m
else
hydr = 0. !m
endif
!! hydraulic radius calculation ends !! tested

!! shear stress calculations begins
taunc = 0.

```

```

    taub = 0.
    tauc = 0.
    taunc = 414. * dp !Pa
    taub = roww * gr * hydr * chs1 !pa
    tauc = taunc * (1. + (a * Exp(b*rows)/(dp * dp)) + (c5/c3*dp)) !! pa
!! shear stress calculations ends

!! resuspension velocity calculation starts
    resvel = 0.
    resvel = Eoa * ((taub - taunc)/(tauc - taunc)**na
!! resuspension velocity calculation ends !! tested

!! settling velocity calculation starts
    setvel = 0.
    setvel = gr * dp * dp * roww * (sr - 1.)/(18. * mu)
!! settling velocity calculation ends !! tested

!! make resuspension and settling velocities zero, when no water
    if(rchdep < 0.01) then !!change3/14/2002
        resvel = 0.
        setvel = 0.
    else
        setvel = setvel
        resvel = resvel
    endif
!!end zeroing

!! calculation bedsediment volume and mass
    bedsedvol = 0.
    bedsedmass = 0.
    bedsedvol = wetp * ch_l2(jrch) * (dtl + dbl) !m3
    bedsedmass = bedsedvol * rows * 1000000. !g
    bedsedmass_tl = 0.
    bedsedmass_bl = 0.
    bedsedmass_tl = wetp * ch_l2(jrch)*dtl*rows*1000000.
    bedsedmass_bl = wetp * ch_l2(jrch)*dbl*rows*1000000.
!! calculation bedsediment volume and mass ends !! tested

!! calculate resuspended mass

```

```

tday = 0.
tday = rtime / 24.
if(tday > 1.) tday = 1.
ressedmass = 0.
ressedmass = wetp *ch_l2(jrch) * resvel * rows * 1000000.
ressedmass = ressedmass * tday * 24. * 3600.

if(ressedmass >= bedsedmass_t1) then
ressedmass_t1 = bedsedmass_t1
ressedmass_b1 = ressedmass - bedsedmass_t1
else
ressedmass_t1 = ressedmass
ressedmass_b1 = 0.
endif
!! resuspended mass calculations ends !! tested

!! initial water volume calculations
watvol = 0.
watvol = varoute(2,inum2) * (1. - rnum1) + rchstor(jrch)
!! water volume calculations ends !! tested
!! conditions when no water, then resuspension would be zero
! if(wetp >0.01) then
! totressedmass = ressedmass_t1 + ressedmass_b1
! ressedmass_t1 = ressedmass_t1
! ressedmass_b1 = ressedmass_b1
! else
! totressedmass = 0.
! ressedmass_t1 = 0.
! ressedmass_b1 = 0.
! endif
!! conditiosn ends

!! this testing done to chek resuspended mass after resuspension
if(watvol .GE. 0.01) then
sussedcon_ar = totressedmass/watvol
else
sussedcon_ar = 0.
endif
!! testing ends

```



```

!! initialisation suspended sediment conc.
  if(curyr == 1. .and. iida == 1. ) then
    sussedcon = 200. !!g/m3 or mg/l
  else
    sussedcon = intss
  endif

!! initialisatoin ends
!! sediment routing, which is estimated on rtsed subroutines
  sedin = 0.
  sedin = varoute(3,inum2) * (1.- rnum1) !!tones
  sedin = sedin * 1000000. !!grams
!! sediment routing ends
!! suspended sediment mass calculations
  sussedmass = 0.
  sussedmass = sedin + rchstor(jrch) * sussedcon + totressedmass
!! conditionn if no water then suspended conc is zero after routing
  if(watvol .LE. 1.) then
    sussedcon_bfs = 0.
  else
    sussedcon_bfs = sussedmass/watvol
  endif
  sussedcon_avr = sussedcon_bfs
!! conditions ends !! tested
!! incorporate settling in suspended mass

  setsedmass = 0.
  setsedmass = sussedmass*(setvel/rchdep)*tday*3600. * 24.
  if(rchdep .LE. 0.02) setsedmass = 0. !!change3/14/2002
!! conditions to include settling of sediment
  If(setsedmass .LE. sussedmass) then
!    setsedmass = setsedmass
    sussedmass = sussedmass - setsedmass
  else
!    setsedmass = setsedmass
!    setsedmass = sussedmass
    sussedmass = 0.
  endif

```

```

!      sussedmass = MAX(10., sussedmass)
!!     conditions to include settling of sediment ends

!!    conditions for no water
      if(watvol .GE. 1.) then
!      sussedcon_bs = sussedmass/watvol
!      sussedcon = sussedmass/watvol
!      sussedmass_bs = sussedmass
      else
!      sussedcon_bs = 0.
!      sussedcon = 0.
!      sussedmass_bs = 0.
      endif
!!    conditions for no water end
!!    suspended sediment conc after settling
      sussedcon_afs = sussedcon
!      sussedcon_t1 = sussedcon
      intss = sussedcon_afs
!!    suspended sediment conc after settling ends
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!

      if(curyr == 1 .and. iida == 1.) then
        bsec_t1 = 700. !! cfu/g
        bsec_b1 = 500. !! cfu/g
      else
        bsec_t1 = ibsedt1 !! insedconct1(jrch)
        bsec_b1 = ibsedb1 !!insedconcb1(jrch)
      endif

      bsen_t1 = 0.
      bsen_b1 = 0.
      bsen_t1 = bsec_t1 * bedsedmass_t1
      bsen_b1 = bsec_b1 * bedsedmass_b1

      bsen_t1 = max(100000000000., bsen_t1)           !! later on 3/6/2012

```

```

bsen_bl = max(100000., bsen_bl)

res_ecn_tl = 0.
res_ecn_bl = 0.

if(rchdep .GE. 0.02) then
res_ecn_tl = ressedmass_tl * bsec_tl
res_ecn_bl = ressedmass_bl * bsec_bl
else
res_ecn_tl = 0.
res_ecn_bl = 0.
endif

bsen_tl = bsen_tl - res_ecn_tl
bsen_bl = bsen_bl - res_ecn_bl

! bsen_tl = max(100000., bsen_tl)      !! later on 3/6/2012
! bsen_bl = max(1E6, bsen_bl)

!! later on 3/6/2012
if(curyr == 1. .and. iida == 1.) then
watlp_conc = 200. !! CFU/100 ml
else
watlp_conc = iwatb      !!inwatconc(jrch)
endif

! if(watvol > 1.) then
!   watlp_conc = watlp_conc
! else
!   watlp_conc = 0.
! endif

totwatlp = 0.
totwatlp = varoute(19,inum2) * varoute(2,inum2)      &
&   *(1.- rnum1)*10000. + watlp_conc * rchstor(jrch)*10000.
totwatlp = max(100000000000., totwatlp)
totwatlp = totwatlp + res_ecn_tl + res_ecn_bl

unat_ecoli = totwatlp * 0.20

```

```

at_ecoli = totwatlp * 0.80
at_ecolicon = at_ecoli/watvol

atcwatlpcon = at_ecolicon / sussedcon_bfs

!   totwatlpcon_ar = totwatlp /watvol !!CFU/m3
!   atcwatlpcon = totwatlpcon_ar /sussedcon_afs  !!CFU/g

!   fa = kp * sussedcon_avr / (1. + kp * sussedcon_avr)
!   setlp = 0.
!   setlp = setsedmass * atcwatlpcon

!   if(setlp .LE. totwatlp) then
!       totwatlp = totwatlp - setlp
!       totwatlp = unat_ecoli + at_ecoli - setlp
!   else
!       totwatlp = 0.
!   endif

!   totwatlp = max(100.,totwatlp) !! later on 3/6/2012

!   wtmp = 0.
!   wtmp = 5.0 + 0.75 * tmpav(jrch)
!   if (wtmp <= 0.) wtmp = 0.1

!   totwatlp = totwatlp * netgrowth_wat(wtmp) !! GROWTH NEW
!pkp_3_13_12   totwatlp = max(100000., totwatlp)

!   watlp_conc = totwatlp /(watvol * 10000.)
!   watlp_conc = max(10., watlp_conc)
!   if(watlp_conc < 1.) watlp_conc = 1.
!   iwatb = 0.
!   iwatb = watlp_conc
!   bsen_t1 = bsen_t1 + setlp * 0.85
!   bsen_t1 = max(1E14, bsen_t1)

!   bsen_t1 = bsen_t1 * netgrowth_sed(wtmp)

```

```

    bsen_bl = bsen_bl + setlp * 0.15

    bsen_bl = bsen_bl * netgrowth_sed(wtmp)
    !   bsen_tl = bsen_tl * netgrowth(wtmp) !! GROWTH NEW
    !   bsen_tl = max(1000000., bsen_tl)
    !   bsen_bl = bsen_bl * netgrowth(wtmp) !! GROWTH NEW
    !   bsen_bl = Max(0., bsen_bl)
    bsec_tl = bsen_tl/bedsedmass_tl
    bsec_bl = bsen_bl/bedsedmass_bl
    !   bsec_tl = max(10., bsec_tl)
    !   if(bsec_tl < 1.) bsec_tl = 1.
    !   if(bsec_bl < 1.) bsec_bl = 1.
    ibsedtl = 0.
    ibsedbl = 0.
    ibsedtl = bsec_tl
    ibsedbl = bsec_bl
!! bedsediment E. coli initializatiotn ends

!! bedsediment E. coli number calculations starts

    rch_bactp(jrch) = 0.
    rch_bactlp(jrch) = 0.
    rch_bactp(jrch) = bsec_tl * 100 !! CFU/100 g
    rch_bactlp(jrch) = watlp_conc
    !   rch_bactp(jrch) = susedcon_bfs
    !   rch_bactlp(jrch) = susedcon_afs
    !   if(watvol > 0.01) then
    !   rch_bactp(jrch) = bsec_tl
    !   rch_bactlp(jrch) = watlp_conc
    !   else
    !   rch_bactp(jrch) = 0.
    !   rch_bactlp(jrch) = 0.
    !   endif

return
end

```

!!end!!

B2. Function for predicting bacteria E. coli net growth in streambed.

!!start!!

```
function netgrowth_sed(tmp)

!!   ~ ~ ~ PURPOSE ~ ~ ~
!!   this function estimate net ecoli growth for a given temperature in streambed
!!   Equation is from Hipsey (2009)

!!   ~ ~ ~ INCOMING VARIABLES ~ ~ ~
!!   name      |units      |definition
!!   ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
!!   tmp       |deg C      |temperature on current day
!!   ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
!!   ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~

!!   ~ ~ ~ LOCAL DEFINITIONS ~ ~ ~
!!   name      |units      |definition
!!   kg        |1/day      |growth rate
!!   mumax     |1/day      |maximum growth rate at 20 deg C
!!   CT1       |-          |species specific constants controlling
!!           |           |the exact shape of the function
!!   CT2       |-          |species specific constants controlling
!!           |           |the exact shape of the function
!!   Tmin      |deg C      |minimum temperature for growth
!!   Tmax      |deg C      |maximum temperature for growth
!!   Theta     |-          |theta controls the sensitivity of kd to temperature
!!   kd        |1/day      |dark death rate
!!   kd20      |1/day      |dark death rate at 20 deg C
!!   ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
!!   ~ ~ ~ OUTGOING VARIABLES ~ ~ ~
!!   name      |units      |definition
!!   netgrowth |1/day      |netgrowth of E. coli corresponding to temperature
```



```

netgrowth_sed = 0.
netgrowth_sed = kg !! - kd
netgrowth_sed = Abs(netgrowth_sed)
return
end

```

!!end!!

B3. Function for predicting bacteria net growth in water column.

!!start!!

```

function netgrowth_wat(tmp)

!!  ~ ~ ~ PURPOSE ~ ~ ~
!!  this function estimate net ecoli growth for a given temperature in water
!!  Equation is from Hipsey (2009)

!!  ~ ~ ~ INCOMING VARIABLES ~ ~ ~
!!  name      |units      |definition
!!  ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
!!  tmp       |deg C      |temperature on current day
!!  ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
!!  ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~

!!  ~ ~ ~ LOCAL DEFINITIONS ~ ~ ~
!!  name      |units      |definition
!!  kg        |1/day      |growth rate
!!  mumax     |1/day      |maximum growth rate at 20 deg C
!!  CT1       |-          |species specific constants controlling
!!           |           |the exact shape of the function
!!  CT2       |-          |species specific constants controlling
!!           |           |the exact shape of the function
!!  Tmin      |deg C      |minimum temperature for growth
!!  Tmax      |deg C      |maximum temperature for growth
!!  Theta     |-          |theta controls the sensitivity of kd to temperature

```



```
!   If (wtemp < Tmin .and. wtemp > Tmax) then
!       kg = 0.
!   end if
```

```
!! netgrowth estimation
```

```
netgrowth_wat = 0.
netgrowth_wat = kg !! - kd
netgrowth_wat = Abs(netgrowth_wat)
return
end
```

```
!!end!!
```

APPENDIX IV: HRU details of SWAT simulation.

SWAT model simulation Date: 3/6/2012 12:00:00 AM Time: 00:00:00							
MULTIPLE HRUS Landuse/Soil/Slope OPTION			THRESHOLDS : 15 / 15 / 15 [%]				
Number of HRUS: 250							
Number of Subbasins: 31							
watershed		Area [ha]	Area[acres]				
		49969.6200	123477.4295				
LANDUSE:		Area [ha]	Area[acres]	%wat.Area			
Corn --> CORN		26847.2678	66340.9410	53.73			
Soybean --> SOYB		21894.4142	54102.1921	43.82			
Range-Grasses --> RNGE		405.2186	1001.3155	0.81			
Forest-Deciduous --> FRSD		411.1675	1016.0155	0.82			
Transportation --> UTRN		167.7610	414.5458	0.34			
Residential-Med/Low Density --> URML		197.7533	488.6583	0.40			
Institutional --> UINS		46.0377	113.7614	0.09			
SOILS:							
IA117		8016.0712	19808.1128	16.04			
IA110		17796.8104	43976.8083	35.62			
IA119		5761.0318	14235.7975	11.53			
IA111		17670.9427	43665.7828	35.36			
IA118		491.2590	1213.9255	0.98			
IA115		233.5050	577.0026	0.47			
SLOPE:							
0-3		21047.6122	52009.7020	42.12			
3-6		19604.9275	48444.7560	39.23			
12-9999		4107.3942	10149.5765	8.22			
6-9		5176.5453	12791.5023	10.36			
9-12		33.1408	81.8927	0.07			
SUBBASIN #		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
1		1098.5400	2714.5473	2.20			
LANDUSE:							
Corn --> CORN		676.0775	1670.6213	1.35	61.54		
Soybean --> SOYB		422.4625	1043.9259	0.85	38.46		
SOILS:							
IA117		1098.5400	2714.5473	2.20	100.00		
SLOPE:							
0-3		659.4010	1629.4129	1.32	60.03		
3-6		439.1390	1085.1344	0.88	39.97		
HRUS							
1		Corn --> CORN/IA117/0-3	413.7136	1022.3070	0.83	37.66	1
2		Corn --> CORN/IA117/3-6	262.3639	648.3143	0.53	23.88	2
3		Soybean --> SOYB/IA117/0-3	245.6874	607.1059	0.49	22.36	3
4		Soybean --> SOYB/IA117/3-6	176.7751	436.8201	0.35	16.09	4
SUBBASIN #		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
2		1898.6400	4691.6344	3.80			
LANDUSE:							
Corn --> CORN		1023.4715	2529.0493	2.05	53.91		
Soybean --> SOYB		875.1685	2162.5851	1.75	46.09		
SOILS:							
IA117		1898.6400	4691.6344	3.80	100.00		
SLOPE:							
3-6		762.5407	1884.2763	1.53	40.16		
0-3		1136.0993	2807.3581	2.27	59.84		
HRUS							

HRUS							
1	Corn --> CORN/IA117/0-3	413.7136	1022.3070	0.83	37.66	1	
2	Corn --> CORN/IA117/3-6	262.3639	648.3143	0.53	23.88	2	
3	Soybean --> SOYB/IA117/0-3	245.6874	607.1059	0.49	22.36	3	
4	Soybean --> SOYB/IA117/3-6	176.7751	436.8201	0.35	16.09	4	
		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
SUBBASIN #	2	1898.6400	4691.6344	3.80			
LANDUSE:							
	Corn --> CORN	1023.4715	2529.0493	2.05	53.91		
	Soybean --> SOYB	875.1685	2162.5851	1.75	46.09		
SOILS:	IA117	1898.6400	4691.6344	3.80	100.00		
SLOPE:							
	3-6	762.5407	1884.2763	1.53	40.16		
	0-3	1136.0993	2807.3581	2.27	59.84		
HRUS							
5	Corn --> CORN/IA117/3-6	399.3559	986.8285	0.80	21.03	1	
6	Corn --> CORN/IA117/0-3	624.1156	1542.2208	1.25	32.87	2	
7	Soybean --> SOYB/IA117/0-3	511.9837	1265.1372	1.02	26.97	3	
8	Soybean --> SOYB/IA117/3-6	363.1848	897.4478	0.73	19.13	4	
		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
SUBBASIN #	3	2868.7500	7088.8247	5.74			
LANDUSE:							
	Corn --> CORN	1745.0725	4312.1615	3.49	60.83		
	Soybean --> SOYB	1123.6775	2776.6632	2.25	39.17		
SOILS:	IA110	281.6093	695.8707	0.56	9.82		
	IA117	2587.1407	6392.9540	5.18	90.18		
SLOPE:							
	0-3	1884.7535	4657.3202	3.77	65.70		
	3-6	983.9965	2431.5045	1.97	34.30		
HRUS							
9	Corn --> CORN/IA110/0-3	162.2780	400.9970	0.32	5.66	1	
10	Corn --> CORN/IA110/3-6	119.3314	294.8737	0.24	4.16	2	
11	Corn --> CORN/IA117/3-6	484.8217	1198.0187	0.97	16.90	3	
12	Corn --> CORN/IA117/0-3	978.6415	2418.2721	1.96	34.11	4	
13	Soybean --> SOYB/IA117/3-6	379.8434	938.6120	0.76	13.24	5	
14	Soybean --> SOYB/IA117/0-3	743.8341	1838.0512	1.49	25.93	6	
		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
SUBBASIN #	4	1166.4900	2882.4551	2.33			
LANDUSE:							
	Corn --> CORN	589.5858	1456.8961	1.18	50.54		
	Soybean --> SOYB	576.9042	1425.5591	1.15	49.46		
SOILS:	IA110	1064.9255	2631.4842	2.13	91.29		
	IA117	101.5645	250.9710	0.20	8.71		
SLOPE:							
	12-9999	315.6270	779.9301	0.63	27.06		
	0-3	316.3577	781.7356	0.63	27.12		
	3-6	381.7088	943.2214	0.76	32.72		
	6-9	133.9738	331.0559	0.27	11.49		
	9-12	18.8228	46.5120	0.04	1.61		
HRUS							
15	Corn --> CORN/IA110/12-9999	161.7036	399.5776	0.32	13.86	1	
16	Corn --> CORN/IA110/0-3	212.4439	524.9594	0.43	18.21	2	

17	Corn --> CORN/IA110/3-6	215.4384	532.3590	0.43	18.47	3
18	Soybean --> SOYB/IA110/0-3	103.9138	256.7762	0.21	8.91	4
19	Soybean --> SOYB/IA110/12-9999	134.4471	332.2255	0.27	11.53	5
20	Soybean --> SOYB/IA110/6-9	100.7725	249.0139	0.20	8.64	6
21	Soybean --> SOYB/IA110/3-6	136.2062	336.5724	0.27	11.68	7
22	Soybean --> SOYB/IA117/3-6	30.0641	74.2900	0.06	2.58	8
23	Soybean --> SOYB/IA117/6-9	33.2013	82.0420	0.07	2.85	9
24	Soybean --> SOYB/IA117/12-9999	19.4763	48.1270	0.04	1.67	10
25	Soybean --> SOYB/IA117/9-12	18.8228	46.5120	0.04	1.61	11
		Area [ha]	Area[acres]	%wat. Area	%Sub. Area	
SUBBASIN #	5	176.7600	436.7828	0.35		
LANDUSE:						
	Corn --> CORN	102.0059	252.0617	0.20	57.71	
	Soybean --> SOYB	74.7541	184.7211	0.15	42.29	
SOILS:						
	IA110	161.6799	399.5192	0.32	91.47	
	IA117	15.0801	37.2636	0.03	8.53	
SLOPE:						
	12-9999	18.9898	46.9247	0.04	10.74	
	6-9	46.1099	113.9398	0.09	26.09	
	0-3	40.3279	99.6522	0.08	22.82	
	3-6	71.3325	176.2661	0.14	40.36	
HRUS						
26	Corn --> CORN/IA110/12-9999	18.9898	46.9247	0.04	10.74	1
27	Corn --> CORN/IA110/6-9	25.9185	64.0459	0.05	14.66	2
28	Corn --> CORN/IA110/0-3	19.3747	47.8759	0.04	10.96	3
29	Corn --> CORN/IA110/3-6	37.7229	93.2153	0.08	21.34	4
30	Soybean --> SOYB/IA110/0-3	16.6631	41.1755	0.03	9.43	5
31	Soybean --> SOYB/IA110/3-6	27.6295	68.2739	0.06	15.63	6
32	Soybean --> SOYB/IA110/6-9	15.3814	38.0081	0.03	8.70	7
33	Soybean --> SOYB/IA117/6-9	4.8100	11.8858	0.01	2.72	8
34	Soybean --> SOYB/IA117/3-6	5.9800	14.7770	0.01	3.38	9
35	Soybean --> SOYB/IA117/0-3	4.2900	10.6009	0.01	2.43	10
		Area [ha]	Area[acres]	%wat. Area	%Sub. Area	
SUBBASIN #	6	687.7800	1699.5388	1.38		
LANDUSE:						
	Corn --> CORN	362.0843	894.7285	0.72	52.65	
	Soybean --> SOYB	325.6957	804.8103	0.65	47.35	
SOILS:						
	IA117	687.7800	1699.5388	1.38	100.00	
SLOPE:						
	0-3	388.0252	958.8297	0.78	56.42	
	3-6	299.7548	740.7090	0.60	43.58	
HRUS						
36	Corn --> CORN/IA117/0-3	205.0715	506.7420	0.41	29.82	1
37	Corn --> CORN/IA117/3-6	157.0128	387.9865	0.31	22.83	2
38	Soybean --> SOYB/IA117/3-6	142.7420	352.7226	0.29	20.75	3
39	Soybean --> SOYB/IA117/0-3	182.9537	452.0877	0.37	26.60	4
		Area [ha]	Area[acres]	%wat. Area	%Sub. Area	
SUBBASIN #	7	2163.8700	5347.0310	4.33		
LANDUSE:						
	Corn --> CORN	1032.9884	2552.5661	2.07	47.74	
	Soybean --> SOYB	1130.8816	2794.4649	2.26	52.26	
SOILS:						
	IA110	1522.5051	3762.1863	3.05	70.36	
	IA117	641.3649	1584.8447	1.28	29.64	
SLOPE:						

		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
SUBBASIN #	7	2163.8700	5347.0310	4.33			
LANDUSE:							
	Corn --> CORN	1032.9884	2552.5661	2.07	47.74		
	Soybean --> SOYB	1130.8816	2794.4649	2.26	52.26		
SOILS:							
	IA110	1522.5051	3762.1863	3.05	70.36		
	IA117	641.3649	1584.8447	1.28	29.64		
SLOPE:							
	6-9	210.8627	521.0523	0.42	9.74		
	0-3	1039.2598	2568.0628	2.08	48.03		
	3-6	913.7475	2257.9159	1.83	42.23		
HRUS							
40	Corn --> CORN/IA110/6-9	148.3110	366.4838	0.30	6.85	1	
41	Corn --> CORN/IA110/0-3	278.9348	689.2618	0.56	12.89	2	
42	Corn --> CORN/IA110/3-6	256.0285	632.6592	0.51	11.83	3	
43	Corn --> CORN/IA117/3-6	141.5046	349.6649	0.28	6.54	4	
44	Corn --> CORN/IA117/0-3	208.2096	514.4963	0.42	9.62	5	
45	Soybean --> SOYB/IA110/3-6	393.8138	973.1337	0.79	18.20	6	
46	Soybean --> SOYB/IA110/0-3	445.4170	1100.6477	0.89	20.58	7	
47	Soybean --> SOYB/IA117/3-6	122.4006	302.4581	0.24	5.66	8	
48	Soybean --> SOYB/IA117/6-9	62.5517	154.5685	0.13	2.89	9	
49	Soybean --> SOYB/IA117/0-3	106.6983	263.6569	0.21	4.93	10	
<hr/>							
		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
SUBBASIN #	8	2344.3200	5792.9319	4.69			
LANDUSE:							
	Corn --> CORN	1244.0592	3074.1326	2.49	53.07		
	Soybean --> SOYB	1100.2608	2718.7994	2.20	46.93		
SOILS:							
	IA110	1358.3589	3356.5728	2.72	57.94		
	IA117	985.9611	2436.3592	1.97	42.06		
SLOPE:							
	3-6	920.6921	2275.0761	1.84	39.27		
	6-9	292.9080	723.7904	0.59	12.49		
	0-3	1130.7199	2794.0654	2.26	48.23		
HRUS							
50	Corn --> CORN/IA110/3-6	251.4294	621.2946	0.50	10.73	1	
51	Corn --> CORN/IA110/6-9	152.0537	375.7323	0.30	6.49	2	
52	Corn --> CORN/IA110/0-3	272.1698	672.5451	0.54	11.61	3	
53	Corn --> CORN/IA117/3-6	204.4833	505.2885	0.41	8.72	4	
54	Corn --> CORN/IA117/0-3	363.9231	899.2721	0.73	15.52	5	
55	Soybean --> SOYB/IA110/3-6	295.5107	730.2216	0.59	12.61	6	
56	Soybean --> SOYB/IA110/0-3	246.3410	608.7210	0.49	10.51	7	
57	Soybean --> SOYB/IA110/6-9	140.8543	348.0581	0.28	6.01	8	
58	Soybean --> SOYB/IA117/3-6	169.2687	418.2714	0.34	7.22	9	
59	Soybean --> SOYB/IA117/0-3	248.2860	613.5272	0.50	10.59	10	
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		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
SUBBASIN #	9	2349.9900	5806.9428	4.70			
LANDUSE:							
	Corn --> CORN	1410.8045	3486.1685	2.82	60.03		
	Soybean --> SOYB	939.1855	2320.7743	1.88	39.97		
SOILS:							
	IA110	2349.9900	5806.9428	4.70	100.00		
SLOPE:							
	12-9999	583.4290	1441.6822	1.17	24.83		
	6-9	504.4590	1246.5435	1.01	21.47		
	3-6	742.0457	1833.6321	1.48	31.58		

HRUS						
60	Corn --> CORN/IA110/12-9999	303.4945	749.9502	0.61	12.91	1
61	Corn --> CORN/IA110/6-9	314.0157	775.9485	0.63	13.36	2
62	Corn --> CORN/IA110/3-6	461.7973	1141.1242	0.92	19.65	3
63	Corn --> CORN/IA110/0-3	331.4970	819.1456	0.66	14.11	4
64	Soybean --> SOYB/IA110/0-3	188.5593	465.9395	0.38	8.02	5
65	Soybean --> SOYB/IA110/12-9999	279.9344	691.7320	0.56	11.91	6
66	Soybean --> SOYB/IA110/6-9	190.4433	470.5950	0.38	8.10	7
67	Soybean --> SOYB/IA110/3-6	280.2484	692.5079	0.56	11.93	8
		Area [ha]	Area[acres]	%wat.Area	%Sub.Area	
SUBBASIN #	10	2490.2100	6153.4334	4.98		
LANDUSE:						
	Corn --> CORN	1460.4154	3608.7595	2.92	58.65	
	Soybean --> SOYB	1029.7946	2544.6739	2.06	41.35	
SOILS:	IA119	2490.2100	6153.4334	4.98	100.00	
SLOPE:						
	12-9999	1228.5626	3035.8396	2.46	49.34	
	3-6	686.5995	1696.6216	1.37	27.57	
	6-9	575.0479	1420.9722	1.15	23.09	
HRUS						
68	Corn --> CORN/IA119/12-9999	752.1078	1858.4961	1.51	30.20	1
69	Corn --> CORN/IA119/3-6	375.9525	928.9975	0.75	15.10	2
70	Corn --> CORN/IA119/6-9	332.3550	821.2659	0.67	13.35	3
71	Soybean --> SOYB/IA119/3-6	310.6469	767.6241	0.62	12.47	4
72	Soybean --> SOYB/IA119/12-9999	476.4547	1177.3435	0.95	19.13	5
73	Soybean --> SOYB/IA119/6-9	242.6929	599.7063	0.49	9.75	6
		Area [ha]	Area[acres]	%wat.Area	%Sub.Area	
SUBBASIN #	11	657.9000	1625.7038	1.32		
LANDUSE:						
	Corn --> CORN	402.3693	994.2748	0.81	61.16	
	Soybean --> SOYB	255.5307	631.4290	0.51	38.84	
SOILS:	IA110	491.8312	1215.3395	0.98	74.76	
	IA119	166.0688	410.3643	0.33	25.24	
SLOPE:						
	0-3	288.4532	712.7822	0.58	43.84	
	6-9	93.3857	230.7608	0.19	14.19	
	3-6	224.5965	554.9893	0.45	34.14	
	12-9999	37.1465	91.7908	0.07	5.65	
	9-12	14.3181	35.3807	0.03	2.18	
HRUS						
74	Corn --> CORN/IA110/0-3	138.1852	341.4626	0.28	21.00	1
75	Corn --> CORN/IA110/6-9	56.9218	140.6567	0.11	8.65	2
76	Corn --> CORN/IA110/3-6	116.9644	289.0248	0.23	17.78	3
77	Corn --> CORN/IA119/0-3	16.6173	41.0623	0.03	2.53	4
78	Corn --> CORN/IA119/3-6	17.4534	43.1283	0.03	2.65	5
79	Corn --> CORN/IA119/12-9999	23.0970	57.0740	0.05	3.51	6
80	Corn --> CORN/IA119/9-12	14.3181	35.3807	0.03	2.18	7
81	Corn --> CORN/IA119/6-9	18.8121	46.4856	0.04	2.86	8
82	Soybean --> SOYB/IA110/0-3	118.7606	293.4634	0.24	18.05	9
83	Soybean --> SOYB/IA110/3-6	60.9992	150.7320	0.12	9.27	10
84	Soybean --> SOYB/IA119/6-9	17.6518	43.6186	0.04	2.68	11
85	Soybean --> SOYB/IA119/0-3	14.8900	36.7939	0.03	2.26	12
86	Soybean --> SOYB/IA119/3-6	29.1796	72.1042	0.06	4.44	13
87	Soybean --> SOYB/IA119/12-9999	14.0494	34.7168	0.03	2.14	14
		Area [ha]	Area[acres]	%wat.Area	%Sub.Area	
SUBBASIN #	12	1927.1700	4762.1334	3.86		

SUBBASIN #	12	1927.1700	4762.1334	3.86	
LANDUSE:					
	Corn --> CORN	1083.8812	2678.3247	2.17	56.24
	Soybean --> SOYB	843.2888	2083.8087	1.69	43.76
SOILS:	IA110	1927.1700	4762.1334	3.86	100.00
SLOPE:					
	3-6	802.0556	1981.9194	1.61	41.62
	6-9	386.9121	956.0791	0.77	20.08
	0-3	738.2023	1824.1349	1.48	38.30
HRUS					
88	Corn --> CORN/IA110/3-6	450.5939	1113.4400	0.90	23.38
89	Corn --> CORN/IA110/6-9	207.7543	513.3714	0.42	10.78
90	Corn --> CORN/IA110/0-3	425.5330	1051.5134	0.85	22.08
91	Soybean --> SOYB/IA110/3-6	351.4617	868.4794	0.70	18.24
92	soybean --> SOYB/IA110/0-3	312.6693	772.6215	0.63	16.22
93	soybean --> SOYB/IA110/6-9	179.1577	442.7077	0.36	9.30
		Area [ha]	Area[acres]	%wat. Area	%Sub. Area
SUBBASIN #	13	3135.3300	7747.5572	6.27	
LANDUSE:					
	Corn --> CORN	1751.5132	4328.0766	3.51	55.86
	Soybean --> SOYB	1383.8168	3419.4806	2.77	44.14
SOILS:	IA110	629.6054	1555.7865	1.26	20.08
	IA119	2505.7246	6191.7707	5.01	79.92
SLOPE:					
	12-9999	1111.8120	2747.3431	2.22	35.46
	0-3	338.1094	835.4853	0.68	10.78
	6-9	753.2711	1861.3705	1.51	24.03
	3-6	932.1375	2303.3584	1.87	29.73
HRUS					
94	Corn --> CORN/IA110/12-9999	89.3033	220.6729	0.18	2.85
95	Corn --> CORN/IA110/0-3	71.5046	176.6913	0.14	2.28
96	Corn --> CORN/IA110/6-9	87.6008	216.4660	0.18	2.79
97	Corn --> CORN/IA110/3-6	131.4012	324.6990	0.26	4.19
98	Corn --> CORN/IA119/3-6	442.6680	1093.8548	0.89	14.12
99	Corn --> CORN/IA119/6-9	380.6188	940.5281	0.76	12.14
100	Corn --> CORN/IA119/12-9999	548.4165	1355.1645	1.10	17.49
101	Soybean --> SOYB/IA110/12-9999	62.2858	153.9114	0.12	1.99
102	soybean --> SOYB/IA110/6-9	56.5790	139.8096	0.11	1.80
103	soybean --> SOYB/IA110/0-3	45.3284	112.0088	0.09	1.45
104	soybean --> SOYB/IA110/3-6	85.6023	211.5274	0.17	2.73
105	soybean --> SOYB/IA119/12-9999	411.8064	1017.5942	0.82	13.13
106	soybean --> SOYB/IA119/3-6	272.4660	673.2772	0.55	8.69
107	soybean --> SOYB/IA119/0-3	221.2764	546.7851	0.44	7.06
108	soybean --> SOYB/IA119/6-9	228.4724	564.5668	0.46	7.29
		Area [ha]	Area[acres]	%wat. Area	%Sub. Area
SUBBASIN #	14	6.3000	15.5676	0.01	
LANDUSE:					
	Corn --> CORN	6.3000	15.5676	0.01	100.00
SOILS:	IA119	6.3000	15.5676	0.01	100.00
SLOPE:					
	3-6	2.0288	5.0133	0.00	32.20
	0-3	4.2712	10.5543	0.01	67.80
HRUS					
109	Corn --> CORN/IA119/3-6	2.0288	5.0133	0.00	32.20
110	Corn --> CORN/IA119/0-3	4.2712	10.5543	0.01	67.80

		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
SUBBASIN #	15	1970.6400	4869.5500	3.94			
LANDUSE:							
	Corn --> CORN	1132.6803	2798.9096	2.27	57.48		
	Soybean --> SOYB	837.9597	2070.6404	1.68	42.52		
SOILS:	IA110	1970.6400	4869.5500	3.94	100.00		
SLOPE:							
	0-3	591.8792	1462.5631	1.18	30.03		
	3-6	908.4749	2244.8868	1.82	46.10		
	6-9	470.2860	1162.1001	0.94	23.86		
HRUS							
111	Corn --> CORN/IA110/0-3	317.7640	785.2107	0.64	16.12	1	
112	Corn --> CORN/IA110/3-6	525.4943	1298.5227	1.05	26.67	2	
113	Corn --> CORN/IA110/6-9	289.4220	715.1762	0.58	14.69	3	
114	Soybean --> SOYB/IA110/6-9	180.8640	446.9239	0.36	9.18	4	
115	Soybean --> SOYB/IA110/0-3	274.1152	677.3523	0.55	13.91	5	
116	Soybean --> SOYB/IA110/3-6	382.9806	946.3641	0.77	19.43	6	
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		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
SUBBASIN #	16	4244.9400	10489.4590	8.50			
LANDUSE:							
	Corn --> CORN	2224.5161	5496.8906	4.45	52.40		
	Soybean --> SOYB	2020.4239	4992.5684	4.04	47.60		
SOILS:	IA111	4244.9400	10489.4590	8.50	100.00		
SLOPE:							
	3-6	1772.8381	4380.7715	3.55	41.76		
	0-3	1614.3767	3989.2055	3.23	38.03		
	6-9	857.7252	2119.4819	1.72	20.21		
HRUS							
117	Corn --> CORN/IA111/3-6	918.2623	2269.0719	1.84	21.63	1	
118	Corn --> CORN/IA111/0-3	855.6780	2114.4231	1.71	20.16	2	
119	Corn --> CORN/IA111/6-9	450.5759	1113.3956	0.90	10.61	3	
120	Soybean --> SOYB/IA111/3-6	854.5758	2111.6996	1.71	20.13	4	
121	Soybean --> SOYB/IA111/0-3	758.6987	1874.7825	1.52	17.87	5	
122	Soybean --> SOYB/IA111/6-9	407.1493	1006.0864	0.81	9.59	6	
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		Area [ha]	Area[acres]	%wat.Area	%Sub.Area		
SUBBASIN #	17	400.9500	990.7675	0.80			
LANDUSE:							
	Corn --> CORN	199.6608	493.3719	0.40	49.80		
	Soybean --> SOYB	201.2892	497.3956	0.40	50.20		
SOILS:	IA111	273.9387	676.9161	0.55	68.32		
	IA119	127.0113	313.8514	0.25	31.68		
SLOPE:							
	3-6	150.9202	372.9314	0.30	37.64		
	0-3	139.3731	344.3978	0.28	34.76		
	6-9	61.8106	152.7370	0.12	15.42		
	12-9999	48.8461	120.7013	0.10	12.18		
HRUS							
123	Corn --> CORN/IA111/3-6	65.2616	161.2646	0.13	16.28	1	
124	Corn --> CORN/IA111/0-3	77.4070	191.2766	0.15	19.31	2	
125	Corn --> CORN/IA119/6-9	11.4257	28.2334	0.02	2.85	3	
126	Corn --> CORN/IA119/12-9999	12.9219	31.9306	0.03	3.22	4	
127	Corn --> CORN/IA119/0-3	20.1309	49.7445	0.04	5.02	5	
128	Corn --> CORN/IA119/3-6	12.5138	30.9223	0.03	3.12	6	
129	Soybean --> SOYB/IA111/3-6	54.5174	134.7153	0.11	13.60	7	
130	Soybean --> SOYB/IA111/6-9	34.9175	86.2829	0.07	8.71	8	

131	Soybean --> SOYB/IA111/0-3	41.8351	103.3767	0.08	10.43	9
132	Soybean --> SOYB/IA119/12-9999	35.9243	88.7707	0.07	8.96	10
133	Soybean --> SOYB/IA119/6-9	15.4674	38.2207	0.03	3.86	11
134	Soybean --> SOYB/IA119/3-6	18.6274	46.0292	0.04	4.65	12
		Area [ha]	Area[acres]	%wat. Area	%Sub. Area	
SUBBASIN #	18	1252.2600	3094.3971	2.51		
LANDUSE:						
	Corn --> CORN	694.3341	1715.7342	1.39	55.45	
	Soybean --> SOYB	557.9259	1378.6628	1.12	44.55	
SOILS:	IA111	1252.2600	3094.3971	2.51	100.00	
SLOPE:						
	3-6	533.5647	1318.4650	1.07	42.61	
	0-3	718.6953	1775.9320	1.44	57.39	
HRUS						
135	Corn --> CORN/IA111/3-6	281.4216	695.4068	0.56	22.47	1
136	Corn --> CORN/IA111/0-3	412.9125	1020.3274	0.83	32.97	2
137	Soybean --> SOYB/IA111/0-3	305.7828	755.6046	0.61	24.42	3
138	Soybean --> SOYB/IA111/3-6	252.1431	623.0582	0.50	20.14	4
		Area [ha]	Area[acres]	%wat. Area	%Sub. Area	
SUBBASIN #	19	575.6400	1422.4352	1.15		
LANDUSE:						
	Corn --> CORN	416.2451	1028.5623	0.83	72.31	
	Soybean --> SOYB	159.3949	393.8729	0.32	27.69	
SOILS:	IA110	575.6400	1422.4352	1.15	100.00	
SLOPE:						
	3-6	272.6259	673.6722	0.55	47.36	
	0-3	162.0886	400.5290	0.32	28.16	
	6-9	140.9255	348.2339	0.28	24.48	
HRUS						
139	Corn --> CORN/IA110/3-6	195.5724	483.2692	0.39	33.97	1
140	Corn --> CORN/IA110/0-3	112.0794	276.9538	0.22	19.47	2
141	Corn --> CORN/IA110/6-9	108.5932	268.3393	0.22	18.86	3
142	Soybean --> SOYB/IA110/6-9	32.3322	79.8946	0.06	5.62	4
143	Soybean --> SOYB/IA110/0-3	50.0092	123.5753	0.10	8.69	5
144	Soybean --> SOYB/IA110/3-6	77.0535	190.4030	0.15	13.39	6
		Area [ha]	Area[acres]	%wat. Area	%Sub. Area	
SUBBASIN #	20	3201.9300	7912.1291	6.41		
LANDUSE:						
	Corn --> CORN	1697.1850	4193.8290	3.40	53.01	
	Soybean --> SOYB	1504.7450	3718.3001	3.01	46.99	
SOILS:	IA111	3201.9300	7912.1291	6.41	100.00	
SLOPE:						
	3-6	1568.8830	3876.7882	3.14	49.00	
	0-3	1633.0470	4035.3409	3.27	51.00	
HRUS						
145	Corn --> CORN/IA111/3-6	859.3146	2123.4094	1.72	26.84	1
146	Corn --> CORN/IA111/0-3	837.8704	2070.4196	1.68	26.17	2
147	Soybean --> SOYB/IA111/0-3	795.1766	1964.9212	1.59	24.83	3
148	Soybean --> SOYB/IA111/3-6	709.5683	1753.3788	1.42	22.16	4
		Area [ha]	Area[acres]	%wat. Area	%Sub. Area	
SUBBASIN #	21	1163.5200	2875.1161	2.33		

LANDUSE:						
	Corn --> CORN	456.0095	1126.8224	0.91	39.19	
	Soybean --> SOYB	707.5105	1748.2937	1.42	60.81	
SOILS:						
	IA110	1163.5200	2875.1161	2.33	100.00	
SLOPE:						
	3-6	561.5341	1387.5788	1.12	48.26	
	0-3	343.5974	849.0463	0.69	29.53	
	6-9	258.3885	638.4910	0.52	22.21	
HRUS						
149	Corn --> CORN/IA110/3-6	217.0384	536.3128	0.43	18.65	1
150	Corn --> CORN/IA110/0-3	142.2765	351.5723	0.28	12.23	2
151	Corn --> CORN/IA110/6-9	96.6946	238.9372	0.19	8.31	3
152	Soybean --> SOYB/IA110/3-6	344.4957	851.2660	0.69	29.61	4
153	Soybean --> SOYB/IA110/6-9	161.6939	399.5538	0.32	13.90	5
154	Soybean --> SOYB/IA110/0-3	201.3209	497.4740	0.40	17.30	6
		Area [ha]	Area[acres]	%wat.Area	%Sub.Area	
SUBBASIN #	22	1352.9700	3343.2565	2.71		
LANDUSE:						
	Corn --> CORN	684.6835	1691.8872	1.37	50.61	
	Soybean --> SOYB	668.2865	1651.3693	1.34	49.39	
SOILS:						
	IA111	1217.9103	3009.5173	2.44	90.02	
	IA110	135.0597	333.7392	0.27	9.98	
SLOPE:						
	0-3	808.2310	1997.1792	1.62	59.74	
	3-6	517.4264	1278.5865	1.04	38.24	
	12-9999	27.3126	67.4909	0.05	2.02	
HRUS						
155	Corn --> CORN/IA111/0-3	417.0321	1030.5072	0.83	30.82	1
156	Corn --> CORN/IA111/3-6	267.6514	661.3800	0.54	19.78	2
157	Soybean --> SOYB/IA110/12-9999	27.3126	67.4909	0.05	2.02	3
158	Soybean --> SOYB/IA110/0-3	70.9126	175.2286	0.14	5.24	4
159	Soybean --> SOYB/IA110/3-6	36.8345	91.0198	0.07	2.72	5
160	Soybean --> SOYB/IA111/3-6	212.9405	526.1867	0.43	15.74	6
161	Soybean --> SOYB/IA111/0-3	320.2863	791.4434	0.64	23.67	7
		Area [ha]	Area[acres]	%wat.Area	%Sub.Area	
SUBBASIN #	23	1432.2600	3539.1861	2.87		
LANDUSE:						
	Range-Grasses --> RNGE	279.7806	691.3518	0.56	19.53	
	Corn --> CORN	661.9522	1635.7170	1.32	46.22	
	Soybean --> SOYB	490.5272	1212.1173	0.98	34.25	
SOILS:						
	IA110	1334.1658	3296.7904	2.67	93.15	
	IA119	98.0942	242.3957	0.20	6.85	
SLOPE:						
	0-3	845.0914	2088.2632	1.69	59.00	
	3-6	541.3543	1337.7136	1.08	37.80	
	12-9999	45.8142	113.2093	0.09	3.20	
HRUS						
162	Range-Grasses --> RNGE/IA110/0-3	116.3167	287.4244	0.23	8.12	1
163	Range-Grasses --> RNGE/IA110/3-6	65.3696	161.5316	0.13	4.56	2
164	Range-Grasses --> RNGE/IA119/3-6	23.4613	57.9741	0.05	1.64	3
165	Range-Grasses --> RNGE/IA119/0-3	28.8186	71.2123	0.06	2.01	4
166	Range-Grasses --> RNGE/IA119/12-9999	45.8142	113.2093	0.09	3.20	5
167	Corn --> CORN/IA110/0-3	377.2548	932.2155	0.75	26.34	6
168	Corn --> CORN/IA110/3-6	284.6974	703.5015	0.57	19.88	7
169	Soybean --> SOYB/IA110/3-6	167.8260	414.7063	0.34	11.72	8
170	Soybean --> SOYB/IA110/0-3	322.7013	797.4110	0.65	22.53	9

SUBBASIN #		Area [ha]	Area[acres]	%wat.Area	%Sub.Area	
24		2083.3200	5147.9879	4.17		
LANDUSE:						
	Corn --> CORN	1287.1402	3180.5879	2.58	61.78	
	Soybean --> SOYB	796.1798	1967.4000	1.59	38.22	
SOILS:						
	IA110	1942.6997	4800.5081	3.89	93.25	
	IA111	140.6203	347.4797	0.28	6.75	
SLOPE:						
	3-6	821.0721	2028.9101	1.64	39.41	
	0-3	1262.2479	3119.0778	2.53	60.59	
HRUs						
171	Corn --> CORN/IA110/3-6	512.1178	1265.4688	1.02	24.58	1
172	Corn --> CORN/IA110/0-3	775.0224	1915.1191	1.55	37.20	2
173	Soybean --> SOYB/IA110/0-3	393.3052	971.8769	0.79	18.88	3
174	Soybean --> SOYB/IA110/3-6	262.2542	648.0433	0.52	12.59	4
175	Soybean --> SOYB/IA111/3-6	46.7000	115.3980	0.09	2.24	5
176	Soybean --> SOYB/IA111/0-3	93.9203	232.0818	0.19	4.51	6

SUBBASIN #		Area [ha]	Area[acres]	%wat.Area	%Sub.Area	
25		1347.3900	3329.4681	2.70		
LANDUSE:						
	Corn --> CORN	642.8289	1588.4624	1.29	47.71	
	Soybean --> SOYB	704.5611	1741.0056	1.41	52.29	
SOILS:						
	IA111	1347.3900	3329.4681	2.70	100.00	
SLOPE:						
	12-9999	250.4811	618.9514	0.50	18.59	
	0-3	356.4520	880.8107	0.71	26.45	
	6-9	290.8817	718.7832	0.58	21.59	
	3-6	449.5752	1110.9228	0.90	33.37	
HRUs						
177	Corn --> CORN/IA111/12-9999	121.2289	299.5626	0.24	9.00	1
178	Corn --> CORN/IA111/0-3	166.2949	410.9231	0.33	12.34	2
179	Corn --> CORN/IA111/6-9	134.7123	332.8807	0.27	10.00	3
180	Corn --> CORN/IA111/3-6	220.5928	545.0959	0.44	16.37	4
181	Soybean --> SOYB/IA111/12-9999	129.2522	319.3887	0.26	9.59	5
182	Soybean --> SOYB/IA111/3-6	228.9824	565.8268	0.46	16.99	6
183	Soybean --> SOYB/IA111/0-3	190.1571	469.8876	0.38	14.11	7
184	Soybean --> SOYB/IA111/6-9	156.1694	385.9025	0.31	11.59	8

SUBBASIN #		Area [ha]	Area[acres]	%wat.Area	%Sub.Area
26		534.0600	1319.6890	1.07	
LANDUSE:					
	Forest-Deciduous --> FRSD	134.8494	333.2197	0.27	25.25
	Range-Grasses --> RNGE	125.4381	309.9638	0.25	23.49
	Soybean --> SOYB	273.7725	676.5055	0.55	51.26
SOILS:					
	IA118	210.8839	521.1048	0.42	39.49

		IA119	162.7614	402.1916	0.33	30.48	
		IA110	66.0200	163.1388	0.13	12.36	
		IA115	94.3946	233.2538	0.19	17.67	
SLOPE :							
		12-9999	217.9581	538.5853	0.44	40.81	
		3-6	169.5305	418.9182	0.34	31.74	
		0-3	106.9030	264.1628	0.21	20.02	
		6-9	39.6684	98.0226	0.08	7.43	
HRUS							
185	Forest-Deciduous -->	FRSD/IA118/12-9999	32.8191	81.0977	0.07	6.15	1
186	Forest-Deciduous -->	FRSD/IA118/3-6	40.6413	100.4267	0.08	7.61	2
187	Forest-Deciduous -->	FRSD/IA118/0-3	30.9486	76.4756	0.06	5.79	3
188	Forest-Deciduous -->	FRSD/IA119/12-9999	30.4404	75.2197	0.06	5.70	4
189	Range-Grasses -->	RNGE/IA110/3-6	8.0486	19.8885	0.02	1.51	5
190	Range-Grasses -->	RNGE/IA110/6-9	3.7779	9.3354	0.01	0.71	6
191	Range-Grasses -->	RNGE/IA110/0-3	8.5414	21.1062	0.02	1.60	7
192	Range-Grasses -->	RNGE/IA115/12-9999	15.5132	38.3340	0.03	2.90	8
193	Range-Grasses -->	RNGE/IA115/3-6	5.5570	13.7316	0.01	1.04	9
194	Range-Grasses -->	RNGE/IA118/12-9999	30.2700	74.7986	0.06	5.67	10
195	Range-Grasses -->	RNGE/IA118/3-6	10.7467	26.5557	0.02	2.01	11
196	Range-Grasses -->	RNGE/IA119/3-6	9.9595	24.6105	0.02	1.86	12
197	Range-Grasses -->	RNGE/IA119/12-9999	24.6367	60.8786	0.05	4.61	13
198	Range-Grasses -->	RNGE/IA119/6-9	8.3870	20.7246	0.02	1.57	14
199	Soybean -->	SOYB/IA110/0-3	30.2964	74.8640	0.06	5.67	15
200	Soybean -->	SOYB/IA110/3-6	15.3557	37.9448	0.03	2.88	16
201	Soybean -->	SOYB/IA115/3-6	27.7910	68.6731	0.06	5.20	17
202	Soybean -->	SOYB/IA115/12-9999	12.7179	31.4267	0.03	2.38	18
203	Soybean -->	SOYB/IA115/0-3	17.5853	43.4541	0.04	3.29	19
204	Soybean -->	SOYB/IA115/6-9	15.2301	37.6344	0.03	2.85	20
205	Soybean -->	SOYB/IA118/6-9	12.2734	30.3282	0.02	2.30	21
206	Soybean -->	SOYB/IA118/3-6	25.5696	63.1838	0.05	4.79	22
207	Soybean -->	SOYB/IA118/12-9999	27.6152	68.2385	0.06	5.17	23
208	Soybean -->	SOYB/IA119/12-9999	43.9455	108.5915	0.09	8.23	24
209	Soybean -->	SOYB/IA119/0-3	19.5313	48.2629	0.04	3.66	25
210	Soybean -->	SOYB/IA119/3-6	25.8609	63.9037	0.05	4.84	26

		Area [ha]	Area[acres]	%wat.Area	%Sub.Area
SUBBASIN #	27	1945.9800	4808.6139	3.89	
LANDUSE :					
	Corn --> CORN	1129.8406	2791.8925	2.26	58.06
	Soybean --> SOYB	816.1394	2016.7213	1.63	41.94
SOILS :					
	IA111	1945.9800	4808.6139	3.89	100.00
SLOPE :					
	0-3	1064.5499	2630.5559	2.13	54.71
	3-6	881.4301	2178.0580	1.76	45.29
HRUS					
211	Corn --> CORN/IA111/0-3	611.6338	1511.3777	1.22	31.43
212	Corn --> CORN/IA111/3-6	518.2068	1280.5148	1.04	26.63
213	Soybean --> SOYB/IA111/3-6	363.2234	897.5432	0.73	18.67
214	Soybean --> SOYB/IA111/0-3	452.9160	1119.1782	0.91	23.27

		Area [ha]	Area[acres]	%wat.Area	%Sub.Area
SUBBASIN #	28	2113.2900	5222.0453	4.23	
LANDUSE :					
	Corn --> CORN	1191.9806	2945.4437	2.39	56.40
	Soybean --> SOYB	921.3094	2276.6016	1.84	43.60
SOILS :					
	IA110	757.8666	1872.7263	1.52	35.86
	IA111	1355.4234	3349.3190	2.71	64.14
SLOPE :					
	0-3	1210.2967	2990.7036	2.42	57.27
	3-6	902.9933	2231.3417	1.81	42.73
HRUS					

		Area [ha]	Area[acres]	%wat.Area	%Sub.Area	
SUBBASIN #	28	2113.2900	5222.0453	4.23		
LANDUSE:						
	Corn --> CORN	1191.9806	2945.4437	2.39	56.40	
	Soybean --> SOYB	921.3094	2276.6016	1.84	43.60	
SOILS:						
	IA110	757.8666	1872.7263	1.52	35.86	
	IA111	1355.4234	3349.3190	2.71	64.14	
SLOPE:						
	0-3	1210.2967	2990.7036	2.42	57.27	
	3-6	902.9933	2231.3417	1.81	42.73	
HRUS						
215	Corn --> CORN/IA110/0-3	309.4777	764.7348	0.62	14.64	1
216	Corn --> CORN/IA110/3-6	190.6028	470.9890	0.38	9.02	2
217	Corn --> CORN/IA111/3-6	320.0358	790.8246	0.64	15.14	3
218	Corn --> CORN/IA111/0-3	371.8643	918.8953	0.74	17.60	4
219	Soybean --> SOYB/IA110/0-3	152.1843	376.0550	0.30	7.20	5
220	Soybean --> SOYB/IA110/3-6	105.6019	260.9475	0.21	5.00	6
221	Soybean --> SOYB/IA111/3-6	286.7528	708.5806	0.57	13.57	7
222	Soybean --> SOYB/IA111/0-3	376.7704	931.0185	0.75	17.83	8
SUBBASIN #	29	531.9000	1314.3515	1.06		
LANDUSE:						
	Forest-Deciduous --> FRSD	206.1455	509.3959	0.41	38.76	
	Transportation --> UTRN	167.7610	414.5458	0.34	31.54	
	Residential-Med/Low Density --> URML	157.9935	390.4098	0.32	29.70	
SOILS:						
	IA115	139.1104	343.7488	0.28	26.15	
	IA118	124.4050	307.4111	0.25	23.39	
	IA119	204.8614	506.2228	0.41	38.52	
	IA110	63.5232	156.9689	0.13	11.94	
SLOPE:						
	3-6	180.6815	446.4730	0.36	33.97	
	12-9999	150.3794	371.5949	0.30	28.27	
	0-3	150.1199	370.9537	0.30	28.22	
	6-9	50.7193	125.3299	0.10	9.54	
HRUS						
223	Forest-Deciduous --> FRSD/IA115/3-6	21.2129	52.4182	0.04	3.99	1
224	Forest-Deciduous --> FRSD/IA115/12-9999	60.5276	149.5666	0.12	11.38	2
225	Forest-Deciduous --> FRSD/IA118/12-9999	59.0126	145.8232	0.12	11.09	3
226	Forest-Deciduous --> FRSD/IA118/3-6	24.3797	60.2436	0.05	4.58	4
227	Forest-Deciduous --> FRSD/IA118/0-3	41.0126	101.3443	0.08	7.71	5
228	Transportation --> UTRN/IA119/0-3	48.6848	120.3026	0.10	9.15	6
229	Transportation --> UTRN/IA119/3-6	77.8337	192.3309	0.16	14.63	7
230	Transportation --> UTRN/IA119/6-9	41.2425	101.9124	0.08	7.75	8
231	Residential-Med/Low Density --> URML/IA110/0-3	41.2459	101.9208	0.08	7.75	9
232	Residential-Med/Low Density --> URML/IA110/3-6	22.2772	55.0481	0.04	4.19	10
233	Residential-Med/Low Density --> URML/IA115/0-3	11.1112	27.4563	0.02	2.09	11
234	Residential-Med/Low Density --> URML/IA115/3-6	15.4196	38.1026	0.03	2.90	12
235	Residential-Med/Low Density --> URML/IA115/12-9999	30.8392	76.2051	0.06	5.80	13
236	Residential-Med/Low Density --> URML/IA119/0-3	8.0653	19.9298	0.02	1.52	14
237	Residential-Med/Low Density --> URML/IA119/6-9	9.4767	23.4175	0.02	1.78	15
238	Residential-Med/Low Density --> URML/IA119/3-6	19.5584	48.3297	0.04	3.68	16
SUBBASIN #	30	2690.5500	6648.4836	5.38		
LANDUSE:						
	Corn --> CORN	1537.5818	3799.4415	3.08	57.15	
	Soybean --> SOYB	1152.9682	2849.0421	2.31	42.85	

SOILS:		IA111	2690.5500	6648.4836	5.38	100.00	
SLOPE:		3-6	1145.4788	2830.5354	2.29	42.57	
		0-3	1545.0712	3817.9481	3.09	57.43	
HRUS							
239	Corn -->	CORN/IA111/3-6	647.3615	1599.6625	1.30	24.06	1
240	Corn -->	CORN/IA111/0-3	890.2203	2199.7789	1.78	33.09	2
241	Soybean -->	SOYB/IA111/0-3	654.8509	1618.1692	1.31	24.34	3
242	Soybean -->	SOYB/IA111/3-6	498.1174	1230.8729	1.00	18.51	4
			Area [ha]	Area[acres]	%wat. Area	%Sub. Area	
SUBBASIN #		31	155.9700	385.4097	0.31		
LANDUSE:							
	Forest-Deciduous -->	FRSD	70.1725	173.3999	0.14	44.99	
	Institutional -->	UINS	46.0377	113.7614	0.09	29.52	
	Residential-Med/Low Density -->	URML	39.7598	98.2484	0.08	25.49	
SOILS:		IA118	155.9700	385.4097	0.31	100.00	
SLOPE:		3-6	64.1690	158.5649	0.13	41.14	
		12-9999	71.0358	175.5330	0.14	45.54	
		0-3	11.5553	28.5536	0.02	7.41	
		6-9	9.2099	22.7581	0.02	5.90	
HRUS							
243	Forest-Deciduous -->	FRSD/IA118/3-6	34.3938	84.9888	0.07	22.05	1
244	Forest-Deciduous -->	FRSD/IA118/12-9999	35.7788	88.4111	0.07	22.94	2
245	Institutional -->	UINS/IA118/0-3	11.5553	28.5536	0.02	7.41	3
246	Institutional -->	UINS/IA118/12-9999	15.0402	37.1651	0.03	9.64	4
247	Institutional -->	UINS/IA118/3-6	19.4422	48.0426	0.04	12.47	5
248	Residential-Med/Low Density -->	URML/IA118/3-6	10.3331	25.5335	0.02	6.63	6
249	Residential-Med/Low Density -->	URML/IA118/12-9999	20.2168	49.9568	0.04	12.96	7
250	Residential-Med/Low Density -->	URML/IA118/6-9	9.2099	22.7581	0.02	5.90	8

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