



Geographic isolation of *Escherichia coli* genotypes in sediments and water of the Seven Mile Creek – A constructed riverine watershed

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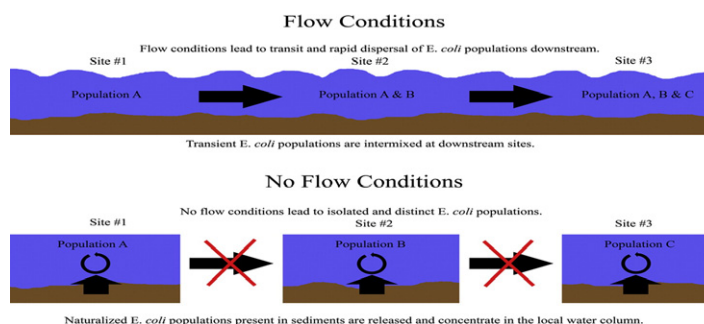
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HIGHLIGHTS

- Sediment *E. coli* are in dynamic equilibrium with the water column.
- Temperature and floods impact *E. coli* populations at the Seven Mile Creek.
- Geographic isolation results in the presence of unique *E. coli* genotypes.
- *E. coli* can grow and become naturalized to sediments and water at Seven Mile Creek.

GRAPHICAL ABSTRACT



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ABSTRACT

Escherichia coli is used to indicate fecal contamination in freshwater systems and is an indicator of the potential presence of human pathogens. However, naturalized *E. coli* strains that persist and grow in the environment confound the use of this bacterium as a fecal indicator. Here we examined the spatial and temporal distribution of *E. coli* in water and sediments of the Seven Mile Creek (SMC), a constructed, ephemeral watershed. *E. coli* concentrations showed variation by site and date, likely due to changes in temperature and rainfall. Horizontal fluorophore enhanced rep-PCR (HFERP) DNA fingerprint analyses indicated that *E. coli* populations were very diverse and consisted of transient and naturalized strains, which were especially prevalent in sediment. *E. coli* fingerprints from water and sediment collected in the same year clustered together with significant overlap, indicating exchange of strains between matrices. Isolates obtained during periods of flow, but not during non-flow conditions, clustered together regardless of sample site, indicating that transport between sites occurred. Naturalized *E. coli* strains were found in the SMC and strains become geographically isolated and distinct during non-flow conditions. Isolates collected during late spring to fall clustered together at each site, suggesting that temperature and growth of naturalized strains are likely factors affecting population dynamics. Results of this study show that newly introduced and naturalized *E. coli* strains are present in the SMC. Results of this study highlight an important concern for resource managers using this species for water quality monitoring.

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1. Introduction

Escherichia coli is a gram negative, rod-shaped, commensal bacterium originally thought to reside only in the lower intestine of warm-blooded animals, including humans (Leclerc et al., 2001; Savageau, 1983). The presence of *E. coli* in water has been used as an indicator of recent fecal contamination, and elevated concentrations may signal the presence of potential human pathogens, such as *Salmonella*, *E. coli* O157:H7, *Shigella*, *Cryptosporidium parvum*, and enteric viruses (U.S.EPA, 1986). Several epidemiological studies have reported a strong correlation between the densities of *E. coli* in water and the risk of gastrointestinal illness (Cabelli et al., 1982; Dufour, 1984b; Marion et al., 2010; U.S.EPA, 1986; Wiedenmann et al., 2006), providing support for the use of this bacterium as an indicator of fecal contamination in freshwater systems. Until recently, however, *E. coli* and other fecal indicator bacteria (FIB), such as enterococci, were thought to survive poorly in the environment. Consequently, elevated levels of *E. coli* in surface waters have often been attributed to runoff from feedlots and manure-amended agricultural land, septic system leakage, untreated sewage from sewer overflows, human recreation, wildlife and urban runoff (Booth et al., 2003; Chalmers et al., 1997; Cox et al., 2005; Coye and Goldoft, 1989; Dufour, 1984a; Haile et al., 1999; Novotny et al., 1985; Wells et al., 1991). However, studies reporting the growth and persistence of *E. coli* in various natural environments have been accumulating in recent years (Byappanahalli et al., 2003; Carrillo et al., 1985; Whitman and Nevers, 2003).

E. coli has been found to survive in tropical and subtropical soils of Hawaii (Byappanahalli, 2000; Byappanahalli and Fujioka, 1998; Hardina and Fujioka, 1991), Florida (Solo-Gabriele et al., 2000) and Guam (Fujioka et al., 1999). In the light of such studies, the potential of temperate environments to act as sinks for *E. coli* has also been examined. Byappanahalli et al. reported the persistence and growth of *E. coli* in soils and riparian sediments of Indiana and also in coastal forest soils from the Great Lakes watershed (Byappanahalli et al., 2003, 2006). Similarly, Ishii and coworkers provided evidence supporting the long-term survival and growth of *E. coli* in Lake Superior watersheds of Minnesota (Ishii et al., 2006a, 2007). In addition to soils and water, *E. coli* can be found to associate with the filamentous macroalga *Cladophora* (Ishii et al., 2006b; Whitman et al., 2003) and periphyton communities (Ksoll et al., 2007) also harbor large concentrations in the Great Lakes. *E. coli* inactivation rates vary depending on temperature, and also differ among types of water sources, such as rivers, streams, and lakes, agricultural waters and wastewaters (Blaustein et al., 2013).

Several field studies have shown that the DNA fingerprints of some *E. coli* strains isolated from soils were unique to specific environments and shared little or no genetic similarity with *E. coli* strains isolated from various host animals (Ishii et al., 2006a, 2007). Additionally, these strains were shown to be present at specific sites over an extended period of time (Ishii et al., 2006a). Taken together, these results strongly suggest that some *E. coli* strains multiply and survive in soils and are present as stable members of the soil microbial community. These strains have been referred to as naturalized *E. coli* (Ishii et al., 2006a). This has obvious ramifications for the use of this bacterium as an indicator for assessing fecal contamination for total maximum daily load (TMDL) determinations and fecal monitoring programs.

The survival of *E. coli* populations in the environment has been shown to depend on various factors such as pH and salinity (Carlucci and Pramer, 1960; Tassoula, 1997), environmental pollutants (Pathak and Bhattacharjee, 1994), temperature (Blaustein et al., 2013; Terzieva and McFeters, 1991), light (Pommepuy et al., 1992; Whitman and Nevers, 2003), predation (Bogosian et al., 1996; Brettar and Hofle, 1992), nutrients (Korhonen and Martikainen, 1991; Shelton et al., 2014), soil moisture content (Byappanahalli and Fujioka, 2004; Solo-Gabriele et al., 2000), organic matter content (Garzio-Hadzick et al., 2010) and soil texture (Desmarais et al., 2002; Ogden et al., 2001). Environmental *E. coli* can enter the water column via run-off

events, resuspension of sediment, and tidal cycles, resulting in an apparent increase in the observed levels of *E. coli* (Desmarais et al., 2002; Jamieson et al., 2005; Piorkowski et al., 2014; Solo-Gabriele et al., 2000). Resuspension of viable soil- or sediment-borne *E. coli* can also cause fecal bacterial counts to exceed state standards (An et al., 2002; Davies et al., 1995) and lead to unnecessary impairment designations and/or closures of recreational waters. Therefore, identifying the source(s) of microbial contamination of waterways requires a clear understanding of the ecology and behavior of fecal indicator bacteria and the various factors (hydro-climatologic conditions, physicochemical parameters and biological interactions) that influence their fate once they enter waterways.

The Seven Mile Creek (SMC) Watershed encompasses 95.3 km² and is located in the Minnesota River Basin, in south-central Minnesota between the small cities of Nicollet and St. Peter. The SMC is 9.8 km long and originates as a series of public drainage ditches. The watershed contains 86% cultivated lands and local interest in protecting the water quality of the Seven Mile Creek from excessive nitrates and fecal bacteria began in the 1980's.

The specific objectives of this study were to: 1) examine the genetic structure of *E. coli* populations obtained from water and sediment in the SMC watershed, 2) determine if these bacteria were newly acquired or naturalized to these environments, and 3) examine the potential factors influencing the distribution of *E. coli* populations present at SMC water and sediment.

2. Materials and methods

2.1. Site description

The SMC watershed is a part of the Middle Minnesota River Basin and is a minor watershed located in south central Minnesota, 22.5 km from Mankato and south of St. Peter, in Nicollet County. The majority of the watershed (about 86%) comprised cultivable lands devoted primarily to corn and soybean production. The remaining area consists of deciduous forest, grassland, wetlands, and animal feedlots. There are 24 feedlots for cattle, dairy cows and hogs located within the watershed. Manure from the feedlot operation is used for fertilizing agricultural fields and ~10–20% of the area within the watershed receives manure every year. Most rainfall to the SMC is received from April to July, and this, combined with snowmelt and runoff, is thought to cause manure from feedlots and fields to enter the creek. The SMC also receives input from three public drainage ditches. After July, the open drainage ditches often dry-up completely and do not supply water to the creek. Temperature and rainfall data for this study were obtained from the Brown Nicollet Cottonwood Water Quality Board and the State of Minnesota Climatology Office at the University of Minnesota (climate.umn.edu). For the purpose of this study, we chose four sites within the SMC watershed, designated as SM1–SM4 (Fig. 1), to examine in detail. These sites were chosen based on ease of sampling access and distance from water inputs along the length of the creek.

2.2. Sampling and *E. coli* enumeration

Sample collection at all four sites (SM1–SM4) took place from April (Spring) to October (Fall) in 2009 and 2010. Water samples were collected 15 to 30 cm below the water surface, in sterile 1 l screw-cap plastic bottles as previously described (Bordner et al., 1978). Depending on the water levels, several volumes (up to 4 l combined) were collected from each site at one sampling point – a portion of which (100 mL) was used for *E. coli* enumeration, and the remaining was shipped to the University of Minnesota for genotyping. Water and sediment samples were collected from a particular spot in each site, and triplicate samples from each 4-liter container were extracted for DNA.

Sediment samples were collected from the top 2 cm of the sediment layer in sterile, 207 ml Whirl-Pak bags (eNasco, Fort Atkinson, WI) using

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