



Seasonal dynamics of tetracycline resistance gene transport in the Sumas River agricultural watershed of British Columbia, Canada

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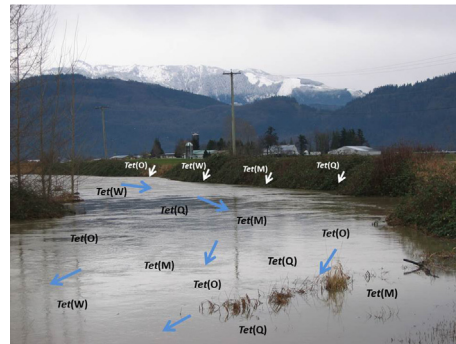
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HIGHLIGHTS

- Four tetracycline resistance genes measured in agricultural river system.
- Seasonal trends of tetracycline resistance genes and water quality were observed.
- Target genes higher in agricultural region than in forest control site.
- High relative abundance of tetracycline resistance genes in wet season months.
- Tetracycline resistance genes can be transported in stream networks.

GRAPHICAL ABSTRACT



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ABSTRACT

Environmental transport of contaminants that can influence the development of antibiotic resistance in bacteria is an important concern in the management of ecological and human health risks. Agricultural regions are locales where practices linked to food crop and livestock production can introduce contaminants that could alter the selective pressures for the development of antibiotic resistance in microbiota. This is important in regions where the use of animal manure or municipal biosolids as waste and/or fertilizer could influence selection for antibiotic resistance in pathogenic bacterial species. To investigate the environmental transport of contaminants that could lead to the development of antibiotic resistance in bacteria, a watershed with one of the highest levels of intensity of agricultural activity in Canada was studied; the Sumas River located 60 km east of Vancouver, British Columbia. This two-year assessment monitored four selected tetracycline resistance genes (*tet(O)*, *tet(M)*, *tet(Q)*, *tet(W)*) and water quality parameters (temperature, specific conductivity, turbidity, suspended solids, nitrate, phosphate and chloride) at eight locations across the watershed. The tetracycline resistance genes (Tc^r) abundances in the Sumas River network ranged between 1.47×10^2 and 3.49×10^4 copies/mL and ranged between 2.3 and 6.9 copies/mL in a control stream (located far from agricultural activities) for the duration of the study. Further, Tc^r abundances that were detected in the wet season months ranged between 1.3×10^3 and 2.29×10^4 copies/mL compared with dry season months (ranging between 0.6 and 31.2 copies/mL). Highest transport rates between 1.67×10^{11} and 1.16×10^{12} copies/s were observed in November 2005 during periods of high rainfall. The study showed that elevated concentrations of antibiotic resistance genes in the order of 10^2 – 10^4 copies/mL can move through stream networks in an agricultural watershed but seasonal variations strongly influenced specific transport patterns of these genes.

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1. Introduction

There is ever-growing concern over the role of the transport and distribution of environmental contaminants that create selective pressures at the genetic level to develop antibiotic resistance in indigenous bacteria. As such, receiving environments are now recognized as both a source and reservoir of genetic determinants of resistance (Alonso et al., 2001; Wright, 2007; Aminov, 2009; Levy, 2012) that can spread throughout wider environmental compartments extending the opportunities for genetic exchange of antibiotic resistance genes (ARGs) between bacteria (Séveno et al., 2002; Martinez, 2008; Davies and Davies, 2010). Some antibiotics and their resistance genes originate in nature (Cantón, 2009; Martinez, 2009); however, low levels of some environmental pollutants can alter selection pressures (Baquero and Coque, 2014) to enrich populations of antibiotic resistant bacteria and increase the risk to public health (Zdziarski et al., 2003; Pruden, 2014; Huijbers et al., 2015). Moreover, environmental contamination with trace metals can play a role in co-selection of antibiotic resistance in bacteria found in soil and water ecosystems (Baker-Austin et al., 2006; Seiler and Berendonk, 2012). Environmental contaminant transport affects processes that regulate the spread of ARGs (Kim et al., 2010) and there is an on-going need to better understand the fate and dynamics of antibiotic resistance in ecosystems (Suzuki et al., 2017). Evidence demonstrates that contaminant exposure can cause genetic stress responses that allow ARGs to be readily exchanged between pathogens and indigenous bacteria present in surface water, groundwater, biofilms, sediments and soils (Mackie et al., 2006; Baquero et al., 2008; Allen et al., 2010). Seasonal variability of non-point source pollutants in agricultural watersheds has been described (Udawatta et al., 2006; Poudel et al., 2013; Zhou et al., 2014).

Therefore, it is important to understand the seasonal variability in the flux of contaminants that could serve as indicators for potential presence of antibiotic resistant bacteria in agricultural regions particularly when the intensity and frequency of rainfall events changes seasonally. Use of antibiotics in veterinary medicine or in fruit tree pest control is an important source of agricultural drug residues (Kümmerer, 2009; McManus et al., 2002; Christiano et al., 2010). Metabolic processes often do not affect the biological activity of antibiotics, and thus land application of animal waste increases the likelihood of contaminant transport (Sarmah et al., 2006). This situation can be amplified by low levels of antibiotics used for the purposes of prophylaxis or growth promotion of food animals (Ghosh and LaPara, 2007). The presence of antibiotic compounds and ARGs in the environment related to agricultural practices have been documented (Halling-Sørensen et al., 1998; Yang and Carlson, 2003; McKinney et al., 2010); however, larger scale combined temporal-spatial studies of resistance gene transport remain limited.

This paper describes the results of a two-year study conducted 2004–2006 that examined the seasonal dynamics and environmental transport of tetracycline resistance (Tc^r) genes (as exemplars) in an agricultural watershed and stream network located near Vancouver, British Columbia, Canada. The overarching hypothesis was that abundances of Tc^r genes are higher in stream networks within agricultural regions and the transport of such genes is influenced by seasonal patterns. Quantitative PCR (qPCR) was used to measure relative abundance of four common Tc^r determinants (*tet(O)*, *tet(M)*, *tet(Q)*, *tet(W)*) and 16S rRNA genes in bacteria from water samples collected 20 cm beneath the water surface on a monthly basis. In addition, standard water quality parameters including temperature, specific conductivity, turbidity, suspended solids, nitrate, phosphate and chloride were monitored in the receiving water bodies to investigate possible correlations of key indicators of contamination with the observed abundance of the selected tetracycline resistance genes. The potential for transport of Tc^r genes along a defined segment of the Sumas River was examined by measuring the flow rate and calculating mass flux of the specific indicator genes under varying stream discharge conditions.

2. Materials and methods

2.1. Study location

This study was conducted in the Sumas watershed located 60 km east of Vancouver, British Columbia, Canada between July 2004 and March 2006. The watershed represents approximately 5700 ha of one of the most economically important areas in Canada for production of poultry, dairy, hogs, fruit and vegetable and nursery farms (Schreier et al., 2001). The segment of the Sumas River investigated here is a second order stream (branch of the Fraser River system which eventually discharges into the Pacific Ocean) that flows northward towards the Fraser River from the Canada–US border (Fig. 1). Some tributaries and canals feed into the Sumas River; eight sampling sites were chosen along an approximately 23 km stretch of the Sumas River stream network at elevations that ranged between 7 and 11 m above sea level (Environment Canada Topographic Data, 2017). The study location was specifically selected to represent agricultural activities in the region with all 8 sampling stations located within 200 m of dairy farms, poultry barns or field food crops. In addition to these sample sites, one control site, situated at 1050 m above sea level on a stream flowing from a forested headwater on the eastern boundary of the watershed (approximately 8 km east of the study location), was designated as a reference site where chemical analyses of chloride, nitrate, phosphate and trace metals revealed negligible possibility of urban or agricultural influence on water quality.

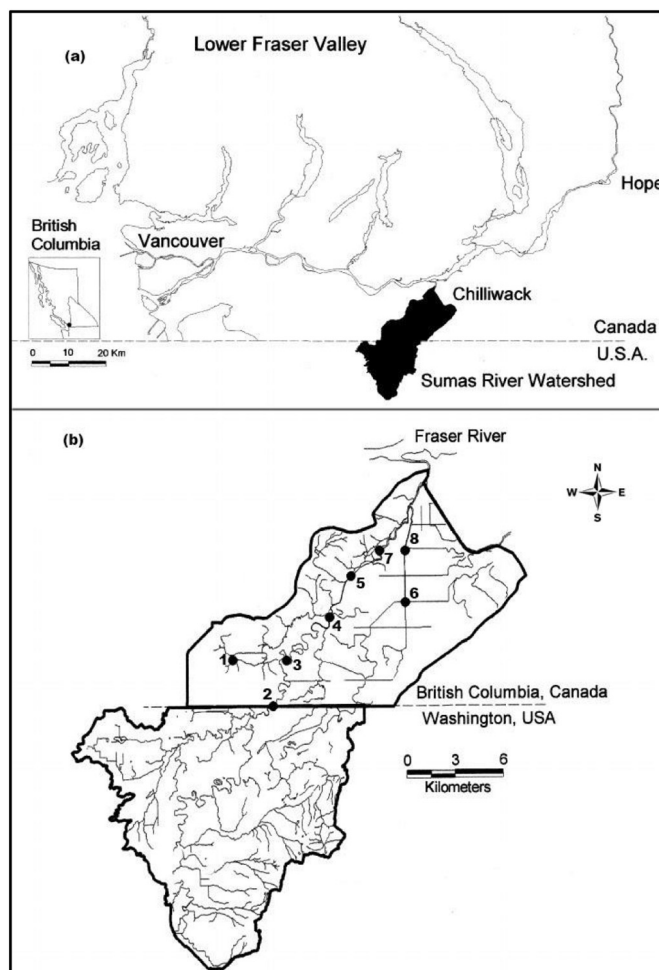


Fig. 1. Map showing (a) geographic location of Sumas River Watershed in British Columbia, Canada with (b) sampling site locations (sites 2–5 used for flux measurements). Map used with permission (Berka et al., 2001).

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