

Keen PL, Knapp CW, Hall KJ, Graham DW.

[Seasonal dynamics of tetracycline resistance gene transport in the Sumas River agricultural watershed of British Columbia, Canada.](#)

Science of the Total Environment 2018, 628-629, 490-498.

Copyright:

© 2018. This manuscript version is made available under the [CC-BY-NC-ND 4.0 license](#)

DOI link to article:

<https://doi.org/10.1016/j.scitotenv.2018.01.278>

Date deposited:

24/02/2018

Embargo release date:

13 February 2019



This work is licensed under a

[Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International licence](#)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19

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

Patricia L. Keen^{1}, Charles W. Knapp², Kenneth J. Hall¹, David W. Graham³*

¹ Department of Civil Engineering, University of British Columbia,
Vancouver, BC, Canada V5L 3B6

² Civil and Environmental Engineering, University of Strathclyde
Glasgow, UK G1 1XJ

³ School of Engineering, Newcastle University
Newcastle upon Tyne, UK NE1 7RU

***Author to whom correspondence may be addressed:** Patricia L. Keen, Department of Civil
Engineering, University of British Columbia, c/o 2049 Graveley Street, Vancouver, British
Columbia, Canada V5L 3B6

Phone +1 604 418-0185 plkeenpl@civil.ubc.ca

20 **ABSTRACT:**

21 Environmental transport of contaminants that can influence the development of antibiotic
22 resistance in bacteria is an important concern in the management of ecological and human health
23 risks. Agricultural regions are locales where practices linked to food crop and livestock
24 production can introduce contaminants that could alter the selective pressures for the
25 development of antibiotic resistance in microbiota. This is important in regions where the use of
26 animal manure or municipal biosolids as waste and/or fertilizer could influence selection for
27 antibiotic resistance in pathogenic bacterial species. To investigate the environmental transport
28 of contaminants that could lead to the development of antibiotic resistance in bacteria, a
29 watershed with one of the highest levels of intensity of agricultural activity in Canada was
30 studied; the Sumas River located 60 km east of Vancouver, British Columbia. This two-year
31 assessment monitored four selected tetracycline resistance genes (*tet(O)*, *tet(M)*, *tet(Q)*, *tet(W)*)
32 and water quality parameters (temperature, specific conductivity, turbidity, suspended solids,
33 nitrate, phosphate and chloride) at eight locations across the watershed. The tetracycline
34 resistance genes (Tc^r) abundances in the Sumas River network ranged between 1.47×10^2 and
35 3.49×10^4 copies/mL and ranged between 2.3 and 6.9 copies/mL in a control stream (located far
36 from agricultural activities) for the duration of the study. Further, Tc^r abundances that were
37 detected in the wet season months ranged between 1.3×10^3 and 2.29×10^4 copies/mL compared
38 with dry season months (ranging between 0.6 and 31.2 copies/mL). Highest transport rates
39 between 1.67×10^{11} and 1.16×10^{12} copies/sec were observed in November 2005 during periods
40 of high rainfall. The study showed that elevated concentrations of antibiotic resistance genes in
41 the order of $10^2 - 10^4$ copies/mL can move through stream networks in an agricultural watershed
42 but seasonal variations strongly influenced specific transport patterns of these genes.

43 **KEYWORDS:** antibiotic resistance genes; tetracycline; environment; transport; agriculture;
44 seasonality.

45

46 **1 INTRODUCTION**

47 There is ever-growing concern over the role of the transport and distribution of environmental
48 contaminants that create selective pressures at the genetic level to develop antibiotic resistance in
49 indigenous bacteria. As such, receiving environments are now recognized as both a source and
50 reservoir of genetic determinants of resistance (Alonso et al., 2001; Wright, 2007; Aminov,
51 2009; Levy, 2012) that can spread throughout wider environmental compartments extending the
52 opportunities for genetic exchange of antibiotic resistance genes (ARGs) between bacteria
53 (Séveno et al., 2002; Martinez, 2008; Davies and Davies, 2010). Some antibiotics and their
54 resistance genes originate in nature (Cantón, 2009; Martinez, 2009); however, low levels of some
55 environmental pollutants can alter selection pressures (Baquero and Coque, 2014) to enrich
56 populations of antibiotic resistant bacteria and increase the risk to public health (Zdziarski et al.,
57 2003; Pruden, 2014; Huijbers et al., 2015). Moreover, environmental contamination with trace
58 metals can play a role in co-selection of antibiotic resistance in bacteria found in soil and water
59 ecosystems (Baker-Austin, 2006; Seiler and Berendonk, 2012). Environmental contaminant
60 transport affects processes that regulate the spread of ARGs (Kim et al., 2010) and there is an on-
61 going need to better understand the fate and dynamics of antibiotic resistance in ecosystems
62 (Suzuki et al., 2017). Evidence demonstrates that contaminant exposure can cause genetic stress
63 responses that allow ARGs to be readily exchanged between pathogens and indigenous bacteria
64 present in surface water, groundwater, biofilms, sediments and soils (Mackie et al., 2006;

65 Baquero et al., 2008; Allen et al., 2010). Seasonal variability of non-point source pollutants in
66 agricultural watersheds has been described (Udawatta et al., 2006; Poudel et al., 2013; Zhou et
67 al., 2014).

68 Therefore, it is important to understand the seasonal variability in the flux of contaminants that
69 could serve as indicators for potential presence of antibiotic resistant bacteria in agricultural
70 regions particularly when the intensity and frequency of rainfall events changes seasonally. Use
71 of antibiotics in veterinary medicine or in fruit tree pest control is an important source of
72 agricultural drug residues (Kümmerer, 2009; McManus et al., 2002; Christiano et al. 2010).
73 Metabolic processes often do not affect the biological activity of antibiotics, and thus land
74 application of animal waste increases the likelihood of contaminant transport (Sarmah et al.,
75 2006). This situation can be amplified by low levels of antibiotics used for the purposes of
76 prophylaxis or growth promotion of food animals (Ghosh and LaPara, 2007). The presence of
77 antibiotic compounds and ARGs in the environment related to agricultural practices have been
78 documented (Halling-Sørensen et al., 1998; Yang and Carlson, 2003; McKinney et al., 2010);
79 however, larger scale combined temporal-spatial studies of resistance gene transport remain
80 limited.

81 This paper describes the results of a two-year study conducted 2004–2006 that examined the
82 seasonal dynamics and environmental transport of tetracycline resistance (Tc^r) genes (as
83 exemplars) in an agricultural watershed and stream network located near Vancouver, British
84 Columbia, Canada. The overarching hypothesis was that abundances of Tc^r genes are higher in
85 stream networks within agricultural regions and the transport of such genes is influenced by
86 seasonal patterns. Quantitative PCR (qPCR) was used to measure relative abundance of four

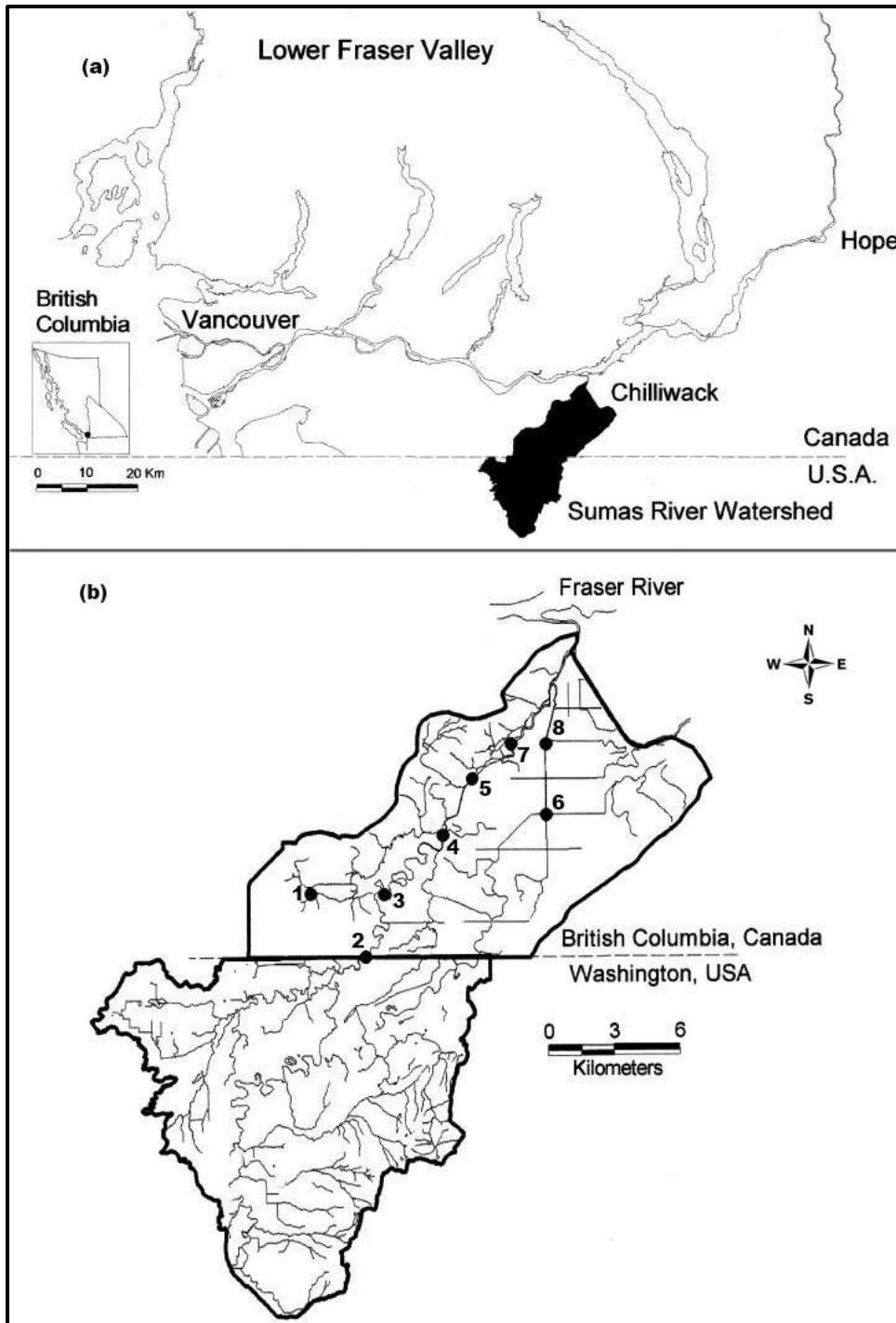
87 common Tc^f determinants (*tet(O)*, *tet(M)*, *tet(Q)*, *tet(W)*) and 16S rRNA genes in bacteria from
88 water samples collected 20 cm beneath the water surface on a monthly basis. In addition,
89 standard water quality parameters including temperature, specific conductivity, turbidity,
90 suspended solids, nitrate, phosphate and chloride were monitored in the receiving water bodies to
91 investigate possible correlations of key indicators of contamination with the observed abundance
92 of the selected tetracycline resistance genes. The potential for transport of Tc^f genes along a
93 defined segment of the Sumas River was examined by measuring the flow rate and calculating
94 mass flux of the specific indicator genes under varying stream discharge conditions.

95 **2 MATERIALS AND METHODS**

96 *2.1 Study Location*

97 This study was conducted in the Sumas watershed located 60 km east of Vancouver, British
98 Columbia, Canada between July 2004 and March 2006. The watershed represents approximately
99 5,700 hectares of one of the most economically important areas in Canada for production of
100 poultry, dairy, hogs, fruit and vegetable and nursery farms (Schreier et al., 2001). The segment
101 of the Sumas River investigated here is a second order stream (branch of the Fraser River system
102 which eventually discharges into the Pacific Ocean) that flows northward towards the Fraser
103 River from the Canada – US border (Figure 1). Some tributaries and canals feed into the Sumas
104 River; eight sampling sites were chosen along an approximately 23 km stretch of the Sumas
105 River stream network at elevations that ranged between 7 and 11 m above sea level
106 (Environment Canada Topographic Data, 2017). The study location was specifically selected to
107 represent agricultural activities in the region with all 8 sampling stations located within 200 m of
108 dairy farms, poultry barns or field food crops. In addition to these sample sites, one control site,

109 situated at 1,050 m above sea level on a stream flowing from a forested headwater on the eastern
110 boundary of the watershed (approximately 8 km east of the study location), was designated as a
111 reference site where chemical analyses of chloride, nitrate, phosphate and trace metals revealed
112 negligible possibility of urban or agricultural influence on water quality.



113
114

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

118 **2.2 Sample collection and processing**

119 Samples for molecular microbial analyses (in quadruplicate) were collected in acid-washed,
120 autoclaved 250 mL amber glass bottles and stored on ice prior to returning to the laboratory. In
121 total, 684 samples were collected and analyzed on an approximately monthly basis over the
122 course of the study period. Field measurements of temperature and conductivity (reported as
123 specific conductivity) were determined at each sampling station using a Yellow Springs
124 Instrument (YSI) Model #30M/50 meter. Dissolved oxygen was measured *in situ* with an YSI
125 Model #58 portable meter and turbidity was measured using a Hach model 2100P portable
126 turbidometer.

127 ***2.3 DNA Extraction***

128 Samples for molecular microbial analyses (transported on ice) were filtered within two hours of
129 sample collection with the filters immediately frozen on dry ice. The filters were shipped within
130 24hrs to the Department of Civil, Architectural and Environmental Engineering at the University
131 of Kansas, Lawrence, Kansas where they remained frozen at -20°C until extraction which was
132 conducted monthly between July 2004 and March 2006. One hundred mL volumes of samples
133 were filtered through pre-sterilized 0.22 µm porosity Nalgene disposable filter funnels (NNI,
134 Rochester NY). Filters from replicates were extracted using MoBio UltraClean Soil DNA kits
135 (Solona Beach, CA) with minor method modifications recommended by the kit manufacturer
136 (MoBio Laboratories, 2004). Filters, beads and extraction buffers were combined, homogenized
137 for 30 seconds (speed 5.5) using a FastPrep (Qbiogene, Irvine, CA) cell disruptor and then
138 incubated at 70°C for 10 minutes to enhance lysis of Gram-positive bacteria. Following
139 incubation, samples were re-agitated for 30 seconds (speed 4.5) and subjected to the further
140 purification steps of the kit manufacturer's protocol. All resulting 50 µL extracts were stored at -

141 20°C prior to analysis. Three replicates were analysed by qPCR and one sample replicate was
142 saved at -80°C for archival purposes.

143 **2.4 qPCR assays**

144 Four common Tc^r genes ((*tet(M)*, *tet(O)*, *tet(Q)*, and *tet(W)*) and 16S rRNA genes were selected
145 for quantification by qPCR analyses. These four Tc^r genes were specifically chosen for
146 comparison with parallel studies examining antibiotic resistance in a US feedlot (Peak et al.,
147 2007; Engemann et al., 2008). The TaqMan probe/primer sets and the plasmid standards used in
148 this investigation have been described previously for *tet(M)* (Peak et al., 2007), *tet(O)*, *tet(Q)* and
149 *tet(W)* (Smith et al., 2004) and for 16S-rRNA gene (Harms et al., 2003). Sample aliquots of 2
150 µL DNA templates were mixed with iQ Supermix PCR reagents (BioRad, Hercules, CA) and
151 500 nM of each primer. A BioRad iCycler equipped with an iCycler iQ fluorescence detector
152 was used for the reactions. Standard curves were constructed from quantification of copy
153 numbers for each gene prepared by 10 –fold serial dilution of the extracted samples for
154 appropriate plasmid DNA that ranged from 1.0 to 1 x 10⁷ copies per reaction in order to optimize
155 PCR reactions and minimize impact of inhibitors that may have been carried over during DNA
156 extraction (Smith et al., 2004). The qPCR efficiencies ranged between 95–105 % as determined
157 by comparing serial dilutions of some selected samples using the 16S-rRNA assay. Reaction
158 specificities were verified using melt curves (55–95°C). All samples were analysed in triplicate
159 and reported abundances represent the arithmetic mean of three measurements.

160

161 **2.5 Water Quality Analyses**

162 Total solids (for determining dry weight sample equivalents where necessary) were measured
163 using method 2540 B (dried at 105°C) (APHA, 1995) and suspended solids were measured by
164 method 2540 C (dried at 105°C) (APHA, 1995). Chloride concentration was measured by
165 mercuric thiocyanate flow injection analysis using standard method 4500-Cl⁻ G (APHA, 1995).
166 Samples collected for nitrate/nitrite and phosphate measurements were preserved in the field
167 immediately with 0.1 g/100 ml phenylmercuric acetate in 20% (v/v) acetone. Cadmium
168 reduction flow injection analysis was used to measure nitrate/nitrite using standard method 4500-
169 NO₃⁻ I (APHA, 1995) and phosphate was measured by flow injection analysis for orthophosphate
170 using method 4500-P G (APHA, 1995). Only for the pre-screening for suitability of candidate
171 control sites, 29 – element ICP – OES (inductively coupled plasma optical emission
172 spectrometry) scans were conducted using an Optima 7300 V ICP-OES (Perkin Elmer).

173 ***2.6 Estimating Transport of Antibiotic Resistance Genes***

174 In order to quantify the mass flux of the four Tc^r genes along a specified ~12.6 km segment of
175 the watercourse network within the Sumas watershed (see Figure 1b), stream velocities were
176 measured monthly at four sentinel stations (sampling sites 2, 3, 4 and 5) between July and
177 December 2005. A Swiffer Model 3000 flow meter was used to measure the velocities at 3-5
178 points across the cross-section of the stream (either by wading across the width of the stream or
179 using an inflatable canoe when stream depth increased) at varying depths (Site 2: 0.47–1.13 m;
180 Site 3: 0.77–0.95 m; Site 4: 0.72–1.07 m; Site 5: 0.39–0.51 m). Volumetric flow (Q) was
181 calculated using the equation $Q \text{ (m}^3\text{/sec)} = vA$ and assuming uniform velocity (v) at 0.3 times
182 depth (Chanson, 2003) and that depth was uniform across estimated cross-sectional area (A).

183

184 **2.7 Data Analyses**

185 All Tc^r quantification values were normalized to 16S rRNA gene abundances to account for the
186 differences in background bacterial abundances and for any variations in the extraction
187 efficiencies. Statistical analyses of the differences between Tc^r gene abundances were performed
188 using SPSS (v 13.01, Chicago, IL) data analysis software. The data were log-transformed with
189 means and 95% confidence intervals calculated for use as the statistical descriptors for resistance
190 gene abundances. The differences between the means were assessed by paired Mann-Whitney U
191 tests and were considered significant at $p < 0.05$. Observations described as occurring during
192 dry season were measured between April and October and those described as occurring in wet
193 season were measured between November and March.

194 Spearman's Rank correlations (2-tailed) were calculated using SPSS statistical software Version
195 13.01 to compare water quality parameters with tetracycline resistance gene abundances over the
196 period of July 2004 and March 2006.

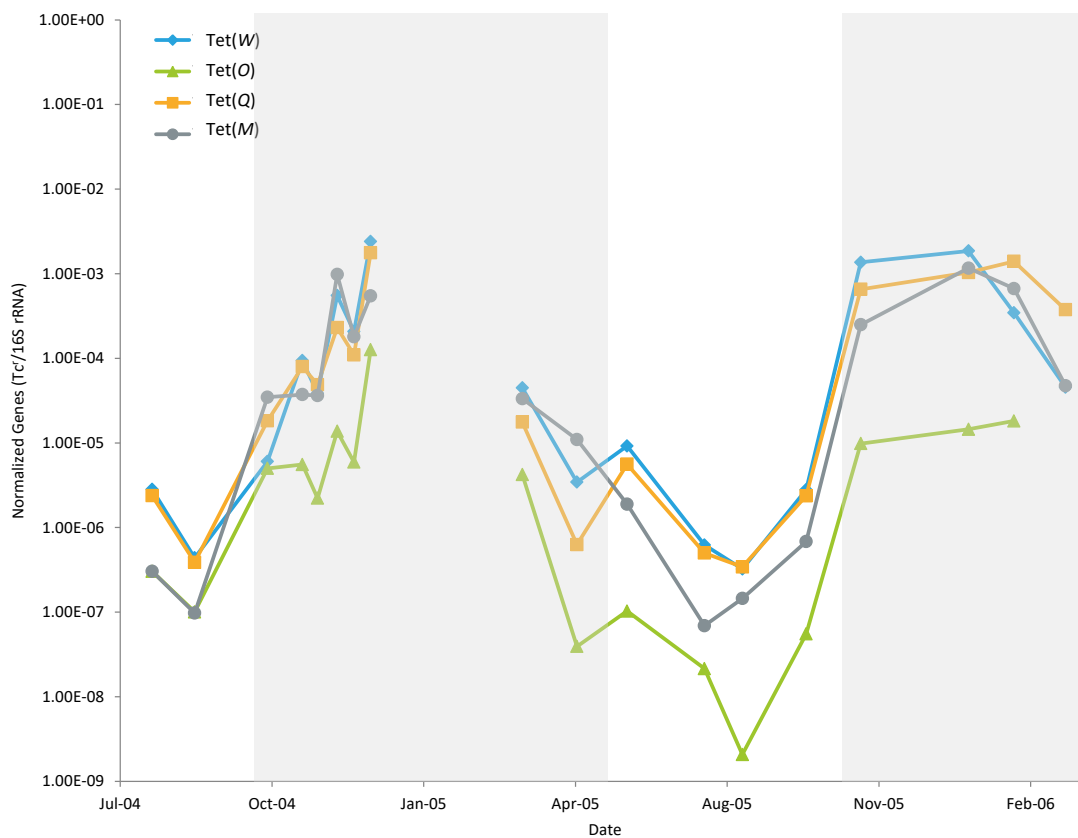
197 **3 RESULTS**

198 ***3.1 Quantification of Tetracycline Resistance Genes***

199 All four of the selected Tc^r genes were detected at the sampling locations along the segment of
200 the Sumas River during every month that was monitored (Figure 2) although the proportion of
201 each gene relative to the total gene abundance was variable (Figure 3). On five sampling
202 occasions (Nov 8, 2004; Dec 2, 2004; Apr 28 2005; Nov 1, 2005 and Feb 10, 2006), Tc^r genes
203 were also detected at the reference control site although the measured abundance of total Tc^r
204 genes was very low (2.3–6.9 gene copies/mL). Microbial abundance, as indicated by

205 measurement of concentration of 16S rRNA genes, ranged between 5.81×10^5 and 1.93×10^7
 206 copies/mL among all stream sites during the monitoring period and was significantly higher ($p <$
 207 0.05) than that at the control site (ranging between 4.47×10^4 and 5.21×10^4 copies/mL).

208
 209



210
 211

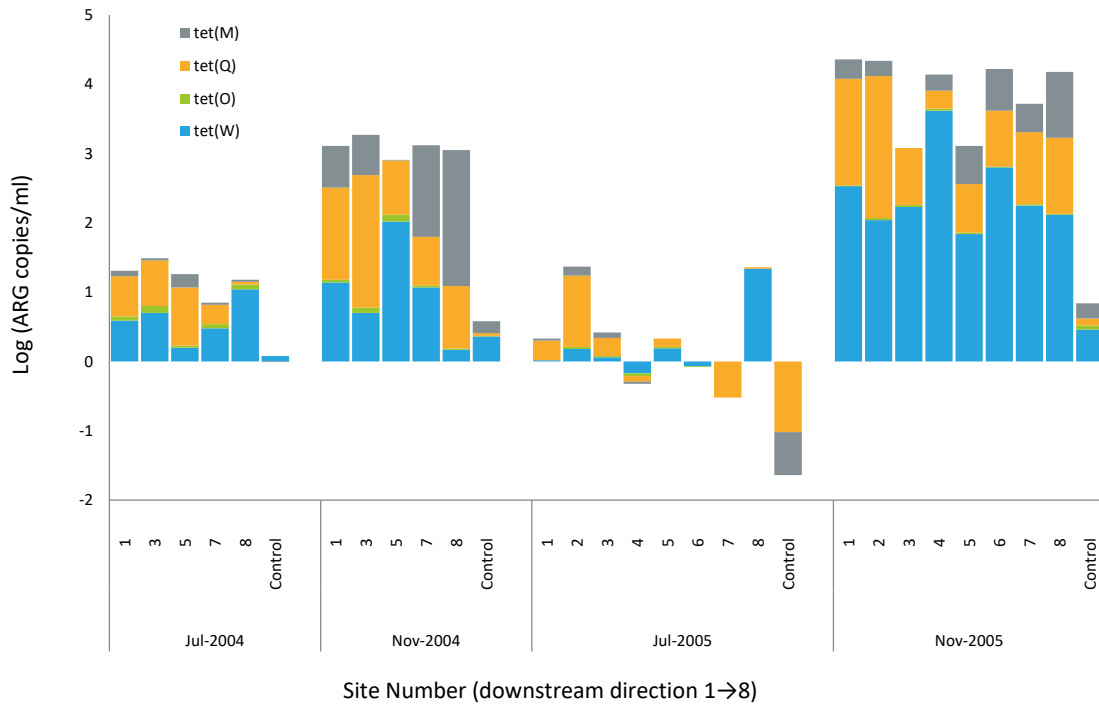
212 Figure 2: Average Tc^f gene relative abundance (Tc^f gene copies per 16S rRNA gene copies) for
 213 the sampling sites for the period of July 2004 – March 2006. Relative abundances of Tc^f genes
 214 were significantly higher ($p < 0.05$) in November 2004 and November 2005 than in other months.
 215 Sites were not monitored in Jan and Feb 2005. Shaded areas represent wet season.

216

217 In the Sumas stream network, seasonal trends of Tc^r gene relative abundances were observed
218 (Figure 2) and occurred similarly each year. The four measured Tc^r genes were normalized to
219 16S rRNA and the average was calculated for all sites along the river segment to demonstrate the
220 observed trend. The lowest gene abundances were observed during the dry season ranging
221 between 3.5 and 28.6 copies/mL in 2004 and ranging between 0.6 and 31.2 copies/mL in 2005.
222 Higher abundance ($p < 0.05$) of total Tc^r genes occurred in wet season months of November (1.3
223 $\times 10^3 - 2.15 \times 10^4$ copies/mL in 2004; $4.76 \times 10^3 - 2.29 \times 10^4$ copies/mL in 2005) and December
224 ($4.89 \times 10^2 - 2.26 \times 10^3$ copies/mL in 2004; $1.47 \times 10^2 - 4.51 \times 10^2$ copies/mL in 2005) and in
225 January to March 2006 ($1.16 \times 10^3 - 3.49 \times 10^4$ copies/mL), representing sampling events that
226 coincided with periods of unusually high precipitation in the Sumas watershed region. For
227 comparison purposes, the official stream discharge rates and rainfall measurements recorded by
228 Environment Canada for the study period are presented in Figure 4.

229 Figure 3 illustrates the abundance profile for the individual Tc^r genes for two specific months of
230 both 2004 and 2005. The proportion of the individual genes relative to the total gene abundance
231 varied from month to month during the study period although some trends were observed. In
232 general, the gene profile was dominated by Tet(*W*), Tet(*Q*) and Tet(*M*) with the abundance of
233 Tet(*O*) being much smaller throughout the duration of the study. November was selected as an
234 example wet season month to demonstrate that despite differences in rainfall and stream
235 discharge rate (Figure 4), some similarity of the Tc^r gene profile could be observed for each
236 consecutive year. July was selected as an example of a dry season month in which no general
237 trend of the abundance profile for individual Tc^r genes could be discerned although the total
238 number of genes measure was low.

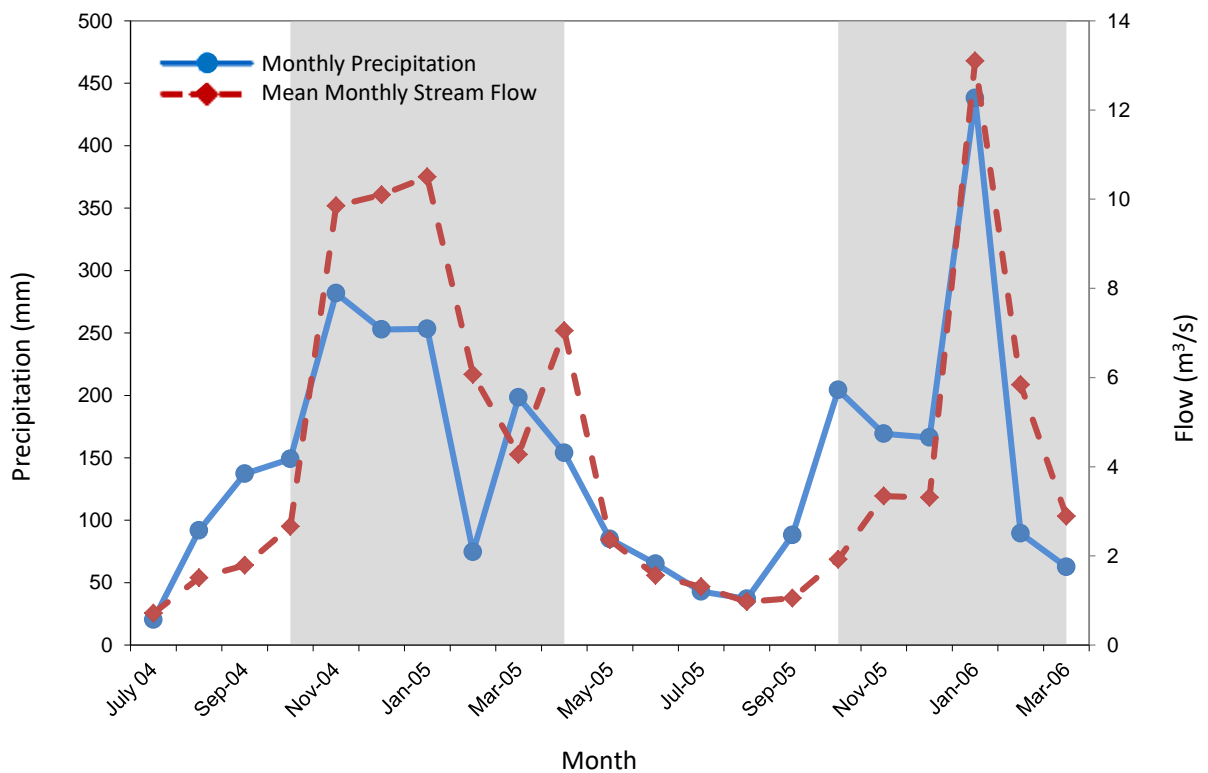
239



240

241 Figure 3: Tetracycline resistance gene abundance profile (average of three replicates) for sites in
 242 the Sumas River stream network comparing July and November in 2004 and 2005. Relative
 243 composition (% total) is represented by segments within each bar (with bar height representing
 244 100% of the log-transformed sum of the four Tc^r genes).

245



246

247 Figure 4: Total monthly precipitation and mean monthly stream discharge in Sumas watershed
 248 (Environment Canada Climate Weather Data; accessed May 2006). Shaded areas represent wet
 249 season.

250

251 3.2 Water Quality Measurements

252 Measurements of water quality are summarized in Table 1. Specific conductivity, nitrate, and
 253 chloride were significantly higher in the Sumas River network ($p < 0.05$) than in the control
 254 stream for both wet season months and dry season months while orthophosphate was
 255 significantly higher during the wet season months. The standard method used for analyses of
 256 nitrogen species in water samples measured both the nitrite and nitrate combined. Nitrite is

257 rarely measured in appreciable concentrations in surface water samples (Ellis, 1989) and thus all
 258 reported concentrations represent nitrate concentrations.

259 Table 1: Concentration of different physico-chemical variables and total tetracycline resistance
 260 genes measured during wet and dry seasons (* indicates statistical significant differences
 261 between the Sumas River network and the control stream at $p < 0.05$).

262

		Wet Season	Control	Dry Season	Control
Temperature (°C)	<i>average</i>	7.7	6.4	19.8	18.7
	<i>Std dev</i>	2.6	2.5	4.6	4.3
Dissolved Oxygen (mg/L)	<i>average</i>	7.8	12.5	9.1	10.5
	<i>Std dev</i>	2.3	1.1	3.3	1.8
Specific Conductivity (µS/cm)	<i>average</i>	236*	72	308*	138
	<i>Std dev</i>	64.5	67.3	24.7	17.1
Turbidity (NTU)	<i>average</i>	19.5	8.1	5.0	1.4
	<i>Std dev</i>	19.3	5.9	4.5	1.2
Suspended Solids (mg/L)	<i>average</i>	15.4	14.1	4.9	2.3
	<i>Std dev</i>	13.0	10.0	4.5	2.1
NO _x -N (mg/L)	<i>average</i>	3.3*	0.4	2.6*	0.7
	<i>Std dev</i>	1.2	0.06	1.3	0.6
PO ₄ -P (µg/L)	<i>average</i>	99.1*	4.89	31.7	6.5
	<i>Std dev</i>	13.0	0.3	25.3	5.9
Cl ⁻ (mg/L)	<i>average</i>	11.6*	1.0	14.7*	0.7
	<i>Std dev</i>	3.9	0.5	2.1	0.2
Total Tc ^f Genes (copies/mL)	<i>average</i>	5540*	0.48	11.4	2.95
	<i>Std dev</i>	6564	0.45	17.8	2.60

263

264 Certain statistical relationships (Spearman's rank correlations) exist between Tc^f gene
 265 abundances and relative Tc^f gene abundances (normalized to 16S rRNA) and selected water
 266 quality parameters, stream discharge and precipitation for sites located along the main stream

267 channel of the Sumas River. Spearman rank correlations were positive ($p < 0.05$) between both
268 Tc^r genes (absolute and relative abundances) and instantaneous discharge, 48 h discharge, 72 h
269 rainfall and turbidity (Table 2) during the monitoring period (illustrated in Figure 4). The range
270 of Spearman's rho calculated for the positive correlations for each of the sites along the stream
271 network comparing abundance of Tc^r genes with instantaneous discharge was $0.762 < \rho < 0.810$;
272 comparing abundance of Tc^r genes with 48 hour discharge was $0.653 < \rho < 0.810$; comparing
273 abundance of Tc^r genes with 72 hour rainfall was $0.502 < \rho < 0.561$; and comparing abundance
274 of Tc^r genes with turbidity was $0.577 < \rho < 0.800$. Significant negative correlations were
275 established for Tc^r genes and chloride concentration, specific conductivity and temperature. The
276 range of Spearman's rho calculated for the negative correlations for each of the sites along the
277 stream network comparing abundance of Tc^r genes with chloride was $-0.870 < \rho < -0.788$;
278 comparing abundance of Tc^r genes with specific conductivity was $-0.832 < \rho < -0.512$; and
279 comparing abundance of Tc^r genes with temperature was $-0.885 < \rho < -0.524$. There were no
280 statistically significant correlations between the average of 16S rRNA genes and any of the
281 measured parameters ($-0.408 < \rho < 0.395$) at the control site. Table 2 summarizes the
282 calculated Spearman's rank correlations for the parameters for which data analyses of the
283 individual sites revealed significant correlations at $p < 0.05$.

284

285 Table 2: Spearman rank correlation table for all sites along the Sumas River between total
286 tetracycline resistance genes and total tetracycline resistance genes normalized to 16S rRNA
287 genes compared with water quality and quantity parameters. Two-tailed significance is indicated
288 in parentheses.

	Instantaneous discharge	48 hour discharge	72 hour rainfall	Temp	Turbidity	Specific conductivity	Chloride	Total Tc^r genes	Tc^r genes normalized to 16S rRNA genes
Instantaneous discharge	1.000								
48 hour discharge	0.959	1.000							
72 hour rainfall	0.553 (0.026)	0.603 (0.013)	1.000						
Temperature	-0.648 (0.009)	-0.741 (0.002)	-0.39 (0.109)	1.000					
Turbidity	0.700 (0.004)	0.743 (0.004)	0.544 (0.020)	-0.520 (0.027)	1.000				
Specific conductivity	-0.579 (0.024)	-0.443 (0.098)	-0.430 (0.075)	No significant correlation	-0.493 (0.038)	1.000			
Chloride	-0.302 (0.029)	-0.609 (0.010)	No significant correlation	No significant correlation	-0.254 (0.032)	0.478 (0.053)	1.000		
Total Tc^r genes	0.621 (0.013)	0.661 (0.007)	0.482 (0.043)	-0.605 (0.008)	0.534 (0.023)	-0.613 (0.007)	-0.513 (0.035)	1.000	
Tc^r genes normalized to 16S rRNA genes	0.578 (0.030)	0.556 (0.039)	0.571 (0.017)	-0.631 (0.007)	0.611 (0.007)	-0.751	-0.683 (0.002)	0.924	1.000

290 **3.3 Estimation of Mass Transport of Tetracycline Resistance Genes**

291 The Table 3 summarizes the calculated mass flux of the total tetracycline resistance genes
292 measured along a finite continuous segment of the Sumas River (approximately 12.6 km). Four
293 sites were chosen (see Figure 1) at locations near to farm facilities or large crop fields. Site 2
294 was located within about 50 m from a dairy barn and site 4 was located near (about 100 m) to a
295 poultry barn. Both site 3 and site 5 were located immediately adjacent to large hay fields. The
296 highest mass flux between 1.67×10^{11} and 1.16×10^{12} copies/sec occurred in November 2005
297 during a period of high total rainfall and statistically significantly higher ($4.16 \times 10^{10} - 2.29 \times$
298 10^{11} copies/m³) abundance of the Tc^r genes.

299

300

301

302

303

304

305

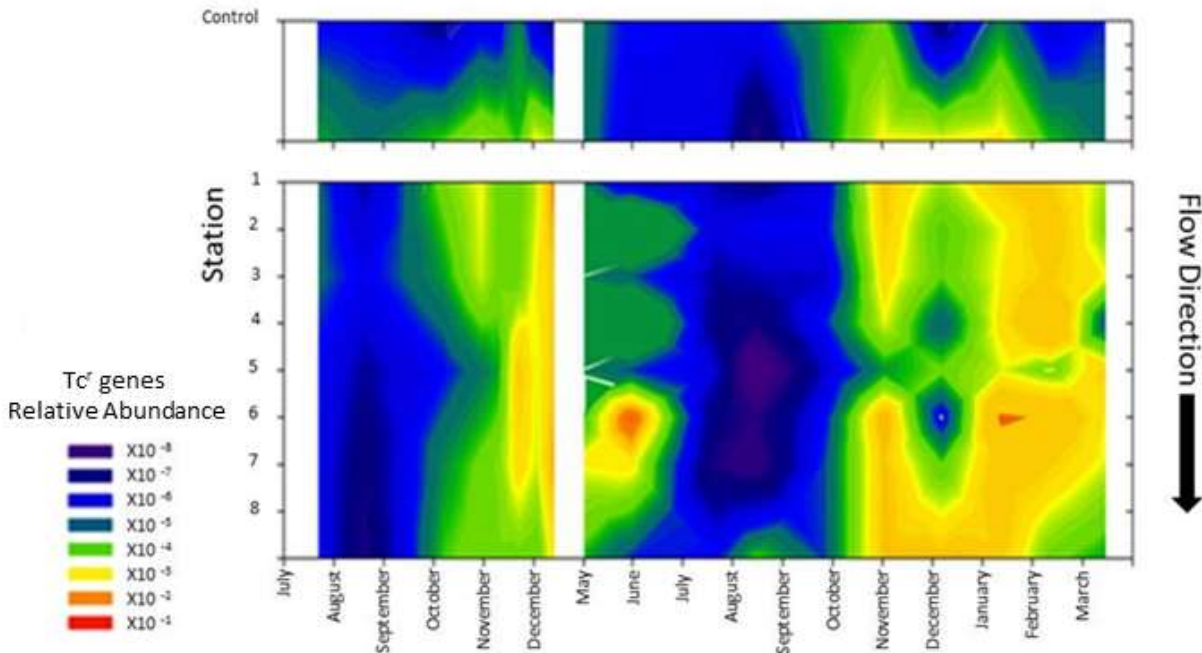
306 Table 3: Calculated mass transport rates of the total tetracycline resistance genes at four stations
307 along one segment of the Sumas River between July and December, 2005 (Site 5 is the furthest
308 downstream). Gene abundance calculated from the average of three replicates. (* indicates
309 significantly higher mass flux at $p < 0.05$ than that measured in other months.)

	Discharge rate (m ³ /s)	Total Tc ^r genes (copies/m ³)	Mass Flux (copies/s)	Sites
July	1.03	2.17 x 10 ⁶	2.24 x 10 ⁶	2
	1.07	2.38 x 10 ⁷	2.55 x 10 ⁷	3
	1.40	2.69 x 10 ⁶	3.76 x 10 ⁶	4
	1.17	4.76 x 10 ⁵	5.56 x 10 ⁵	5
August	2.00	1.09 x 10 ⁶	2.18 x 10 ⁶	2
	0.61	2.86 x 10 ⁷	1.74 x 10 ⁷	3
	0.62	1.11 x 10 ⁶	6.90 x 10 ⁵	4
	0.98	9.60 x 10 ⁵	9.43 x 10 ⁵	5
September	0.82	1.81 x 10 ⁶	1.48 x 10 ⁶	2
	1.10	1.19 x 10 ⁷	1.31 x 10 ⁷	3
	1.11	4.45 x 10 ⁶	4.94 x 10 ⁶	4
	0.89	2.28 x 10 ⁷	2.04 x 10 ⁷	5
November	4.08	2.29 x 10 ¹¹	9.34 x 10 ¹¹ *	2
	5.38	2.16 x 10 ¹¹	1.16 x 10 ¹² *	3
	4.56	6.32 x 10 ¹⁰	2.88 x 10 ¹¹ *	4
	4.00	4.16 x 10 ¹⁰	1.67 x 10 ¹¹ *	5
December	2.58	4.15 x 10 ⁸	1.07 x 10 ⁹	2
	2.89	1.47 x 10 ⁸	4.24 x 10 ⁸	3
	4.46	1.80 x 10 ⁸	8.06 x 10 ⁸	4
	2.34	1.54 x 10 ⁷	3.59 x 10 ⁷	5

312 4 DISCUSSION

313 Land use factors likely contributed to the observed higher abundances of Tc^f genes in the Sumas
314 River stream network when compared with the control site (Figure 6). Agricultural land use
315 along the stream reach in the immediate proximity of all sampling locations of this study
316 included dairy operations, poultry and swine farms and large crops fields. Of 27 individual land
317 parcels observed within 500 m of the stream network under study, 14 of these farming operations
318 produced poultry or livestock while 9 other farms produced corn or hay. Popowska et al. (2012)
319 detected *tet(M)*, *tet(O)* and *tet(W)* by qPCR analyses in soils collected from farmland in the area
320 of Lesznawola, Poland and demonstrated that more diverse populations of bacteria with
321 resistance were present in the agricultural soil samples. Higher relative abundance of Tc^f genes
322 (some in the order of 10⁵ to 10⁷ gene copies /mL) in soils collected near livestock farms in
323 agricultural regions in China have been described (Wu et al., 2010; Ji et al., 2012; Zhu 2013).
324 The water quality trends described in this study (with the exception of the Tc^f genes that had not
325 previously been measured) had been observed for the same sampling sites (Berka et al., 2001)
326 and differences in flow conditions for the Sumas stream network (often slower discharge rates)
327 had been recorded (Shead, 2004). Italian researchers have demonstrated correlations between
328 ARGs and water quality parameters, specifically nitrogen and phosphorus compounds and total
329 organic carbon, in aquatic ecosystems following rainfall events (Di Cesare et al., 2015).
330 Observations presented in this study suggest that agricultural land use activities likely
331 contributed to the potential for runoff of ARGs into the stream network.

332



333

334 Figure 6: Seasonal and spatial trends in total tetracycline resistance genes normalized to 16S
 335 rRNA genes along all sites of the investigated segment of the Sumas River stream network for
 336 the period of July 2004 – March 2006.

337

338 The patterns of Tc^r abundances illustrated in Figure 6 likely reflected seasonal weather and land-
 339 use patterns. Previous research in the Sumas watershed has demonstrated that nutrient levels,
 340 fecal coliform counts, concentrations of suspended particulate matter and other environmental
 341 contaminants (including metals) increased during the wet season (Berka, 1996; MacDonald,
 342 2005, Ross, 2006; Solano 2006). It is likely that rainfall influenced the runoff and soil erosion
 343 from agricultural fields that, in turn, contributed to the higher relative abundance of Tc^r genes
 344 observed during wet seasons in this study. Similar patterns of gene abundances and upstream
 345 agricultural activity have been observed (Pruden et al., 2012; Heuer et al., 2011; Luby et al.,
 346 2016; Nölvak et al., 2016) and increased abundances occur regardless of antibiotic usage in
 347 animals (Udikovic-Kolic et al., 2014). Coupled with seasonal variation of stream flow (e.g.

348 Knapp et al., 2012), agricultural activities can dramatically alter the transport of ARGs through
349 the aquatic receiving environment in the Sumas watershed. Positive Spearman's Rank
350 correlation between turbidity and Tc^r genes and the seasonal increase in turbidity as rainfall
351 events become more frequent and intense during the year suggests that Tc^r genes associated with
352 particulates can be transported in receiving waters. The results of this study combined with
353 evidence gathered from researchers in several regions of the world support the conclusion that
354 soil, manure and water in agricultural locations are possible hotspots of antibiotic resistance
355 genes and antibiotic resistant bacteria (Thanner et al., 2016) and that environmental factors
356 influence abundance and transport of ARGs in agricultural watersheds.

357 As with most agricultural regions, land application of manure on fields is used for soil
358 conditioning, crop fertilization and agricultural waste disposal in the Sumas watershed.
359 Composted manure is distributed on fields using tractor-drawn equipment and liquid manure is
360 spread through irrigation systems when soils are not excessively saturated. In the province of
361 British Columbia, there are no explicit regulations that limit field application of manure between
362 specific dates although most field fertilization occurs between April and October. The BC
363 Ministry of Agriculture Code of Farm Practice (2014) states that spreading of manure "is not
364 advised during periods of high rainfall or on snow-covered ground." Manure management
365 practices conducted during various times of the year are likely to play a role in the introduction
366 of contaminants to the stream network of the Sumas watershed during periods of higher rainfall.

367 Manure application on land is a likely contributor to elevated abundance of tetracycline
368 resistance genes in bacteria collected from the Sumas River. Tetracycline resistance genes

369 conferring ribosomal protection (i.e. *tet* (W) and *tet* (Q)) were most abundant, which reflect
370 profiles of genes from animal fecal samples (Patterson et al., 2007; Peak et al., 2007).

371 In this study, the abundances of the four Tc^r genes measured in the stream network varied with
372 season, with significantly higher abundances observed during rainy months (October – January),
373 and this is reflected in water quality patterns. This and previous research in the Sumas watershed
374 (MacDonald, 2005, Ross, 2006; Solano 2006) demonstrated that seasonal variation in nitrate
375 concentrations indicate agricultural non-point source pollution and elevated measurement levels
376 occurred during the wet winter months, whereas lower nitrate values were recorded in summer
377 low flow conditions (Berka et al., 2001; Schreier et al., 2001; Ross, 2006; Solano, 2006).

378 Significantly higher orthophosphate concentrations were measured in the Sumas stream network
379 during the wet season months than those in the control stream also suggesting that surface run-
380 off fertilizers contributed to the contaminant load in receiving waters. Conversely, specific
381 conductivity and measurements of chloride were higher (although not statistically significant) in
382 the dry season months than during wet season months in the stream network, which reflects
383 considerable contribution of groundwater to the stream at low surface flows (Berka, 1996;
384 Solano, 2006) and dilution during periods of precipitation.

385 Rainfall events influenced the transport of Tc^r genes in surface waters. Relationships between
386 the abundance of Tc^r genes and specific water quality parameters were observed at instantaneous
387 discharge, 48 h discharge, 72 h rainfall suggesting that intensity and duration of rainfall events
388 influenced the abundance of Tc^r genes in the flowing water. Manure-borne bacteria have been
389 shown to be transported by rainfall or irrigation into surface waters via soil and organic particles

390 (Jeng et al., 2005; Pachepsky et al., 2008) and higher discharge rates related to stormwater runoff
391 have been associated with higher turbidity in urban and rural streams (Mallin et al., 2009).

392 Transport of tetracycline resistance genes through the environment could be observed during wet
393 fall and winter seasons and the gene flux estimates of the indicator Tc^r genes mirrored the stream
394 discharge profiles and gene concentrations (Table 3). The location of dairy farms, poultry
395 operations and large crop fields together with the timing of manure application on the adjacent
396 land along the specific stream reach could have influenced observed seasonal Tc^r gene flux. The
397 highest Tc^r gene mass flux was measured in November which correlates to a period immediately
398 after the time when most manure was spread on land in the fall and the beginning of more
399 intense winter rainfall. It is likely that local variation in Tc^r gene flux resulted from differences
400 in inputs with space and time. Field fertilization with animal manure combined with seasonal
401 precipitation patterns likely contributed to the Tc^r gene flux via soil erosion and surface runoff.

402 **5 CONCLUSIONS**

403 The presence of ARGs at elevated abundance in streams and transport of these genes through
404 water courses could potentially provide opportunities for *de novo* induction of resistance in
405 environmental bacteria. Changes in weather patterns, such as shorter duration high intensity
406 rainfall events, increase the potential for soil erosion and elevate the concentration of particulate
407 matter and associated contaminants in rivers and streams. This research confirms that increased
408 abundance of some specific tetracycline resistance genes were present in the agricultural stream
409 network of the Sumas watershed and has demonstrated that ARGs can be co-lineated with the
410 suspended material in aquatic systems. Therefore higher intensity rainfall events are likely to
411 increase both suspended particulates and ARGs in agricultural watersheds as a result of soil

412 erosion. Equally important, results of this case study demonstrate that during periods of
413 considerably lower rainfall in warm summer months, the concentrations of particulate matter and
414 bacteria were lower while the abundance of ARGs remained proportionately high. It is likely
415 that agricultural activities and land use practises in the watershed contributed to elevated
416 concentrations of nitrate, phosphate, chloride and tetracycline resistance genes that were
417 measured. These observations underscore the importance of making mindful decisions in land
418 use and management in agricultural watersheds and that minimizing risks linked to the spread of
419 antibiotic resistance requires limiting the circulation of ARGs into and through the environment.

420 **ACKNOWLEDGMENTS**

421 The authors wish to thank Paula Parkinson, Susan Harper, and Raphaël Fugère for their
422 assistance with laboratory analyses and fieldwork. Financial assistance for this research was
423 supported by contributions from a Natural Science and Engineering Research Council (NSERC)
424 grant to Dr. Ken Hall, from a U.S. Environmental Protection Agency STAR Grant to Dr. David
425 Graham, and from the Canadian Water Network Centres of Excellence.

426 **REFERENCES**

427 Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the
428 wild: Antibiotic resistance genes in natural environments. *Nat Rev Microbiol* 2010; 8: 251–
429 59.

430 Alonso A, Sánchez P, Martínez J-L. Environmental selection of antibiotic resistance genes.
431 *Environ Microbiol* 2001; 3: 1–9.

432 American Public Health Association (APHA). Eaton AD, Clesceri LS, Greenberg AE
433 (editors). Standard Methods for the Examination of Water and Wastewater. 1995. 19th
434 Edition. American Public Health Association. Washington, DC.

435 Aminov RI. The role of antibiotics and antibiotic resistance in nature. *Environ Microbiol*
436 2009; 11: 2970–88.

437 Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV (2006). Co-selection of
438 antibiotic and metal resistance. *Trends Microbiol* 2006; 14: 176–82.

439 Baquero F, Martinez J-L, Cantón R. Antibiotics and antibiotic resistance in water
440 environments. *Curr Opin Biotech* 2008; 19: 260–65.

441 Baquero F, Coque TM. Widening the spaces of selection: Evolution along sublethal
442 antimicrobial gradients. *MBio* 2014; 5(6):e02270.

443 Berka C. Relationships between Agricultural Land Use and Surface Water Quality Using a
444 GIS. Sumas River Watershed, Abbotsford, BC. 1996. Master of Science thesis, Resource
445 Management and Environmental Studies, University of British Columbia. 174 pp.

446 Berka C, Schreier H, Hall K. Linking water quality with agricultural intensity in a rural
447 watershed. *Water Air Soil Pollut* 2001; 127: 389–401.

448 British Columbia Ministry of Agriculture, Food and Fisheries. Strengthening Farming: Right
449 to Farm. Agricultural Code of Practice. May 2014.

450 Cantón R. Antibiotic resistance genes from the environment: a perspective through newly
451 identified antibiotic resistance mechanisms in the clinical setting. *Clin Microbiol Infect*
452 2009; 15 (Suppl 1): 20–25.

453 Chanson H. Application of the Bernoulli equation to open channel flows. Chapter 3 in: The
454 Hydraulics of Open Channel Flow. 2003. Published by Hydrology Bureau of Yellow River
455 Conservancy Committee. China. 405 pp.

456 Christiano RSC, Reilly CC, Miller WP Scherm H. Oxytetracycline dynamics on peach leaves
457 in relation to temperature, sunlight, and simulated rain. *Plant Dis* 2010; 94: 1213–18.

458 Davies JE, Davies D. Origins and evolution of antibiotic resistance. *Microbiol Mol Biol Rev*
459 2010; 74(3): 417–433.

460 Di Cesar A, Eckert EM, Teruggi A, Fontaneto D, Bertoni R, Callieri C, Corno G.
461 Constitutive presence of antibiotic resistance genes within the bacterial community of a large
462 subalpine lake. *Molecular Ecol* 2015; 24(15): 3888–3900.

463 Ellis KV. Surface water pollution and its control. 1989. MacMillan Publisher Ltd.

464 Engemann CA, Keen PL, Knapp CW, Hall KJ, Graham DW. Fate of tetracycline resistance
465 genes in aquatic systems: migration from the water column to peripheral biofilms. *Environ*
466 *Sci Technol* 2008; 42(14): 5131–5136.

467 Environmental Canada Weather Data. Accessed May, 2006 at:
468 http://www.climate.weatheroffice.ec.gc.ca/advanceSearch/searchHistoricData_e.html

469 Environment Canada Climate Weather Data. Accessed May 7, 2016 at:
470 http://climate.weather.gc.ca/climate_data/monthly_data_e.html

471 Environment Canada Topographic Data. Accessed Oct 2, 2017 at: <http://en-ca.topographic->
472 [map.com/places/Sumas-River-813291/](http://en-ca.topographic-map.com/places/Sumas-River-813291/)

473 Ghosh S, LaPara TM. The effects of subtherapeutic antibiotic use in farm animals on the
474 proliferation and persistence of antibiotic resistance among soil bacteria. *ISME J* 2007; 1(3):
475 191–203.

476 Halling-Sørensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lützhof HC, Jørgensen
477 SE. Occurrence, fate and effects of pharmaceutical substances in the environment – A
478 review. *Chemosphere* 1998; 36(2): 357–393.

479 Harms G, Layton AC, Dionisi HM, Gregory IR, Garrett VM, Hawkins SA, Robinson KG,
480 Sayler GS. Real-time PCR quantification of nitrifying bacteria in a municipal wastewater
481 treatment plant. *Environ Sci Technol* 2003; 37(2): 343–351.

482 Heuer H, Schmitt H, Smalla K. Antibiotic resistance gene spread due to manure application
483 on agricultural fields. *Curr Opin Microbiol* 2011; 14(3): 236–43.

484 Huijbers PM, Blaak H, de Jong MC, Graat EA, Vandenbroucke-Grauls CM, de Roda
485 Husman AM. Role of the environment in the transmission of antimicrobial resistance to
486 humans: A review. *Environ Sci Technol*. 2015; 49(20):11993–12004.

487 Jeng HC, England AJ, Bradford HB. Indicator organisms associated with stormwater
488 suspended particles and estuarine sediment. Part A: Toxic/Hazardous Substances. *Environ*
489 *Eng J Environ Sci Health*. 2005; 40: 779–791.

490 Ji X, Shen Q, Liu F, Ma J, Xu G, Wang Y, Wu M. Antibiotic resistance gene abundances
491 associated with antibiotics and heavy metals in animal manures and agricultural soils
492 adjacent to feedlots in Shanghai; China. *J Hazard Mater* 2012; 235–236: 178–85.

493 Kim SC, Davis JG, Truman CC, Ascough JC 2nd, Carlson K. Simulated rainfall study for
494 transport of veterinary antibiotics – mass balance analysis. *J Hazard Mater* 2010; 175(1-3):
495 836–43.

496 Knapp CW, Lima L, Olivares-Rieumont S, Bowen E, Werner D, Graham DW. Seasonal
497 variations in antibiotic resistance gene transport in the Almendares River, Havana, Cuba.
498 *Front Microbiol* 2012; 3: 396.

499 Kümmerer K. Antibiotics in the aquatic environment – A review Part 1. *Chemosphere*
500 2009; 75: 417–34.

501 Levy SB. Introduction. In *Antimicrobial Resistance in the Environment*. Keen, PL,
502 Montforts, MHMM, editors. 2012. Wiley-Blackwell, Hoboken, New Jersey USA

503 Luby EM, Moorman TB, Soupier ML. Fate and transport of tylosin-resistant bacteria and
504 macrolide resistance genes in artificially drained agricultural fields receiving swine manure.
505 *Sci Total Environ* 2016; 550: 1126–33. doi:10.1016/j.scitotenv.2016.01.132.

506 MacDonald JR. Impacts of Urban Hillslope Development and Agriculture on Hydrology and
507 Water Quality in the Chilliwack Creek Watershed, British Columbia. 2005. Master of
508 Science thesis, Resource Management and Environmental Studies. University of British
509 Columbia.

510 Mallin MA, Johnson VL, Ensign SH, Comparative impacts of stormwater runoff on water
511 quality of an urban, a suburban and a rural stream. *Environ Monit Assess* 2009; 159: 475–
512 91.

513 Martinez J-L. Antibiotics and antibiotic resistance genes in natural environments. *Science*
514 2008; 321: 365–367.

515 Martinez J-L. The role of natural environments in the evolution of resistance traits in
516 pathogenic bacteria. *Proc Biol Sci* 2009; 276(1667): 2521–30.

517 Mackie RI, Koike S, Krapac I, Chee-Sanford J, Maxwell S, Aminov RI. Tetracycline
518 residues and tetracycline resistance genes in groundwater impacted by swine production
519 facilities. *Anim Biotechnol* 2006; 17(2):157–176.

520 McKinney CW, Loftin KA, Meyer MT, Davis JG, Pruden A. *Tet* and *sul* antibiotic resistance
521 genes in livestock lagoons of various operation type, configuration, and antibiotic
522 occurrence. *Environ Sci Technol* 2010; 44: 6102–6109.

523 McManus PS, Stockwell VO, Sundin GW, Jones AL. Antibiotic use in plant agriculture.
524 *Annu. Rev. Phytopathol.* 2002; 40: 443–65.

525 MoBio Laboratories. Alternative protocol for maximum yields. In: UltraClean™ Soil DNA
526 Isolation Kit Instruction Manual. 2004. MoBio Laboratories. Solona, CA.

527 Nölvak H, Truu M, Kanger K, Tampere M, Espenberg M, Loit E, Raave H, Truu J.
528 Inorganic and organic fertilizers impact the abundance and proportion of antibiotic resistance
529 and integron-integrase genes in agricultural grassland soil. *Sci Total Environ* 2016; 562:
530 678–89. doi: 10.1016/j.scitotenv.2016.04.035.

531 Pachepsky YA, Yu O, Karns JS, Shelton DR, Guber AK, van Kessel JS. Strain-dependent
532 variations in attachment of *E. coli* to soil particles of different sizes. *Int Agrophysics* 2008;
533 22: 61–66.

534 Patterson AJ, Colangeli R, Spigaglia P, Scott KP. Distribution of specific tetracycline and
535 erythromycin resistance genes in environmental samples assessed by macroarray detection.
536 *Enviro. Microbiol* 2007; 9(3): 703–715.

537 Peak N, Knapp CW, Yang RK, Hanfelt MM, Smith MS, Aga DS, Graham DW. Abundance
538 of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different
539 antibiotic use strategies. *Environ Microbiol* 2007; 9 (1): 143–151.

540 Popowska M, Rzczycka M, Miernik A, Krawczyk-Balska A, Walsh F, Duffy B. Influence
541 of soil use on prevalence of tetracycline, streptomycin, and erythromycin resistance and
542 associated resistance genes. *Antimicrob Agents Chemother* 2012; 56(3): 1434–43.

543 Poudel DD, Lee T, Srinivasan R, Abbaspour K, Jeong CY. 2013. Assessment of seasonal and
544 spatial variation of surface water quality, identification of factors associated with water
545 quality variability, and the modeling of critical nonpoint source pollution areas in an
546 agricultural watershed. *J Soil and Water Conservation* 2014; 68(3): 15 –71.

547 Pruden A, Arabi M, Storteboom HN. Correlation between upstream human activities and
548 riverine antibiotic resistance genes. *Environ Sci Technol* 2012; 46 (21): 11541–49.

549 Pruden A. Balancing water sustainability and public health goals in the face of growing
550 concerns about antibiotic resistance. *Environ Sci Technol* 2014; 48(1): 5–14.

551 Ross DJ. Influence of Climate and Agricultural Land Use on Nutrient and Bacterial Cycling
552 in Surface Waters of the Lower Fraser Valley, British Columbia. 2006. PhD thesis in
553 Resource Management and Environmental Studies. University of British Columbia.

554 Sarmah AK, Meyer MT, Boxall AB. A global perspective on the use, sales, exposure
555 pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment.
556 *Chemosphere* 2006; 65: 725–59.

557 Schreier H, Bestbier R, Brown S, Hall K, von Westarp S, Elliot L. Agricultural Watershed
558 Management. 2001. Multimedia CD ROM. Institute for Resources and Environment.
559 University of British Columbia. Vancouver, Canada.

560 Seiler C, Berendonk TU. Heavy metal driven co-selection of antibiotic resistance in soil and
561 water bodies impacted by agriculture and aquaculture. *Front Microbiol* 2012; 3: 399.

562 Séveno, N.A.; Kallifidas, D.; Smalla, K.; van Elsas, J.D.; Collard, J.-M.; Karagouni, A.D.;
563 Wellington, E.M.H. Occurrence and reservoirs of antibiotic resistance genes in the
564 environment. *Rev Med Microbiol* 2002; 13(1): 15–27.

565 Shead R. Summary of surface water quality sampling on Sumas River and tributaries
566 Abbotsford, British Columbia. British Columbia Ministry of Water, Land and Air
567 Protection. Technical Report 2004; 47 p.

568 Smith MS, Yang RK, Knapp CW, Niu YF, Peak N, Hanfelt MM, Galland JC, Graham DW.
569 Quantification of tetracycline resistance genes in feedlot lagoons by real-time PCR. *Appl*
570 *Environ Microbiol* 2004; 70(12): 7372–7377.

571 Solano MG. Land Use Impacts on Ground and Surface Water Quality in the Bertrand Creek
572 Watershed (Township of Langley, B.C.) 2006. Master of Science Thesis, Resource
573 Management and Environmental Studies. University of British Columbia.

574 Suzuki S, Pruden A, Virta M, Zhang T. Editorial: Antibiotic resistance in aquatic systems.
575 *Front Microbiol* 2017; 8: 14.

576 Thanner S, Drissner D, Walsh F. Antimicrobial resistance in agriculture. 2016; *mBio* 7(2):
577 e02227-15

578 Udawatta RP, Motavalli PP, Garrett HE. Nitrogen losses in runoff from three adjacent
579 agricultural watersheds with claypan soils. *Agriculture, Ecosystems & Environment* 2006;
580 117: 39–48.

581 Udikovic-Kolic N, Wichmann F, Broderick NA, Handelsman J. Bloom of resident
582 antibiotic-resistant bacteria in soil following manure fertilization. *Proc Natl Acad Sci*
583 2014; 111(42): 15202–07.

584 Wright GD. The antibiotic resistome: The nexus of chemical and genetic diversity. *Nat Rev*
585 *Microbiol* 2007; 5:175–186.

586 Wu N, Qiao M, Zhang B, Cheng W-D, Zhu Y-G. Abundance and diversity of tetracycline
587 resistance genes in soils adjacent to representative swine feedlots in China. *Environ Sci Tech*
588 2010; 44: 6933–39.

589 Yang S, Carlson K. Evolution of antibiotic occurrence in a river through pristine, urban and
590 agricultural landscapes. *Water Res* 2003; 37: 4645–56.

591 Zdziarski P, Simon K, Majda J. Overuse of high stability antibiotics and its consequences in
592 public and environmental health. *Acta Microbiol Pol* 2003; 52(1): 5–13.

593 Zhou X, Helmers MJ, Asbjornsen H, Kolka R, Tomer MD, Cruse RM. Nutrient removal by
594 prairie strips in agricultural landscapes. *J Soil and Water Conservation* 2014; 69(1): 54–64.

595 Zhu Y-G, Johnson TA, Su J-Q, Qiao M, Guo G-X, Stedtfeld RD; Hashsham SA, Tiedje JM.
596 Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc Natl Acad*
597 *Sci* 2013; 110(9): 3435–40.