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Variation of an indicator of *Escherichia coli* persistence from surface waters of mixed-use watersheds, and relationship with environmental factors

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Abstract – *Escherichia coli* is an indicator of fecal pollution used to mandate recreational and drinking water quality. Concentrations of culturable *E. coli* following contamination of surface water are determined by three factors: dilution; cell attachment to particulate material and settling or resuspension in the water column; and the net rate of change in viability. This study evaluated the variability in the latter parameter, and how predictive variation in death rate was of culturable population densities at the time of sampling. Water samples ($N = 232$) with varying levels of *E. coli* contamination were collected from 46 discrete locations in four watersheds across Canada over a three-month period and enumerated for culturable *E. coli* by membrane filtration plate counting ($T0_{EC}$). Water samples were again enumerated following a laboratory 24 h holding period at 30 °C in the dark, and the difference considered the death rate (Δ_{EC}). Relationships of $T0_{EC}$ and Δ_{EC} with environmental and water chemistry factors were explored using step-wise multiple regression. The model predicting $T0_{EC}$ indicated that stream order, total rainfall seven days in advance of sampling day, total phosphorus, and Δ_{EC} were the most significant contributors. The model predicting Δ_{EC} indicated that turbidity and $NH_3 + NH_4$ were the most important contributors. A model suggests that the persistence factor is less important than dilution (*i.e.* stream order) in describing *E. coli* densities, followed by factors that influence the loading of *E. coli* into watersheds.

Key words: *Escherichia coli* / surface water / persistence / environmental factors

Introduction

Escherichia coli is ubiquitous in the gastrointestinal tracts of humans and other warm-blooded animals (Leclerc *et al.*, 2001). In secondary habitats, such as water, the detection of *E. coli* is considered to be evidence of recent fecal contamination, and therefore an indicator of contamination with pathogenic microorganisms of human health concern (Dufour, 1984). *E. coli* serves as a basis on which the microbiological quality of recreational and drinking water is measured and mandated to minimize public health risks (USEPA, 2000).

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The concentration of *E. coli* in surface water at any one time will depend on the rate of fecal contaminant input, and on the rates of three processes that redistribute, remove, or augment culturable cell densities in the water, namely: (i) dilution; (ii) cell attachment to particulate material and subsequent settling or resuspension in the primary water column; and (iii) the net rate of change in viability due to cell death, or proliferation. Environmental factors that affect *E. coli* viability in natural waters include water temperature (Faust *et al.*, 1975), predation (McCambridge and McMeekin, 1980), antibiotics (Carlucci and Pramer, 1960a), organic matter (Bouteleux *et al.*, 2005), viral lysis (Carlucci and Pramer, 1960b), nutrients (Topp *et al.*, 2003;

Table 1. Watershed designation and location, number of sampling sites, number of samples collected, period of time covered by sampling, initial and final abundance of *E. coli* in samples, and abundance variation during laboratory holding period for each of the watershed sampled (Bras d'Henri (BDH), South Nation (SN), Oldman (OLD), and Sumas (SU)). Average, standard deviation (SD), minimum (min), and maximum (max) values are given for initial ($T0_{EC}$) and final ($T24_{EC}$) abundance in water (CFU.100 mL^{-1}), and for abundance variation (Δ_{EC} (%)).

	BDH	SN	OLD	SU
Province	Québec	Ontario	Alberta	British Columbia
N° sampling sites	5	23	12	5
N° samples	19	134	63	20
Sampling period (2007)	4-Jun to 30-Jul	4-Jun to 13-Aug	28-May to 13-Aug	28-May to 7-Aug
$T0_{EC}$ (average ± SD [min–max])	258 ± 319 [6–1090]	282 ± 700 [1–6400]	84 ± 98 [0–440]	332 ± 486 [12–1770]
$T24_{EC}$ (average ± SD [min–max])	114 ± 154 [4–530]	103 ± 361 [0–3900]	34 ± 37 [0–154]	152 ± 167 [7–670]
Δ_{EC} (average ± SD [min–max])	− 48 ± 37 [− 95 to 50]	− 58 ± 49 [− 100 to + 250]	− 39 ± 83 [− 100 to + 413]	− 29 ± 49 [− 93 to + 77]

Cook and Bolster, 2007), and irradiation with UV and visible light (Noble *et al.*, 2004).

Given the widespread use of *E. coli* as an indicator of fecal contamination, understanding environmental conditions that influence the persistence of this microorganism in surface water is critical for improving our capacity to predict human health threats from waterborne disease. *E. coli* was originally thought to persist for only a limited time outside the gastrointestinal tract, where conditions differ drastically from the intestines of warm-blooded animals (Ishii and Sadowsky, 2008). However, recent studies have demonstrated the ability of some strains to survive for several months in soil, sediments and sand in tropical and temperate environments (Anderson *et al.*, 2005; Ishii *et al.*, 2007). In freshwater, *E. coli* was found to persist up to 260 days in sterile river water (Flint, 1987), and between 270 days to more than 450 days in filter-sterilized groundwater with varying nutrient composition (Cook and Bolster, 2007). *E. coli* cells in isolation chambers placed directly into streams and springs persisted *in situ* for up to four months (Davis *et al.*, 2005).

Microorganism environmental fate models used as a framework for the establishment of Total Maximum Daily Limits (TMDLs) or Quantitative Microbial Risk Assessments (QMRAs) generally employ rudimentary descriptions of cell die-off kinetics and transport physics (Haas *et al.*, 1999; Benham *et al.*, 2006) but other models have also been described in the literature (Canale *et al.*, 1993; Wilkinson *et al.*, 1995; Kashefipour *et al.*, 2002; Collins and Rutherford, 2004; Servais *et al.*, 2007). The complexities of the processes that govern *E. coli* concentrations in surface waters complicate the prediction of the fate of microorganisms in natural systems, and therefore the capacity to predict fecal pollution impacts on human and animal health (Pachepsky *et al.*, 2006). These assessments would be improved by a greater understanding of the ecology of *E. coli* in natural systems.

The primary purpose of this work was to examine if an indicator of "net persistence" of culturable *E. coli* in surface water samples was related to observed *E. coli* densities at the time of sampling. We enumerated by membrane filtration plate count *E. coli* populations in 232 water samples collected from the water column of rivers and streams within four river basins geographically dispersed within agriculturally dominated regions in Canada (Johnson *et al.*, 2003; van Bochove *et al.*, 2005; Smith *et al.*, 2007; Wilkes *et al.*, 2009). From these sampled waters, we defined a bulk indicator of *E. coli* persistence by determining the rate of change in these populations during a 24-h incubation under controlled laboratory conditions. In an attempt to establish the significance of this persistence indicator relative to other factors likely to impact directly *E. coli* densities in water courses (*e.g.*, nutrients, temperature, water course size), we employed step-wise multiple regression to predict initial *E. coli* densities from the persistence indicator, and other environmental factors. Finally, we determined if water properties at the time of sampling were associated with *E. coli* persistence characteristics measured in the laboratory.

Material and methods

Sample sites and surface water sampling

Surface water sampling sites were located within four watersheds widely dispersed across Canada: the Bras d'Henri watershed in Québec (BDH; van Bochove *et al.*, 2005), the South Nation River watershed in Ontario (SN; Wilkes *et al.*, 2009), the Oldman River watershed in Alberta (OLD; Johnson *et al.*, 2003), and the Sumas River watershed in British Columbia (SU; Smith *et al.*, 2007). Water was sampled from 46 discrete locations (5, 23, 12, and 5 sampling sites in BDH, SN, OLD, and SU, respectively).Water samples (N total = 232; 19, 130, 63, and 20 samples from BDH, SN, OLD, and SU, respectively) were collected between May 28 and August 13, 2007 (Table 1). For bacterial analysis, one liter of surface water was collected at a depth of 0.5 m from the surface directly into sterile containers (Systems Plus, Woodstock, ON,

Canada). For low water conditions, a water sample was retrieved halfway between the surface and the bottom. The samples (packed with cold packs) were shipped by overnight courier to Agriculture and Agri-Food Canada (AAFC) laboratories in London, ON, Canada.

Weather and water physical and chemical quality

Climate and water quality properties were determined for the SN, and not the other sites. Turbidity was measured using a Global Water WQ770 hand held unit of range 0–1000 nephelometric turbidity units (NTU) with an accuracy of $\pm 5\%$ of scale (Global Water Instrumentation Inc., Gold River, CA). Solar radiation was recorded using a solar radiation sensor on a HOBO Weather Station Logger (Onset part # H21-001, Onset Computer Corporation, Bourne, MA), with a 30 min interval, and were expressed in $W.m^{-2}$. At time of sampling, water temperature, pH, conductivity, dissolved oxygen and oxidation reduction potential were measured using a YSI 556 Multi Probe System unit with YSI sensor 5563 Probe module (Yellow Springs Instruments Inc., Yellow Springs, OH), with accuracies of $\pm 0.15\,^{\circ}C$, ± 0.2 pH units, ± 0.001 $mS.cm^{-1}$, $\pm 6\%$ dissolved oxygen units, and ± 20 mV (YSI 556 Multi Probe System Operations Manual). The YSI sensor(s) were suspended approximately 1 m below the surface of the water before reading, or if suspension of the sensor was not possible, in a retrieved bucket sample. For nutrient analysis, 1 L of water was taken from the sample site and refrigerated overnight at AAFC laboratories in Ottawa, Canada. On the following day, 300 mL of sample water was partitioned, stored in a cooler with icepacks, and delivered to the City of Ottawa, Robert O. Pickard Environmental Center (ROPEC) Laboratory for analysis of ammonia + ammonium and reactive phosphorus (Standard Methods 4500-NH3, and 4500-P), nitrite and nitrate (Standard Method 4110B), and total Kjeldahl nitrogen and total phosphorus (Standard Methods 4500-Norg and 4500-PF) (American Public Health Association, 1999). Rainfall, maximum, minimum and mean temperatures (°C) for Russell Ontario were obtained from Environment Canada's Meteorological Service (Environment Canada, 2007). Variables summarising rainfall (mm) on day of water sample, on day of water sample and one (Rain 1d), two (Rain 2d), three (Rain 3d) and seven (Rain 7d) days in advance were calculated. Mean temperature was expressed as the average of the maximum and minimum temperature on the day of sample collection. Strahler (1952) stream order for sample sites were calculated using a Geographic Information System and the Spatial Analyst package of ArcMap 9.1 (Environmental Systems Research Institute, Redlands, CA) and a digital elevation model (DEM). The DEM was "filled", removing small defects and/or sinks (areas of no outward flow), and flow direction and flow accumulation were calculated on the filled DEM, to determine the direction of flow on a cell by cell basis and the number of upstream cells contributing to a cell, in this case, the sampling locations. Stream thresholds were identified, and Strahler order was calculated using the "Stream Order" tool of the Spatial Analyst package.

Escherichia coli enumeration and death rate determination

Water samples were shipped on ice packs and received within 24 h of sampling by the microbiology laboratory. Water samples were enumerated for *E. coli* ($T0_{EC}$) by membrane filtration as described in Wilkes *et al.* (2009). Briefly, 10- to 100-mL portions of water were filtered through sterile, 0.45-μm pore-size, 47-mm-diameter cellulose acetate filters (Pall Gelman GN-6; VWR International, Mississauga, ON, Canada), and the filters were plated onto mFC basal medium (Difco, Fisher Scientific, Ottawa, ON, Canada) supplemented with 100 $mg.L^{-1}$ of 3-bromo-4-chloro-5-indolyl-β-D-glucopyranoside (BCIG) (hexylammonium salt; Inverness Medical, Ottawa, ON, Canada) and then incubated overnight at 44.5 °C. Samples were then placed in a 30 °C incubator where they were held statically in the dark for 24 h, following which culturable *E. coli* were again enumerated ($T24_{EC}$).

Data analysis

E. coli abundance was expressed in colony forming units (CFU).100 mL^{-1}. The change in abundance of culturable cells during the 30 °C holding period is expressed as follows: $\Delta_{EC} = [(T24_{EC} - T0_{EC}) / T0_{EC}] \times 100$, where a value of -100 indicates a complete disappearance of cultivable *E. coli*, a value of 0 would indicate no change in abundance, and a value of 100 would indicate that *E. coli* abundance doubled during the 24-h holding period. Abundance variation values were compared using Wilcoxon matched-pairs test and the Mann-Whitney U test. The SPSS 11.0 for Windows program (SPSS, Inc., Chicago, IL) was used for all statistical analyses; all significance tests and correlations were considered significant statistically at a p value of ≤ 0.05. In some cases data were log transformed (base 10).

Main effects forward-based multiple stepwise regression analyses were conducted (Statistica, StatSoft Inc., Tulsa, OK) using data derived from the South Nation River watershed in Ontario. Maximum allowable tolerance settings (0.6) were used in order to maximally reduce variable contribution redundancy in the model. Two separate regression analyses were conducted. The first regression analyses was used to predict $T0_{EC}$ from the persistence indicator (Δ_{EC}) and a suite of water and weather variables that we hypothesized could be associated with the initial concentrations of *E. coli* (Table 2). The second analyses was designed to predict Δ_{EC} from water quality properties of the sampled water in order to determine if there were distinct properties of the water that were predictive of the persistence of *E. coli* resulting from the incubation challenge.

Table 2. Forward stepwise multiple linear regression (MLR) dependent and independent variables used for SN watershed samples.

Variable	Unit	Description	Log transformed for analysis	Used to predict $T0_{EC}$	Used to predict ΔEC
Δ_{EC}	%	Percent change of *E. coli* concentration as measured prior and post incubation $\Delta_{EC} = ([T24_{EC} - T0_{EC}]/ T0_{EC})*100$		X	
$T0_{EC}$	CFU.100 mL^{-1}	*E. coli* concentration before incubation	X		
Water temperature	°C	Temperature of water at time of sample collection		X	X
pH	No unit	pH of water at time of sample collection	X	X	X
Electrical conductivity (EC)	$mS.cm^{-1}$	Ability of water at time of sample collection to conduct electrical current	X	X	X
Dissolved oxygen (DO)	%	Amount of gaseous O_2 dissolved in sample water at time of collection		X	X
Oxidation reduction potential (ORP)	mV	Potential of water to oxidize at time of sample collection	X	X	X
Turbidity	NTU (nephelometric turbidity units)	Cloudiness of sample water as measured with a nephelometer sensor	X	X	X
Strahler order	Numeric	Ranges from 1 (first order) to 7 (main branch of South Nation River). See Strahler (1952)		X	
$NH_3 + NH_4$	$mg.L^{-1}$	Ammonia + ammonium concentration of sample water	X	X	X
NO_2^-	$mg.L^{-1}$	Nitrite concentration in sample water	X	X	X
NO_3^-	$mg.L^{-1}$	Nitrate concentration in sample water	X	X	X
Reactive phosphorus (RP)	$mg.L^{-1}$	Reactive phosphorus concentration in sample water	X	X	X
Total Kjeldahl nitrogen (TKN)	$mg.L^{-1}$	Organic nitrogen concentration in sample water	X	X	X
Total phosphorus (TP)	$mg.L^{-1}$	Total phosphorus concentration in sample water	X	X	X
Rainfall; Rain 1_d; Rain 2_d; Rain 3_d; Rain 7_d	mm	Total rainfall on day of sample and 1, 2, 3 and 7 days in advance		X	
Max. temp.	°C	Daily maximum air temperature on day of sample		X	
Min. temp.	°C	Daily minimum air temperature on day of sample		X	
Mean temp.	°C	Mean daily air temperature on day of sample		X	
Insolation	$MJ.m^{-2}$	Incoming solar radiation per day at HOBO weather station in sample basin		X	

Results

Initial culturable *E. coli* abundance in water samples ($N = 232$) ranged from undetectable (< 1 CFU.100 mL^{-1}) to 1.7×10^3 CFU.100 mL^{-1} (average ± SD of $1.7 \pm 2.6 \times 10^2$ CFU 100.mL^{-1}). After a 24-h holding period at 30 °C, *E. coli* abundance was generally significantly reduced (Wilcoxon matched-pairs test, $p < 0.0001$) and ranged between undetectable to 6.7×10^2 CFU.100 mL^{-1} (average ± SD of $0.6 \pm 1.0 \times 10^2$ CFU.100 mL^{-1}) (Table 1). Abundance decreased from 528 ± 319 to 114 ± 154 CFU.100 mL^{-1} for BDH, from 282 ± 700 to 103 ± 361 CFU.100 mL^{-1} for SN, from 84 ± 98 to 34 ± 37 CFU.100 mL^{-1} for OLD, and from 332 ± 486 to 152 ± 167 CFU.100 mL^{-1} for SU over the incubation period (Table 1). Calculated Δ_{EC} ranged between -100 and 413% with an average ± SD of -50 ± 60%. A total of 18 samples exhibited an increase in their *E. coli* densities after the incubation period (positive Δ_{EC} values): nine of these samples had $T0_{EC}$ densities below the 1st quartile of the dataset, indicating that these high increases might be related to the reproducibility of the enumeration method when the *E. coli* concentrations are low. Sample Δ_{EC} values were compared between ranges of initial *E. coli* abundance values ($T0_{EC}$) as defined based on quartile of the initial abundance distribution (group 1: < 20 CFU.100 mL^{-1}, $N = 60$; group 2: 20–70.5 CFU.100 mL^{-1}, $N = 56$; group 3: 70.5–220 CFU.100 mL^{-1}, $N = 59$; and group 4: > 220 CFU.100 mL^{-1}, $N = 57$; with samples from the four watersheds parsing out evenly within all

Table 3. Pearson product moment correlation coefficients ($p < 0.05$ underlined) for persistence analysis of SN watershed samples. Inputs as specified in Table 2 were log transformed prior to calculation of the correlation coefficients.

	$T0_{EC}$	Δ_{EC}	Water temp.	DO	Strahler order	Rain fall	Rain 1_d	Rain 2_d	Rain 3_d	Rain 7_d	Max. temp.	Min. temp.	Mean temp.	Insolation	pH	EC	ORP	Turbidity	$NH_3 + NH_4^+$	NO_2^-	NO_3^-	RP	TKN	TP
$T0_{EC}$	1.00																							
Δ_{EC}	−0.15	1.00																						
Water temp.	−0.53	0.06	1.00																					
DO	−0.37	0.05	0.63	1.00																				
Strahler order	−0.58	−0.02	0.59	0.23	1.00																			
Rainfall	0.26	0.02	−0.32	−0.19	0.05	1.00																		
Rain 1_d	0.21	−0.03	−0.30	−0.19	0.07	0.95	1.00																	
Rain 2_d	0.26	−0.11	−0.24	−0.19	0.10	0.80	0.78	1.00																
Rain 3_d	0.26	−0.11	−0.22	−0.19	0.10	0.73	0.68	0.99	1.00															
Rain 7_d	0.27	−0.11	−0.25	−0.21	0.10	0.80	0.75	0.99	0.99	1.00														
Max. temp.	−0.20	0.03	0.35	0.25	−0.06	−0.88	−0.89	−0.69	−0.62	−0.70	1.00													
Min. temp.	0.13	−0.10	0.00	−0.09	0.08	0.35	0.41	0.77	0.77	0.71	−0.27	1.00												
Mean temp.	−0.01	−0.08	0.23	0.10	0.04	−0.27	−0.20	0.26	0.31	0.21	0.42	0.76	1.00											
Insolation	−0.25	−0.07	0.30	0.21	−0.02	−0.89	−0.80	−0.72	−0.68	−0.73	0.92	−0.33	0.31	1.00										
pH	−0.40	0.01	0.76	0.63	0.62	−0.03	−0.01	−0.11	−0.13	−0.12	0.02	−0.11	−0.08	0.04	1.00									
EC	0.07	0.12	−0.01	−0.24	−0.08	0.00	0.00	−0.03	−0.04	−0.03	−0.01	−0.04	−0.05	0.00	0.08	1.00								
ORP	0.10	−0.13	−0.09	−0.25	0.18	0.32	0.34	0.36	0.36	0.38	−0.47	0.17	−0.16	−0.38	0.05	0.10	1.00							
Turbidity	0.27	0.26	−0.03	−0.23	−0.34	−0.07	−0.05	−0.11	−0.13	−0.12	0.10	−0.03	0.04	0.09	−0.10	0.27	−0.15	1.00						
$NH_3 + NH_4^+$	0.14	−0.02	−0.24	−0.48	−0.13	−0.07	−0.05	0.07	0.10	0.09	−0.08	0.06	0.00	0.00	−0.33	0.27	0.14	0.48	1.00					
NO_2^-	0.03	0.19	−0.10	−0.44	0.38	0.15	0.25	0.25	0.22	0.22	−0.09	0.33	0.24	0.01	−0.22	0.33	0.39	0.17	0.27	1.00				
NO_3^-	−0.22	−0.06	0.30	0.23	0.56	0.38	0.35	0.32	0.30	0.31	−0.27	0.22	0.01	−0.32	0.36	−0.18	0.07	−0.20	−0.15	0.30	1.00			
RP	0.36	0.05	−0.31	−0.45	−0.48	−0.12	−0.14	−0.10	−0.08	−0.09	0.09	−0.07	0.00	0.07	−0.43	0.16	0.04	0.41	0.30	0.30	−0.50	1.00		
TKN	0.19	0.08	0.05	−0.17	−0.16	−0.11	−0.11	−0.06	−0.04	−0.05	0.12	0.00	0.08	0.12	−0.04	0.30	0.03	0.64	0.70	−0.02	−0.03	0.24	1.00	
TP	0.41	0.05	−0.20	−0.35	−0.47	−0.15	−0.15	−0.09	−0.07	−0.08	0.16	−0.03	0.08	0.16	−0.29	0.25	0.04	0.61	0.49	0.26	−0.50	0.80	0.65	1.00

Table 4. Stepwise multiple regression results and coefficients using parameters described in Table 2, for SN watershed samples. Independent variables are listed in order of forward step model contribution. Bolded values are significant at 0.05 level.

Ind. variable or intercept	Beta	Std. error of beta	B	Std. error of B	*p*-Level	Adjusted R^2
Predicting $T0_{EC}$						
Intercept			2.895232	0.170118	**0.0000**	
Strahler order	−0.522682	0.071253	−0.208986	0.028489	**0.0000**	0.33
Rain 7	0.317954	0.063639	0.015749	0.003152	**0.0000**	0.43
Total phosph.	0.197214	0.071196	0.446386	0.161149	**0.0064**	0.46
Δ_{EC}	−0.132763	0.063368	−0.002081	0.000993	**0.0381**	0.48
Predicting Δ_{EC}						
Intercept			−15.0811	61.08581	0.8053	
Turbidity	0.345710	0.094199	21.9649	5.98502	**0.0003**	0.06
$NH_3 + NH_4$	−0.194238	0.094458	−15.0387	7.31335	**0.0417**	0.08

density groups: χ^2 test, $p = 0.084$) (Fig. 1). Differences in Δ_{EC} were observed between low and high abundance levels (Δ_{EC} average ± SD of $-29 \pm 85\%$ and $-64 \pm 23\%$, for group 1 and 4 respectively; Mann-Whitney test, $p < 0.05$). Between watersheds, *E. coli* abundance variations following holding period were significantly different between SN and SU, with culturable populations in SU samples being more persistent than those in SN samples (Δ_{EC} average ± SD of $-29 \pm 49\%$ and $-58 \pm 49\%$, respectively; Mann-Whitney test, $p < 0.001$). No difference was observed between any of the other watersheds.

A multiple linear regression (MLR) analysis was carried out on SN samples as a case study: the correlation matrix among the variables listed in Table 2, indicated that logged-transformed $T0_{EC}$ ($T0_{EC}$ was initially not normally distributed) was significantly correlated at the 0.05 level with a wide variety of environmental and water quality properties (Table 3). Logged $T0_{EC}$ was insignificantly ($p > 0.05$) but negatively correlated with Δ_{EC}. The persistence indicator (Δ_{EC}) was found to be significantly correlated with only turbidity and NO_2^- concentration. The stepwise regression results for the SN data set are presented in Table 4. Briefly, the model predicting $T0_{EC}$ from the independent variables given in Table 2, indicated that stream order, total rainfall seven days in advance of sampling day, total phosphorus, and Δ_{EC} (persistence indicator) were the most important and significant ($p < 0.05$) model contributors. For this model, the adjusted R^2 was 0.48 ($p < 0.000$) with a standard error of the estimate of 0.545. The variance reduction associated with Δ_{EC} was small, but significant (0.02 of adjusted R^2). The stepwise multiple regression model predicting Δ_{EC} from the variables given in Table 2, indicated that turbidity and $NH_3 + NH_4^+$ were the most important model contributors, but variance reductions by these variables were very small. The adjusted R^2 of the model is 0.08 ($p < 0.00$) with a standard error of the estimate of 45.88.

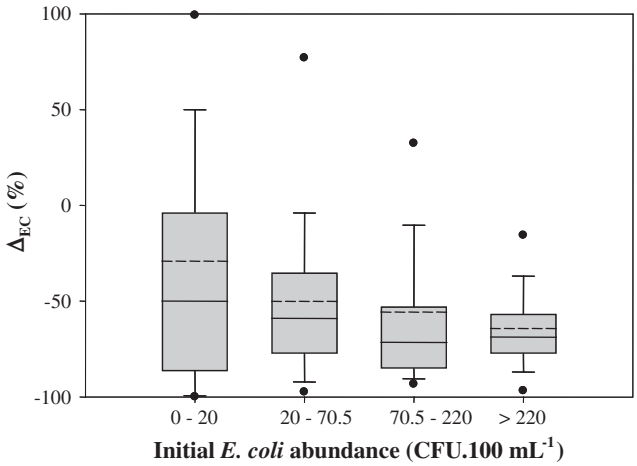

Fig. 1. Boxplots of *E. coli* persistence values (Δ_{EC}, %) during laboratory holding periods for four ranges of initial *E. coli* abundance (0–20; 20–70.5; 70.5–220; > 220 CFU.100 mL^{-1}). Boxplots indicate the first and third quartile (bottom and top lines of the box), the median (middle line of the box) and the smallest and largest observations (bottom and top whiskers) of a data distribution. The outside dots indicate the 5th and the 95th percentile. The average value is indicated by the dashed line.

Discussion

The persistence indicator exhibited less variability and more negative values for the heavily *E. coli* contaminated samples than in less heavily contaminated samples (Fig. 1). Furthermore, stepwise regression modelling with SN data found that $T0_{EC}$ was linked statistically with the persistence indicator, suggesting that *E. coli* in water samples that were more heavily contaminated were less persistent than in water samples that were less heavily contaminated. We propose two possible reasons for these observations: recently some *E. coli* strains have been found to be environmentally adapted or naturalized, and shown to grow and maintain their populations in secondary habitats, soils, sediment, sand, or algal surfaces (Ishii and Sadowsky, 2008). In the present study, we speculate that heavily contaminated water samples could be fecally contaminated, and are characterized by isolates that survive poorly in secondary habitats. In contrast, *E. coli* populations detected in more pristine samples could be dominated by naturalized *E. coli* that survive relatively

better in secondary habitats. Confirmation of this hypothesis would require that *E. coli* in the more pristine water samples be found to be distinct from those in the more heavily contaminated, using for example Multilocus Sequence Typing or Repetitive Extragenic Palindromic PCR (Ishii *et al.*, 2006; Walk *et al.*, 2007). A second possibility is that the *E. coli* concentrations in the range examined here did not saturate prey density with respect to (for example) planktonic grazing or viral lysis. The *E. coli* population density would then be rate limiting for the frequency of contact and efficiency of prey interception by grazers, or the production and availability of lytic viruses (Weinbauer and Höfle, 1998; Wommack and Colwell, 2000). Confirmation of this hypothesis would require a systematic analysis of grazing and bacteriophage-mediated lysis at varying *E. coli* densities.

The laboratory incubations (holding periods) reported here would capture some, but not all of the factors that are going to determine persistence *in situ*; yet, the incubations were devised to provide a standardized environment to gauge relative density changes. These would include biologically-mediated factors such as predation (McCambridge and McMeekin, 1980) and viral lysis (Carlucci and Pramer, 1960b); and water quality parameters including the presence of antibiotics or other inhibitors (Carlucci and Pramer, 1960a), organic and particulate matter (Bouteleux *et al.*, 2005), and nutrients (Cook and Bolster, 2007). Laboratory incubations were carried out at 30 °C, which corresponded to the maximum water temperature recorded in the field during the sampling period (data not shown). Unlike most published persistence studies, we did not inoculate the water with defined concentrations of pure cultures, so our results would not be biased by the genetic characteristics of a given isolate, its physiological status according to how the inoculum was prepared, or by the initial cell density chosen.

Two key environmental variables that could influence persistence *in situ* that were absent in the *in vitro* incubations were incident radiation, and water temperature (Faust *et al.*, 1975; Whitman *et al.*, 2008). The relationship between these two parameters and the abundance of *E. coli* at time of sampling was evaluated in the SN and OLD datasets. There was little variability in the solar radiation during the sampling period (maximal daily irradiance average ± SD: 786 ± 248 W.m^{-2}), and this could not account for the wide range in density of culturable *E. coli* in the water (Table 1). Furthermore, the absence of a relationship between irradiance and Δ_{EC} suggests that potential variation in sublethal irradiation damage prior to sampling did not compromise the cells during the *in situ* incubation (Muela *et al.*, 2000). Water temperature increased gradually over the sampling period from 13 to 29 °C in SN and from 8 to 25 °C in OLD. *E. coli* survival was shown to be negatively linked to temperature ranges between 10 and 30 °C in various environments (Faust *et al.*, 1975; Servais *et al.*, 1985; Craig *et al.*, 2004). However no evidence in the present work suggests that an increase in water temperature was linked to lower *E. coli* concentrations at sampling time, or beneficially influenced persistence during the holding period (Table 3).

E. coli abundance change (Δ_{EC}) was most strongly correlated with turbidity, and turbidity provided the greatest contribution to variance reduction in the multiple regression modelling. The beneficial effect of turbidity on *E. coli* survival could be attributed to several mechanisms depending on the type of suspended particles. When suspended particles are of algal nature, it was found that *E. coli* cells can take advantage of the higher nutrient concentrations surrounding algal cells because of the release of organic substances (Brettar and Höfle, 1992). Particles can also represent ecological shelters against grazing pressure (Brettar and Höfle, 1992). Suspended solids such as sand, clay mineral or organic matter were also demonstrated to provide protection against UV light by limiting the depth of light penetration in the water column in estuarine or freshwater environments (Marshall, 1968; Craig *et al.*, 2004; Beversdorf *et al.*, 2007).

Stepwise regression modelling with SN data found that $T0_{EC}$ was linked statistically, in order of model contribution, to stream order (a surrogate variable for dilution), rainfall for one week prior to sampling (a variable describing pollution driving mechanisms), total phosphorus (a parameter commonly correlated with fecal pollution due to runoff), and the persistence indicator (Δ_{EC}); the latter suggesting that *E. coli* in water samples that were more heavily contaminated were less persistent than in water samples that were less heavily contaminated. Overall, based on the presented dataset collected on the SN watershed, these results indicate that when developing models predicting levels of *E. coli* contamination, in order of importance, dilution, climate drivers, and conditions influencing persistence of culturable cells are factors that need to be considered.

In summary, an indicator of *E. coli* persistence was poorly, but inversely related to initial *E. coli* concentrations in water from a variety of watersheds across Canada. For a subset of data obtained from one watershed, the indicator was a significant ($p < 0.05$) contributor to a multiple regression model predicting *E. coli* concentrations. The work presented here concerned flowing systems, and we would anticipate that the characteristics of persistence and growth of *E. coli* in lentic environments, stagnant drainage, lakes, or reservoirs, will undoubtedly differ from our observations. Overall, based on these results, we would suggest that the persistence factor is less important than dilution in describing *E. coli* densities, followed by factors that influence the loading of *E. coli* into watersheds (Roser *et al.*, 2006). With respect to environmental fate models used for TMDL determinations or QMRAs, these results provide interesting insights that should improve the prediction of *E. coli* concentrations in natural systems and in surface waters, and should be helpful for prediction of fecal pollution impacts on human and animal health.

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