Supplementary Materials for

Application of coagulation and foam concentration method to quantify waterborne pathogens in river water samples

Yoshihiro Suzuki[1]*, Atsushi Jikumaru[1], Soichiro Tamai[1], Kei Nukazawa[1], Yoshifumi Masago[2], Satoshi Ishii[3, 4]

[1]Department of Civil and Environmental Engineering, Faculty of Engineering, University of Miyazaki, Miyazaki, Japan

[2]Center for Social and Environmental Systems Research, National Institute for Environmental Studies, Ibaraki, Japan

[3]Department of Soil, Water, and Climate, University of Minnesota, Minnesota, USA

[4]BioTechnology Institute, University of Minnesota, Minnesota, USA

*Correspondence: ysuzuki@cc.miyazaki-u.ac.jp; Tel.: +81-985-58-7339

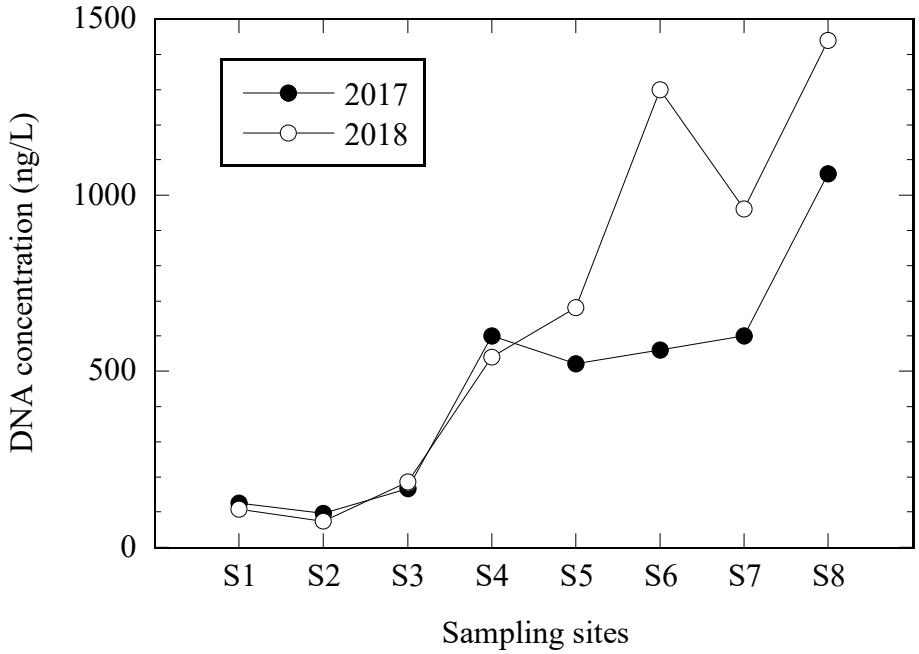

Figure S1. Changes in the DNA concentration at each sampling site in the Kiyotake River.

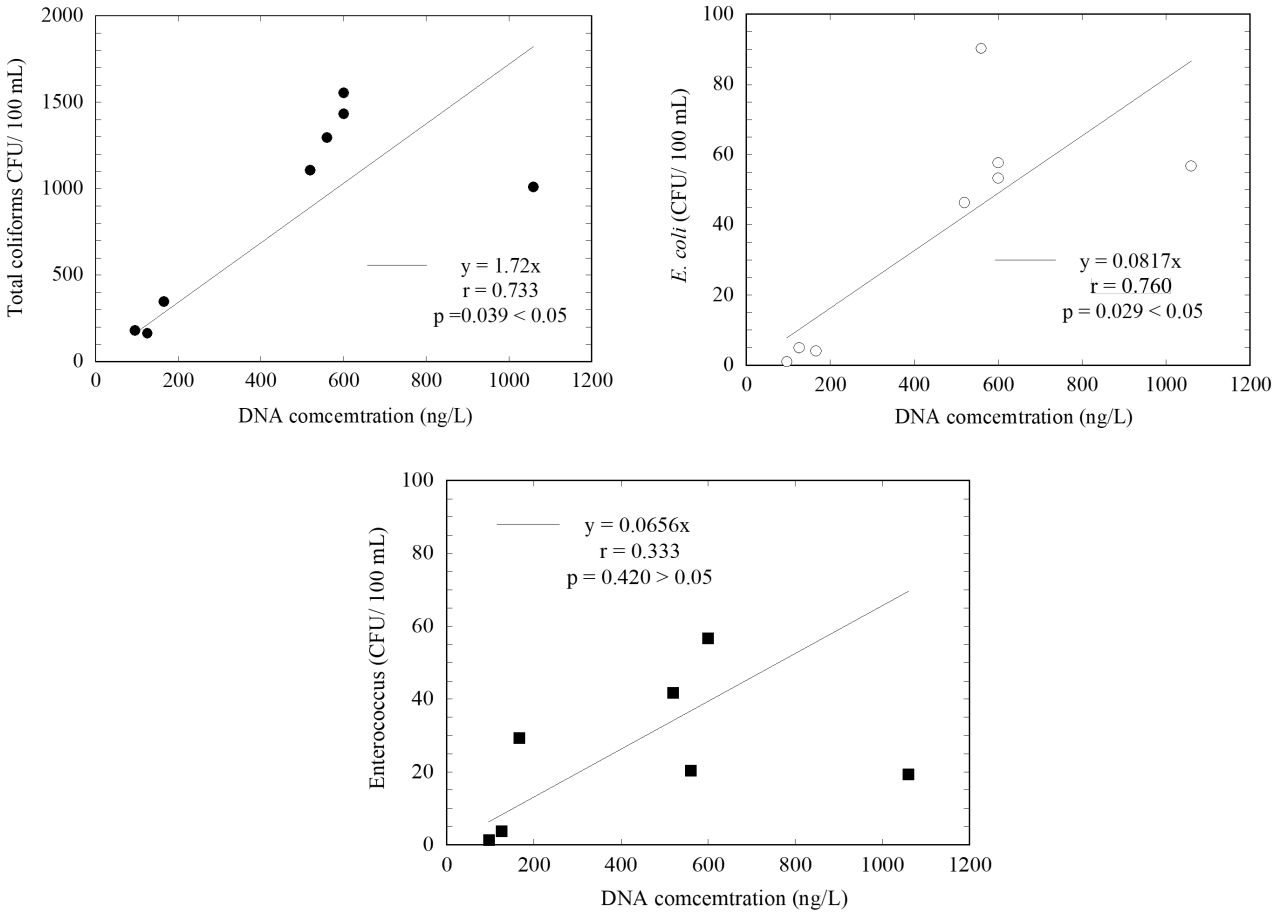

Figure S2. Correlations between DNA concentration and the counts of fecal indicator bacteria (total coliforms, E. coli, and Enterococcus) along the river.

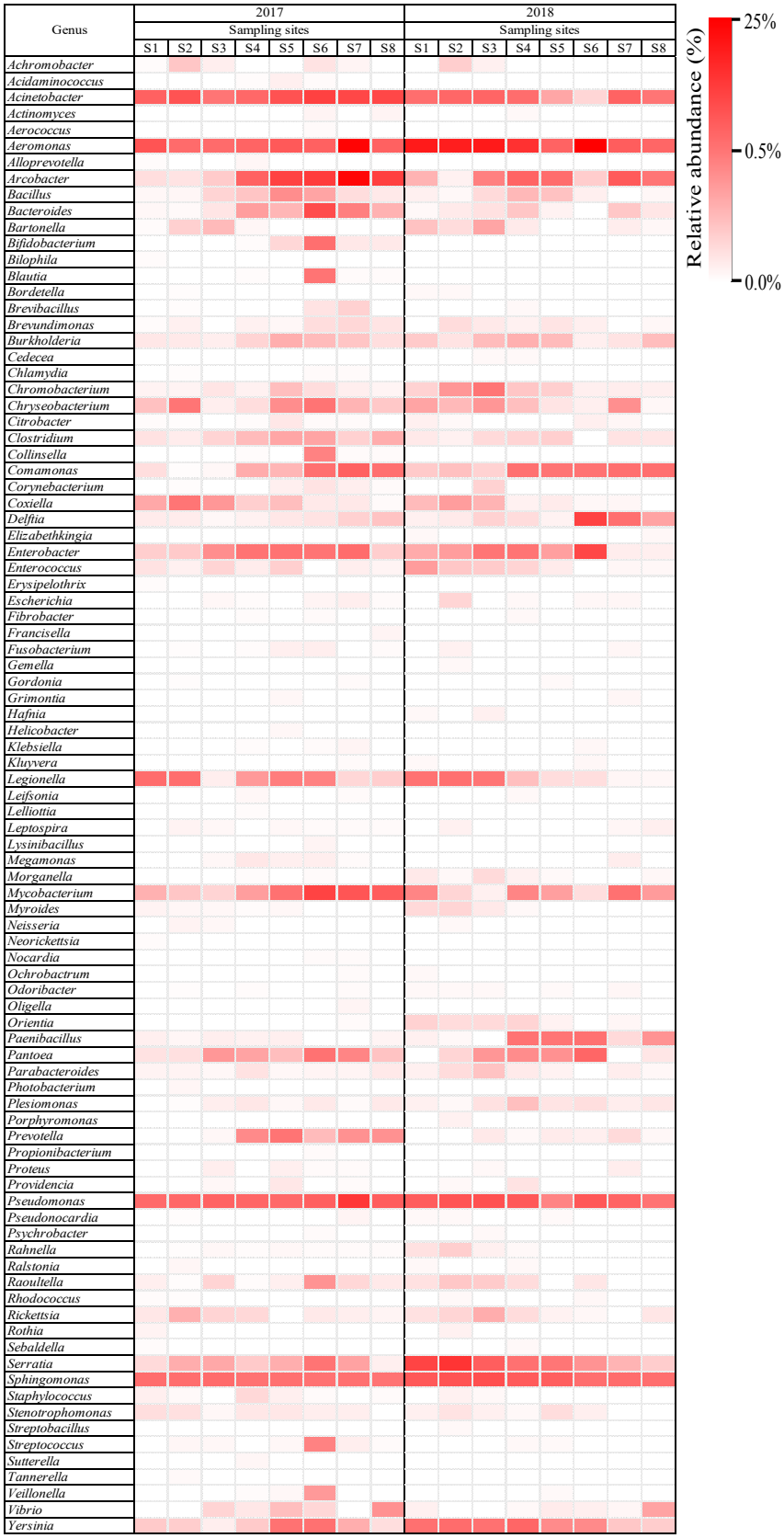

Figure S3. Heatmap showing the relative abundances of 35 potentially pathogenic genera identified.

Only genera that were present at >0.1% of the population are shown.

Species	2017								2018							
	Sampling sites								Sampling sites							
	S1	S2	S3	S4	S5	S6	S7	S8	S1	S2	S3	S4	S5	S6	S7	S8
Bordetella bronchiseptica																
Bordetella pertussis																
Brevundimonas vesicularis																
Enterobacter hormaechei																
Fusobacterium nucleatum																
Fusobacterium varium																
Legionella sainthelensi																
Leifsonia aquatica																
Morganella morganii																
Proteus vulgaris																
Providencia stuartii																
Pseudomonas alcaligenes																
Vibrio fluvialis																
Vibrio mimicus																
Yersinia kristensenii																
Yersinia pestis																
Yersinia rohdei																
Yersinia ruckeri																

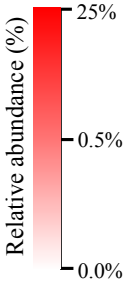

Figure S4. Heatmap showing the relative abundances of 18 potentially pathogenic species identified. Only species that were present at >0.1% of the population are shown.

Table S1. Primers, probes, and thermal conditions used for dPCR in this study.

Target bacteria	Target genes	primers, probes	Sequence (5'-3')	µM	Thermal conditions	Rerferences
Shiga-toxin producing *E. coli* (STEC)	*stx1*	*Stx1* - f	CATCGCGAGTTGCCAGAAT	0.9	50°C, 2 min > 95°C, 30 sec > 95°C, 3 sec > 60°C, 30 sec; 40 cycles	[42] Chui et al. (2010)
		Stx1 - r	GCGTAATCCCACGGACTCTTC	0.9		
		Stx1 - pro	FAM-CTGCCGGACACATAGAAGGAAACTCATCA-TAMRA	0.25		
	stx2	*Stx2* - f	CCGGAATGCAAATCAGTC	0.9	50°C, 2 min > 95°C, 30 sec > 95°C, 3 sec 56°C, 30 sec ; 40 cycles	[42] Chui et al. (2010)
		Stx2 - r	CAGTGACAAAACGCAGAACT	0.9		
		Stx2 - pro	FAM-ACTGAACTCCATTAACGCCAGATATGA-TAMRA	0.25		
Enteroinvasive *E. coli* (EIEC)/*Shigella* spp.	*ipaH*	*ipaH* - f	CCTTTTCCGCGTTCCTTG	0.2	95°C, 10 min > 95°C, 30 sec >60°C, 1 min; 40 cycles	[43] Vu et al.(2004)
		ipaH - r	CGGAATCCGGAGGTATTG C	0.2		
		ipaH - pro	FAM - CGCCTTTCCGATACCGTCTCTGCA - TAMRA	0.04		
Campylobacter jejuni	*hipO*	*hipO* - f	TGCACCAGTGACTATGAATAACGA	0.9	95°C, 10 min > 95°C, 20 min > 60° C, 1 min; 40 cycles	[44] Vondrakova et al.(2014)
		hipO - r	TCCAAAATCCTCACTTGCCATT	0.9		
		hipO - pro	FAM - TTGCAACCTCACTAGCAAAATCCACAGCT-TAMRA	0.25		
E. coli	*uidA*	*uidA* - f	CGGAAGCAACGCGTAAACTC	0.3	95°C, 10 min > 95°C, 20 sec > 60° C, 1 min; 50 cycles	[45] Silkie et al.(2008)
		uidA - r	TGAGCGTCGCAGAACATTACA	0.9		
		uidA - pro	FAM-CGCGTCCGATCACCTGCGTC-TAMRA	0.3		