Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

1 Flow cytometer comparison

Two flow cytometers were used for these sampling campaigns, an Accuri C6 flow cytometer (Accuri; BD Biosciences, San Jose, CA) and a BD FACSCanto cell analyzer (Canto; BD Biosciences, San Jose, CA). DWDS F is the only location for which the Canto was used because the Accuri broke down during field sampling (the supplemental data files include which cytometer was used to produce which data point). Once the Accuri was repaired and returned, a comparison experiment was completed. For both cytometers, we started with the publicly available gate developed by researchers at the Swiss Federal Institute of Aquatic Science and Technology (Eawag gate; 38) and then results from Sperotech nano fluorescent size standard kit (Spherotech, Catalog #NFPPS-52-4K) were used to adjust the gate for the Canto (adjusted gate; Figure S6).The Spherotech beads were all at a concentration of $1x10^6$ beads per mL. The Accuri was able to quantify the larger beads with under 10% error (Table S6). The Canto with adjusted gate was most accurate for the largest bead (less than 1% error), but still had an error of 18.5% for the 0.88 µm beads, which improved from the standard error with the Eawag gate, 25.5%.

The beads that were 0.22 µm and 0.45 µm were not accurately quantified by either cytometer (Table S6), but the small beads were detected outside of the gate by the Accuri and not by the Canto. This result might be evidence that the Accuri has a lower limit of quantificaiton than the Canto (Figure S6), and the quantification limit was not determined specifically for the Canto. However, the Eawag method we use recommends working with cell counts of 10^2-10^7 cells per mL (38). If the detection limit for samples taken with the Canto was 10^2 cells per mL for both total cell counts and intact cell counts instead of the limits of 12 and 22 cells per mL respectively determined by Miller et al. with the Accuri (40), nothing would be impacted because the lowest Canto total cell count datapoint is 350 cells per mL and that of intact cell count is 304 cells per mL.

Bottled Evian water was used to verify that adjusting the Eawag gate led to comparable cell counts of a microbial community from the same source. Three bottles of Evian purchased from the same location were analyzed in biological triplicate with the Accuri on June 12, 2019 and in biological duplicate with the Canto on June 14, 2019. Adjusting the gate brought the Canto intact or total cell count value closer to that of the Accuri than the Eawag values in most cases (Figure S7). This pattern did not hold for total cell count of bottle 1, which had an adjusted average greater than the Accuri average. However, this sample had the largest standard deviation (Table S7), and the paired intact cell count measurement was brought much closer to that of the Accuri. Overall, we concluded that the differences associated with cytometers following adjustment were minor as compared to differences associated even with the same site in a distribution system over time, which can range orders of magnitude (e.g., site_ut in Figure 1A).

2 Supplemental Tables

Table S1: Summary of sample locations and parameters measured. Each system was sampled in either 2016 and/or 2018 in the distribution system (where n is the number of sites sampled each year). The parameters measured were intact cell counts (ICC), total cell counts (TCC), residual disinfectant concentration, pH, temperature, adenosine triphosphate concentration (ATP), and/or heterotrophic plate counts (HPC).

System	distribution system	n	TCC & ICC	Residual disinfectant	pH	temperature	ATP	HPC
A	2016	12	2016	2016	2016	-	-	2016
	2018	12	2018	2018	2018	2018	2018	2018
B	2016	12	2016	2016	-	-	-	-
	2018	10	2018	2018	2018	2018	2018	2018
C	2016	12	2016	2016	2016	-	-	-
	-	-	-	-	-	-	-	-
D	2016	7	2016	2016	2016	2016	-	-
	-	-	-	-	-	-	-	-
E	2016	5	2016	2016	2016	2016	-	-
	-	-	-	-	-	-	-	-
F	-	-	-	-	-	-	-	-
	2018	24	2018	2018	2018	2018	2018	2018

Table S2: Ranges in parameter values for samples taken in all drinking water distribution systems sampled for this study by type of secondary disinfectant applied in the system, including intact cell counts (ICC), total cell counts (TCC), adenosine triphosphate concentration (ATP), and heterotrophic plate counts (HPC).

Assay	secondary disinfectant	n	min	median	max	geometric mean	geometric sd	arithmetic mean	arithmetic sd
ICC (cells/mL)	Chloramine	112	1.18E+02	2.42E+03	1.52E+05	3.62E+03	6.16	-	-
TCC (cells/mL)	Chloramine	112	3.50E+02	9.76E+03	6.23E+05	1.32E+04	5.33	-	-
intracellular ATP (nM)	Chloramine	94	<1.83E-05	1.05E-04	2.85E-02	2.10E-04	11.6	-	-
total ATP (nM)	Chloramine	94	1.85E-04	1.45E-03	3.12E-02	1.69E-03	4.10	-	-
HPC (MPN/100 mL)	Chloramine	67	<1	24.7	2.42E+03	20.6	10.0	-	-
free chlorine (mg/L)	Chloramine	96	<0.02	0.050	0.540	-	-	0.084	0.095
total chlorine (mg/L)	Chloramine	109	<0.02	1.88	2.90	-	-	1.71	0.782
pH	Chloramine	84	7.67	8.05	8.45	-	-	8.03	0.142
temperature (°C)	Chloramine	82	13.7	18.6	28.8	-	-	20.0	3.99
ICC (cells/mL)	Free chlorine	54	<22	3.53E+03	1.58E+05	2.58E+03	12.9	-	-
TCC (cells/mL)	Free chlorine	54	31.7	7.13E+03	1.58E+05	4.97E+03	10.0	-	-
intracellular ATP (nM)	Free chlorine	21	<1.83E-05	1.53E-03	1.26E-02	9.13E-04	6.84	-	-
total ATP (nM)	Free chlorine	21	3.08E-03	8.25E-03	1.54E-02	7.41E-03	1.73	-	-
HPC (MPN/100 mL)	Free chlorine	31	<1	2.03	2.30E+02	3.26	4.02	-	-
free chlorine (mg/L)	Free chlorine	54	0.100	0.730	2.14	-	-	0.790	0.472
total chlorine (mg/L)	Free chlorine	32	0.240	0.710	1.22	-	-	0.722	0.276
pH	Free chlorine	44	7.40	8.22	8.74	-	-	8.23	0.303
temperature (°C)	Free chlorine	35	15.7	22.9	26.1	-	-	22.2	2.26

Table S3: Corrected Akaike information criterion (AICc) values for backward stepwise model selection (all are generalized linear mixed models with site as a random intercept) shown in order of AICc.

model	Int	free chlorine	pH	temp	total chlorine	free chlorine: pH	free chlorine: temp	total chlorine: pH	total chlorine: temp	df	AICc
2	8.63		0.40	0.35	-1.31	0.39			-0.24	8	1,526.38
12	8.46	-0.40	0.52	0.36	-1.30	0.53			-0.21	9	1,527.46
20	8.46	-0.40	0.52	0.36	-1.30	0.53			-0.21	9	1,527.46
11	8.24	-0.87	0.57		-1.23	0.59	-0.70		-0.13	9	1,527.57
18	8.47	-0.51	0.52	0.30	-1.33	0.74				8	1,527.97
10	8.64		0.42	0.40	-1.32	0.41	0.12		-0.24	9	1,528.81
13	8.29	-0.86	0.56	0.05	-1.26	0.71	-0.57			9	1,528.89
5	8.34	-0.65	0.55	0.19	-1.26	0.55	-0.36		-0.18	10	1,529.50
15	8.34	-0.65	0.55	0.19	-1.26	0.55	-0.36		-0.18	10	1,529.50
19	8.71	-0.003	0.34	0.39	-1.30				-0.33	8	1,529.63
22	8.71	-0.003	0.34	0.39	-1.30				-0.33	8	1,529.63
6	8.43	-0.40	0.48	0.33	-1.26	0.46		0.08	-0.21	10	1,529.83
4	8.24	-0.85	0.55		-1.21	0.56	-0.68	0.03	-0.13	10	1,530.16
3	8.61		0.37	0.39	-1.28	0.33	0.16	0.09	-0.24	10	1,531.09
7	8.29	-0.84	0.53	0.06	-1.24	0.67	-0.52	0.04		10	1,531.45
14	8.62	-0.19	0.35	0.26	-1.27		-0.28		-0.30	9	1,531.83
1	8.34	-0.62	0.52	0.20	-1.24	0.51	-0.31	0.04	-0.18	11	1,532.13
8	8.51	-0.24	0.30	0.27	-1.18		-0.08	0.21	-0.26	10	1,532.21
16	8.51	0.02		0.23	-1.14	0.24	-0.17		-0.20	9	1,534.46
21	8.51	0.02		0.23	-1.14	0.24	-0.17		-0.20	9	1,534.46
9	8.48	0.01		0.27	-1.09	0.08	0.06	0.23	-0.20	10	1,534.76
17	8.52	-0.33	0.40		-1.34	0.73			-0.05	8	1,537.39

Table S4: Ranges in parameter values for samples taken in distribution system F, including intact cell counts (ICC), total cell counts (TCC), adenosine triphosphate concentration (ATP), and heterotrophic plate counts (HPC).

Assay	n	min	median	max	geometric mean	geometric sd	arithmetic mean	arithmetic sd
ICC (cells/mL)	100	118	2.53E+03	1.52E+05	3.95+03	5.59	-	-
TCC (cells/mL)	100	350	1.18E+04	6.22E+05	1.40+04	5.29	-	-
intracellular ATP (nM)	94	2E-05	1E-04	0.029	2E-04	11.6	-	-
total ATP (nM)	94	2E-04	0.001	0.031	0.002	4.10	-	-
HPC (MPN/100 mL)	67	1.0	24.7	2.42E+03	20.6	10.0	-	-
free chlorine (mg/L)	96	<0.02	0.05	0.54	-	-	0.084	0.095
total chlorine (mg/L)	97	<0.02	1.88	2.90	-	-	1.70	0.77
pH	84	7.67	8.05	8.45	-	-	8.03	0.142
temperature (°C)	82	13.7	18.6	28.8	-	-	19.9	3.99

Table S5: number of samples (n) and percentage of those samples that were quantifiable by drinking water distribution system sampled, including intact cell counts (ICC), total cell counts (TCC), adenosine triphosphate concentration (ATP), and heterotrophic plate counts (HPC).

	distribution system A		distribution system B		distribution system C		distribution system D		distribution system E		distribution system F	
	n	percent quantifiable	n	percent quantifiable	n	percent quantifiable	n	percent quantifiable	n	percent quantifiable	n	percent quantifiable
ICC	22	100	20	100	12	100	7	85.7	5	40.0	100	100
TCC	22	100	20	100	12	100	7	100	5	100	100	100
intracellular ATP	11	90.9	10	90.0	0	-	0	-	0	-	94	64.9
total ATP	11	100	10	100	0	-	0	-	0	-	94	100
HPC	21	76.2	10	90.0	0	-	0	-	0	-	71	81.7

Table S6: Result of calibration bead experiments with beads of four different diameters measured on Accuri flow cytometer with Eawag gate and Canto flow cytometer with Eawag gate and adjusted gate. Accuri results are biological triplicates of geometric averages from technical triplicates and Canto results are biological duplicates of geometric averages from technical triplicates.

Flow cytometer and gate	measurement	0.22 µm	0.45 µm	0.88 µm	1.35 µm
Accuri Eawag gate	Arithmetic average count (beads/mL)	7.8E+05	4.5E+03	1.1E+06	1.1E+06
	Arithmetic standard deviation	1.7E+04	8.3E+02	2.2E+04	1.2E+04
	Percent error (%)	22.3	99.6	6.20	9.82
Canto adjusted gate	Arithmetic average count (beads/mL)	3.7E+03	2.4E+03	8.2E+05	9.9E+05
	Arithmetic standard deviation	2.2E+03	1.6E+03	4.0E+04	2.4E+04
	Percent error (%)	99.6	99.8	18.5	0.77
Canto Eawag gate	Arithmetic average count (beads/mL)	1.2E+03	9.1E+02	7.5E+05	9.9E+05
	Arithmetic standard deviation	8.1E+02	5.4E+02	6.1E+04	2.5E+04
	Percent error (%)	99.9	99.9	25.5	0.96

Table S7: Result of the same three bottles of Evian water measured on Accuri flow cytometer with Eawag gate and Canto flow cytometer with Eawag gate and adjusted gate. Accuri results are biological triplicates of geometric averages from technical triplicates and Canto results are biological duplicates of geometric averages from technical triplicates. Data for intact cell count assay (ICC) and total cell count assay (TCC) are shown.

Flow cytometer and gate	measurement	Evian bottle 1		Evian bottle 2		Evian bottle 3	
		TCC	ICC	TCC	ICC	TCC	ICC
Accuri Eawag gate	Arithmetic average count (cells/mL)	1.9E+05	1.5E+05	1.6E+05	1.1E+05	1.2E+05	9.5E+04
	Arithmetic standard deviation	2.5E+04	9.9E+03	6.2E+03	3.9E+03	3.8E+03	3.3E+03
Canto adjusted gate	Arithmetic average count (cells/mL)	2.7E+05	1.5E+05	1.5E+05	9.8E+04	1.1E+05	8.6E+04
	Arithmetic standard deviation	1.2E+05	1.6E+04	8.2E+03	2.0E+04	4.7E+03	7.6E+03
Canto Eawag gate	Arithmetic average count (cells/mL)	2.1E+05	1.2E+05	1.2E+05	8.9E+04	9.8E+04	7.5E+04
	Arithmetic standard deviation	8.8E+04	1.6E+04	9.6E+03	2.1E+04	2.3E+03	8.2E+03

3 Supplemental Figures

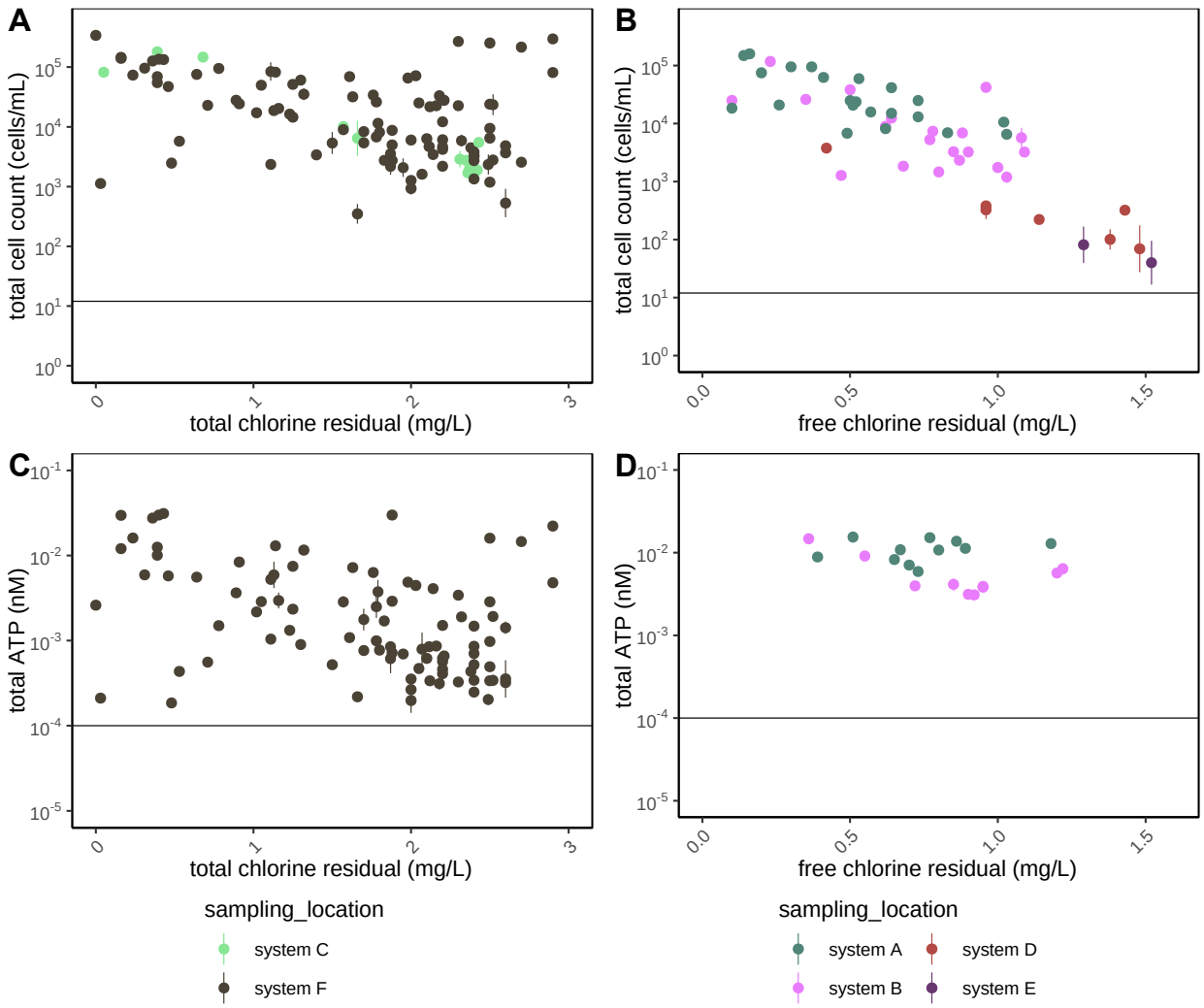

Figure S1: total cell counts (A-B) and total ATP (C-D) in drinking water distribution systems sampled in this study. Horizontal lines denote quantification limits. Points are the geometric mean of the technical replicates and error bars represent geometric standard deviation for technical triplicates.

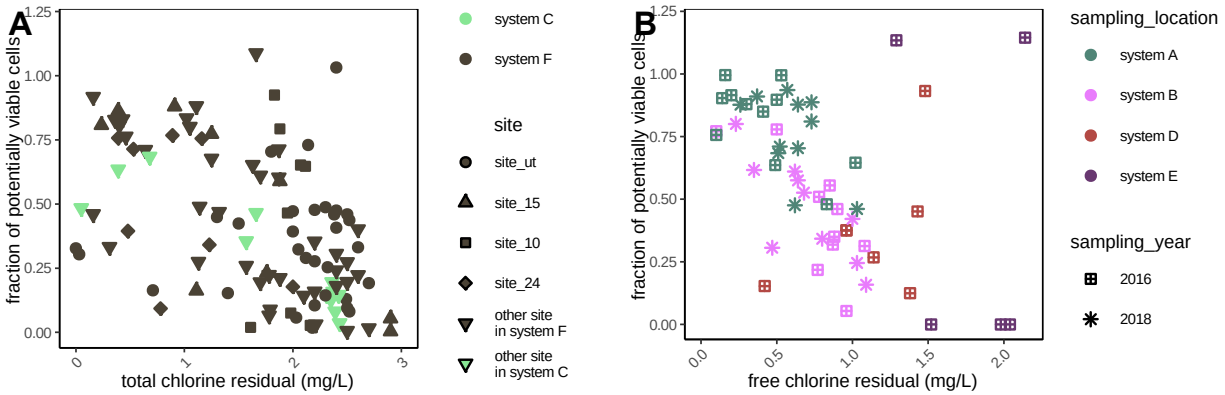

Figure S2: Fraction of potentially viable cells (intact cell counts/total cell counts) in chloraminated (A) and chlorinated (B) drinking water distribution systems sampled in this study. Shapes in A denote locations in distribution system F that were sampled at least six times between August 2017 and April 2018. Shapes in B denote locations in distribution system A and distribution system B that were sampled once in 2016 and repeated in 2018.The four samples with intact cell counts/total cell counts > 1 had intact cell counts and total cell counts values within 15% of each other.

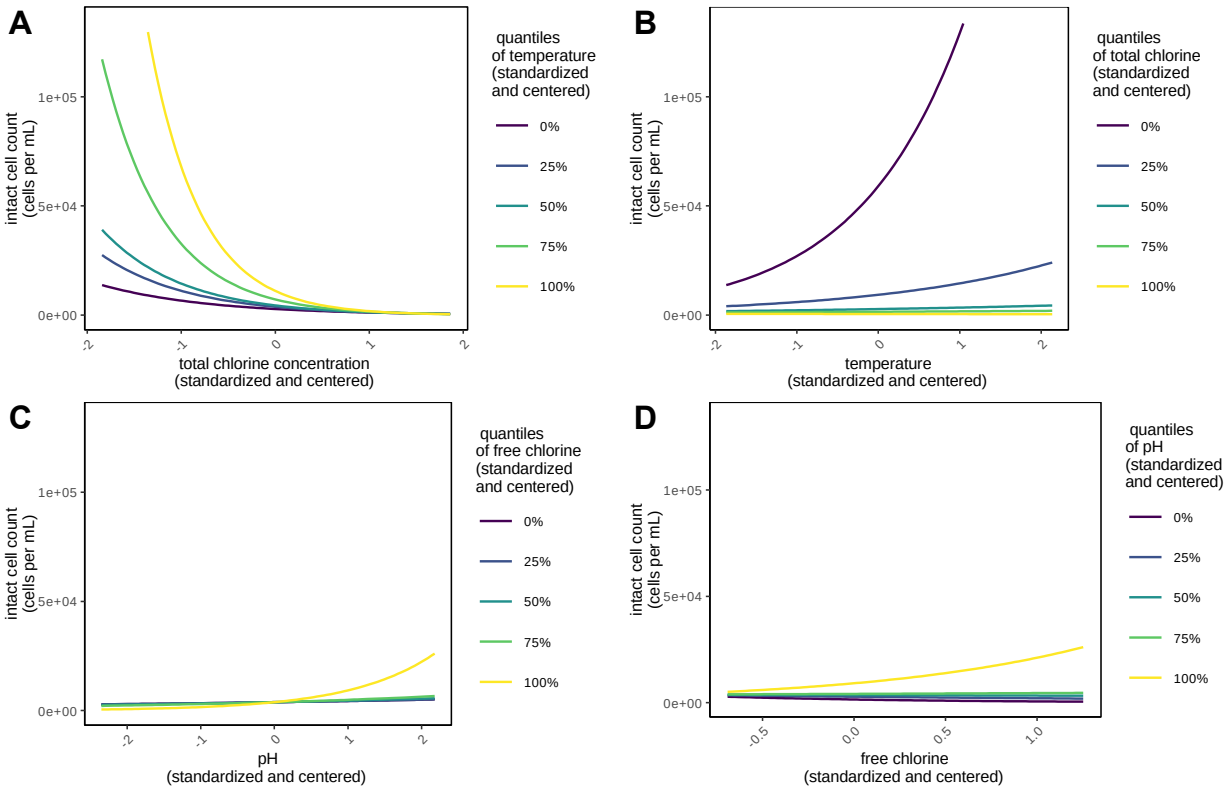

Figure S3: Visual representation of the most optimal model (Equation 2).To generate lines, all fixed effects were held constant at its mean except (A) Figure 3 in the main text included for comparison: total chlorine is on the x axis and temperature is varied in the model at each quantile value (-1.9, -0.10, -0.53, 0.87, and 2.1), (B) temperature is on the x axis and total chlorine is varied in the model at each quantile value (-1.8, -0.43, 0.51, 0.96, and 1.9), (C) pH is on the x axis and free chlorine is varied in the model at each quantile value (-0.69, -0.69, -0.58, -0.38, and 1.3), and (D) free chlorine is on the x axis and pH is varied in the model at each quantile value (-2.3, -0.78, -0.17, -0.20, and 2.2).

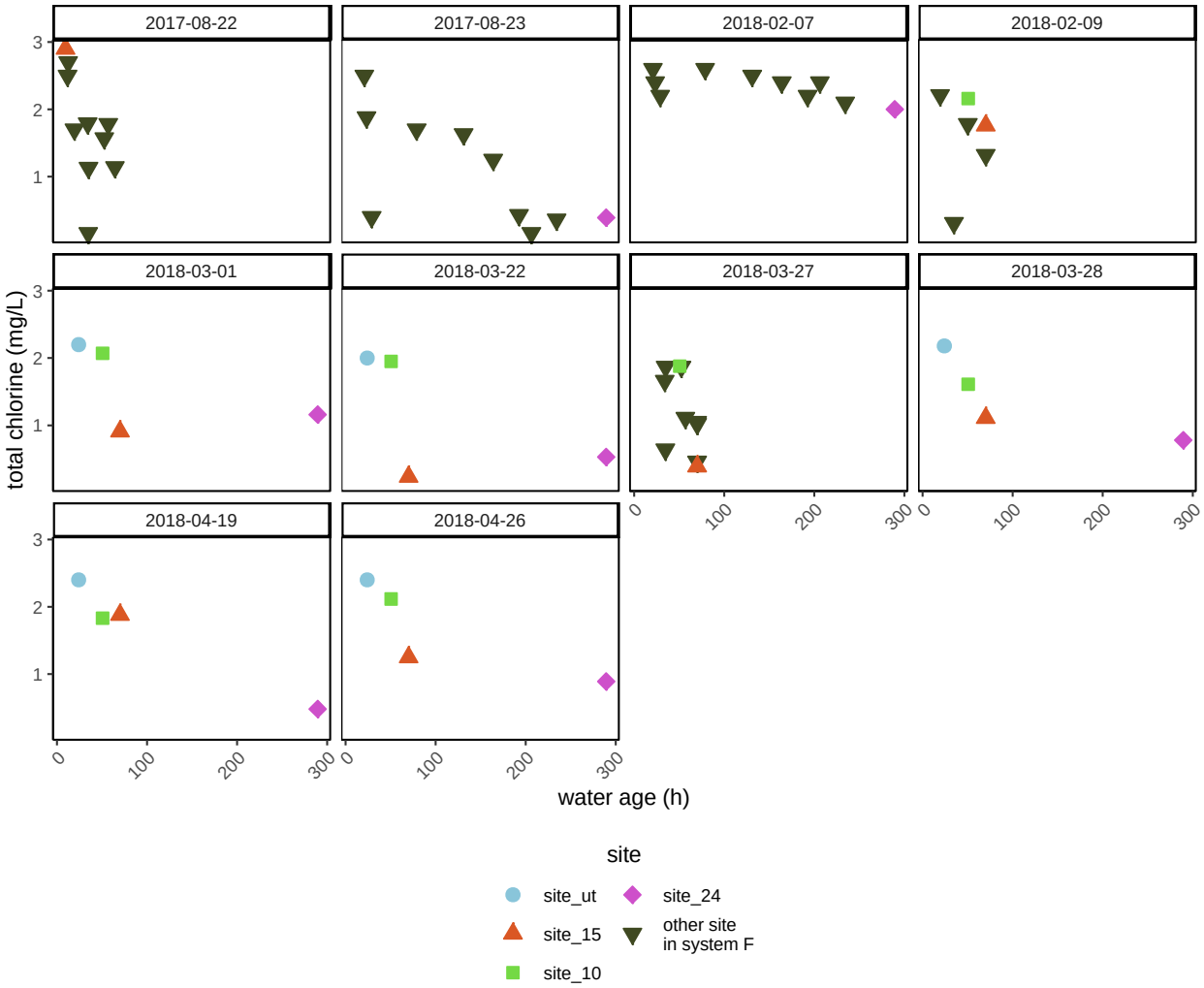

Figure S4: total chlorine concentration by water age (hours) in distribution system F for sampling dates with at least two samples collected. Shapes denote locations in distribution system F that were sampled at least six times between August 2017 and April 2018.

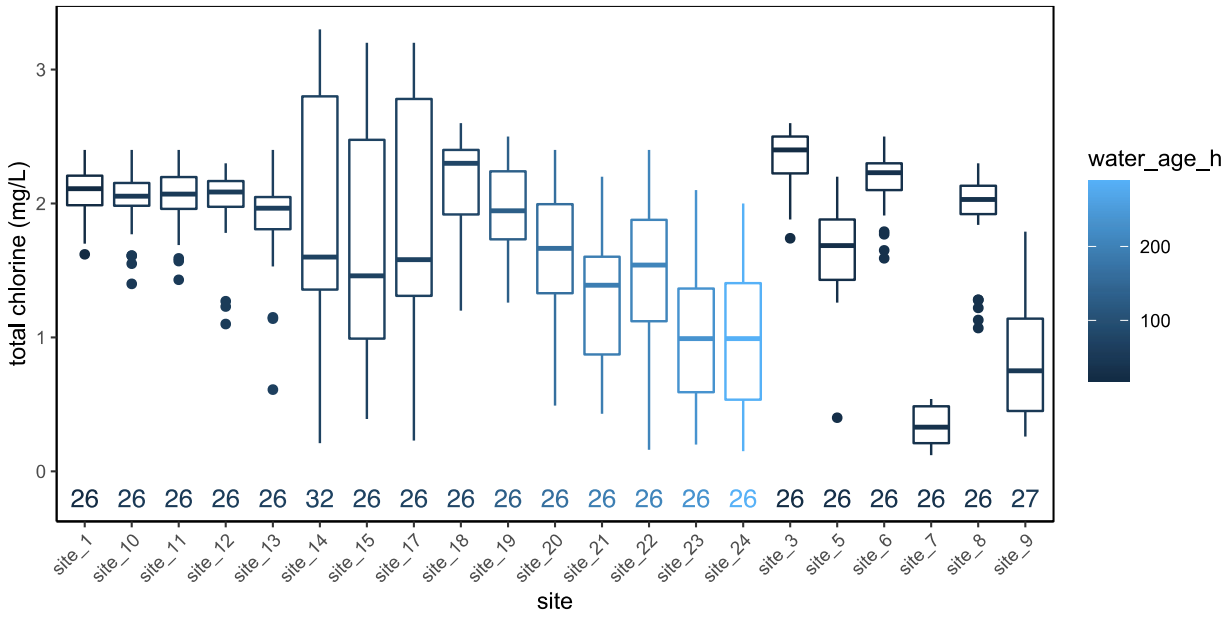

Figure S5: Ranges in total chlorine concentration by location in distribution system F from July 2017 to July 2018. Numbers underneath each box represent the number of sample measurements for that site.

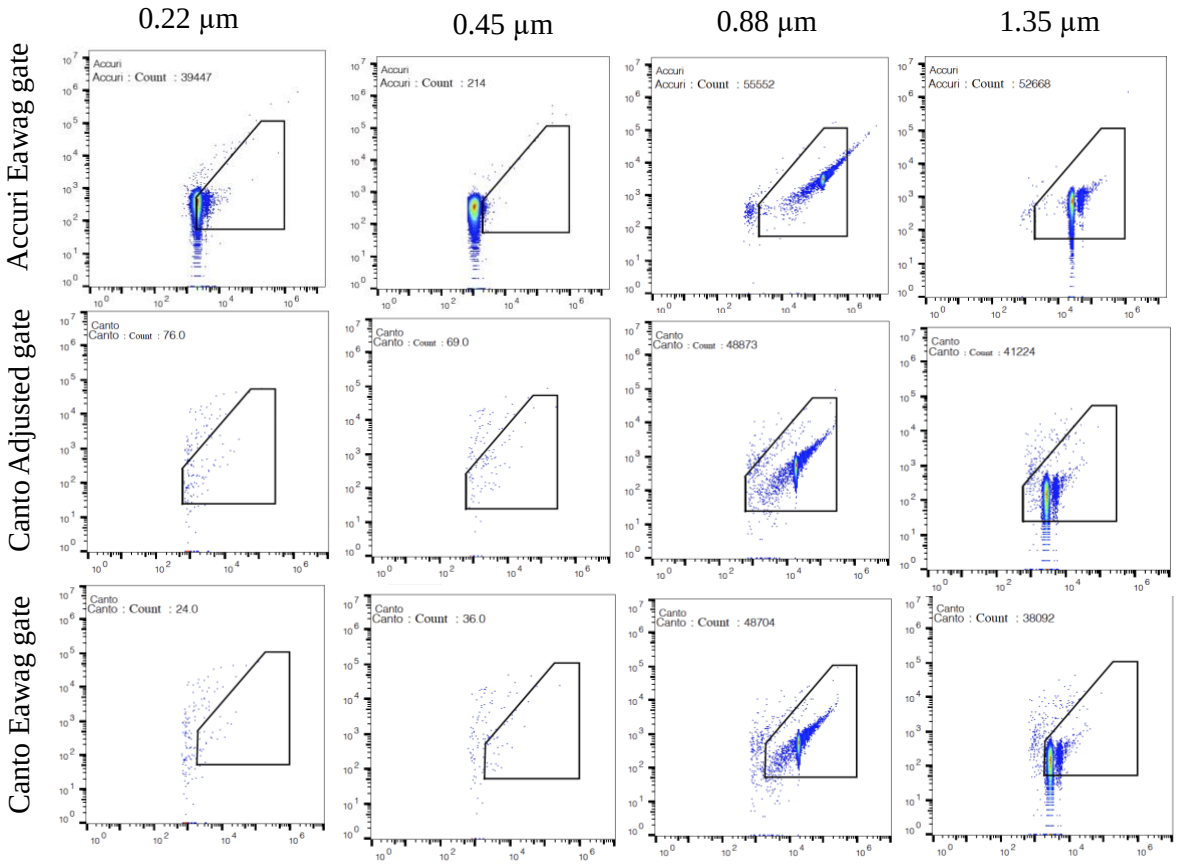

Figure S6: Result of calibration bead experiments with beads of four different diameters measured on Accuri flow cytometer with Eawag gate and Canto flow cytometer with Eawag gate and adjusted gate. One technical replicate is shown for each particle size with green fluorescence on the y-axis and red fluorescence on the x-axis.

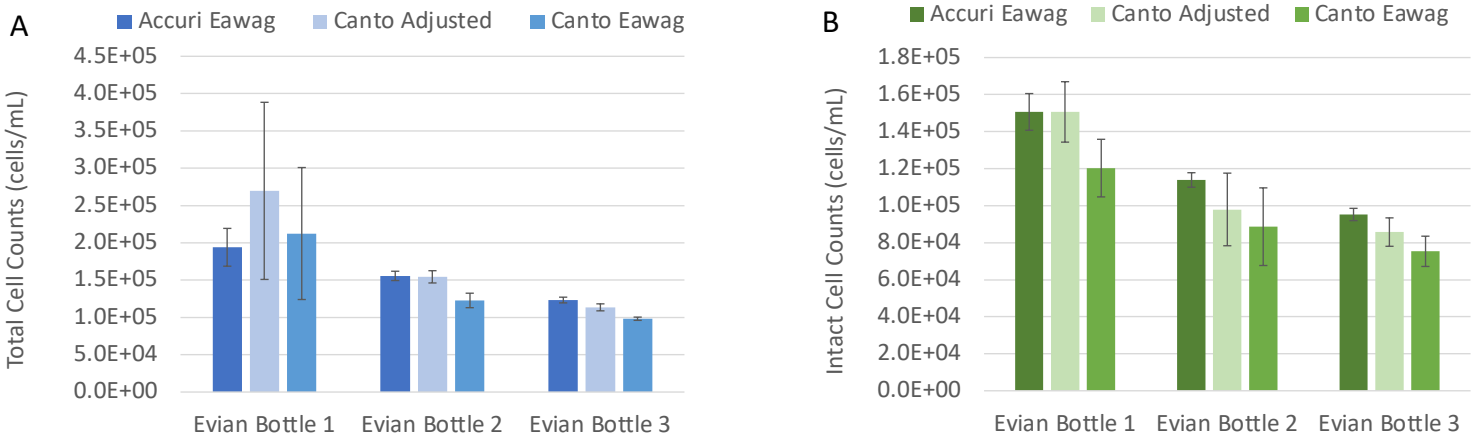

Figure S7: Result of the same three bottles of Evian water measured on Accuri flow cytometer with Eawag gate and Canto flow cytometer with Eawag gate and adjusted gate. Accuri results are biological triplicates of geometric averages from technical triplicates and Canto results are biological duplicates of geometric averages from technical triplicates. Error bars represent spread associated with standard deviation of biological replicates. Data for total cell count assay (A) and intact cell count assay (B) are shown.

4 Author Contributions

- Lauren C. Kennedy: Experimental design, sampling, and lab work for systems A (2016 & 2018), B (2016 & 2018), C (2016), F (2018); data analysis; manuscript writing; manuscript editing
- Scott E. Miller: Experimental design, sampling, & lab work for systems A, B, C, D, E in 2016; manuscript editing
- Rose S. Kantor: Sampling and lab work for systems A and F in 2018; manuscript editing
- Kara L. Nelson: Experimental design, data analysis, manuscript writing, and manuscript editing