Spring 2021

Effect of Intermittent Water Supply on Water Quality in a Model Pipeloop

Mariam Alkattan

Effect of Intermittent Water Supply on Water Quality in A Model Pipeloop

A Project Presented

by

MARIAM ALKATTAN

Master of Science in Civil Engineering

Department of Civil and Environmental Engineering
University of Massachusetts
Amherst, MA 01003

May 2021

EFFECT OF INTERMITTENT WATER SUPPLY ON WATER QUALITY IN A MODEL PIPELOOP

A Masters Project Presented

by

MARIAM ALKATTAN

Approved as to style and content by:

DocuSigned by:
Emily Kumpel
E4F23C5B4E8A4D1...
Emily Kumpel, Chairperson

DocuSigned by:
01D6091B23DA416...
Caitlyn S. Butler, Member

DocuSigned by:
Jessica Schi
D6723FBF52F54C2...
Jessica Schiffman, Member

DocuSigned by:
01D6091B23DA416...
Caitlyn S. Butler
Civil and Environmental Engineering Department

ACKNOWLEDGEMENTS

This work was supported by a National Science Foundation (CBET-1804232) grant and the Department of Civil and Environmental Engineering in the College of Engineering at the University of Massachusetts Amherst (UMass Amherst).

Special thanks to Sherrie Webb-Yagodzinski, Patrick Wittbold, Emma Guertin, Isaac Reyes, Stephen Stamegna, and the Advanced Digital Design and Fabrication Facility at UMass Amherst for making the construction and operation of the experimental pipeloops possible and to Kat Fish, Vanessa Speight, Gabrielle String, and the Light Microscope Facility at UMass Amherst for providing guidance and biological methods.

ABSTRACT

Intermittent water supply (IWS) is defined as a piped drinking water distribution system that operates for less than 24 hours per day. Water quality is found to be negatively impacted in IWS, which creates a human health risk. There are still may gaps in our understanding of pathways of contamination in IWS, which has been a limitation in creating appropriate solutions to maintain water quality in IWS systems. To characterize these pathways, we ran a study to investigate the impact of intermittency on water quality, biofilms, and water pressure in IWS, which consisted of constructing two identical model drinking water distribution systems. One was operated as an IWS and the other a continuous water supply (CWS), as a control. Water samples were taken for water quality analysis, biofilms were sampled, and pressure was monitored continuously in these systems. Key finding included a significant decrease in chlorine residual and increases in turbidity, TOC, and microbial concentration as in the water that was first flushed through the IWS pipeloop. However, IWS water quality parameters matched those is the CWS pipeloop or were better over the course of an IWS supply period. This implies the need for management of flush water in IWS systems. In addition, the biofilms in the IWS pipeloop before a supply period were found to have a larger spread than those after a supply period. Lastly, negative pressures were found in the IWS system. These results have implications for future research and IWS operation.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Cities around the world are struggling to keep up with demands on their infrastructure due to population growth, urbanization, displacement, and changes in natural resources due to climate change. When demands on piped water systems exceed supply, an intermittent water supply (IWS) is created. IWS is defined as a piped drinking water distribution system that operates for less than 24 hours per day. The practice of IWS is widespread, impacting over one-third of the piped water supplies in Asia, Africa, and Latin America. In South East Asia, 95% of water utilities operate intermittently (Taylor, Slocum, and Whittle 2018). In India alone, it is estimated that 200 million people access piped water that is an IWS. Ultimately, IWS is estimated to be responsible for 17 million infections, 4 million cases of diarrhea, and 1560 deaths per year globally (Bivins et al. 2017). The importance of our piped water infrastructure is being felt acutely now as many people are relying on water delivered to homes during the COVID-19 pandemic. Besides the inconveniences and challenges of receiving water on an intermittent basis, water quality is found to be negatively impacted in IWS, which creates a human health risk (Ercumen, Gruber, and Colford 2014; Ercumen et al. 2015; Adane et al. 2017; Jeandron et al. 2015; Cifuentes Enrique et al. 2002).

Water being supplied intermittently can adversely affect water quality by allowing inorganic and organic contamination to enter the water supply through multiple mechanisms (Kumpel and Nelson 2016; 2014). Contamination can occur from intrusion of liquid and other material that surround the distribution systems pipes from loss of pressure during non-supply periods. Contamination can also occur from bacteriological regrowth from loss of chlorine residual due to long retention times and stagnation. Biological material can also enter the water supply due to the intermittent operation of a drinking water distribution system during system start-up. For example, settled material in the distribution pipes can become mobilized. Also, biofilms, communities of microorganisms that grow on the pipe walls, can shear off during system start up and enter the water distribution system. Biofilms play an important role in distribution system water quality as they can harbor and become reservoirs for pathogens (Flemming, Percival, and Walker 2002; Wingender and Flemming 2011). Studies on IWS are often case studies as they are done on water distribution systems in the field which can be highly variable.

Stagnation, which increases water retention times and reduces chlorine residuals, in continuous water supplies (CWSs) is known to reduce water quality and induce bacterial growth (Lautenschlager et al. 2010; Zlatanović, van der Hoek, and Vreeburg 2017; Manuel, Nunes, and Melo 2009). Although there are studies investigating stagnation in CWS and its impact on water quality (often overnight stagnation in premise plumbing or dead ends in water distribution systems), it is not clear whether these effects are transferable to IWS hydraulics. In an IWS, pipes lose pressure and may empty of or be only half full of water between supply-cycles, in contrast to pipes stagnating but otherwise pressurized in a CWS. Little is known about the relative importance of mechanisms of contamination in an IWS, the magnitude of observed impacts, and how they change throughout a supply cycle. Such knowledge could inform the use and development of interventions to improve water quality in IWS. Prior studies of IWS have largely include been cross-sectional samples taken at a single time; however, water quality in an IWS varies throughout

an IWS supply cycle. In addition, there is no information on the impact of IWS on the structure, growth, and changes in the biofilms that grow within water distribution systems, and whether they play an important role in IWS water quality.

The objective of this study is to design an experiment to measure the impact of IWS on water quality in a controlled setting. The goal of the study will be to compare physical, chemical, and biological water quality in an IWS to a CWS throughout supply periods in a lab-scale setting. In addition, the study will investigate the impact of IWS on the biofilms growing within a water distribution system. These findings can be used to help identify the impact of IWS on water quality and identify potential recommendations for improving water quality in IWS systems.

2. MATERIALS AND METHODS

2.1 Pilot-scale Distribution System

For this study, we constructed two model drinking water distribution systems (DWDS) consisting of two identical piped recirculation systems. One was operated intermittently as an IWS (experiment) and the other operated continuously as a CWS (control) (Figure 1). The pipes were made of 2-inch diameter Schedule 80 PVC, as PVC piping is commonly used throughout regions experiencing IWS. Each pipeloop was 28-ft in total length (8.5 meters). The model was attached to a slotted metal framing strut channel hung vertically in the lab. The set-up was located in a temperature-controlled location with a setpoint temperature of 72 degrees C. Each pipeloop included a 0.06 HP circulation pump, 38 L cylindrical polypropylene reservoir tank with cover, and 2 sample taps. Water flow was maintained at 3gpm (0.1 m/s) and at a setpoint pressure of 5 PSI in the CWS at all times, and in the IWS during supply hours. A 12-hr hydraulic residence time (HRT) was created by using a dual channel chemical feed pump to pump fresh tap water into each tank at a rate that would replace the system volume (34 liters) twice per day. Inflowing fresh tap water from the chemical feed pump displaced water in the tank causing excess water volume to flow out of the system via an overflow valve in the reservoir tanks.

Municipal (Amherst, MA) tap water was used to supply the system. The municipal tap water was treated at the Atkins Water Treatment Plant through pre-treatment with ozone followed by mixed-media filtration, ozonation, GAC filtration, and disinfection residuals through chloramination. (“Atkins Reservoir | Amherst, MA - Official Website” n.d.).

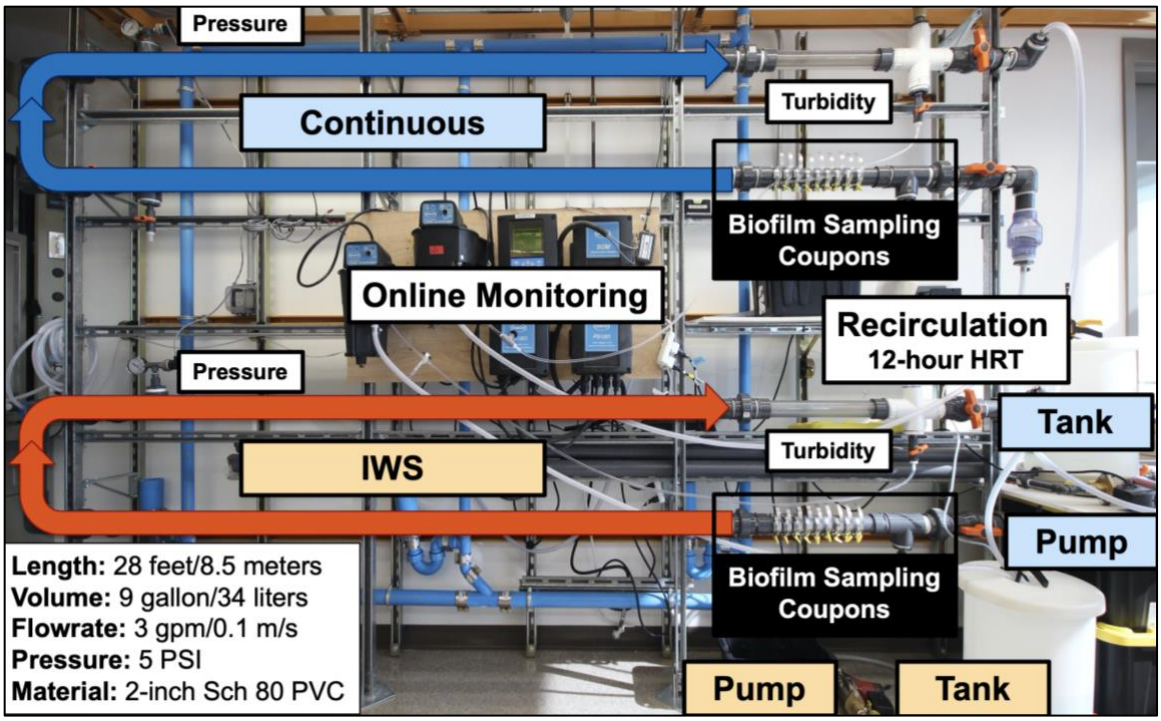


Figure 1. Both experimental pipeloops were supplied with water from the Amherst, MA town water distribution system.

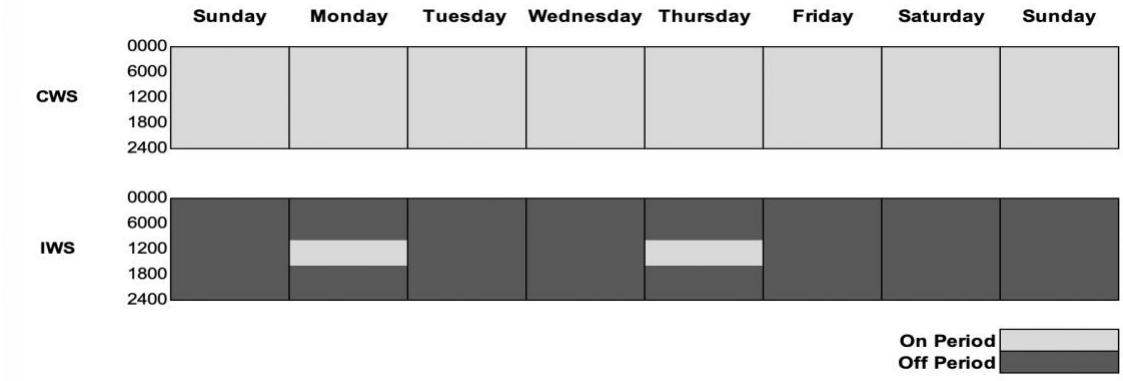


Figure 2. Visual diagram of the operational schedule used for the continuous and intermittent operation of the pipeloops.

2.2 Pipeloop Operations

To disinfect the pipeloop prior to experimentation, deionized water containing a 20 mg/L hydrochloride concentration was recirculated in the pipeloop for 24 hours at double the regular operating flow rate. After, the pipeloop was flushed with tap water until chlorine levels in the pipeloop returned to baseline. Both pipeloops were operated continuously for 5 weeks prior to one pipeloop being switched to an intermittent flow schedule. This allowed the time for microorganisms from the tap water to recolonize the system.

The schedule for the intermittent water supply was an 'on' period of 6-hrs (~10am – 4pm) a day for two days a week, which were almost always Monday and Thursday, with a few exceptions (Figure 2). The system was operated by hand by turning the pump on and off. Water was drained from the intermittent system during 'off' periods in the supply schedule. The water was allowed to completely drain during IWS off periods to represent the draining of water in full scale distribution systems through leakage points that occur when the water supply is turned off. Water was drained through two sample ports at the bottom of each pipeloop. The continuous water supply was always 'on' at a steady flow rate, apart from several off periods needed for maintenance of the system.

2.3 Water Quality Analysis

500 mL samples of bulk water were collected from sample taps in the pipeloops during the days when the IWS loop was operational. Each sample was collected in a sterile glass beaker. Sample taps were located close to the beginning on the pipeloop, as the other sample tap was used for online turbidity monitoring. Prior to sampling, taps were sterilized with 70% ethanol solution.

From the IWS loop, three samples were taken during each "on" period: one at the very beginning of an intermittent supply cycle, five minutes later, and just prior to turning it off. When the first and last samples from the IWS were collected, samples were collected at the same time from the CWS loop. The source water (tap water) was also sampled soon after the start of an IWS supply cycle. In total, six water samples were analyzed on each analysis day twice a week for 22 weeks.

Sample analysis included measurement of conductivity and temperature (Oakton PCTSTestr 50 hand-held multi-probe (Oakton Research, Vernon Hills, IL)), turbidity (HACH 2100 benchtop turbidimeter (HACH, Loveland, CO)), pH (benchtop pH meter and probe (Fisher Scientific, Waltham, MA)), and free chlorine, total chlorine, free ammonia, monochloramine, and total iron (DR900 multiparameter portable colorimeter (HACH, Loveland, CO) and HACH reagents). Analysis also included total organic carbon (TOC) and total nitrogen (TOC analyzer with a total nitrogen measuring unit (Shimadzu, Columbia, Maryland)). TOC and total nitrogen samples were preserved and prepared by first filtering the sample through a 47mm 0.45 um membrane filter (Fisherbrand) and adjusting pH to below 2 using a 6N solution of Hydrochloric Acid. Samples were typically stored for no longer than two weeks before analysis.

We measured the cATP (cellular adenosine tri-phosphate) concentration from intact cells in a sample. ATP (cATP) concentrations were measured using a commercial test kit specified for use with drinking water (LuminUltra, New Brunswick, Canada). The commercial cATP test kit used is an enzyme-based assay conducted on intact cells collected from the passing of a 50 mL sample though a .45 μm pore syringe filter. Samples for cATP samples were collected in a sterile container right after grab samples were collected. Sample size for cATP samples was 100 mL.

2.4 Pressure and Turbidity Sensing and Monitoring

Pressure was monitored continuously via pressure transducers installed into each pipeloop. Pressure transducers were periodically verified via calibrated analog pressure gauges installed beside each pressure transducer in the pipeloop. Turbidity was measured continuously through a HACH 1720D Turbidimeter that was connected to each pipeloop through a sample tap located near the end of the pipeloop. All turbidimeters and pressure transducers were connected to a data logger and control software (DATAQ, Akron, Ohio) that calibrated and logged pressure data and turbidity data continuously every 10 seconds to an excel spreadsheet.

2.5 Biofilm Analysis

Figure 3. Biofilm sampler on left with an insert partially removed, with dime positioned above for scale. Biofilm samplers fit into holes drilled into pipe walls as demonstrated on the right. This design is based on the biofilm sampling coupon from the Pennine Water Group at the University of Sheffield (Deines et al. 2010).

Holes were drilled in a section of each pipeloop and biofilm samplers were inserted. The biofilm sampling coupons matched the design of Pennine Water Group (PWG) biofilm sampling coupons (Deines et al. 2010) (Figure 3). The biofilm samplers were cut from the same pipes used in the construction of the pipeloop so that they would match the internal curvature of the pipe. The biofilm samplers included a flat insert to be used for microscopic imaging and an outer surface for the later removal of biofilm to be used for future extraction of DNA for genetic analysis. Each pipeloop had a pipe section with 15 biofilm samplers: 9 samplers arranged at the bottom of the piped sections, 3 in the middle, and 3 at top. Biofilm samplers were arranged at the top, middle, and bottom of a pipe to provide information on potential variability of the biofilm at various positions around the pipe circumference. Biofilm samplers were wiped with 70% ethanol before being inserted into the pipeloop.

Biofilms were developed over the 28 weeks the pipeloops were in operation. For analysis, biofilm samplers were removed from each pipeloop at the end of 6 months. To visualize cells in the biofilm using microscopy, the biofilms was stained with DAPI using a protocol adapted from String et al. (String et al. 2021). In summary, the biofilm sampler inserts were fixed to a microscope slide and stained with a 6000uM solution of DAPI (Sigma Aldrich). The inserts were then covered with a layer of aqueous fluorescent mounting media (Sigma Aldrich) to protect them from drying out and to provide a barrier between the sample and the coverslip that was placed over it. Stained biofilm sampler inserts were stored at 4°C and imaged over the course of 19 weeks (7 months).

2.6 Microscopy and Image Processing

DAPI stained biofilm inserts were imaged using a confocal laser scanning microscope (Nikon A1R: Nikon A1 Resonant Confocal with TIRF Module) at the University of Massachusetts Amherst Nikon Center of Excellence. 405 nm violet laser and a x20 objective was used to produce lambda-Z-stacks. The Z-stack limits were investigator-selected by manually viewing the biofilm under the microscope and selecting the topmost and bottommost cells in the FOV and configuring the microscope to scan that portion of the sample. This was done due to variations in stage height in the biofilms in each sample.

Five images from each biofilm sample were taken and averaged per sample. Field of view (FOV) locations were evenly spaced lengthwise across the biofilm sampling insert and results from the replicates from each sample were averaged. Each FOV had an image area of 19600 um^2. Microscope images were analyzed using the Nikon NIS-Elements universal imaging software. Features of the Nikon NIS-Elements software allow for the cleaning of 'noise' from a microscope image and the detection of objects in the image. The software is able to extract the accumulated volume of objects detected in an image and provide the location of the centroid of each object. In this way the topmost and bottommost cells were able to be detected.

2.7 Data Analysis

Water quality parameter and biofilm data were tested for normality using the Shapiro-Wilk test. Data sets were considered normal at $p < 0.05$. The Kruskal-Wallis H test, a rank-based nonparametric test, was used to determine statistical significance between groups. Groups were considered significantly different at $p < 0.05$. Correlations were analyzed by calculating the Pearson's correlation coefficient. Graphing and data analysis were carried out using R (R Core Team 2020). Transient water pressure decreases lasting for less than 3 seconds were removed by hand from online pressure data prior to analysis.

Staining of the biofilm revealed only the cells that composed the biofilm and not the proteins and carbohydrates that also make up the biofilm. With this considered, a parameter termed spread was used as a proxy for thickness as explained in Fish 2015 (Fish et al. 2015). Cell spread is a measurement in the z-direction between the topmost and bottom most cells identified in the biofilm. Biofilms were analyzed for cell volume, cell spread, and cell coverage. Cell coverage was calculated by dividing the cell volume by the cell spread.

3. RESULTS

3.1 Source Water Quality

Table 1. Descriptive statistics of source water (tap water) from June 18 – November 22, 2019. Number of samples (n), median concentrations, of samples collected for tap water sampled at the start of IWS "on period", which occurred twice per week. Total iron, pH, DO, conductivity, and temperature sometimes only sampled during only one of the IWS on periods per week, creating variation in sample number. Geometric mean reported for Total Nitrogen.

		Summer (June 18 – Aug 29, 2019)		Fall (Sept 2 – 22 Nov 22, 2019)
Parameter	**n**	**Median**	**n**	**Median**
Temperature (°C)	14	21.60	17	20.70
Conductivity (µS/cm)	14	88.00	17	87.90
pH	14	7.87	17	7.69
Turbidity (NTU)	25	0.35	18	0.38
Total Iron (mg/L)	24	0.09	10	0.09
Free Cl (mg/L Cl2)	24	0.07	18	0.49
Total Cl (mg/L Cl2)	24	0.67	18	0.88
Monochloramine (mg/L Cl2)	25	0.60	18	0.83
Free Ammonia (mg/L NH3-N)	25	0.30	18	0.28
Total Nitrogen (mg/L)	18	0.5852	14	0.7805
TOC (mg/L)	18	1.903	14	2.860
cATP (pg/mL)	16	3.510	15	1.780

Multiple physical, chemical, and biological parameters in the Amherst tap water used to supply both pipeloops were analyzed. Using the Shapiro–Wilk test for normality the data for all water quality parameters measured were found to be non-normally distributed, except TOC which was found to be distributed log-normally. Thus, median values were reported except for Total Nitrogen, where geometric mean was reported.

Water quality exhibited variation by season: Summer (June 18 – Aug 29, 2019) and Fall (Sept 2 - Nov 22, 2019). The median measured free chlorine concentration of 0.07 mg/L Cl_2 in the summer was lower than the median 0.49 mg/L Cl_2 concentration measured in the fall and likely impacted measured biological concentrations. Correspondingly, the median total chlorine concentration of 0.88 mg/L Cl_2 was higher in the fall than the median 0.67 mg/L Cl_2 concentration in the summer.

Utilities add ammonia to a solution containing free residual chlorine to create monochloramine in the water supply. Ammonia is added in excess, and that excess can be assessed by measuring free ammonia concentrations. The median free ammonia concentrations were similar across the two seasons (0.30 and 0.28 mg/L Cl_2 in the summer and fall, respectively). The median monochloramine concentration was 0.60 mg/L Cl_2 in the summer and 0.83 mg/L Cl_2 in the fall and followed the same trends as those from free and total chlorine concentrations.

Biological activity was assessed by measuring cellular cATP concentration in the water samples. In the summer season, the cATP concentration of 3.51 pg/mL was almost twice as high than the 1.78 pg/mL concentration in the fall. The fall concentration of ATP is within range for other ATP concentrations found in distributed drinking water (Vang et al. 2014; Vital et al. 2012; Liu et al.

2013), however the summer cATP concentrations were somewhat elevated. Median water temperature was 21.6°C in the summer and 20.7°C in the fall, and this may have impacted biological concentrations as well.

Median TOC concentrations were 1.903 mg/L in the summer and 2.860 mg/L in the fall, close to and above the EPA recommendation for TOC concentration (of <2.0 mg/L for treated water). The geometric mean of Total Nitrogen concentrations was 0.5852 mg/L in the summer and 0.7805 mg/L in the fall. The median TOC and the geometric mean of the total nitrogen concentrations were elevated in the fall, which may have been due to increased leaf litter in the surface waters that supplied the drinking water in the town of Amherst (Duan et al. 2014).

Several water quality parameters were similar across the seasons. The total iron median value was 0.10 mg/L, well below the 0.3 mg/L MCL for drinking water, and similar between the seasons. Median turbidity levels and conductivity concentration were relatively low, and median pH values were typical for drinking water; all remained similar across the two seasons.

3.2 Bulk Water Quality in IWS and CWS

Table 2. Descriptive statistics including the number of samples (n) and median of samples collected from Source; CWS at time 0 and end; and IWS at time 0, 5 minutes, and end. Sampling ranged from June 18 – November 19, 2019.

Parameter	n	Source	n	CWS T=0 Median	n	IWS T=0 Median	n	IWS T=5min Median	n	CWS T=end Median	n	IWS T=end Median
Temperature (°C)	31	21.1	29	21.2	28	19.5	28	20.5	24	22.1	27	22.2
Conductivity (µS/cm)*	31	87.9	29	87.0	28	96.2	27	88.2	24	96.3	27	87.3
pH*	31	7.75	29	7.47	28	7.14	28	7.60	24	7.25	27	7.45
Turbidity (NTU)	43	0.387	42	0.318	42	0.566	42	0.382	37	0.305	41	0.363
Total Iron (mg/L)*	24	0.10	12	0.06	11	0.09	10	0.12	7	0.05	10	0.10
Free Cl (mg/L Cl2)	42	0.30	42	0.06	43	0.02	42	0.15	37	0.05	42	0.05
Total Cl (mg/L Cl2)	42	0.70	42	0.20	42	0.07	41	0.44	36	0.18	42	0.16
Monochloramine (mg/L Cl2)	43	0.78	24	0.16	24	0.11	22	0.34	17	0.17	23	0.18
Free Ammonia (mg/L NH3-N)	43	0.27	23	0.39	23	0.33	21	0.34	16	0.36	22	0.37
Total Nitrogen (mg/L)**	32	0.7000	32	0.7168	32	0.6769	32	0.6994	30	0.7314	33	0.7273
NPOC (mg/L)	32	2.441	32	2.157	32	4.142	32	2.409	30	2.493	33	2.544
cATP (pg/mL)	31	2.79	31	30.93	31	200.89	31	15.32	29	18.03	31	18.24

*Measured weekly versus measured twice weekly during the twice weekly supply cycles.
**Geometric mean

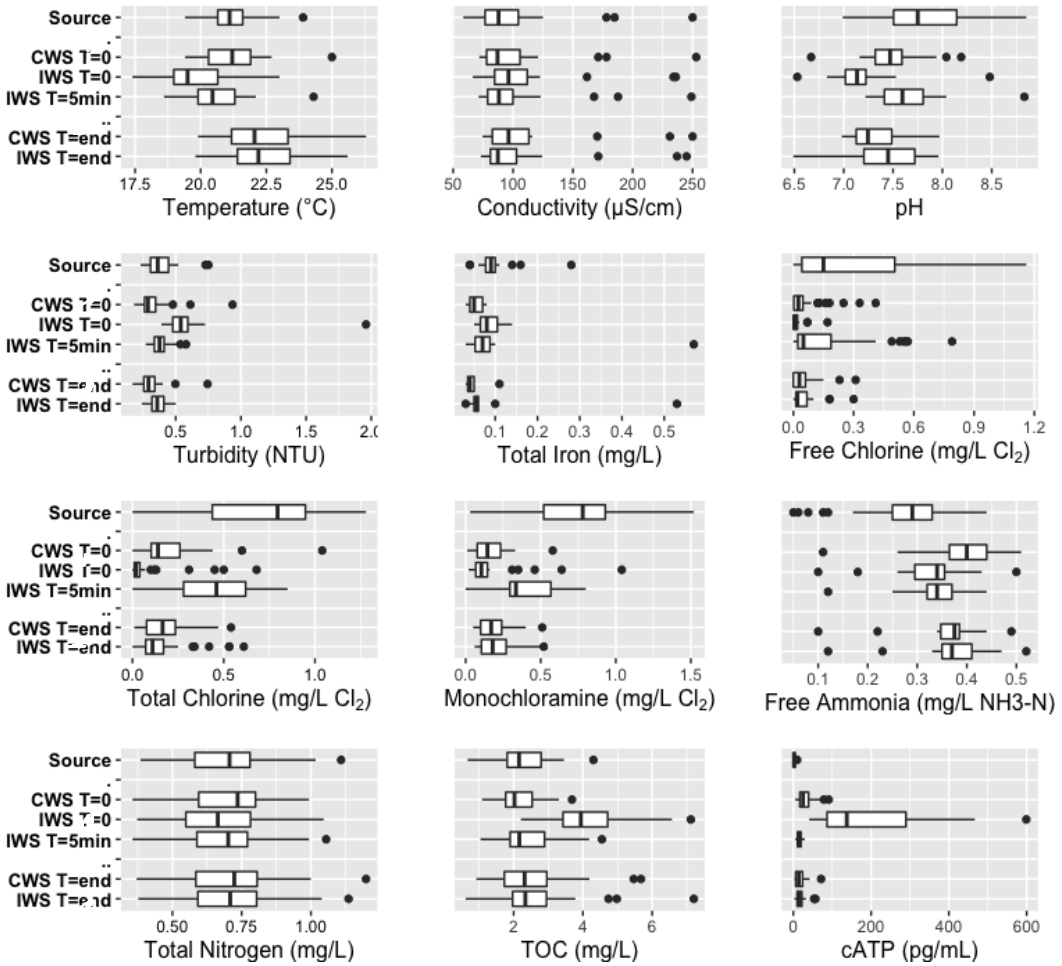


Figure 4. Box and whisker plots show the median, lower and upper quartiles, range, and outliers of water quality parameters of samples collected from Source; CWS at time 0 and end; and IWS at time 0, 5 minutes, and end. Sampling ranged from June 18 – November 19, 2019.

Results of water quality analysis from source water, the IWS pipeloop, and the CWS pipeloop are presented in Table 2 and Figure 3. Differences between the sample groups were found to be statistically significant across all parameters except conductivity (0.6100) and total nitrogen ($p = 0.6473$). There was very little change in the median between the different groups for conductivity concentrations.

The median turbidity in the source water was 0.387 NTU. The median turbidity in the CWS at both the beginning (T=0) and end of the cycle (T=end) was significantly lower than the source water ($p = 0.0002$ and $p = 0.3e\text{-}05$). In addition, CWS (T=0) and (T=end) had significantly similar median values (0.318 and 0.305 NTU; $p = 0.8404$). The median turbidity in the IWS (T=0) was 0.566 NTU, which decreased to 0.382 NTU at IWS (T=5) and was 0.363 NTU at IWS (T=end). Turbidity levels in the CWS and IWS, except for the turbidity at the beginning of the IWS supply cycle, were lower than the turbidity in the source water. This may indicate settling in the pipes, especially in the CWS. The IWS supply cycles saw higher turbidity than in the source water, suggesting possible mobilization of settled material present in the pipeloop. The turbidity decreased by the end of the IWS supply cycle but remained higher than in the CWS at the same

relative timepoint. A similar trend was observed with total iron, suggesting that some particles in the IWS that would otherwise have settled may have remained suspend at the end of the 6-hour supply period.

The median free chlorine concentration in the source water was 0.30 mg/L. The median free chlorine in CWS at both the beginning (T=0) and end (T=end) were statistically lower than the source water, and the two values were similar to each other (0.06 and 0.05 mg/L) and showed no statistical difference ($p = 0.7922$). The median free chlorine in the IWS was 0.02 mg/L at the beginning (T=0), rose to 0.15 mg/L after 5 minutes, and dropped to 0.05 mg/L; the end was similar to CWS (T=end) with no statistical difference between the free chlorine concentration at IWS=end and CWS=end ($p = 0.792$). There were no significant differences in free chlorine concentrations for IWS=5min and source water ($p = 0.064$). Total chlorine, monochloramine, and free ammonia concentrations followed a similar trend. This aligns with the hydraulic operation of the pipes: there is a 12-hour residence time in the CWS pipe loop. With IWS, first the pipes are empty with perhaps some stagnant water, but then fresh (source water) enters, eventually reaching a steady state similar to that in the CWS. This same trend is seen in temperature and pH, as these indicate the change between stagnant water, recirculated water with an increased water age, and fresh water coming from the source.

The median TOC in the source water was 2.2174 mg/L. The median TOC in the CWS at the beginning (T=0) and end were similar (2.157 mg/L and 2.493 mg/L, respectively; ($p = 0.720$), and neither differed from the source water ($p = 0.750$ and $p = 0.950$, respectively). The median TOC at the IWS beginning (T=0) was 4.142 mg/L, which decreased to 2.409 mg/L after 5 minutes (T=5min) and to 2.544 NTU at the end (T=end) and showed statistical differences between IWS T=0 and IWS T=5min, and IWS T=5min and IWS=end ($p = 0.7.9e-08$ and $p = 1.3e07$). There was no significant difference between mean TOC at the end of the IWS and CWS supply cycles ($p = 0.910$), nor at IWS T=end and source water ($p = 0.820$). Except for the IWS samples at T=0, all the samples had a similar median value. The increase in the IWS (T=0) may be due to the increase in particulate containing organic carbon suspended during system start-up.

The median cATP in the source water was 2.79 pg/mL. The cATP in the CWS at the beginning (T=0) (30.93 pg/mL) was significantly lower than the end (T=end) (18.03 pg/mL) ($p = 0.0091$). The median cATP in the IWS at the beginning (T=0) was 200.89 pg/mL, which decreased to a median of 15.32 pg/mL after 5 minutes (T=5) and 18.03 pg/mL at the end (T=end). Median cATP was significantly higher in the CWS and IWS samples as compared to the source water. The median cATP concentrations in the IWS sample taken at T=0 was 72 times higher than the cATP median concentration measured in the source water and at least a 6 times higher concentration than the cATP concentration taken in the CWS pipeloop at the same time (CWS T=0).

Results of the Pearson parametric correlation test between the various water quality parameters showed a positive correlation between turbidity and TOC ($r^2=0.37$) and turbidity and cATP ($r^2=0.51$), indicating that increases in turbidity and TOC were correlated with increases in biological concentrations. In addition, cATP was negatively correlated with free chlorine ($r^2=-0.22$), total chlorine ($r^2=-0.33$), and monochloramine($r^2=-0.33$), indicating that disinfectant was impacting biological concentrations in the water samples. Monochloramine was negatively

correlated with temperature (r^2=-34) and may be impacted by seasonal temperature changes. Similar results were reported in study comparing the effects of water quality parameters on microbial abundance in disinfected drinking water systems (Kennedy et al. 2020).

3.3 Online Pressure and Turbidity

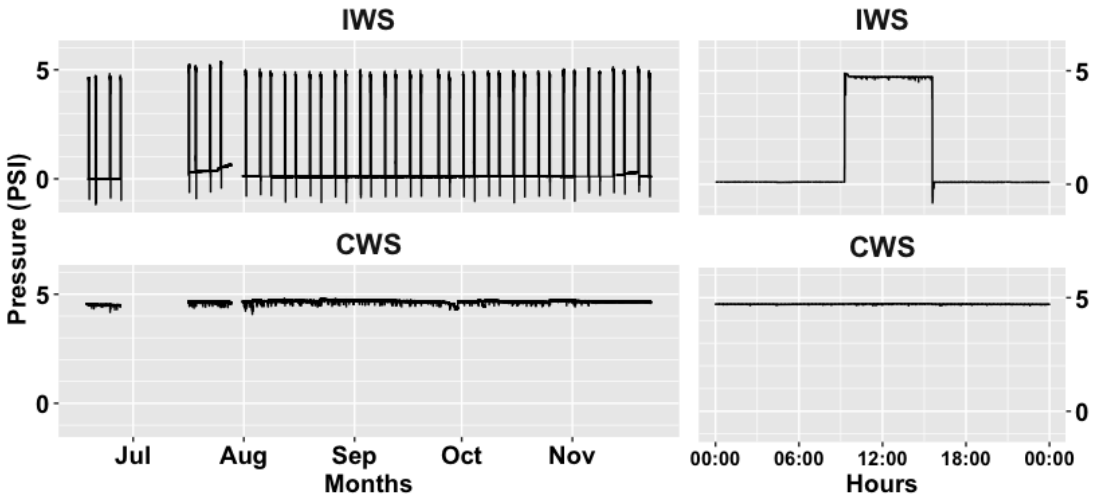


Figure 5. Pressure measured every 10 seconds over the 6-month experimental period (June 18 – November 19, 2019) in the CWS and IWS pipeloop (left). Pressure reading over a single day (September 5, 2019) in the CWS and IWS pipeloop (right). Gaps in data represent data lost due to equipment failures.

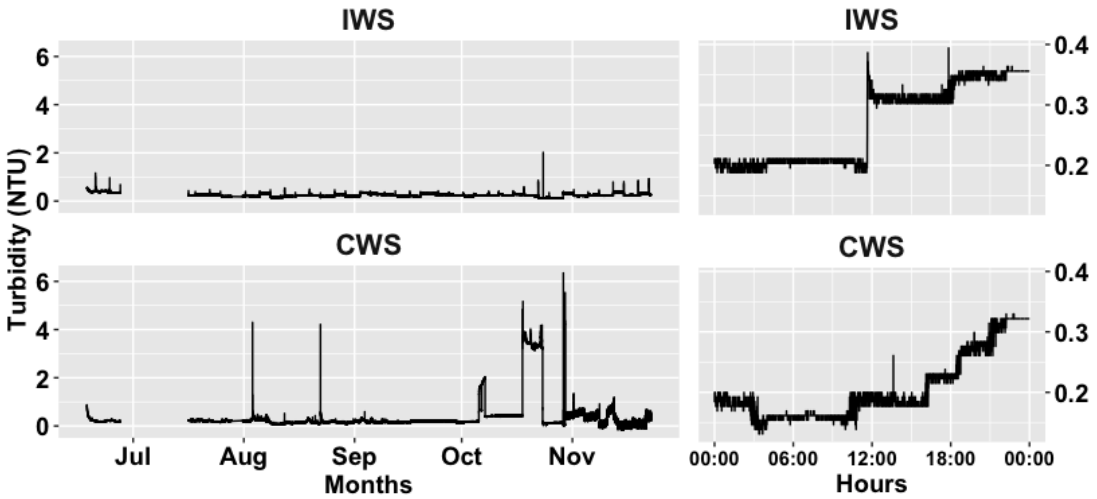


Figure 6. Turbidity measured every 10 seconds over the 6-month experimental period (June 18 – November 19, 2019) in the CWS and IWS pipeloop (left). Turbidity readings in a single day (September 5, 2019) in the CWS and IWS pipeloop (right). Gaps in data represent lost data.

Pressure and turbidity were logged in each experimental pipeloop every 10 seconds. Mean IWS supply duration during the experimental period was 6hrs and 21 minutes. Pressure in the CWS remained at around 5 PSI. During an IWS "on" period, pressure rose almost immediately to approximately 5 PSI (Figure 5), similar to the CWS operating pressure, and remained until the

end. When the pump was switched off and water from the pipeloop was allowed to drain, pressure reached negative values of around -1 PSI. Negative pressures observed in the pipeloop may have important implications: in a full-scale distribution system, negative pressures can draw contaminated water or other substances surrounding the distribution system into the distribution system via backflow or intrusion. This intruded material may introduce pathogens and other contaminants.

Turbidity in the IWS pipeloop was higher during the IWS on period than the CWS supply (Figure 6), although, notably, the levels are low. Turbidity was higher during the first 5-7 minutes of supply (Figure S4) and remained so compared to the turbidity in the CWS.

3.4 Biofilm

Table 3. Biofilm sample number, cell volume, cell spread, and cell coverage from biofilms sampled from the CWS and before and after an IWS supply period at the end of the experimental period. Biofilm samplers were in three positions in the pipeloop (top, middle, and bottom).

Position	Supply	*n*	Median Cell Volume (um^3)	Median Cell Spread (um)	Median Cell Coverage (um^2)
All	CWS	15	1303.442	7.2	9.2E-03
	IWS Before	5	1372.372	9.0	7.8E-03
	IWS After	8	1345.097	8.1	8.5E-03
	Average		**1340.304**	**8.1**	**8.5E-03**
Bottom	CWS	9	1040.630	7.2	7.4E-03
	IWS Before	3	1027.616	9.0	5.8E-03
	IWS After	4	1345.097	8.1	8.5E-03
	Average		**1137.781**	**8.1**	**7.2E-03**
Middle	CWS	3	1303.442	6.0	1.1E-02
	IWS Before	1	1748.410	7.8	1.1E-02
	IWS After	2	1055.793	7.8	6.9E-03
	Average		**1369.215**	**7.2**	**9.8E-03**
Top	CWS	3	2127.792	9.0	1.2E-02
	IWS Before	1	1122.141	9.0	6.4E-03
	IWS After	2	1537.736	9.3	8.4E-03
	Average		**1595.890**	**9.1**	**9.0E-03**

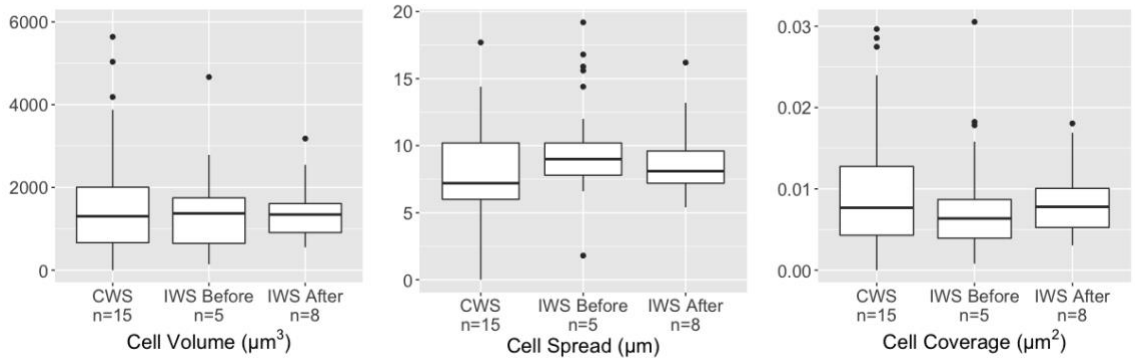


Figure 7. Comparison of biofilms sampled from the CWS supply and before and after an IWS supply cycle. Box and whisker plots show the median, lower and upper quartiles, range, and outliers of biofilm cell volume, biofilm cell spread, and biofilm density.

Results of biofilm analysis are presented in Table 3 and Figure 7. Biofilm samples were taken from the CWS pipeloop (n = 15) and from the IWS pipeloop before (n = 5) and after the IWS supply cycle (n = 8). Biofilm samples were also designated by position in the pipeloop pipe wall; bottom (n = 16), middle (n = 6), and top (n = 6). Samples were stained with DAPI to identify the cells that made up the biofilm and five fields of view, as replicates, were taken from each sample. Examples of images taken of the biofilms are presented in Figure 8.

Data collected on biofilms sampled from both pipeloop were not normally distributed. Cell volume ($p = 0.9713$) and cell coverage ($p = 0.5696$) were found to be not statistically significant between the CWS and IWS groups, while cell spread was found to be significantly different ($p = 0.0209$). In turns of the position of the biofilms sampled, statistical significance was found between the cell spread ($p = 0.0041$) and cell coverage ($p = 0.0336$), however there was no statistical significance in the cell volume ($p = 0.0590$). Lack of statistical significance in cell volume in biofilms sampled from different positions within a pipeloop were found to be consistent with a similar study (Fish et al. 2015).

Cell spread appeared to be the greatest in the IWS Before group among all the biofilms sampled from the various positions, with a median of 9.0 um (Figure 7). This compared with a median of 8.1 um in the CWS group and a median of 7.2 um in the IWS After group. Both the IWS Before and IWS After groups had a larger cell spread than the CWS group. Among the IWS groups, the IWS Before had a thicker spread. This suggests that part of the biofilm in the IWS system may have been sheared off during IWS startup and operation and resulted in a loss of ‘thickness’ in the biofilm.

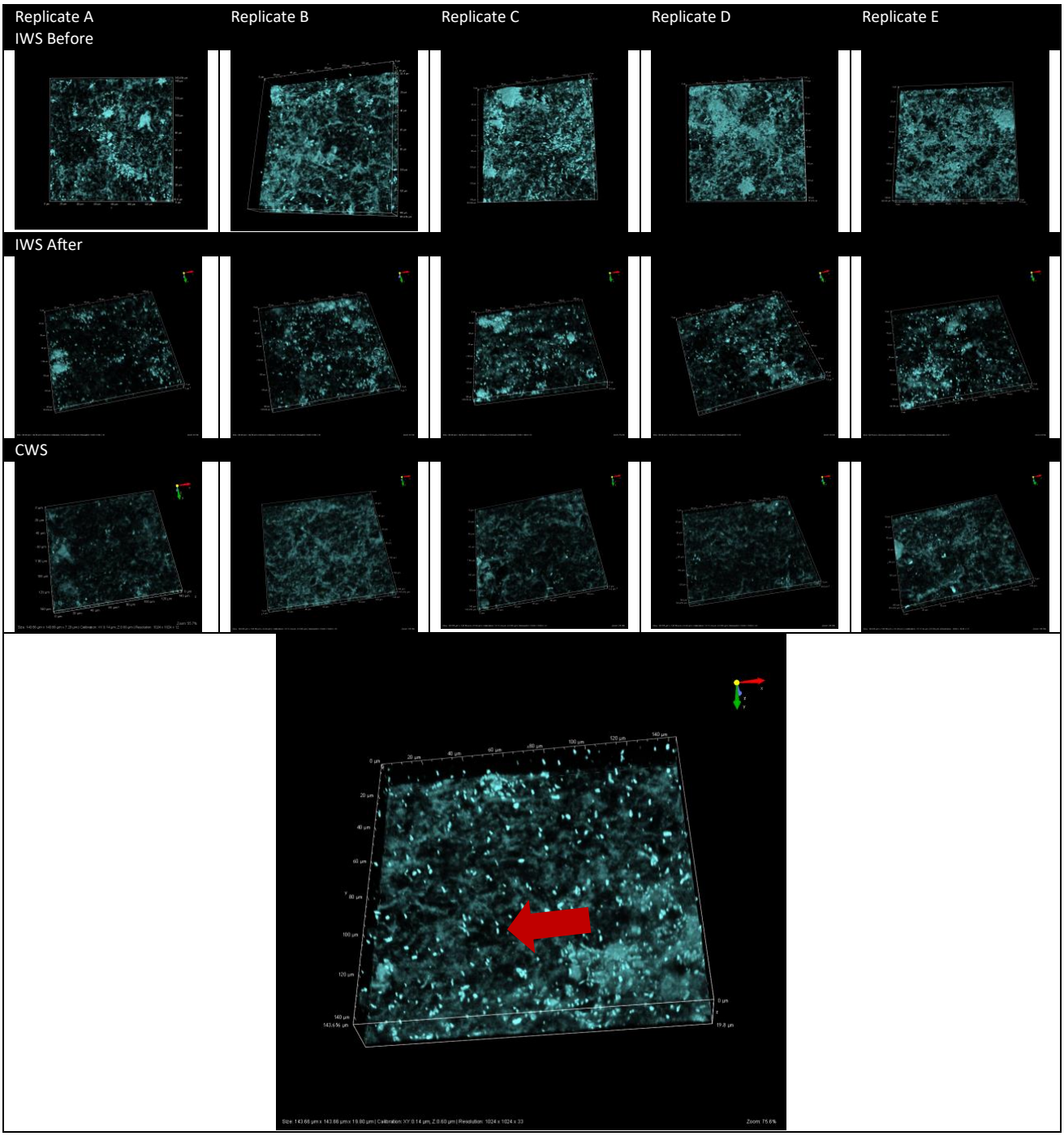


Figure 8: Selection of biofilm Z-stack images taken on a confocal scanning microscope that represent biofilms taken from the IWS before and after a supply cycle and from the CWS. At bottom is a zoomed in image for better illustration. Bright round objects are cells, an example of which is pointed out by the red arrow. Shading/noise on the bottom plane is autofluorescence from the plastic substrate of the biofilm insert. Cell spread was calculated by measuring the distance in the z-direction from the center of the bottom most and topmost cell in each image. This image was taken from a biofilm sampling coupon insert from an IWS system before the start of a supply cycle.

4. DISCUSSION

Measurements taken 5 minutes into the IWS supply cycle showed lower concentration of chlorine and higher concentrations of biomass and turbidity as compared to CWS. The water quality by 5 minutes into the IWS supply cycle was similar to the water quality at the end of the IWS supply cycle. Notably, chlorine residuals at the end of the IWS supply cycle were similar to that of the CWS, although the IWS system had slightly lower cATP concentrations but higher turbidity. The higher turbidity in the IWS pipeloop at the end of the supply period may be due to there not being adequate time for particles to settle after system start up. These results imply that lower water quality in IWS is concentrated in the startup period when the system is first turned on. Otherwise, IWS water quality compares to and might even be an improvement to CWS water quality at the end of a supply period, likely related to the lower water age in the IWS system.

This compares with what we know about CWSs that experience unsteady flow and stagnation. Stagnation and variable demand patterns can decrease biological stability in the drinking water (Manuel, Nunes, and Melo 2009). Results also show negative pressures in the IWS system when it is turned off. This is due to the negative pressures created by water leaving the IWS system during draining. This is important to consider because it suggests that materials surrounding the IWS system could be drawn into the system, and then would be left inside the pipes until the re-start of the next supply cycle. Therefore, IWS systems should be kept away and protected from areas of contamination such as sewer lines, latrines, stormwater drains, and any industry that might pose a risk to contamination. It has been suggested in another study that an IWS protected from sources of contamination have better water quality (Erickson et al. 2017).

Overall, biofilms were found to have the greatest spread in the biofilms sampled from the IWS pipeloop before an IWS supply period versus after an IWS supply period. This suggests that the biofilms may have been sheared off during supply operation (which could be associated with biomass entering the bulk water supply and can also be observed in the cATP elevated IWS concentrations measured in the IWS during start up. If there are pathogens in the biofilms that are sheared off this may create an additional public health risk. These results are consistent with previous studies conducted on biofilms that show that biofilms subjected to shear stress are subject to removal while biofilms that are subjected to continuous flow are thinner and more dense like those found in the CWS pipeloop (Melo 2005; Paris, Skali-Lami, and Block 2007).

There are limitations to studying IWS using a lab-based model and additional limitations pertaining to our experimental setup. Water circulating in the pipeloops may not be the best representation of water flowing through a distribution system in the field. The water retention time of 12hrs used in this study may have been insufficient and the water source supplying the experimental pipeloop was monochlorinated, unlike the chlorinated systems in low- and middle-income countries. In addition, the water velocity may have been too low and steady as compared to distribution systems in the field with higher and more variable water velocities. Additionally, sample sizes for the biofilms sampled were low and a larger sample size would better validate the part of the study that included the analysis of the biofilms. Studies conducted in the lab will not replicate completely the conditions found in full-scale distribution systems in the field. A particular limitation of the study includes lack of intrusion that would occur in full-scale water distribution

systems. However, it is important to limit intrusion for this study to better understand the other factors related to IWS. Otherwise, the experimental pipeloop included many factors that would be critical for understanding IWS mechanisms.

Often resource-limited settings get lower quality of drinking water, but this does not have to be so. We can implement drinking water solutions that are appropriate to these settings. In doing so we ensure that public health is also protected for these populations. Recommendations based on the conclusions of this study include future research and suggestions for IWS system operation.

These are several results of the study that have implications for the operation of IWS systems to better maintain water quality. Water flushed through the IWS during start-up can be somehow managed by being diverted when the IWS system is first turned on for a supply period. In addition, high chlorine residual can be maintained in the system to prevent the regrowth of biomass and protect against biological contamination. In addition, better input water quality in terms of lower turbidity and TOC concentrations as well as higher chlorine residual concentrations may result in lower levels of biological activity in IWS systems. In addition, because of the negative pressures experienced in IWS, it is important to keep the area surrounding the water distribution system clear of any potential contaminants that can enter the system during negative or lower pressure periods.

An important research question to explore is the impact of frequency and duration of the IWS supply period on IWS water quality. Also, studies that include the impact of intrusion on IWS should be conducted. Another area to be explored is the impact of input water quality on changes in water quality in IWS. Further studies are needed to explore the possible transport of biomass between the biofilms in IWS and water supply in IWS.

5. RECOMMENDATIONS FOR FUTURE STUDIES

The experimental setup had several challenges during early days of operation. The first was leakage, mainly from the biofilm sampling coupons. This issue was resolved by adding a second hose clamp to secure the sampling coupons to the pipe, and the backing material for the coupon sampler was changed to another plastic material that was more resilient to cracking. Regardless of the material used for the biofilm sampling coupon backings, the coupons were found to crack from the pressure of being secured to the pipe. We also found that it was important to not over-tighten the biofilm sampling coupons, as this caused the backing to crack and increase leakage. The system still experienced some leakage during the experimental period; however, it was minimal.

The leakage from the system influenced the operation parameters that were chosen for the study. The following are recommendations could improve the future pipeloop studies or if the experiment is repeated:

- **Pump:** Future experiments should use a stainless-steel pump to protect the pump from rusting when it is off during the IWS off periods. A cast iron recirculation pump was used in a preliminary study prior to this study and produced an excess of rust that entered and stained the pipeloop system. Also, we recommend choosing a pump that could provide greater flow velocities and

pressures, as well as the ability to be programmed to operate at variable speeds to better replicate the variable velocities created by demand patterns in full-scale water distribution systems.

- **Hydraulic Retention Time:** Without the early challenge of leakage and the need to quickly replace lost water, increasing the hydraulic retention time to a rate that more resembles the hydraulic retention times found in full-scale water distribution systems would be beneficial. A more representative hydraulic retention time would likely change the values found in the water quality parameters measured as part of this study, such as chlorine residual and biological activity.

- **Biofilm Sampling:** We recommend increasing the number of biofilm samplers installed in the pipeloop. We would recommend placing these additional biofilm samplers not only in various vertical positions but in horizontal positions along the pipeloop. In addition, we recommend removing and analyzing the coupons throughout the experimental period, rather than only at a single time point at the end of the experimental period.

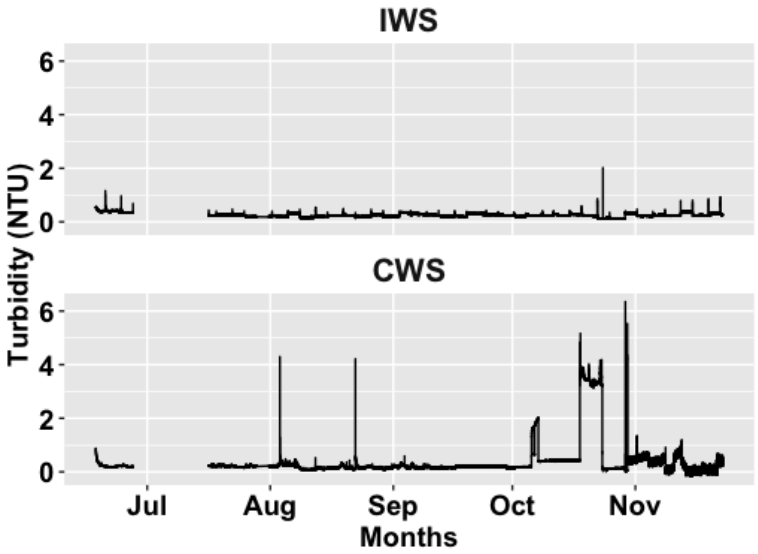
- **Source Water Supply:** In future experiments, it would be ideal to use a water supply that was chlorinated, as opposed to chloraminated, to better represent the type of chlorine residual used in drinking water distribution systems that are intermittently supplied. Other impacts on water supply changes should also be explored in future studies, such as the impact that highly treated vs contaminated water supplies would have on distribution system water quality.

- **Supply Schedule and Operation:** In future experiments, impacts of IWS supply duration and frequency should be explored as it may impact results. This is especially important since it is an unexplored area in IWS and represents a major gap in IWS knowledge and research.

- **Experimental Variables:** In future IWS and/or pipeloop experiments, it would be beneficial to explore experimental variables that were beyond the scope of this experiment. For example, it would be beneficial to examine the role pipe material plays on water quality characteristics, as well as the impact of seasonality on water quality.

APPENDIX: Supplementary Information

a)



b)

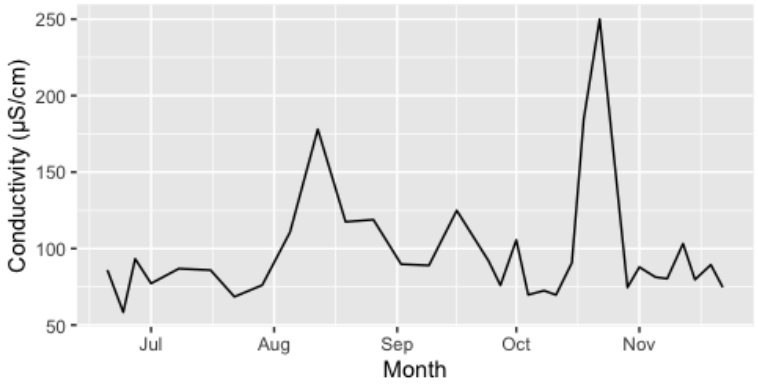


Figure S1. Comparison of online turbidity in the IWS and CWS pipeloops from June 18 – Nov 22, 2019 (a) compared to conductivity in the measured tap water coming into both of the pipeloop systems (b). Changes in turbidity may be due to water supply changes in the Town of Amherst, MA tap water as indicated by changes in conductivity over the same period.

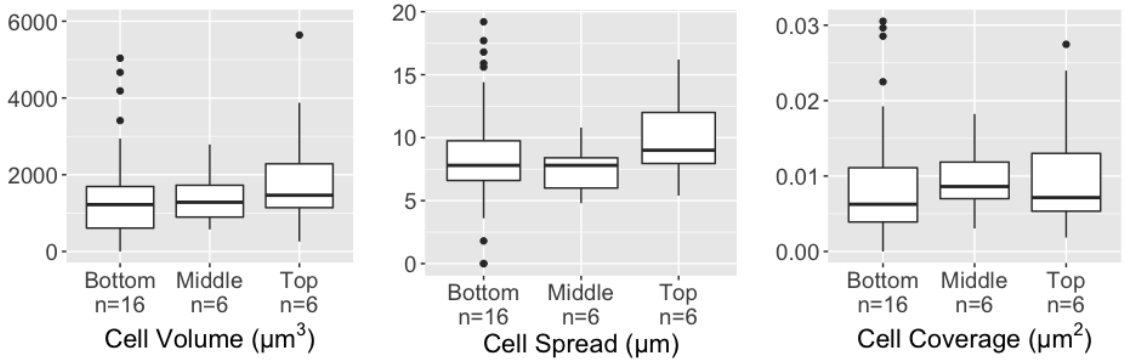


Figure S2. Comparison of biofilms sampled from biofilm samplers from the top, middle, and bottom position of the pipe wall. Box and whisker plots show the median, lower and upper quartiles, range, and outliers of biofilm cell volume, biofilm cell spread, and biofilm density.

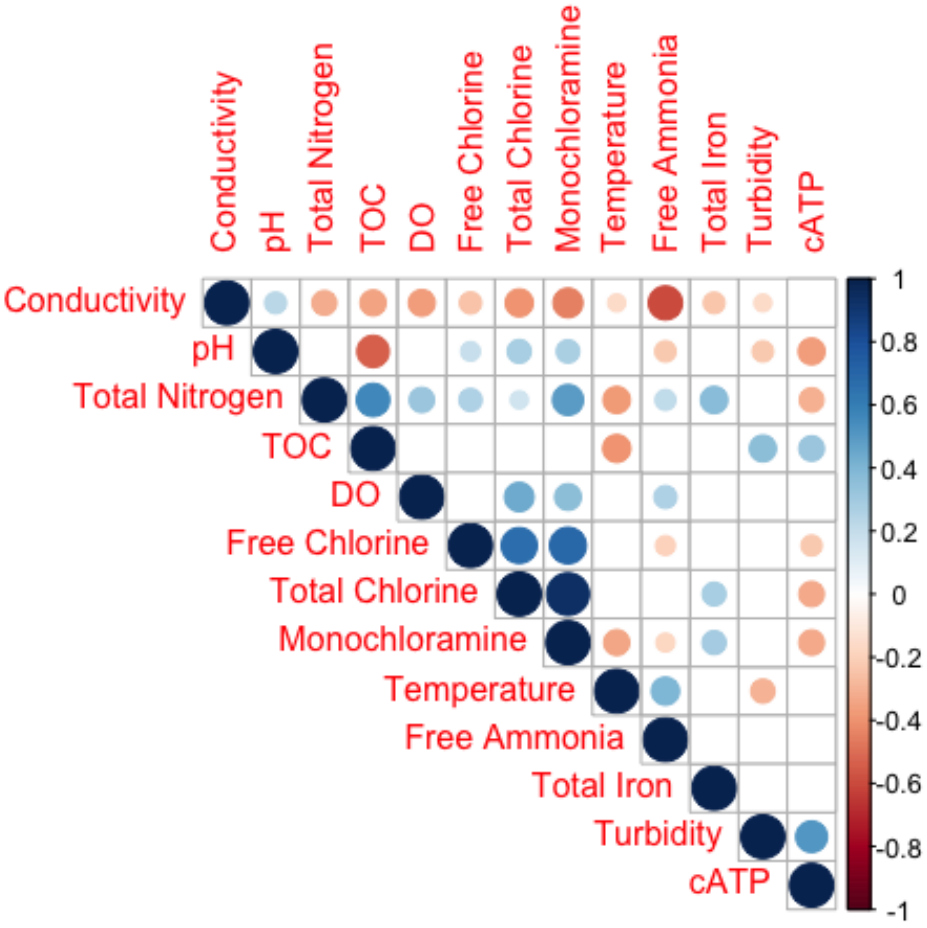


Figure S3. Correlation matrix of the water quality parameters measured from the bulk water samples. Positive correlations are displayed in blue and negative correlations in red. Color intensity and size of circle correspond proportional to the correlation coefficients. Insignificant correlations are blank ($p > 0.05$).

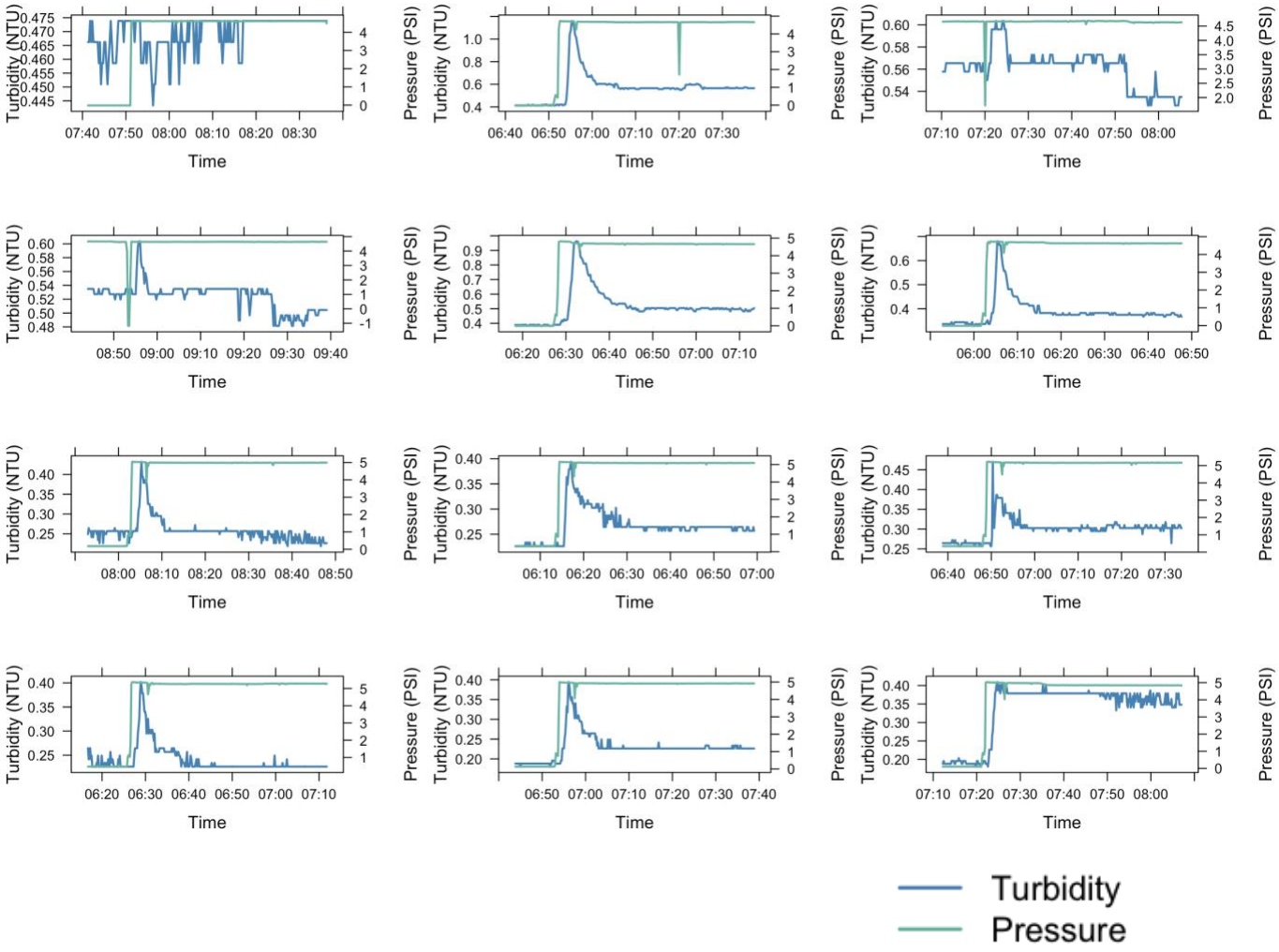


Figure S4. Matrix of plots of the first 12 instances of IWS supply cycle. Plotted are online pressure versus online turbidity for the first 5 minutes before an IWS on cycle and 1 hour after the IWS supply cycle. It is noted that turbidity spikes in about the first 5 minutes during IWS start up and then rapidly decreases and stabilize over the next 10 minutes after that.

REFERENCES

Adane, Metadel, Bezatu Mengistie, Girmay Medhin, Helmut Kloos, and Worku Mulat. 2017. "Piped Water Supply Interruptions and Acute Diarrhea among Under-Five Children in Addis Ababa Slums, Ethiopia: A Matched Case-Control Study." *PLOS ONE* 12 (7): e0181516. https://doi.org/10.1371/journal.pone.0181516.

"Atkins Reservoir | Amherst, MA - Official Website." n.d. Accessed June 15, 2020. https://www.amherstma.gov/180/Atkins-Reservoir.

Bivins, Aaron W., Trent Sumner, Emily Kumpel, Guy Howard, Oliver Cumming, Ian Ross, Kara Nelson, and Joe Brown. 2017. "Estimating Infection Risks and the Global Burden of Diarrheal Disease Attributable to Intermittent Water Supply Using QMRA." *Environmental Science & Technology* 51 (13): 7542–51. https://doi.org/10.1021/acs.est.7b01014.

Cifuentes Enrique, Suárez Leticia, Solano Maritsa, and Santos René. 2002. "Diarrheal Diseases in Children from a Water Reclamation Site in Mexico City." *Environmental Health Perspectives* 110 (10): A619–24. https://doi.org/10.1289/ehp.021100619.

Deines, Peter, Raju Sekar, P. Stewart Husband, Joby B. Boxall, A. Mark Osborn, and Catherine A. Biggs. 2010. "A New Coupon Design for Simultaneous Analysis of in Situ Microbial Biofilm Formation and Community Structure in Drinking Water Distribution Systems." *Applied Microbiology and Biotechnology* 87 (2): 749–56. https://doi.org/10.1007/s00253-010-2510-x.

Duan, Shuiwang, Katie Delaney-Newcomb, Sujay S. Kaushal, Stuart E. G. Findlay, and Kenneth T. Belt. 2014. "Potential Effects of Leaf Litter on Water Quality in Urban Watersheds." *Biogeochemistry* 121 (1): 61–80. https://doi.org/10.1007/s10533-014-0016-9.

Ercumen, Ayse, Benjamin F. Arnold, Emily Kumpel, Zachary Burt, Isha Ray, Kara Nelson, and John M. Colford. 2015. "Upgrading a Piped Water Supply from Intermittent to Continuous Delivery and Association with Waterborne Illness: A Matched Cohort Study in Urban India." Edited by Clarissa Brocklehurst. *PLOS Medicine* 12 (10): e1001892. https://doi.org/10.1371/journal.pmed.1001892.

Ercumen, Ayse, Joshua S. Gruber, and John M. Colford. 2014. "Water Distribution System Deficiencies and Gastrointestinal Illness: A Systematic Review and Meta-Analysis." *Environmental Health Perspectives*, March. https://doi.org/10.1289/ehp.1306912.

Erickson, John J., Charlotte D. Smith, Amador Goodridge, and Kara L. Nelson. 2017. "Water Quality Effects of Intermittent Water Supply in Arraiján, Panama." *Water Research* 114 (May): 338–50. https://doi.org/10.1016/j.watres.2017.02.009.

Fish, Katherine E., Richard Collins, Nicola H. Green, Rebecca L. Sharpe, Isabel Douterelo, A. Mark Osborn, and Joby B. Boxall. 2015. "Characterisation of the Physical Composition and Microbial Community Structure of Biofilms within a Model Full-Scale Drinking Water Distribution System." *PLOS ONE* 10 (2): e0115824. https://doi.org/10.1371/journal.pone.0115824.

Flemming, H.-C., S. L. Percival, and J. T. Walker. 2002. "Contamination Potential of Biofilms in Water Distribution Systems." *Water Science and Technology: Water Supply* 2 (1): 271–80. https://doi.org/10.2166/ws.2002.0032.

Jeandron, Aurélie, Jaime Mufitini Saidi, Alois Kapama, Manu Burhole, Freddy Birembano, Thierry Vandevelde, Antonio Gasparrini, Ben Armstrong, Sandy Cairncross, and Jeroen H. J. Ensink. 2015. “Water Supply Interruptions and Suspected Cholera Incidence: A Time-Series Regression in the Democratic Republic of the Congo.” *PLOS Medicine* 12 (10): e1001893. https://doi.org/10.1371/journal.pmed.1001893.

Kennedy, Lauren C., Scott E. Miller, Rose S. Kantor, and Kara L. Nelson. 2020. “Effect of Disinfectant Residual, PH, and Temperature on Microbial Abundance in Disinfected Drinking Water Distribution Systems.” *Environmental Science: Water Research & Technology*, November. https://doi.org/10.1039/D0EW00809E.

Kumpel, Emily, and Kara L. Nelson. 2014. “Mechanisms Affecting Water Quality in an Intermittent Piped Water Supply.” *Environmental Science & Technology* 48 (5): 2766–75. https://doi.org/10.1021/es405054u.

———. 2016. “Intermittent Water Supply: Prevalence, Practice, and Microbial Water Quality.” *Environmental Science & Technology* 50 (2): 542–53. https://doi.org/10.1021/acs.est.5b03973.

Lautenschlager, Karin, Nico Boon, Yingying Wang, Thomas Egli, and Frederik Hammes. 2010. “Overnight Stagnation of Drinking Water in Household Taps Induces Microbial Growth and Changes in Community Composition.” *Water Research* 44 (17): 4868–77. https://doi.org/10.1016/j.watres.2010.07.032.

Liu, G., E. J. Van der Mark, J. Q. J. C. Verberk, and J. C. Van Dijk. 2013. “Flow Cytometry Total Cell Counts: A Field Study Assessing Microbiological Water Quality and Growth in Unchlorinated Drinking Water Distribution Systems.” *BioMed Research International* 2013: 1–10. https://doi.org/10.1155/2013/595872.

Manuel, C. M., O. C. Nunes, and L. F. Melo. 2009. “Unsteady State Flow and Stagnation in Distribution Systems Affect the Biological Stability of Drinking Water.” *Biofouling* 26 (2): 129–39. https://doi.org/10.1080/08927010903383448.

Melo, L.F. 2005. “Biofilm Physical Structure, Internal Diffusivity and Tortuosity.” *Water Science and Technology* 52 (7): 77–84. https://doi.org/10.2166/wst.2005.0184.

Paris, Tony, Salaheddine Skali-Lami, and Jean-Claude Block. 2007. “Effect of Wall Shear Rate on Biofilm Deposition and Grazing in Drinking Water Flow Chambers.” *Biotechnology and Bioengineering* 97 (6): 1550–61. https://doi.org/10.1002/bit.21321.

String, Gabrielle M., Marta Domini, Hanaa Badr, Hannah Brodsky, Yarmina Kamal, Tyler Tatro, Miranda Johnston, et al. 2021. “Efficacy of Locally-Available Cleaning Methods and Household Chlorination at Inhibiting Biofilm Development in Jerricans Used to Store Household Drinking Water.” *Environmental Science: Water Research & Technology* 7 (2): 367–83. https://doi.org/10.1039/D0EW00748J.

Taylor, David D. J., Alexander H. Slocum, and Andrew J. Whittle. 2018. “Analytical Scaling Relations to Evaluate Leakage and Intrusion in Intermittent Water Supply Systems.” Edited by Zhi Zhou. *PLOS ONE* 13 (5): e0196887. https://doi.org/10.1371/journal.pone.0196887.

Vang, Óluva K., Charlotte B. Corfitzen, Christian Smith, and Hans-Jørgen Albrechtsen. 2014. “Evaluation of ATP Measurements to Detect Microbial Ingress by Wastewater and Surface Water in Drinking Water.” *Water Research* 64 (November): 309–20. https://doi.org/10.1016/j.watres.2014.07.015.

Vital, Marius, Marco Dignum, Aleksandra Magic-Knezev, Petra Ross, Luuk Rietveld, and Frederik Hammes. 2012. “Flow Cytometry and Adenosine Tri-Phosphate Analysis: Alternative Possibilities to Evaluate Major Bacteriological Changes in Drinking Water Treatment and Distribution Systems.” *Water Research* 46 (15): 4665–76. https://doi.org/10.1016/j.watres.2012.06.010.

Wingender, Jost, and Hans-Curt Flemming. 2011. “Biofilms in Drinking Water and Their Role as Reservoir for Pathogens.” *International Journal of Hygiene and Environmental Health*, The second European PhD students workshop: Water and health ? Cannes 2010, 214 (6): 417–23. https://doi.org/10.1016/j.ijheh.2011.05.009.

Zlatanović, Lj., J. P. van der Hoek, and J. H. G. Vreeburg. 2017. “An Experimental Study on the Influence of Water Stagnation and Temperature Change on Water Quality in a Full-Scale Domestic Drinking Water System.” *Water Research* 123 (October): 761–72. https://doi.org/10.1016/j.watres.2017.07.019.