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Monitoring Adenosine Triphosphate Concentrations in a Chloraminated Drinking Water Distribution System for Risk and Asset Management

Rasha Maal-Bared [1,*], Michael McCracken [2], Bharatee Busawon [3] and Darlyce Simpson [4]

[1] Process Development Team, Quality Assurance and Environment, EPCOR Water Services Inc., Edmonton, AB T5H 0E8, Canada
[2] Edmonton Drinking Water Plants, EPCOR Water Services Inc., Edmonton, AB T5J 3Y3, Canada; mmccracken@epcor.com
[3] Analytical Operations, Quality Assurance and Environment, EPCOR Water Services Inc., Edmonton, AB T5H 0E8, Canada; bbusawon@epcor.com
[4] Quality Assurance, Quality Assurance and Environment, EPCOR Water Services Inc., Edmonton, AB T5H 0E8, Canada
* Correspondence: rmaal-bared@epcor.com; Tel.: +1-780-412-7617

Abstract: Utilities rely on reliable and robust monitoring systems to inform decisions around asset operation and management in the drinking water distribution system (DWDS) to deliver high quality, biologically stable drinking water to consumers. However, traditional culture-based testing methods present challenges that make the timely detection of regrowth in the DWDS difficult. This study reports the results of an extensive adenosine triphosphate (ATP) monitoring campaign—a non-regulated parameter—in an urban, chloraminated drinking water system that analyzed over 5000 samples from two drinking water treatment plants (DWTPs), associated DWTP reservoirs, twelve outlying reservoirs and the DWDS between 2019–2022. ATP concentrations increased significantly between the two DWTP reservoirs and outlying reservoirs but decreased between the outlying reservoirs and DWDS samples. Relationships between ATP concentrations and other water quality variables varied depending on sampling location. Heterotrophic plate counts (HPC) were mainly non-detects (<1 CFU/mL) providing limited operational guidance compared to ATP. ATP concentrations exhibited temporal and spatial variation but did not exceed the proposed 10 pg/mL corrective action limit suggested by the manufacturer. ATP concentrations were also able to inform outlying reservoir management decisions. Monitoring ATP could serve as a useful indicator of biological stability in the DWDS for the utility of the future.

Keywords: ATP; HPC; operational thresholds; chlorine residual; asset management; treatment plants; reservoirs; distribution system

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1. Introduction

Effective drinking water treatment is an essential component of the multibarrier approach for the management of contaminants in drinking water and the protection of public health [1,2]. However, due to the nature and complexity of centralized drinking water distribution systems (DWDS), by the time water reaches the consumer tap it has often been exposed to microbiological, chemical or physical factors that render it different from the quality of the finished water leaving the drinking water treatment plant (DWTP) [1,3–7]. Utilities aim to provide customers with biologically stable water that has been subject to minimal regrowth and changes in microbiological concentrations and composition in the DWDS [8,9]. To accomplish this, utilities rely on good practices in the construction, operation, monitoring and maintenance of all parts of the DWDS network [10–12]. Despite utility efforts, the detection of microbiological changes in drinking water presents some unique challenges related to microbiological testing methodology and the identification of thresholds that trigger operational or corrective actions to mitigate risk to the consumers.

Most utilities rely on fecal indicator bacteria and heterotrophic plate counts (HPCs) to assess risk to public health and changes in microbiological water quality, respectively. Fecal indicator bacteria tend to be regulated and part of routine testing. HPCs are often unregulated. Different jurisdictions use different HPC limits for taking corrective actions in the DWDS (e.g., 100 CFU/mL versus 500 CFU/mL). HPCs refer to culture-based tests that are designed to recover a wide range of microorganisms that use organic carbon as an energy source (e.g., bacteria, yeasts, moulds). Although HPC testing is included in the Standard Methods for the Examination of Water and Wastewater [13], there is no universally accepted, standardized HPC method (i.e., different incubation conditions and culture media can be used) making the interpretation of HPC results challenging for utilities [14–16]. It has become standard practice to perform two different tests, one at 20–22 °C for 3–5 days and a second at 35–37 °C for 1–2 days [17] but that remains time-consuming, laborious and limits the opportunity for rapid corrective action in the DWDS [18,19]. HPCs do not allow for the identification of specific organisms or pathogens and cannot be used as an indicator of public health risk, but if tested in parallel with disinfectant residual can provide an indication of a change in water quality [20]. These limitations have led to the adoption of adenosine triphosphate (ATP) monitoring by some utilities.

ATP is the principal energy carrier that fuels biosynthesis, motility and other maintenance functions in all living cells in unicellular and multicellular organisms. Regardless of the source, all energy is transformed into ATP by living cells [21]. Thus, the detection of ATP in any environmental sample reflects the presence of living organisms. ATP has been used for a wide variety of environmental surveillance purposes including monitoring food safety [22,23], nitrification [24] and disinfection of surfaces in health care setting [21]. Many researchers have also advocated for its use to monitor biological stability in the DWDS [15,25–29]. ATP monitoring provides an estimate of total biomass in a water sample in 15 min compared to a minimum of 48 h required for HPC methods [30]. The method also relies on the use of much large volumes (approximately 50–100 mL) to estimate the concentration of ATP in pg/mL (equivalent to ng/L). The method is easily adapted to inform utility decision making in a more timely manner, provided utilities can identify operational thresholds that are specific to the systems' source water (groundwater and surface water) and treatment systems (non-chlorinated, chlorinated and chloraminated) [31].

Utilities are required to sample a wide range of locations in their service areas to monitor regulated parameters to ensure water quality is maintained across the DWDS. Utilities only tend to monitor unregulated parameters if they provide operational value to decision-making. While numerous studies have explored ATP concentrations in non-chlorinated and chlorinated systems [15], there are currently no large, long-term monitoring datasets that provide information on the biological stability or the factors impacting ATP concentrations throughout a chloraminated DWDS. Several authors discuss the roles of growth-promoting nutrients (e.g., assimilable organic carbon) [32–34], temperature [35–37], retention time and stagnation [35,37,38], fluctuations in flow velocity [32,33], pipe diameter [38] and iron concentrations [35,37,38] on ATP concentrations. While physico-chemical water quality data are commonly collected in real-time (e.g., turbidity, particle counts, chlorine), used for decision-making and transmitted to the utility's supervisory control and data acquisition (SCADA) system, online microbiological sensors are uncommon [33]. An increasing number of studies have identified microbial parameters, including ATP and cell counts by flow cytometry, as potential online monitoring targets for DWDS early warning systems [25,33,39,40]. Regardless of whether microbial data are collected in real-time or by analysis of grab samples, utilities still need to understand what ATP concentration fluctuations are considered acceptable and which should require operational intervention. At this moment, this information does not exist. A recent AWWA report exploring the use of ATP for infrastructure release post-repairs showed large variations in ATP concentrations at different utilities, but the study was only conducted over a limited period of time and at a limited number of locations [18]. Prest et al. provided a large dataset of 3000 data points but only included five sampling locations along the distribution network starting with the treatment plant over a five-week period [33]. No study to date has provided a comprehensive, long-term dataset that characterizes ATP concentrations by DWDS monitoring sites (e.g., treatment plants, plant reservoirs, field or outlying reservoirs, random distribution system, hydrants and customer complaints) over multiple years. These data would be useful for utilities to identify operational action thresholds by sampling location and conduct a full cost–benefit assessment of introducing ATP testing (e.g., cost, labour and workflow associated with resamples or corrective actions based on number of samples above threshold values). It would also provide more guidance to researchers exploring real-time monitoring sensors by identifying required testing ranges and potential sites for sensor installations (e.g., treatment plants, reservoirs).

The main objective of this paper is to evaluate the ATP concentrations in a chloraminated DWDS between 2019 and 2022 to achieve the following objectives:

1. Characterize ATP concentrations both at the DWTP and in the DWDS, as well as the factors influencing those concentrations.
2. Compare ATP and HPC results collected in 2019 to assess each parameters value to decision making.
3. Define ATP concentration thresholds that should result in preventative or corrective action to manage risk and water quality changes.
4. Evaluate the use of ATP monitoring for management of treated water storage facilities using two outlying reservoir case studies.

We show that ATP concentrations exhibit an increase between the DWTP and the DWDS displaying both temporal and seasonal heterogeneity. We also confirm that ATP values provide better operational decision support than HPCs for asset management and proactive water quality management in the DWDS.

2. Materials and Methods

2.1. Drinking Water Treatment, Storage and Distribution

Sampling and testing were conducted in the City of Edmonton between 2019 and 2022. It included samples from the following locations (referred to as sampling locations): (1) the two DWTPs (i.e., treated final effluent from DWTP1 and DWTP2) supplying over 1.4 M residents with drinking water, (2) two treatment plant reservoirs (DWTP1 Reservoir and DWTP2 Reservoir), (3) all twelve outlying field reservoirs divided by pressure zone (primary, secondary and tertiary), (4) a large number of DWDS samples that include 30 fire stations and random distribution system samples, (5) customer complaint samples and (6) a very limited number of infrastructure renewal samples. Raw water samples were also included for reference.

The two treatment plants produce an average total volume of 350 ML/day (i.e., megaliters per day) and rely on coagulation/sedimentation, anthracite/sand filters, UV disinfection and chloramination. The chlorine target and average concentration for water leaving both plants are 2.5 mg/L and 2.2 mg/L, respectively. The reservoirs store a combined gross volume of 800 ML in the DWDS. The Edmonton DWDS is a highly complex gridded 3900 km network in which the average water residence time ranges dramatically, especially when incorporating outlying reservoir storage times. Because of the complexity of the network, it is impossible to clearly determine linear portions on which there could be a succession of sampling points at fully identified distances and residence times. The two DWTP reservoirs are located underground at the plants and are therefore the closest to the treated water source, while outlying reservoirs are father away and have longer residence times. The tertiary outlying reservoir only came online in 2021 and therefore has the smallest sample size. Figure 1 provides a schematic of the water treatment process at both DWTPs in this study.

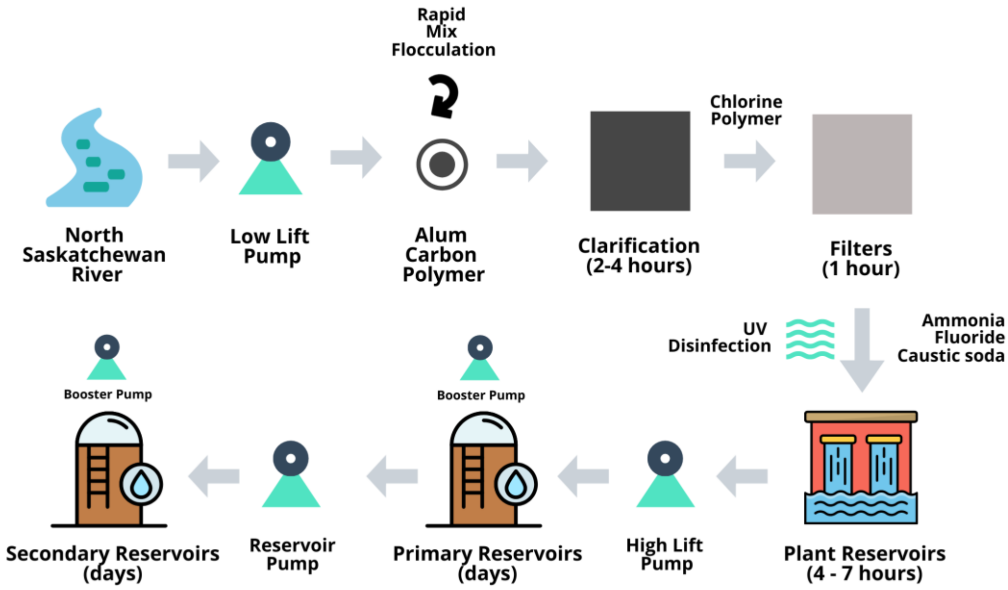


Figure 1. Drinking water treatment, storage and distribution process in Edmonton between 2019–2022.

2.2. Sampling Protocol for Water Samples

Treated water samples were all collected in sterile 200 mL bottles for microbiological analyses and in 1 L glass bottles for physico-chemical tests as per Standard Methods for the Examination of Water and Wastewater [13]. Chlorine-containing microbiological water samples were first quenched with 0.1 mM $Na_2S_2O_3$, transported to the laboratory, kept between 2–8 °C and tested within less than 20 h from collection time. Plant and reservoir samples were collected at dedicated taps that are continuously running, thus not requiring flushing. Distribution system samples were collected at hydrants or customer and fire station taps. These were disinfected using 25 ppm bleach solution and allowed to run for 5–10 min until turbidity and chlorine values stabilize before sample collection.

For the additional exploration of ATP data uses for operational decision making, two case studies were provided. The first case study assessed changes in water quality in an outlying primary reservoir (gross storage: 64.6 ML; available storage: 50.5 ML) where ingress was identified in 2020. Chlorine, turbidity and ATP concentrations and online trends were monitored. The reservoir was cleaned and repaired in September 2021 (e.g., edge repair of roof membrane, membrane extension). The reservoir was returned to service in April 2022. Data before and after repair and cleaning were compared.

The second case study assessed ATP, total chlorine and turbidity values at an outlying primary reservoir (available storage: 17.3 ML) during draining and overnight stagnation in August 2022. Draining was performed over three consecutive days. Starting height of water in reservoir was 14.3 m. Water samples were collected every 0.3 m and analyzed onsite for all three parameters. No flushing was conducted to assess suction pipe conditions at the reservoir.

2.3. Analytical Testing

All analyses were performed by the utility laboratory in Edmonton, which is accredited by the Canadian Association for Laboratory Accreditation (CALA). HPC and ATP tests are not regulated parameters or part of the utility's Approval to Operate, while all other chemical and physical parameters are regulated. Testing was conducted in accordance with Standard Methods for the Examination of Water and Wastewater [13] unless otherwise stated.

HPCs were collected using the spread plate method (100 µL on Plate Count Agar for 48 h at 35 °C) outlined in Standard Method (SM) 9215. Total ATP concentration was determined using the Luminultra QGA Kit (Luminultra Technologies, Fredericton, NB, Canada) as per manufacturer's instructions. Briefly, 50 mL of sample are syringe-filtered using a sterile 0.45 µm filter. The material collected onto the filter is lysed using 1-mL lysing buffer. The lysate is diluted and mixed with an equal volume of luciferin-luciferase enzyme that binds to all available ATP resulting in the emission of light. Light emission is measured using the PhotonMaster™ (Luminultra Technologies, Fredericton, NB, Canada) in Relative light units (RLU). The value is converted using a standard concentration of 1 pg ATP to concentration of cellular ATP (cATP) per millilitre of sample (cATP/mL). The method used for total ATP measurement here is conservative in that both intra- and extracellular ATP are measured. We will refer to total cellular ATP as ATP from hereon. Due to practical constrains, the samples were not run in duplicate, however one duplicate was run per batch (every 24 samples), along with a negative control (sterile Type II water) and a positive ATP standard (known concentration of 1 pg ATP/mL). The Photonmaster™ detection limit was 10 RLU and overall detection limit was 0.1 pg ATP/mL. Overall, %CV for all ATP testing ranged between 4% and 10% depending on the sample matrix tested.

Total residual chlorine was determined using SM 4500-Cl using a Metrohm 905 Titrando autotitrator (Metrohm, Herisau, Switzerland). Turbidity was determined using a Hach TL2300 turbidimeter as per SM 2130B, Turbidity by Nephelometric Method. Conductivity was determined by using a conductivity meter (ORION™ D 16010, Vernon Hills, IL, USA) with a built-in temperature sensor as per SM 2510. Colour was analyzed using a Shimadzu UV Mini-1240V. pH was determined electrometrically using a modified version of SM 4500H. Total organic carbon (TOC) was measured using Standard Method 5310B by Shimadzu V analyser as per SM 2120.

2.4. Data Processing and Statistical Analyses

All analyzed data were downloaded from the utility's quality-controlled Laboratory Information Management System (LIMS). For the case studies, online monitoring data were imported from the SCADA system. For all analyses, non-detects were re-entered as half the value of the detection limit following the EPA recommended approach for analytes that are likely to be present in concentrations between zero and the detection limit [41,42]. The data analyzed were broken down by sampling location or water quality variable. A Shapiro–Wilk test was used to assess normality of the datasets. The results revealed that ATP and other water quality data were normally distributed. Thus, summary statistics, such as averages and standard deviations were used to interpret and visualize the data. However, since the data were autocorrelated, non-parametric Kruskal–Wallis tests were used to determine significant differences between sampling locations.

The relationships between ATP concentrations and other water quality variables were examined using response screening, which relies on bivariate regressions but adjusts for false discovery rates (FDR), outliers and missing values. ATP concentrations were used as the Y variables, while total chlorine, TOC, turbidity, ambient temperature, colour and conductivity were used as the predictors. Reported results include the sample size, *p*-value associated with the regressions, the FDR Log Worth, effect size and R-squared. The LogWorth is the quantity $-\log10(p\text{-value})$. A value that exceeds 2 is significant at the 0.01 *p*-value level ($-\log10(0.01) = 2$). This value is then used to create the FDR LogWorth (the quantity $-\log10(\text{FDR } p \text{ Value})$) and the rank fraction. FDR LogWorth values greater than two (*p*-values less than 0.01) are considered significant. All statistical analyses were performed in JMP™ 16.0 (SAS Corp., Cary, NA, USA).

3. Results

3.1. Mean ATP Concentrations and Water Quality Parameters by Sampling Location

Table 1 provides a detailed list of water quality parameters in addition to ATP concentrations by sampling location. ATP values below the detection limit were reported as <0.1 pg ATP/mL. The results include sample size per test, mean values and standard deviations. ATP values were highest for Primary outlying reservoirs > Tertiary outlying reservoir > Secondary outlying reservoirs > Fire stations = Complaints > Random DWDS samples > DWTP reservoir and treated samples. There was no statistically significant difference between ATP concentrations at DWTP1 and DWTP2 for both treated and plant reservoir water highlighting that plant performance was comparable. Treated DWTP water samples exhibited a statistically significant reduction in ATP concentrations from raw samples at both plants highlighting the efficacy of the process achieving 2.76 and 3.27 in ATP log reductions. No statistically significant differences between ATP concentrations in DWTP treated effluent and plant reservoir water were seen at both plants (p-value > 0.5). In comparison, ATP concentrations increased significantly between plant reservoirs and outlying reservoirs (DWTP1 z-score = 9. 54, $p < 0.0001$ and DWTP2 reservoir z-score = 14. 28, $p < 0.0001$), and decreased again between outlying reservoirs and DWDS samples (z-score = −518, $p < 0.0001$). Figure 2 shows that total chlorine residuals decreased after leaving the DWTP reservoirs but did not drop below 1.2 mg/L. The difference in chlorine between outlying reservoirs and DWDS samples was not statistically significant (p-value > 0.05). Similarly, turbidity values increased after leaving the DWTP reservoirs but the difference between the outlying reservoirs and DWDS samples was not statistically significant.

Table 1. Mean concentrations and standard deviations of adenosine triphosphate (ATP) and other water quality parameters in Edmonton by sampling location in 2019–2022.

	ATP [1]			Total Chlorine			Turbidity			Conductivity			TOC		
Sampling Location	**N**	**Mean**	**Std Dev**	**N**	**Mean**	**Std Dev**	**N**	**Mean**	**Std Dev**	**N**	**Mean**	**Std Dev**	**N**	**Mean**	**Std Dev**
DWTP1 Raw	94	80.96	77.45		NT [2]		1204	26.56	85.00	1204	14.14	17.67	40	8.2	0.1
DWTP1 Treated	598	0.14	0.23		NT		1383	0.05	0.02		NT			NT	
DWTP1 Reservoir	570	0.13	0.12	1451	1.99	0.21	1430	0.05	0.12	1287	1.07	0.40	1451	7.8	0.8
DWTP2 Raw	101	93.47	85.78		NT		1203	29.64	126.22	1203	14.27	18.19	40	8.2	0.1
DWTP2 Treated	519	<0.1	<0.1		NT		1341	0.05	0.01		NT			NT	
DWTP2 Reservoir	496	0.10	0.08	1445	2.04	0.23	1336	0.04	0.01	1285	1.04	0.36	1445	7.8	0.8
Primary Reservoirs	481	0.44	0.79	1071	1.62	0.22	1066	0.10	0.14	272	379.1	53.3	134	1.63	0.81
Secondary Reservoirs	622	0.28	0.52	1469	1.79	0.19	1458	0.09	0.24	180	394.6	37.0	180	1.67	0.58
Tertiary Reservoirs	62	0.39	0.83	92	1.21	0.18	91	0.10	0.04	14	383.4	34.6	14	1.24	0.57
Fire Stations	1335	0.24	1.04	2833	1.70	0.25	2817	0.17	0.33		NT			NT	
Random DWDS	85	0.22	0.36	4179	1.73	0.26	4181	0.15	0.58		NT		13	1.75	0.56
Renewals	4	<0.1	<0.1	589	1.76	0.26	589	0.43	0.58		NT			NT	
Complaints	190	0.24	0.30	459	1.67	0.27	458	0.44	0.69		NT			NT	
WHO Parameter Limit		N/A			Minimum 0.2 mg/L Maximum 5 mg/L [3]			Water entering the distribution system ≤ 1 NTU [4]			N/A			N/A	

Note(s): [1] Values less than the detection limit are reported as <0.1 pg ATP/mL. [2] Not tested (NT) because the parameter is not regulated and provides no operational value. [3] WHO suggested limits are for free chlorine at point of delivery not total chlorine which is measured here. For effective disinfection, residual free chlorine concentration must be ≥0.5 mg/L after at least 30 min contact time at pH < 8.0. [4] Large well-run municipal supplies should be able to achieve turbidities < 0.5 NTU and on average turbidities ≤ 0.2 NTU.

3.2. Variability in ATP Concentrations and Water Quality by Season and Sampling Location

3.2.1. Temporal and Spatial Variability in ATP Concentrations

ATP concentrations followed a seasonal pattern with values being higher in the warm months than in the cold months regardless of the sampling location. The effect of temperature was more pronounced in some sampling locations than in others, as can be seen in Figure 3. Concentrations in the warm months were highest for primary outlying reservoirs, followed by secondary outlying reservoirs and fire stations. However, sample sizes for the tertiary reservoir, random DWDS samples and complaints were smaller potentially making the trend less obvious. Similarly, Figure 4 shows the spatial variability in ATP concentrations for all outlying reservoirs (panel A) and fire stations (panel B). Mean ATP concentrations differed by sampling location highlighting the need for additional exploration in some cases. In addition, some sampling locations had recurring ATP outliers with concentrations higher than 1 pg/mL. Gaining a better understanding of the distribution of outliers can provide insight into action limits the utility could adopt and their potential operational impacts.

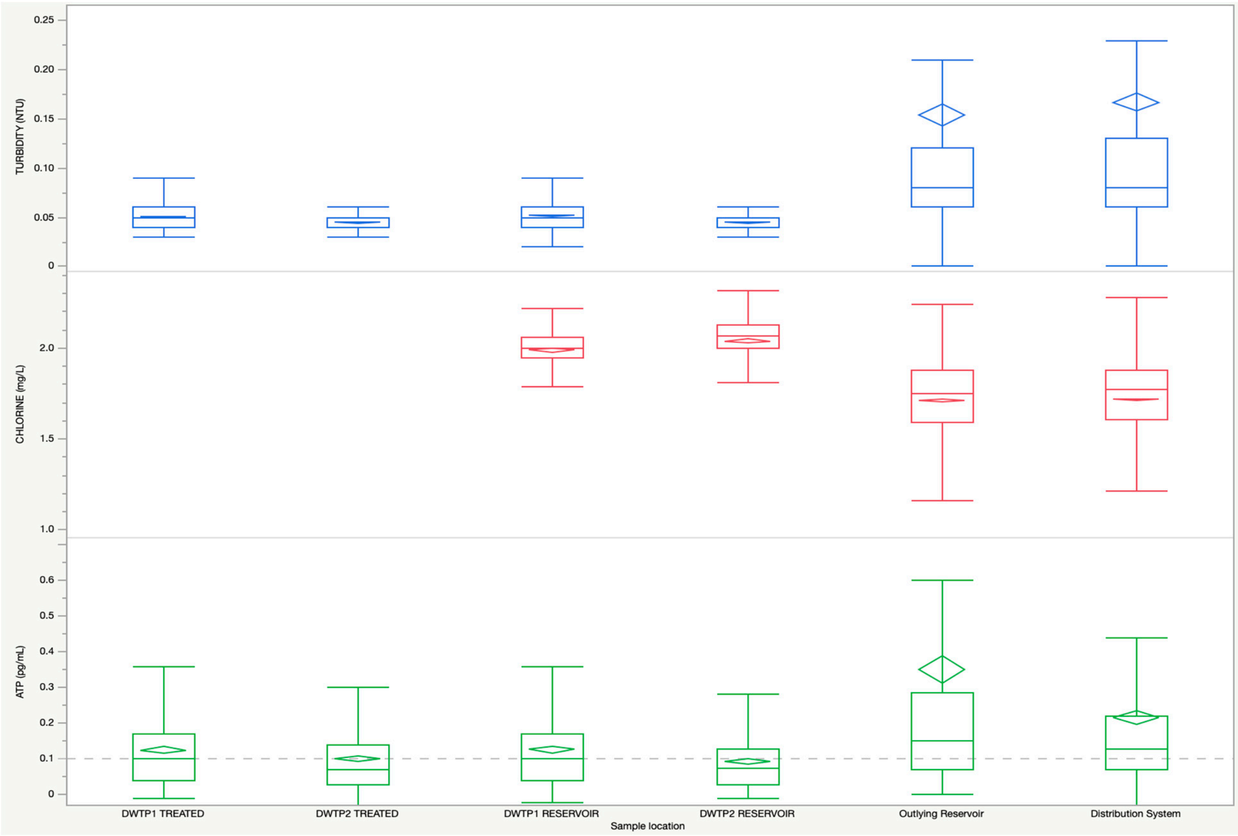


Figure 2. Box plot with mean concentrations of ATP (pg/mL), total chlorine (mg/L) and turbidity (NTU) for both DWTP treated effluent and DWTP reservoirs, outlying reservoirs and DWDS samples. Diamonds depict 95% confidence internals.

3.2.2. Relationships between ATP Concentrations and Water Quality Variables by Sampling Location

A detailed exploration of variables impacting ATP concentrations was performed using response screening. Results are provided in Table 2 and only list statistically significant relationships where the FDR *p*-values are less than 0.01. Raw water ATP concentrations showed strong relationships with turbidity, colour and ambient temperatures confirming the importance of seasonality. Treated water ATP concentrations had the strongest relationships with turbidity. Even though ATP concentrations between both plant reservoirs were not statistically different, relationships with water quality variables at each plant were different. DWTP1 reservoir water ATP concentrations had strong relationships with turbidity and color, while DWTP2 reservoir water ATP concentrations had strong relationships with colour, ambient temperature and turbidity. This may be explained by the fact that DWTP1 is located upstream of DWTP2 and in a less urbanized area. Finally, the strongest relationships between ATP concentrations and other water quality variables in outlying reservoirs and in the DWDS were seen with total chlorine residual and ambient temperatures. However, ATP concentrations in outlying reservoirs also exhibited strong relationships with conductivity, TOC and turbidity, unlike DWDS samples. In addition, the strength of these relationships with ATP concentrations was much more pronounced in outlying reservoirs, which exhibited an effect size of 37% and 34% with chlorine and ambient temperature, respectively (versus 8% and 7% for all DWDS samples). This highlight the importance of microbiological testing in the DWDS given that relationships with variables assumed to predict microbial regrowth may not be robust.

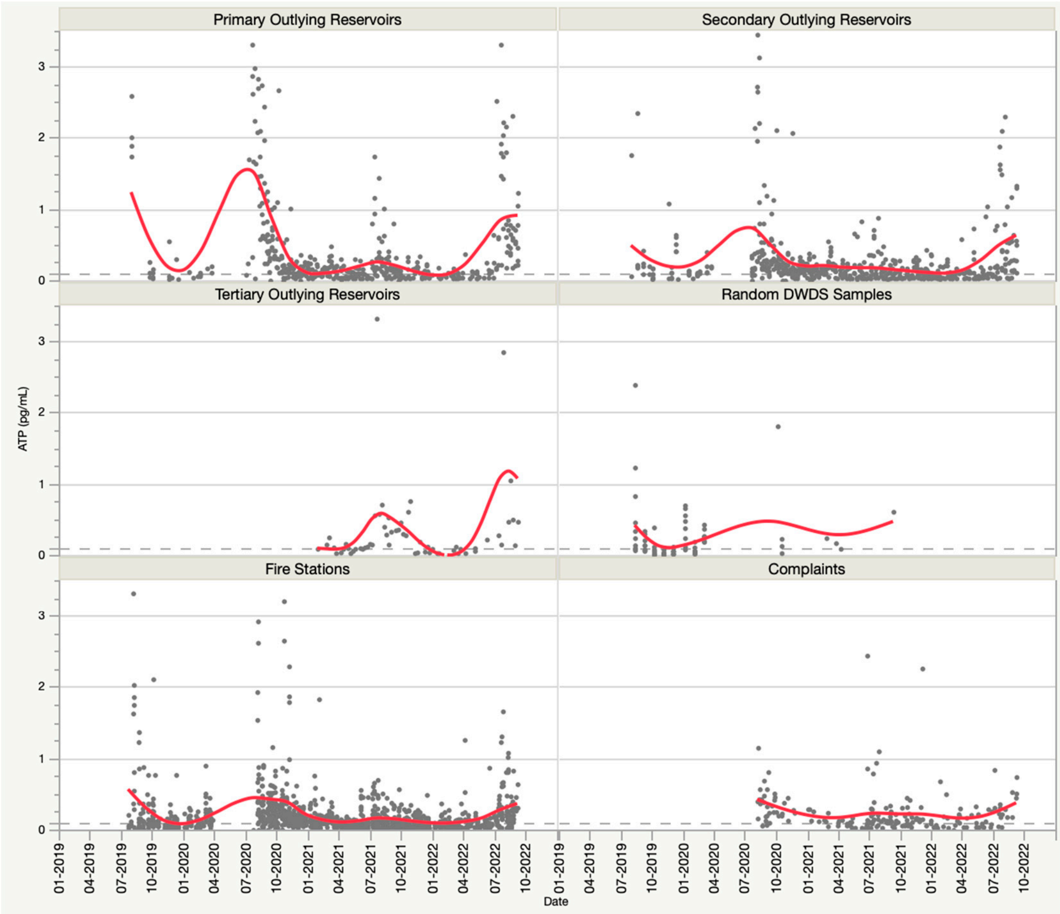
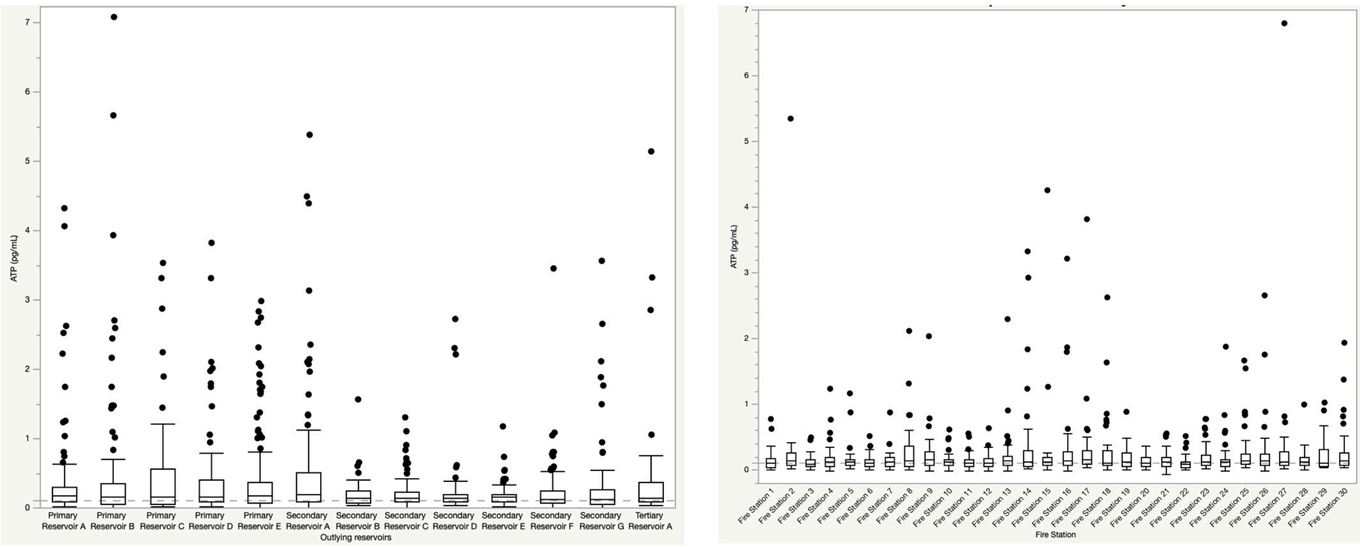


Figure 3. Seasonal changes in ATP concentrations (pg/mL) for outlying primary, secondary and tertiary reservoirs, random DWDS, fire station and complaint samples between 2019 and 2022. Black dots represent data points and red line is the smoothed moving average.



(**a**) (**b**)

Figure 4. Comparison of mean concentrations of ATP (pg/mL) between 2019 and 2022 in the DWDS. (**a**) Mean ATP concentration at outlying and DWTP reservoirs; (**b**) mean ATP concentrations at fire stations.

Table 2. Response screening assessing the relationships between various water quality parameters and ATP concentrations by sampling location. All listed relationships are statistically significant with FDR *p*-values < 0.01.

Y	X	Count	*p*-Value	FDR *p*-Value	FDR LogWorth	Effect Size	RSquare
DWTP1 Raw							
ATP	Turbidity	94	1.20×10^{-34}	5.95×10^{-34}	33.23	0.8937	0.807
ATP	Colour	94	2.30×10^{-16}	5.77×10^{-16}	15.24	0.7178	0.521
ATP	Ambient Temp	91	1.80×10^{-4}	3.07×10^{-4}	3.51	0.3859	0.146
DWTP2 Raw							
ATP	Colour	101	2.90×10^{-21}	1.45×10^{-20}	19.84	1.501	0.597
ATP	Turbidity	101	6.44×10^{-9}	1.61×10^{-8}	7.79	1.045	0.290
ATP	Ambient Temp	97	8.73×10^{-7}	1.46×10^{-6}	5.84	0.939	0.226
DWTP1 Treated							
ATP	Turbidity	558	1.80×10^{-4}	3.55×10^{-4}	3.45	0.192	0.025
DWTP2 Treated							
ATP	Turbidity	481	2.00×10^{-4}	4.08×10^{-4}	3.39	0.214	0.028
ATP	Ambient Temp	472	1.34×10^{-3}	1.34×10^{-3}	2.87	0.183	0.022
DWTP1 Reservoir							
ATP	Turbidity	560	4.53×10^{-7}	2.72×10^{-6}	5.57	0.266	0.045
ATP	Colour	493	2.30×10^{-4}	6.93×10^{-4}	3.16	0.215	0.027
DWTP2 Reservoir							
ATP	Colour	439	3.06×10^{-6}	1.84×10^{-5}	4.74	0.244	0.049
ATP	Ambient Temp	455	2.00×10^{-5}	5.83×10^{-5}	4.24	0.225	0.040
ATP	Turbidity	463	5.14×10^{-5}	1.03×10^{-4}	3.99	0.212	0.035
ATP	Chlorine	495	5.00×10^{-4}	7.53×10^{-4}	3.12	0.175	0.024
Outlying Reservoirs							
ATP	Chlorine	1169	7.90×10^{-40}	4.76×10^{-39}	38.32	0.373	0.139
ATP	Ambient Temp	1013	1.50×10^{-26}	4.37×10^{-26}	25.36	0.344	0.107
ATP	Conductivity	248	5.00×10^{-5}	1.01×10^{-4}	4.00	0.272	0.065
ATP	TOC	147	1.60×10^{-4}	1.97×10^{-4}	3.71	0.347	0.094
ATP	Turbidity	1168	1.50×10^{-4}	1.97×10^{-4}	3.71	0.110	0.012
DWDS Samples (Fire stations, Randoms, Complaints)							
ATP	Chlorine	1608	7.00×10^{-19}	2.79×10^{-18}	17.56	0.088	0.048
ATP	Ambient Temp	1414	3.02×10^{-9}	6.04×10^{-9}	8.22	0.066	0.025

3.3. Comparing ATP Concentrations and HPC Counts

The comparison between HPCs and ATP concentrations was only conducted on samples collected in 2019 and is seen in Figure 5. During that period, HPC results for the drinking water plants samples (n = 163), outlying reservoir samples (n = 51) and DWDS samples (n = 267) were 91%, 98% and 92% negative providing non-detects (<1 CFU/mL), respectively. Only one tested sample provided HPC results higher than the internal operational limit of 60 CFU/mL. In comparison, ATP tests provided actionable results that can be used to support decision-making.

3.4. Proposed Operational Thresholds for Utilities

A significant number of ATP measurements fell below the detection limit (<0.1 pg/mL) for all sampling locations, especially for DWTP reservoir samples. Values in the 1–3 pg/mL range were also common providing higher granularity and potential ability to detect early changes and microbial regrowth throughout the system as can be seen in Figure 6. While some sampling locations had ATP values in the 3–5 pg/mL range, many did not exceed

3 pg/mL. Only two sampling location, both outlying reservoirs, had ATP concentrations above 5 pg/mL and none had concentrations above 10 pg/mL. The results suggest that for this chloraminated system an operational threshold value of 10 pg ATP/mL that results in utility intervention (e.g., flushing, maintenance, disinfection) is realistic and practical. It also suggests that an internal utility action limit of 5 pg ATP/mL could be a useful metric to trigger additional testing and resampling. The higher ATP values in outlying reservoirs compared to other sampling locations indicate the importance of reservoir management practices and the usefulness of a robust and accurate monitoring parameter. Two case studies follow that provide additional examples of the value of ATP monitoring.

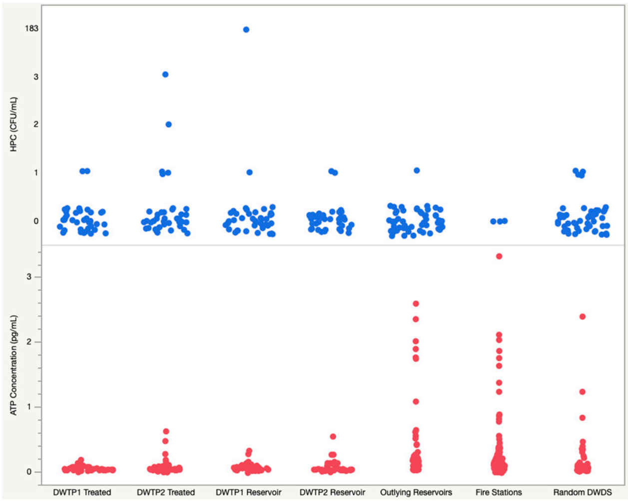


Figure 5. Mean concentrations of ATP (pg/mL) and HPCs (CFU/mL) for both DWTP treated effluent and DWTP reservoirs, outlying reservoirs and DWDS samples. Blue circles represent heterotrophic plate counts (HPC) in CFU/mL and red circles represent ATP concentrations in pg/mL.

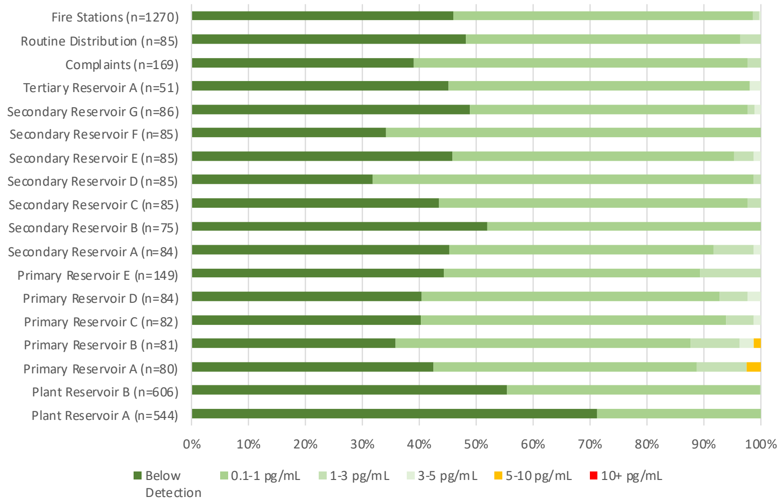
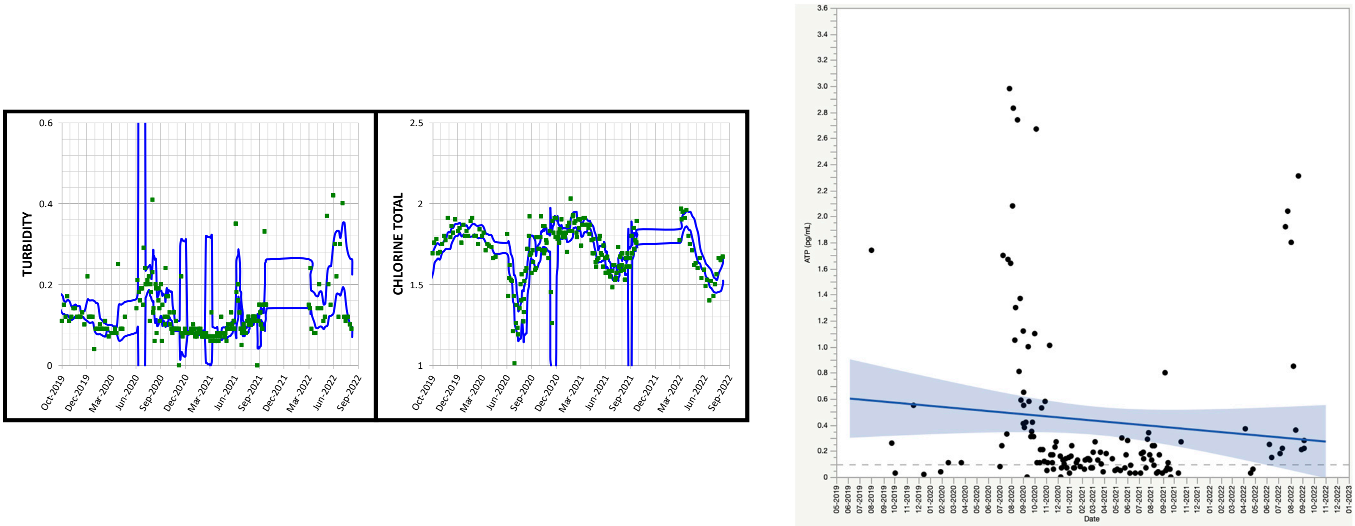


Figure 6. ATP concentration value ranges at the DWTPs, reservoirs and in the DWDS between 2019 and 2022.

3.5. Outlying Reservoir Case Studies

3.5.1. Case Study 1: Outlying Primary Reservoir Maintenance

Following an inspection in 2020, ingress into one of the outlying primary reservoirs was noted. Historical chlorine and turbidity monitoring data in SCADA were reviewed (Figure 7a) but no changes were observed that indicate a potential concern or need for action. In comparison, monitoring ATP concentrations provided some indication of water quality fluctuations. Capital repairs and reservoir cleaning were initiated and repairs were completed in 2022. Figure 7b shows the statistically significant reduction in ATP signal in 2022 after repairs were performed (F-ratio = 347.76; *p*-values = 0.0002).



(**a**) (**b**)

Figure 7. Case study 1—Outlying primary reservoir water quality between 2019 and 2022 before and after repairs. (**a**) Online total chlorine and turbidity measurements between 2019 and 2022 (green dots represent measurements, blue line represents moving average) (**b**) Mean ATP concentrations (pg/mL) between 2019 and 2022, with the blue line showing the line of fit and the shading highlighting 95% confidence intervals.

3.5.2. Case Study 2: Outlying Primary Reservoir Draining

Outlying primary reservoir draining results are shown in Figure 8. This scenario replicates what happens over summer months when water use is high over the daytime, but stagnation occurs overnight when most consumers are asleep. While ATP concentrations were relatively low throughout the draining process, at the beginning of draining each morning following stagnation, ATP results were higher and chlorine concentrations lower than the rest of the day. This indicated that the issue resulting in high ATP and low chlorine was localized in the suction pipe since results stabilized after 5 purge volumes of the suction pipe. The trial results were contrary to what operations expected due to the assumption that below ground piping would be subject to minimal microbiological activity due to relatively low temperatures compared to above ground reservoirs in the DWDS. Given the relatively high biological activity of the suction pipe (though still well within action guidelines), operations initiated remedial actions.

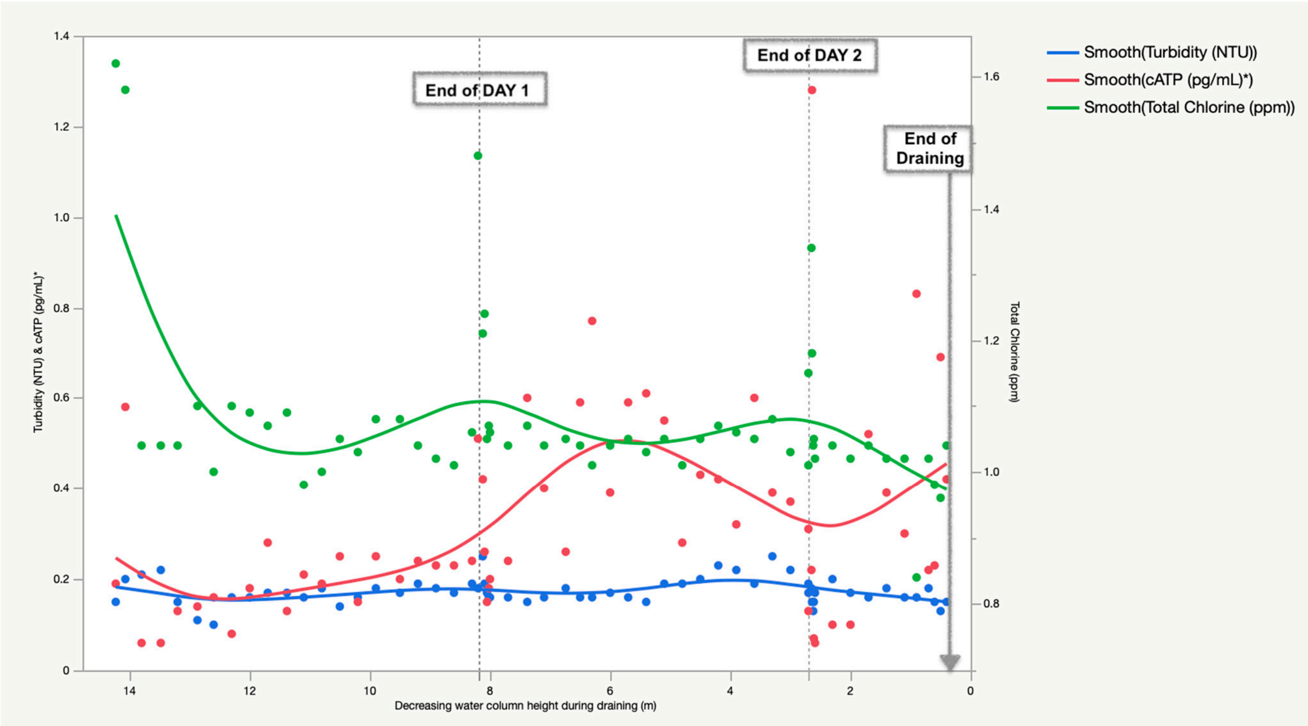


Figure 8. Case study 2—Outlying primary reservoir draining and overnight stagnation experiment in August 2022. Graph shows ATP (red), total chlorine (green) and turbidity (blue) measurements on the y-axis as water height (x-axis) in the reservoir is decreasing. Lines show end of Day 1, Day 2 and end of draining the full reservoir. The line represents the smoothed moving averages for each of the three parameters.

4. Discussion

Utilities rely on reliable and robust monitoring systems to inform decisions around asset management in the DWDS network to deliver high quality, biologically stable drinking water to consumers [8–12]. However, traditional microbiological testing methods present some unique challenges that make the timely detection of regrowth and microbiological changes in the DWDS difficult. In the present study, we provide the results of an extensive ATP monitoring campaign—a non-regulated parameter—in an urban center that includes ATP concentrations at two DWTPs, DWTP reservoirs, outlying reservoirs in three pressure zones and in the DWDS between 2019 and 2022. The ATP data are provided along with traditional water quality parameters and supports the premise that ATP is both superior to HPCs and more likely to provide sensitive, actionable data that result in timely intervention at the DWTPs and in the DWDS.

Since the highest microbiological risk in drinking water is often associated with the ingestion of fecal pathogens, detection of fecal indicator bacteria (*E. coli,* specifically) remains the gold standard for evaluation of public health risk from drinking water. While this is without doubt of utmost importance, many changes in water quality can occur before we start detecting coliforms. In addition, monitoring for fecal indicator bacteria does not take into consideration that feces can be a source of non-bacterial pathogenic organisms (e.g., viruses, protozoa, helminths) which do not always corelate with coliforms [43] and that some free living amoebae can act as reservoirs for bacterial pathogens hindering their detection [8,44,45]. Additionally, opportunistic pathogens, such as *Pseudomonas aeruginosa*, *Legionella pneumophila* and *Mycobacterium avium* complex, are not fecal in origin and are being reported as causes for public health concern [34,37,46]. These limitations, as well as the recognition that uncontrolled regrowth of bacteria in the DWDS can lead to a range of aesthetic (e.g., deterioration of taste, odor, color) and operational challenges (e.g., fouling or biocorrosion of pipes), has led many utilities to continue testing for HPCs to detect changes in water quality.

HPCs were test first introduced in 1883 by Robert Koch to monitor water quality changes in point-of-use filtered water over time. The test recovered a wide range of microorganisms grown on non-selective culture media (e.g., bacteria, yeasts, moulds) that use organic carbon as an energy source. Due to the use of non-selective media, HPCs are unable to identify specific organisms and should not be used as an indicator of public health risk [47]. However, if tested in parallel with disinfectant residual, HPCs can provide an indication of a change in water quality [20]. HPCs have no standard universally accepted analytical method [13], no accepted threshold value that requires intervention by utilities and at a minimum require 1–2 days to produce results making timely intervention unlikely [17]. In comparison, ATP testing presents an attractive alternative for monitoring biological water stability in 15 min [15,25–29], provided utilities can identify actionable thresholds for operational intervention and asset management.

In this study, average ATP concentrations in treated plant effluent and plant reservoir water were 0.12 and 0.10 pg/mL, respectively. The ATP concentrations in outlying reservoirs ranged between 0.28–0.44 pg/mL and then decreased to 0.22–0.24 in DWDS, fire station and complaint samples. This corresponds with the wide range of ATP values reported in the literature over the last three decades. De Vera and Wert (2019) reported a low 14-day cumulative biomass production (CBP_{14}) of 6–8 d.ng ATP/L in a chlorinated, ozonated drinking water system, despite elevated assimilable organic carbon (AOC) levels [9]. In comparison, van der Wielen et al. detected a CBP_{14} of 110 d·ng ATP/L (range: 6.8–174.8 d·ng ATP/L) in Dutch, non-chlorinated DWDSs [31] confirming their previously published results (ATP < 6 ng/L in treated, non-chlorinated water; range: 1.5–69.1 ng/L) [34]. Ghazali et al. used a two-tier drinking water surveillance system that monitored organic carbon, free chlorine, turbidity, pH, and conductivity, followed by a confirmatory ATP test and reported concentrations between 0.98 and 98.0 pg/mL [48]. Nescerecka et al. monitored a full-scale, chlorinated, surface water-fed DWDS using fire hydrant samples and reported mean ATP values of 0.015 ± 0.005 nM (range: 0.021–0.063 nM) [15]. In their report for the American Water Works Association on using ATP concentrations to release distribution system infrastructure, Stoddart et al. reported that the three participating chloraminated utilities and the two chlorinated utilities had mean ATP concentrations of 0.4, 0.4 and 13 pg/mL and 0.5 and 2.2 pg/mL, respectively [18]. We reported our results in pg/mL and did not use cumulative biomass indices or microbial equivalents to interpret ATP results. The use of cumulative biomass indices, while useful for result smoothing, would still require a utility to have monitoring results for 7–14 days to draw conclusions. The microbial equivalent calculation assumes that all *E. coli*-sized cells in a sample contain 0.001 pg (1 fg) of ATP, which is inaccurate in many cases.

Our results correspond with other studies that suggest that while ATP concentrations in the DWDS will vary with source water (e.g., groundwater, surface water, recharged aquifer) and with disinfectant choice (non-chlorinated, chlorinated and chloraminated) [31], ATP seems more influenced by operational practices in the DWDS. Prest et al. reported that high ATP concentrations (range 20–40 ng/L, max. value of 120 ng/L) were frequently recorded at higher flow velocities [33]. Similarly, Fish et al. demonstrated that hydraulic regimes influence biofilm biomass concentration, which can influence planktonic ATP concentrations after biofilm sloughing events [49]. Reports around influence of pipe materials on ATP are contradictory with some authors reporting its significance [6,38], while other suggest the lack of a relationship [34]. Some reports suggest that planktonic bacteria and bacteria associated with loose deposits could be managed more effectively through optimization of treatment processes and cleaning practices [6].

Our results correspond with observations by others that many variables result in temporal and spatial fluctuations in ATP concentrations. Variables reported in the literature include changing seasons and high temperature, main breaks, corrosion, reduced disinfectant residuals, ingress and fluctuating hydraulic conditions (e.g., stagnation, biofilm detachment) [8,9,16,50]. We noted a clear seasonal pattern in ATP concentrations, which has been reported by others, especially at consumer taps [15,18,35–37]. Hallam et al. reported a 50% decrease in ATP concentrations as temperature decreased from 17 °C to 10 °C, which may have been related to decreased disinfectant decay at lower temperatures [36]. Stoddart et al. reported similar findings but cautioned that the relationship is not linear [18]. Temperatures in Edmonton during the study period ranged between −36 °C and 27 °C. In the present work, based on a linear regression between temperature and ATP concentrations, on average we noted a 0.2 pg ATP/mL increase with a 10 °C increase in ambient temperatures, possibly influencing the significant number of non-detects in the winter season.

Unlike the results reported by Prest et al. [33], spatial heterogeneity in ATP could not easily be explained by distance from the DWTPs or by residence time due to the complexity and lack of linearity of the system. The increase in ATP concentrations between DWTP reservoirs and outlying reservoirs corresponds with reports by others [15,16,33] but in our study chlorine residual remained above 1.2 mg/L, highlighting that ATP concentrations can still be high when chlorine residual is high [35,37]. We did, however, observe a decrease in ATP concentrations between outlying reservoirs and samples collected in the DWDS. One possible explanation for this based on the response analysis we conducted was that TOC levels were still a significant variable in outlying reservoirs impacting ATP concentrations. This was not the case for fire station, random DWDS or complaint samples. Most likely, the consumption of assimilable organic carbon by microorganisms following water release from outlying reservoirs into the pipes of the DWDS limits regrowth and increases competition in the microbial community thus reducing ATP concentrations [9,15,51]. According to Prest et al., even a concentration of 1 µg/L of organic carbon can stimulate the growth of 10^3 to 10^4 cells/mL in the DWDS [8]. Learbuch et al. distinguished the effect growth potential on planktonic versus biofilm organisms and found a significant relationship between planktonic growth potential and ATP concentration in water but not in biofilm [34]. Regardless, we were able to effectively compare fire stations and outlying reservoir microbiological water quality and distinguish locations that may require follow-up.

Managing drinking water storage facilities remains a challenge for many utilities [11,12,52]. Utilities rely on regular monitoring, maintenance and inspection programs to ensure that reservoir water quality meets regulatory standards. While clear testing requirements exist to monitor reservoir water quality, maintenance and inspection programs are less standardized. For facilities that are inspected, the most frequently documented interval between inspections in 1999 was six to eight years, deviating from the three-year inspection frequency recommended by AWWA [53]. The AWWA Research Foundation study even concluded that many storage facilities were not inspected at all [54]. We were able to effectively use ATP concentrations to inform asset management decisions related to outlying reservoirs. The use of ATP allowed the identification of ingress impacts on water quality when turbidity and total chlorine residuals did not provide operational value, similar to what has been reported by Vang et al. [55]. In addition, ATP provided a useful tool in the field during reservoir draining that was able to pinpoint to suction pipe condition deterioration, corresponding with work by Gosi et al. [56].

While there is no consensus on ATP action limits in the DWDS, the manufacturer of the ATP kit used in this study recommends using values between 0.1–1.0 pg/mL as an indicator of acceptable microbiological drinking water quality and values above 10 pg/mL as a trigger for corrective actions (e.g., reservoir cleaning, unidirectional flushing, inspections, resampling). In our system, this guideline resulted in no interruption to service and still allowed for timely intervention when water quality changes are noted. Utilities could use a proactive threshold 5–10 pg ATP/mL to trigger additional sampling in the DWDS but these values should be confirmed in different jurisdictions. In the long-run, ATP testing is conducive to adoption in the field to monitor other operational activities, such as reducing duration of unidirectional flushing to green the practice [25] and testing water prior to release post-infrastructure repair and main breaks [18]. It could also become part of an online, real-time microbial contamination warning system to assess deterioration of water quality at sensitive and critical locations in the distribution system (e.g., reservoirs, treated plant effluent) [25,33,39,40]. Regardless of whether microbial data are collected in real-time or by analysis of grab samples, ATP could potentially become a critical monitoring parameter for the proactive and forward-thinking utility of the future.

5. Conclusions

Below, we provide a summary of take-home messages surmised from this study:

- Using large, long-term ATP concentration datasets by sampling multiple locations in the DWDS starting with the treatment plant allows utilities and researchers to draw reliable and scientifically sound conclusions around the factors that may affect drinking water quality and microbial regrowth in the real world.
- ATP concentrations exhibited an increase between the DWTP and the outlying reservoirs and a decrease after leaving the outlying reservoirs potentially signaling the importance of TOC removal in controlling microbial regrowth in this chloraminated DWDS.
- The higher ATP values in outlying reservoirs compared to other sampling locations indicate the importance of reservoir management practices and the usefulness of a robust and accurate monitoring parameter.
- ATP concentrations were highest in the summer and exhibited significant spatial heterogeneity within sampling locations (e.g., outlying reservoirs, fire stations).
- ATP values provide better operational decision support than HPCs, which were negative (<1 CFU/mL) more than 90% of the time.
- ATP concentrations were used effectively for outlying reservoir management decisions and detected both ingress and suction pipe condition deterioration when chlorine and turbidity did not signal significant water quality changes.
- While a significant number of ATP measurements fell below the detection limit (<0.1 pg/mL), values in the 1–3 pg/mL range were also common providing higher granularity and potential ability to detect early changes and microbial regrowth throughout the system.
- The results suggest that an ATP operational threshold concentration of 10 pg/mL could be used to trigger utility intervention (e.g., flushing, maintenance, disinfection). It also suggests that an internal utility action limit of 5 pg/mL could be a useful metric to trigger additional testing and resampling.
- Given its granularity and speed of testing, researchers should continue to explore potential sensors and real-time monitoring tools that enable continuous monitoring of ATP at sensitive locations at the DWTP and in the DWDS.

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Data Availability Statement: Providing location of drinking water plants, reservoirs and other assets is considered a potential threat to public health and should be avoided. Research data is unavailable due to privacy or ethical restrictions.

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