Canadian Journal of Microbiology
Revue canadienne de de microbiologie

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Journal:	*Canadian Journal of Microbiology*
Manuscript ID	cjm-2015-0375.R1
Manuscript Type:	Article
Date Submitted by the Author:	28-Sep-2015
Complete List of Authors:	Scott, Daniel B.; University of Waterloo, Civil and Environmental Engineering Van Dyke, Michele I.; University of Waterloo, Civil & Environmental Engineering Anderson, William B.; University of Waterloo, Civil and Environmental Engineering Huck, Peter M.; University of Waterloo, Civil and Environmental Engineering
Keyword:	chloramine, distribution system, drinking water, nitrifier, ammonia-oxidizing

Influence of Water Quality on Nitrifier Regrowth in Two Full-scale Drinking Water Distribution Systems

Daniel B. Scott[†], Michele I. Van Dyke[*], William B. Anderson and Peter M. Huck

NSERC Chair in Water Treatment, Department of Civil and Environmental Engineering, University of Waterloo, Waterloo, Ontario, Canada

[*]Corresponding author. 200 University Avenue West, Waterloo, Ontario, Canada N2L 3G1.
Tel: 519-888-4567 ext. 36193, Email: mvandyke@uwaterloo.ca.
William B. Anderson, wbanderson@uwaterloo.ca
Peter M. Huck, pm2huck@uwaterloo.ca

[†]Current address: ADI Systems Inc., Fredericton, New Brunswick, Canada.
Email: daniel.b.scott@adi.ca

ABSTRACT

The potential for regrowth of nitrifying microorganisms was monitored in two full-scale chloraminated drinking water distribution systems in Ontario, Canada, over a nine month period. Quantitative PCR was used to measure *amoA* genes from ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), and these values were compared with water quality parameters that can influence nitrifier survival and growth including total chlorine, ammonia, temperature, pH, and organic carbon. Although there were no severe nitrification episodes, AOB and AOA were frequently detected at low concentrations in samples collected from both distributions systems. A culture-based presence/absence test confirmed the presence of viable nitrifiers. AOB were usually present in similar or greater numbers than AOA in both systems. As well, AOB showed higher regrowth potential compared with AOA in both systems. Statistically significant correlations were measured between several water quality parameters of relevance to nitrification. Total chlorine was negatively correlated with both nitrifiers and heterotrophic plate count (HPC) bacteria, and ammonia levels were positively correlated with nitrifiers. Of particular importance was the strong correlation between HPC and AOB, which reinforces the usefulness of HPC as an operational parameter to measure general microbiological conditions in distribution systems.

KEYWORDS: ammonia-oxidizing, chloramine, distribution system, drinking water, nitrifier.

INTRODUCTION

Monochloramine is used as a secondary disinfectant by many drinking water utilities in North America due to its lower potential for forming disinfection by-products (DBPs), longer persistence in distribution systems, and improved biofilm penetration compared with free chlorine (LeChevallier et al. 1990; Zhang and Edwards 2009). However, the presence of ammonia can also promote the growth of nitrifiers, which can lead to operational or regulatory-compliance challenges including a decline in the disinfectant residual, increased biofilm production and corrosion. Nitrification is a widespread issue in chloraminated drinking water distribution systems. For example, Wilczak et al. (1996) found that two thirds of U.S. utilities that applied chloramination experienced some degree of nitrification.

Ammonia-oxidation, the first and rate-limiting step of nitrification (Francis et al. 2005), is performed by autotrophic microorganisms from Bacteria (ammonia-oxidizing bacteria, AOB) and Archaea (ammonia-oxidizing archaea, AOA). This first step of nitrification is of concern in distribution system environments (Skadsen 1993; Lipponen et al. 2002) and is the subject of the present research. Some free ammonia will typically be available to ammonia-oxidizing microorganisms in treated water, and this amount will increase as monochloramine undergoes decay in the distribution system. This creates a positive feedback loop, since nitrification products (nitrite, and increased organic matter from nitrifier growth) accelerates chloramine decay, providing more free ammonia, and thus further promoting the growth of nitrifying microorganisms (Oldenburg et al. 2002). Factors that can affect distribution system nitrification include ammonia concentration, monochloramine concentration, temperature, dissolved oxygen (DO), pH, and organic carbon levels (Zhang et al. 2009).

Consequences of distribution system nitrification include an accelerated decay of the chloramine residual, a rise in nitrite and/or nitrate concentrations, and the potential for an increase in heterotrophic bacteria levels, and these have been recommended as indicators that nitrification is occurring (Odell et al. 1996; AWWA 2006). The consequences of nitrification are not likely to be a direct risk to public health; rather, they may lead to operational or regulatory-compliance challenges. However, a loss of the disinfectant residual can potentially decrease the robustness of the distribution system as the final barrier for safe drinking water before it is delivered to consumers (Health Canada 2002).

In this study, water samples were collected from two full-scale chloraminated distribution systems in southern Ontario, and parameters relevant to nitrification were monitored. The potential for regrowth of both AOA and AOB was measured using quantitative PCR assays that target the *amoA* gene. The *amoA* gene encodes subunit A of the ammonia monooxygenase enzyme, required for the oxidation of ammonia to hydroxylamine. The traditional technique for enumerating nitrifying bacteria is the culture-based most probable number (MPN) method (Standard Methods 2005). However, culture-independent techniques are more rapid and accurate than culture-based methods because nitrifiers are very slow-growing and growth media shows some selectivity (i.e. Hoefel et al. 2005). Molecular techniques have been used to assess the relative occurrence of AOA and AOB in various environments, but few studies have applied these methods to drinking water (de Vet et al. 2009; van der Wielen et al. 2009; Kasuga et al. 2010b). The correlation between nitrifier concentrations and potential indicators or predictors of nitrification was also evaluated.

MATERIALS AND METHODS

Systems studied

The water utilities of the City of Toronto (Toronto Water) and the Region of Waterloo participated in this study. Both systems are located in southern Ontario, Canada, and use monochloramine as a secondary disinfectant. The Toronto Water distribution system is served by four water treatment plants that draw raw water from Lake Ontario. The plant that serves the part of the distribution system included in this study is a conventional water treatment plant with a capacity of 615 ML/d. The process train includes pre-chlorination (for zebra mussel control), coagulation, flocculation, sedimentation, dual-media (anthracite/sand) filtration, and chlorine disinfection, followed by chloramination for secondary disinfection. The Region of Waterloo distribution system is served by a blend of surface water (27%) and groundwater (73%), and these combined sources have a capacity of 72 ML/d. The distribution system included in this study receives surface water from the Grand River, and the process train at the treatment plant consists of coagulation, flocculation, sedimentation, ozonation, multi-media filtration, UV disinfection, and chlorine disinfection, followed by chloramination for secondary disinfection. Treated surface water is blended with groundwater prior to entering the distribution system. The groundwater was treated using either UV or chlorine for primary disinfection, followed by chloramines for secondary disinfection. Table 1 summarizes the average values of the physical-chemical parameters measured over the course of the study at the entrance to each distribution system (sites T-WTP and W-WTP). The DOC concentration, conductivity, background nitrate levels and temperature range were higher in Waterloo, whereas the values for the other parameters were similar.

Sample sites used in the study were spatially dispersed, had some historical differences in total chlorine and heterotrophic plate count (HPC) concentrations, and were used by each utility

as part of their regular monitoring program. Sample sites in the Toronto Water distribution system included one treatment plant (T-WTP) and 6 locations in the distribution system (T-1 to T-6), and 12 samples were collected from each site (approximately every 2-4 weeks from November 2009 to August 2010). Sample sites in the Region of Waterloo included the treatment plant (W-WTP) and 7 locations in the northern area of the regional distribution system (W-1 to W-7), and 9 samples were collected from each site (every 2-4 weeks from February to August 2010). Site W-5 was near a free chlorine booster station and thus had a free chlorine residual instead of a chloramine residual. The distance between each distribution system site and the drinking water treatments plant increased with increasing sample designation.

Physical-chemical analyses

Samples collected at the treatment plants were taken from continuous-flow sampling taps. Distribution system samples were taken after the taps were flushed to a steady water temperature. Temperature, conductivity, pH and chlorine were measured on site. Temperature and conductivity were measured with a Hach CO150 conductivity meter, and pH with an Orion 290A pH meter with an Ag/AgCl electrode probe. Total chlorine was analyzed by Hach method 8167 (Hach 2008), which is based on Standard Method 4500-Cl G. Samples for further analyses (DOC, ammonia, nitrite, nitrate) were collected in 300 mL glass bottles and transported to the lab on ice. Samples for DOC and ion chromatography were filtered through a 0.45 µm Pall Supor 450 membrane filter and stored at 4°C until analysis. DOC was quantified by automated wet oxidation (OI Analytical 1010), and nitrite and nitrate using a Dionex ICS-series ion chromatograph with an AS4A-SC 4 mm anion exchange column. Monochloramine and free ammonia were measured according to Hach method 10200 (Hach 2008).

125

126 **Microbiological analyses**

127 Distribution system samples for microbiological analyses were collected before the tap
128 was flushed, and although this was a departure from standard sampling procedures (Method 9060
129 A, Standard Methods 2005), it was considered more appropriate for this study because it
130 provided a better measure of microbial regrowth and biofilm formation potential. In addition,
131 stagnant water represents a more critical condition for nitrification, and thus was of greater
132 interest in this project. Samples for nitrifier analysis were collected in sterile 1 L plastic bottles
133 containing 1 mL of 3% sodium thiosulfate to quench the disinfectant residual. A separate 250
134 mL water sample collected in a sterile bottle containing sodium thiosulfate (Systems Plus, Baden,
135 ON) was taken for HPC. Sample bottles were transported on ice, held at 4°C and analyzed within
136 48 h. For HPC analysis, 1 mL and 10 mL volumes were separately passed through a sterile 0.45
137 µm Pall GN-6 membrane filter by vacuum filtration, placed on R2A agar and incubated at 28^{o}C
138 for 5–7 days. Colonies were then counted at 20× magnification. This method is based on
139 Standard Method 9215 (Standard Methods 2005).

140 Ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) were
141 enumerated by quantitative PCR (qPCR) of the *amoA* gene. One L of each sample was
142 concentrated by vacuum filtration through a sterile 0.2 µm Pall Supor 200 membrane filter. Each
143 filter was placed in a 3 mL plastic vial containing 1.5 mL of GITC buffer (Cheyne et al. 2010)
144 and frozen at -80^{o}C. Samples were then subjected to three freeze-thaw cycles (-80^{o}C followed by
145 50^{o}C for 15 minutes), followed by DNA extraction and purification as described by Cheyne et al.
146 (2010). qPCR was performed using AOB primers that target the β-Proteobacterial group of
147 ammonia-oxidizing bacteria as described by Rotthauwe et al. (1997) and AOA primers that

target strains from the domain Archaea as described by de la Torre et al. (2008). Each 50 µL PCR reaction contained 20 µL of concentrated DNA (corresponding to 200 mL of distribution system sample), 300 nM of each primer, 25 µL of 2x SsoFast EvaGreen Supermix (BioRad), and 20 µg BSA (Sigma). The cycling conditions for the AOA assay were 1 cycle at 95°C for 2 minutes; 40 cycles at 95°C for 15 s, 54°C for 30 s and 72°C for 60 s. The same conditions were used for the AOB assay, but with an annealing temperature of 59°C. PCR templates used *amoA* genes cloned into the pCR 4-TOPO vector (Invitrogen). The bacterial *amoA* gene was obtained from a pure culture of *Nitrosomonas europaea*. The archaeal *amoA* gene was amplified from a river water sample. The vector was linearized using the PstI enzyme, and template concentrations were determined using the Quant-iT PicoGreen kit (Invitrogen). The specificity of each PCR reaction was confirmed by melt curve analysis and agarose gel electrophoresis.

A culture-based detection method for ammonia-oxidizing bacteria based on Standard Method 9245 (Standard Methods 2005) was applied to samples collected from the Toronto distribution system on 17 August 2010, and to samples collected from the Region of Waterloo distribution system on 25 August 2010. One L of each sample was filtered through a 0.2 µm Pall Supor 200 membrane. The membrane was then placed in 50 mL of growth media and incubated at 28°C for 28 days. The media was tested for nitrite and nitrate using the Hach NitriVer and NitraVer reagents. Samples with detectable nitrite (> 0.002 mg/L as N) and nitrate (> 0.1 mg/L as N) were considered positive for ammonia-oxidizing microorganisms.

Data analyses

Statistical analysis was performed using the software "R" (R Development Core Team 2009). The relationship between distribution system parameters was determined using the non-

parametric Spearman correlation coefficient (ρ). The Spearman correlation coefficient is often used in distribution system research (Cunliffe 1991; Rice et al. 1991; Lipponen et al. 2002, 2004) when the parameters measured are not normally distributed or have a large number of values below the detection level. To determine if the correlation between certain parameters was due to their mutual correlation with a third factor, partial correlation coefficients were calculated. The Kruskal-Wallis test was used to determine if there was a difference in nitrifier concentrations between sites. Significant differences in nitrifier and HPC concentrations between water leaving the treatment plants (T-WTP and W-WTP) and each distribution system site were measured using the Mann-Whitney U test ($p < 0.05$; 2 tailed).

RESULTS AND DISCUSSION

Occurrence of nitrifying microorganisms

Few studies (van der Wielen et al. 2009; Kasuga et al. 2010b, 2010a; Wang et al. 2014) have examined both AOB and AOA in drinking water. Since most existing information on ammonia-oxidizing microorganisms is for bacteria (AOB), the occurrence of AOA in distribution systems is of interest, as they may have different growth and survival properties. For example, Martens-Habbena et al. (2009) found a half-saturation coefficient for ammonia in a strain of AOA that was lower than any reported for AOB, suggesting that some AOA strains may be better adapted for growth at low ammonia concentrations. According to Kasuga et al. (2010b), AOA and AOB might differ in their resistance to chlorine. Indeed, questions on the relative abundance and respective roles of AOA and AOB in nitrification have been topics of recent research (Hatzenpichler 2012; Prosser and Nicol 2008; Schleper 2010).

193 This study used a PCR approach targeting the *amoA* genes of either AOB or AOA to
194 detect putative ammonia-oxidizing microorganisms in two chloraminated drinking water
195 distribution systems. PCR assays that target the *amoA* gene are frequently used in environmental
196 studies, since conserved but distinct groups of this functional gene have been detected in
197 bacterial and archaeal microorganisms (Francis et al. 2005). To date, AOA strains have been
198 shown to contain one copy of the gene (Hatzenpichler 2012), while AOB strains have between 2
199 to 3 copies (Norton et al. 2002). By using a gene that encodes a key metabolic enzyme, assays
200 have certain advantages over phylogenetic markers including improved specificity and
201 sensitivity (Junier et al., 2010). Results showed that *amoA* genes from both groups of ammonia-
202 oxidizing microorganisms were frequently detected in samples collected from the two systems
203 (Figures 1 and 2). The data are summarized as boxplots, showing the median and central 50%
204 (interquartile range), with whiskers that extend to the minimum and maximum values. Sample
205 sites are ordered on the x-axis according to distance from the water treatment plant (WTP), but
206 the cumulative hydraulic retention time for each site was not available.
207 In the Waterloo distribution system, AOB and AOA concentrations were significantly
208 correlated (Table 2). Site W-5 was located by a reservoir with a free chlorine booster station, and
209 therefore was the only site to use free chlorine and not chloramines. W-5 had low levels of both
210 types of ammonia-oxidizing microorganisms relative to other sites, showing that nitrification is
211 an issue primarily when chloramines are used as a disinfectant. In the Toronto system, AOB
212 were typically more numerous than AOA, and the concentration of the two groups was not
213 correlated (Table 2). AOA concentrations following treatment at Waterloo were consistently
214 higher than at Toronto, presumably due to higher source water concentrations. However,
215 nitrifiers in the raw water were not measured in this study.

In both systems, AOB concentrations increased from low levels in water leaving the treatment plants to 2-3 log higher concentrations at sites in the distribution system. This increase was significant at each site (except W-5) in both the Toronto and Waterloo systems (Figure 1). However, AOA did not show the same regrowth potential. There were increases in the AOA concentration (up to 1 log) at some sites, and in Toronto this increase was significant at sites T-2 and T-4, and in Waterloo at sites W-1, W-3 and W-4 (Figure 2). Overall, the increase in AOB from the entrance to each distribution system to the site with the highest median value was greater than the increase in AOA. This suggests that AOB may be better adapted for growth in distribution systems. Further research is recommended to investigate differences between the optimal niches for AOB and AOA, and whether there is any difference in their roles in distribution system nitrification.

To complement the nitrifier occurrence data obtained from qPCR of the *amoA* genes, a culture-based test for ammonia-oxidizing microorganisms was also employed. This was done because PCR detects intact DNA from both live and dead cells, whereas a culture-based test will only measure if viable cells are present. Table 3 shows that for samples collected on a day with typical water quality, the majority of sites were positive for autotrophic ammonia-oxidation, indicating that viable ammonia-oxidizing microorganisms were present. However, it is possible that the concentration of *amoA* genes detected by PCR was influenced by the presence of dead cells, since the samples were taken following water treatment processes that included disinfection.

Relationship between nitrifiers and water quality parameters

Water quality parameters were monitored in both full-scale distribution systems, and the relationship between these parameters and nitrifier concentrations was determined (Table 2). This information is useful for understanding conditions that can influence nitrifier survival and growth in drinking water systems. In addition, this assessment can identify if factors that are easily and routinely measured in drinking water systems can predict nitrifier occurrence and potential nitrifying conditions.

General microbiological water quality is typically measured in drinking water using the HPC method (National Research Council 2006; Health Canada 2012), and it has been suggested that an increase in HPC bacteria may be associated with nitrification (Skadsen 1993; Odell et al. 1996; Wilczak et al. 1996; Zhang et al. 2009). Two mechanisms by which nitrification can promote the growth of heterotrophic bacteria are (1) accelerating the decay of the disinfectant residual and (2) increasing organic carbon availability through the formation of soluble microbial products (SMP) (Rittmann et al. 1994). Figure 3 shows HPC levels measured at the entrance to each distribution system were low (less than 1 CFU per mL on most sampling dates), and that HPC concentrations were 1-2 log higher in distribution system samples. This suggests that conditions were favorable for bacterial regrowth in both distribution systems. The site with a free chlorine residual (W-5) had HPC levels similar to nearby sites, whereas AOB and AOA concentrations at W-5 were much lower than at nearby chloraminated sites. This suggests that nitrifier regrowth was controlled by limitation of the ammonia substrate, and not by disinfection with free chlorine, which would have had a similar effect on HPC.

AOB were positively correlated with HPC levels in both distribution systems (Table 2). As discussed further below, AOB and HPC were also significantly correlated with total chlorine, and therefore partial correlation coefficients were calculated to determine if the correlation

between AOB and HPC was due to their sensitivity to chlorine. The partial correlation coefficients of AOB and HPC with total chlorine as the third factor were 0.48 and 0.54 for Toronto and Waterloo, respectively (results not shown). Since these partial correlation coefficients were similar to the original values (0.51 for Toronto and 0.58 for Waterloo), the correlation between AOB and HPC was not a statistical artefact of their mutual correlation with total chlorine. However, HPC levels were not correlated with AOA in either system (Table 2). This suggests that factors affecting survival and regrowth of AOB and HPC in the distribution systems did not similarly promote regrowth of AOA.

The fact that HPC and AOB were correlated reinforces that HPC is a good indicator for the regrowth of nitrifying bacteria. Although there was some loss of DOC after each WTP (12-16% in Toronto, 22-37% in Waterloo), there was no correlation between DOC and HPC or nitrifiers (Table 2). However, DOC levels may not have been correlated with the fraction of organic carbon that is readily available to microorganisms (i.e. assimilable organic carbon (AOC)). A correlation between nitrifying bacteria and heterotrophic bacteria has been previously observed in bulk water samples (Lipponen et al. 2002) and biofilms (Lipponen et al. 2004). Health Canada (2012) favours HPC as a useful operational parameter to monitor general bacteriological water quality, including bacterial regrowth, and this study provides support for that position. HPCs have also been recommended as a possible nitrification indicator by Zhang et al. (2009), but they caution that other factors can lead to high HPC aside from nitrification, and so cannot be used in isolation.

Disinfectant residual concentration is an important factor that can affect the survival and growth of microorganisms including nitrifiers (Odell et al. 1996; Pintar and Slawson 2003), and sites with a lower disinfectant residual will be more vulnerable to nitrification events (Yang et al.

2007). However, some studies have found that AOB have long inactivation times with monochloramine, so maintaining a disinfectant residual does not always prevent their growth (Oldenburg et al. 2002; Wahman et al. 2009). Monitoring results in Figure 4 show that in both systems there was a significant decrease in the disinfectant residual. There was some variation in disinfectant residual between and within sites, and the variability was less in the Toronto system compared with Waterloo. The total chlorine residual in Waterloo decreased by about one third from the entrance of the distribution system (site W-WTP) to more distant sites. As mentioned earlier, W-5 was the only site to use free chlorine, and therefore had a lower free chlorine residual target. Combined chlorine residuals in both systems were always within the 0.25–3.0 mg/L range required for distribution systems in Ontario (Ontario Ministry of the Environment 2006). Chlorine concentration had a statistically significant negative correlation with AOB and AOA ($p < 0.01$), and HPC bacteria ($p < 0.1$) in the Toronto distribution system, and with AOB ($p < 0.1$) and HPC ($p < 0.01$) in the Waterloo distribution system. The negative correlation between total chlorine residual and microorganism abundance were expected, since previous studies have shown similar findings for biofilm bacteria (Lipponen et al. 2004) and nitrifying bacteria (Cunliffe 1991).

Ammonia concentration is also an important parameter, as it is the substrate for nitrifying microorganisms. However, ammonia can be released by the decay of chloramine and consumed by microbial activity, so its concentration in chloraminated drinking water distribution systems can be difficult to interpret. In the Toronto system, average ammonia levels were similar among sampling sites (Figure 5). In the Waterloo system, many sites had a high variability of ammonia levels, and the concentration at site W-7 was significantly higher than at the WTP, likely due to monochloramine decay. Site W-4 is a dead-end site and had the highest range of ammonia values

and a lower than average chlorine residual concentration (Figure 4). Since W-5 had free-chlorine instead of chloramine, it had a significantly lower ammonia concentration. In the Toronto and Waterloo distribution systems, there was a decrease in the total chlorine residual and a rise in ammonia at some sites in the warmer summer months, even though the chlorine residual at the entrance to each distribution system remained stable (data not shown). Seasonal variations in the stability of chloramines are expected, because the decay rate of monochloramine increases at higher temperatures (Vikesland et al. 2001).

Ammonia concentration had a weak but significant ($p < 0.1$) positive correlation with AOB in both distribution systems, and with AOA in the Waterloo distribution system (Table 2). Even though ammonia is the substrate for the first phase of nitrification, it is unclear from previous studies how ammonia affects the risk of nitrification. High ammonia concentrations have been identified as a possible cause of nitrification episodes (Skadsen 1993), and Lipponen et al. (2002) found that ammonia and AOB levels were correlated in drinking water distributions systems (Lipponen et al. 2002). However, other studies have reported that the ammonia concentration was not a significant risk factor for nitrification (Odell et al. 1996; Yang et al. 2007). One possible explanation for these mixed results is that the ammonia concentration initially rises and then declines as nitrification progresses (Liu et al. 2005). Therefore, ammonia measurements taken during different stages of a nitrification event may show opposite trends (increasing or decreasing). Also, if chloraminated distribution systems are inhabited by species of AOA and AOB with very low half-saturation coefficients for ammonia (Bollmann et al. 2002; Martens-Habbena et al. 2009) then ammonia concentrations may not be the main growth limiting factor. The results of this study indicate that the ammonia concentration may be related to the

abundance of nitrifying microorganisms, but the relatively low Spearman correlation coefficients (0.21–0.29) suggest a weak relationship.

In both distribution systems, there was a negative correlation between temperature and the total chlorine residual, and a positive correlation between temperature and ammonia. This is reasonable, as monochloramine decays more quickly at higher temperatures (Vikesland et al. 2001). Nitrification has been reported across a wide range of water temperatures (Wilczak et al. 1996; Lipponen et al. 2002; Pintar et al. 2005), although higher temperatures can increase the growth rate of nitrifying bacteria (Rittmann and Snoeyink 1984; Antoniou et al. 1990). The ability of AOB to survive at low temperatures (e.g. 6^{o}C; Pintar and Slawson 2003) could explain why no correlation between AOB and temperature was observed in this study.

A rise in nitrite levels is a commonly used indicator for nitrification, since it is normally present at very low levels in distribution systems. Figure 6 shows that the average nitrite levels were below 0.01 mg-N/L and there were no significant increases at sites in both systems, although they were slightly higher at most Waterloo sites compared with Toronto. As well, there was no relationship between nitrite concentrations and ammonia-oxidizing microorganisms (Table 2). The exceedance of a threshold level of nitrite can be used as an indication of nitrification, and various values have been proposed. Odell et al. (1996) suggested 0.05 mg/L NO_2-N, but Pintar et al. (2005) found that this was too high to serve as an effective early warning indicator. Other thresholds for identifying nitrification are 0.025 mg-N/L (Fleming et al. 2005) and 0.015 mg-N/L (AWWA 2006). On some sample dates, the nitrite threshold of 0.015 mg-N/L was exceeded at sites T-5 and T-6 in the Toronto distribution system and at sites W-1, W-3, W-4, W-6, and W-7 in the Waterloo distribution system. However, overall, results showed that there were no large or prolonged increases in nitrite, indicating that no serious nitrification episodes

occurred during the period of the sampling campaign. Pintar et al. (2005) conducted an earlier study on nitrification in the Waterloo distribution system, including some sites that were sampled in this study. Compared to their findings, much greater stability (less seasonal variation) of the chloramine residual was observed in the present work. Even though nitrification was generally well-controlled in both systems over the course of the study, the ubiquity of *amoA* genes suggests the presence of nitrifying microorganisms and the potential for nitrification if conditions that limit nitrifier growth and activity are not well controlled.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the tremendous support, access to facilities and data provided by Abhay Tadwalkar, Susan Atlin, David Scott and Robert Kozlowski at Toronto Water, and Nancy Kodousek, Tim Walton and Alex Lee at the Region of Waterloo. We also thank Shoeleh Shams at the University of Waterloo for technical assistance. Funding for this project was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) in the form of an Industrial Research Chair in Water Treatment at the University of Waterloo. Chair partners included: Associated Engineering Group Ltd., the cities of Barrie, Brantford, Guelph, Hamilton and Ottawa, Conestoga-Rovers & Associates Limited, EPCOR Water Services, GE Water & Process Technologies Canada, Lake Huron and Elgin Area Water Supply Systems, the Ontario Clean Water Agency (OCWA), the Regions of Durham, Halton, Niagara and Waterloo, RAL Engineering Ltd., Toronto Water, and the Walkerton Clean Water Centre. Lead author Daniel Scott was the recipient of an NSERC Canada Graduate Scholarship.

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497 **TABLES**

498 Table 1. Average water quality values measured at the entrance to each distribution system

Parameter	**Toronto** (mean ± standard deviation)	**Waterloo** (mean ± standard deviation)
Source	Lake Ontario	Surface water blended with groundwater
pH	7.5 ± 0.2	7.5 ± 0.2
Temperature (°C)	10 ± 2	12 ± 6
Dissolved organic carbon (mg/L)	2.64 ± 0.86	3.57 ± 1.32
Conductivity (µS/cm)	316 ± 34	706 ± 62
Total chlorine (mg-Cl_2/L)	1.28 ± 0.11	1.47 ± 0.12
Ammonia (mg-N/L)	0.13 ± 0.10	0.15 ± 0.05
Nitrate (mg-N/L)	0.43 ± 0.12	3.48 ± 0.52
Nitrite (mg-N/L)	0.001 ± 0.002	0.004 ± 0.004

499

Table 2. Correlation coefficient values (*p*) for parameters in each full-scale distribution system

	Toronto				Waterloo			
	AOB	**AOA**	**HPC**	**Temp**	**AOB**	**AOA**	**HPC**	**Temp**
AOB		-0.01	**0.51****	0.18		**0.71****	**0.58****	0.00
AOA			-0.06	**0.22***			0.15	-0.03
Total chlorine	**-0.45****	**-0.28****	**-0.21***	**-0.23***	**-0.25***	0.01	**-0.50****	**-0.51****
DOC	0.04	0.14	-0.15	**0.58****	-0.07	0.02	0.02	**0.54****
Ammonia	**0.21***	0.13	0.06	**0.51****	**0.27***	**0.29***	0.16	0.3
Nitrite	0.12	0.06	0.17	0.2	0.17	0.15	-0.03	-0.11

* significant at $p < 0.1$; ** significant at $p < 0.01$

Table 3. Nitrifiers measured using a culture-based (presence/absence) method

Toronto		Waterloo	
Site	**Nitrifiers**	**Site**	**Nitrifiers**
T-WTP	Absent	W-WTP	Present
T-1	Present	W-1	Present
T-2	Present	W-2	Present
T-3	Absent	W-3	Present
T-4	Present	W-4	Present
T-5	Present	W-5	Present
T-6	Present	W-6	Present
		W-7	Present

FIGURE CAPTIONS

Figure 1. Concentrations of bacterial *amoA* gene copies in the Toronto and Waterloo distribution systems. Significant differences between the water treatment plant (T-WTP and W-WTP) and distribution system sites are marked as * (n = 12 for each Toronto site and n = 9 for each Waterloo site).

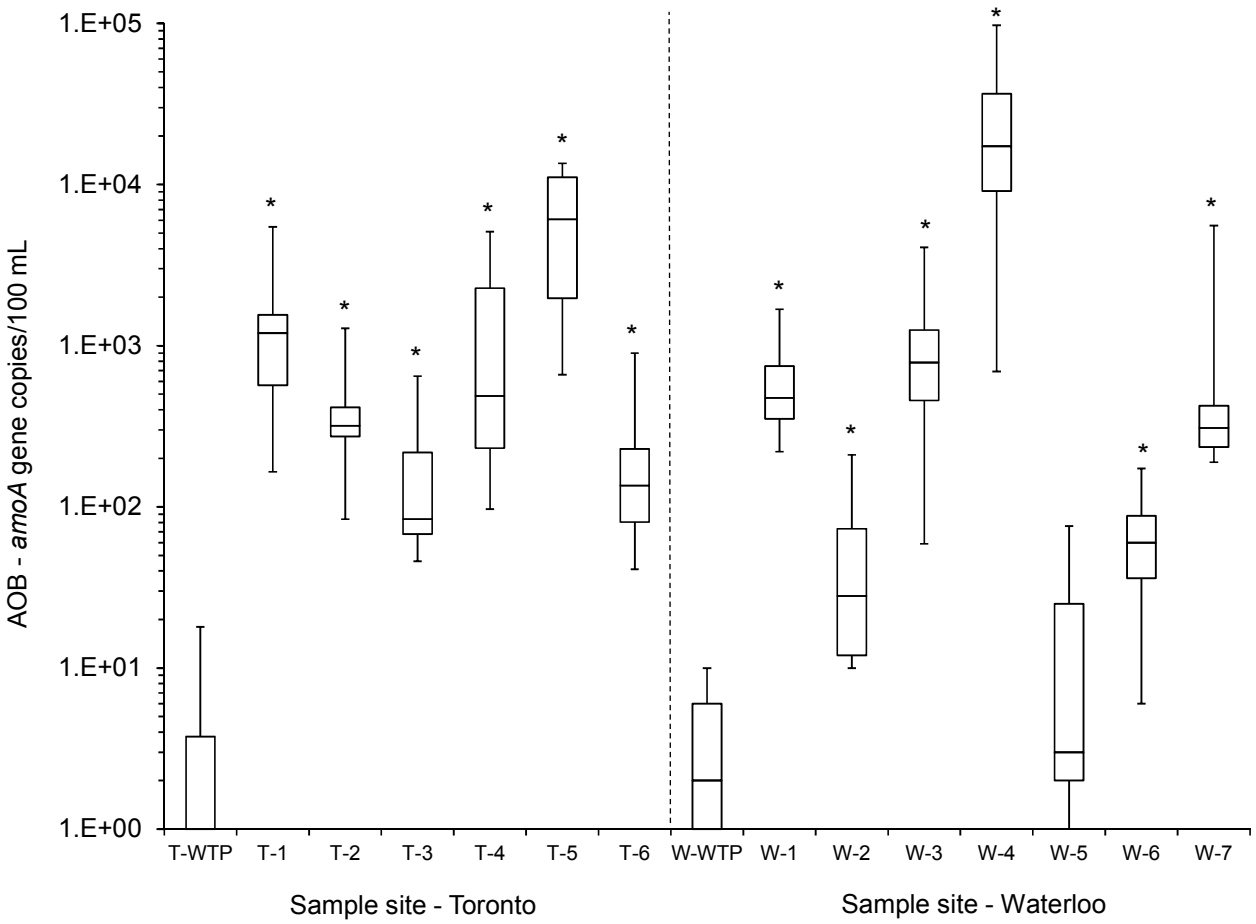
Figure 2. Concentrations of archaeal *amoA* gene copies in the Toronto and Waterloo distribution systems. Significant differences between the water treatment plant (T-WTP and W-WTP) and distribution system sites are marked as * (n = 12 for each Toronto site and n = 9 for each Waterloo site).

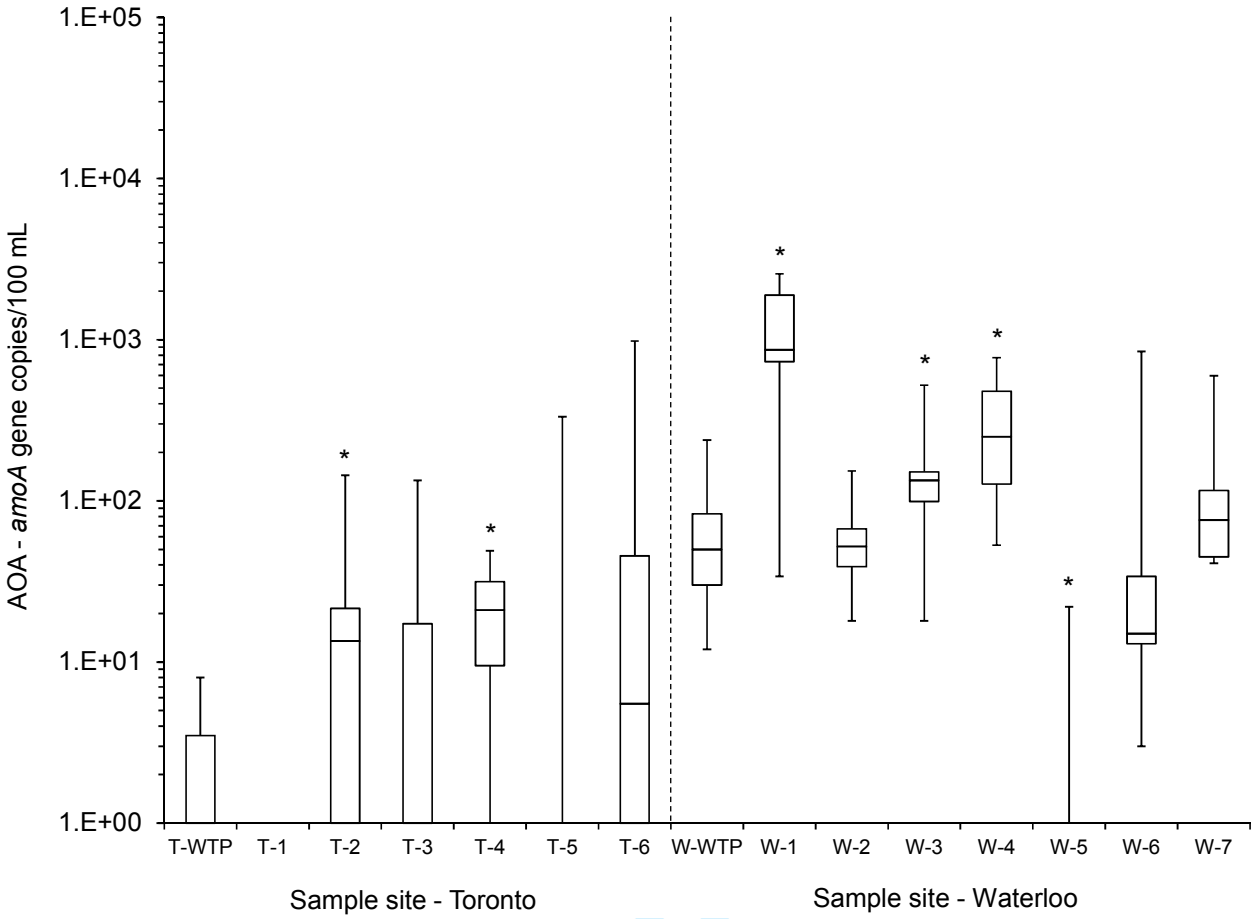
Figure 3. First flush HPC levels at each site in the Toronto and Waterloo distribution systems. Significant differences between the water treatment plant (T-WTP and W-WTP) and distribution system sites are marked as * (n = 12 for each Toronto site and n = 9 for each Waterloo site).

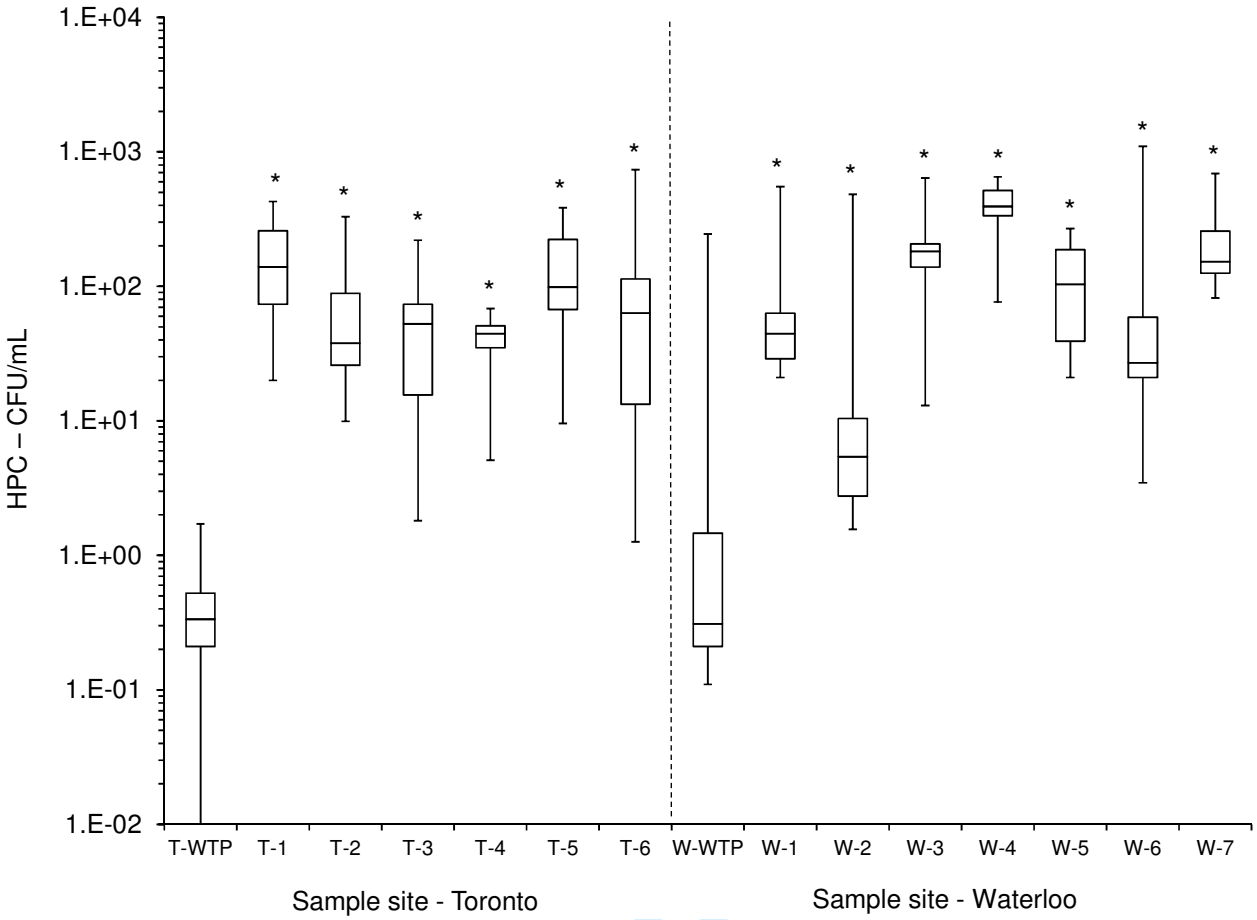
Figure 4. Total chlorine residual concentrations at sample locations in each distribution system. Significant differences between the water treatment plant (T-WTP and W-WTP) and distribution system sites are marked as * (n = 12 for each Toronto site and n = 9 for each Waterloo site).

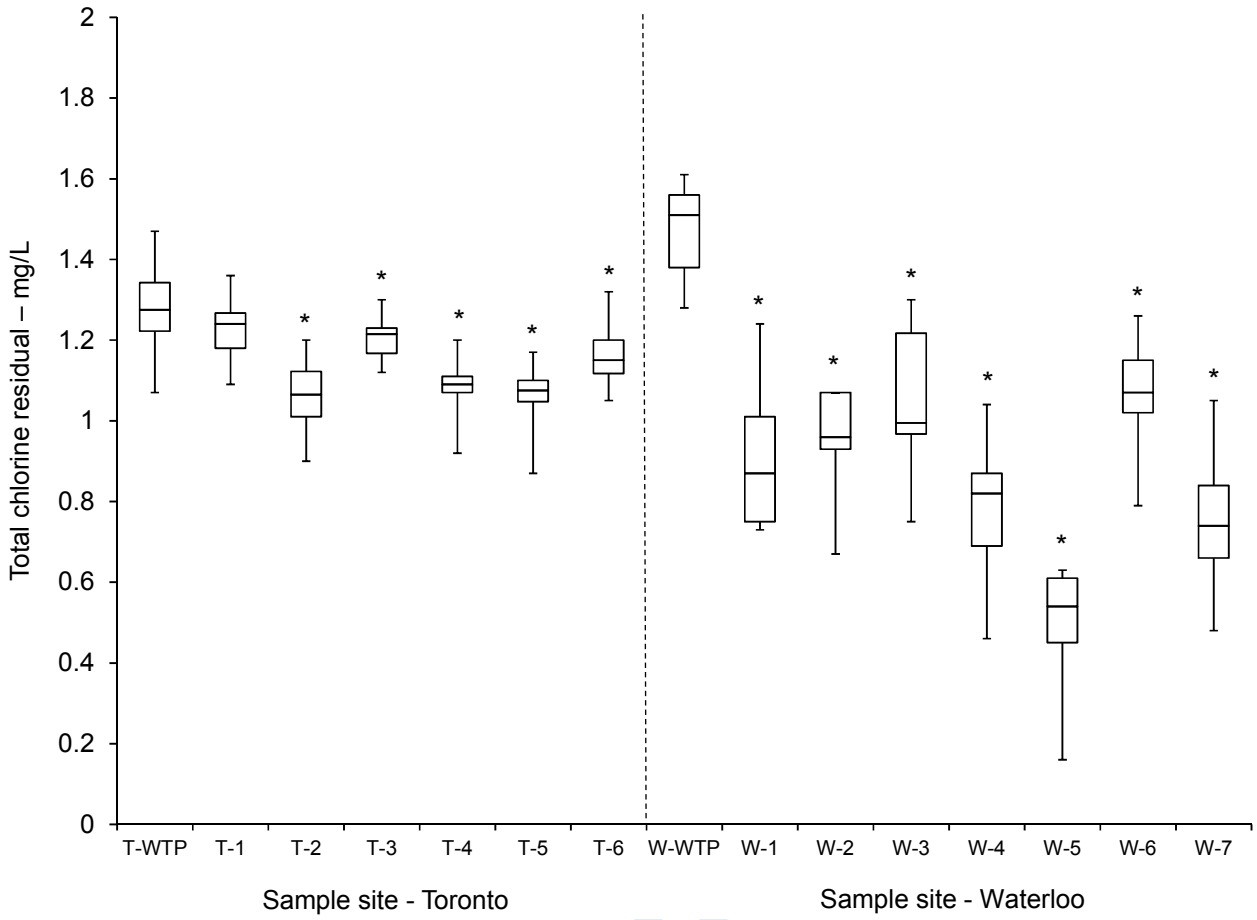
Figure 5. Ammonia concentrations at sample locations in each distribution system. Significant differences between the water treatment plant (T-WTP and W-WTP) and distribution system sites are marked as * (n = 12 for each Toronto site and n = 9 for each Waterloo site).

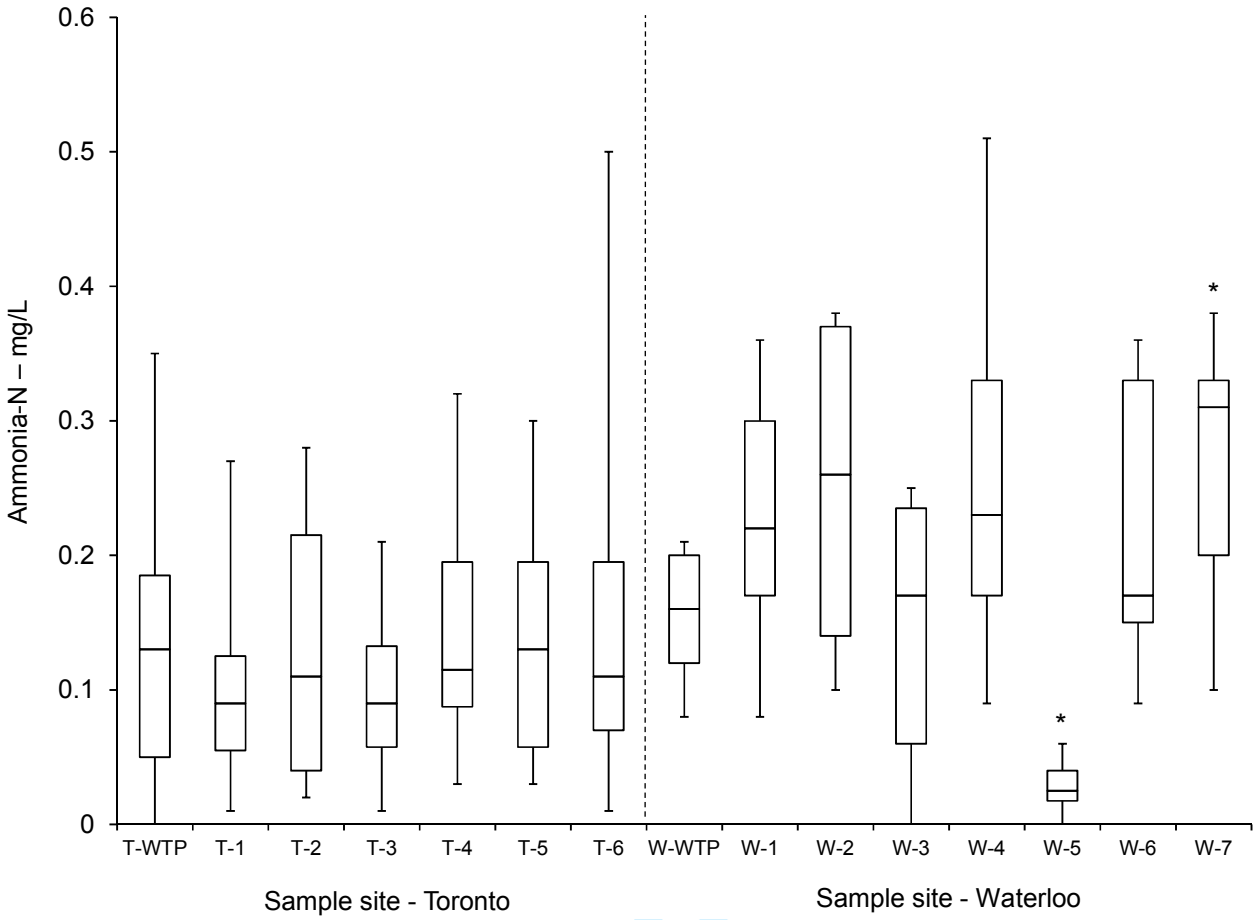
Figure 6. Nitrite concentrations at sample locations in each distribution system (n = 12 for each Toronto site and n = 9 for each Waterloo site).

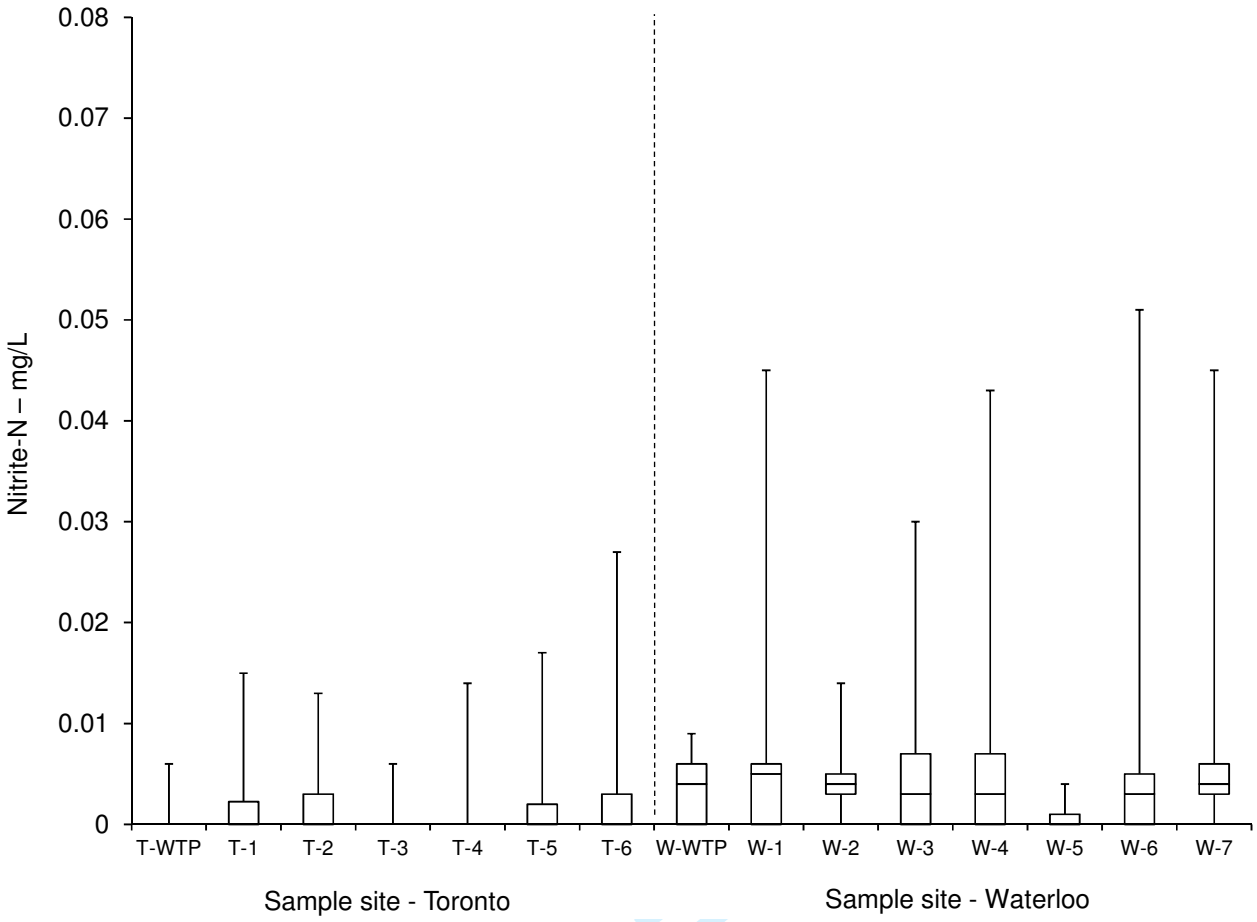

1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
AOB - amoA gene copies/100 mL
T-WTP
T-1
T-2
T-3
T-4
T-5
T-6
W-WTP
W-1
W-2
W-3
W-4
W-5
W-6
W-7
Sample site - Toronto
Sample site - Waterloo


1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
AOA - amoA gene copies/100 mL
T-WTP
T-1
T-2
T-3
T-4
T-5
T-6
W-WTP
W-1
W-2
W-3
W-4
W-5
W-6
W-7
Sample site - Toronto
Sample site - Waterloo


1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
1.E-01
1.E-02
HPC – CFU/mL
T-WTP
T-1
T-2
T-3
T-4
T-5
T-6
W-WTP
W-1
W-2
W-3
W-4
W-5
W-6
W-7
Sample site - Toronto
Sample site - Waterloo


Total chlorine residual – mg/L
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
T-WTP
T-1
T-2
T-3
T-4
T-5
T-6
W-WTP
W-1
W-2
W-3
W-4
W-5
W-6
W-7
Sample site - Toronto
Sample site - Waterloo


0.6
0.5
0.4
0.3
0.2
0.1
0
Ammonia-N – mg/L
T-WTP
T-1
T-2
T-3
T-4
T-5
T-6
W-WTP
W-1
W-2
W-3
W-4
W-5
W-6
W-7
*
*
Sample site - Toronto
Sample site - Waterloo


0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Nitrite-N – mg/L
T-WTP
T-1
T-2
T-3
T-4
T-5
T-6
W-WTP
W-1
W-2
W-3
W-4
W-5
W-6
W-7
Sample site - Toronto
Sample site - Waterloo