Detection of viable bacterial pathogens in a drinking water source using propidium monoazide-quantitative PCR
Avid Banihashemi, Michele I. Van Dyke and Peter M. Huck

ABSTRACT
A cell viability assay was applied to measure bacterial enteric pathogens in a river in southern Ontario, Canada that is used as a source of drinking water. Pathogen concentrations were measured using both propidium monoazide (PMA)-quantitative polymerase chain reaction (PCR) and quantitative PCR (qPCR) without PMA pretreatment to compare viable and total (live and dead) cells. The pathogens evaluated were Salmonella enterica, thermophilic Campylobacter, and Escherichia coli O157:H7, and the suspected enteric pathogen Arcobacter butzleri was also investigated. Results showed that for all strains dead cells were detected in few river water samples, and the difference between total and viable cell concentrations for each pathogen group was always less than 0.5 log. A. butzleri was detected at concentrations 2–3 log higher than the other pathogens. S. enterica, Campylobacter, and E. coli O157:H7 were detected at low concentrations at one sample location and at higher concentrations at a second sampling location. Results from this study show qPCR with PMA pretreatment can provide reliable enumeration of viable pathogens in surface water, and that dead cells were rarely present in water used as a source for drinking water treatment.

Key words | Arcobacter butzleri, PCR, propidium monoazide, river water, viability, waterborne pathogen

INTRODUCTION
An accurate assessment of pathogens in drinking water sources provides valuable information for safety planning and risk management of water treatment systems. Data on pathogen types and concentrations present in source water are needed to establish and evaluate new and current treatment processes, and to evaluate corrective actions towards improved source water quality. For example, risk assessment models can be used to measure pathogen removal through water treatment systems and to quantify the public health risk of finished water. These models often assess specific groups of pathogens that are known to be present in source water, but are often limited by a lack of reliable data.

Pathogenic bacteria have long been detected using classical culture-based methods, which include growth on selective media followed by morphological and biochemical/immunological confirmation. However, culture methods are time-consuming and many waterborne bacteria can enter a viable but non-culturably state in the environment without losing their virulence (Oliver 2010). To address this problem, DNA-based methods such as polymerase chain reaction (PCR) amplification were developed, and can improve the detection of pathogens in the environment. For example, Campylobacter in surface waters have been detected at a higher frequency using molecular methods compared with culture-based methods (Rothrock et al. 2009; Van Dyke et al. 2010). The problem with PCR-based methods is that the signal obtained can originate from both live and inactive/dead cells. Therefore, it is important to develop techniques that can rapidly detect viable cells only, in order to more accurately measure human health risk.

Propidium monoazide-PCR (PMA-PCR) is a modified PCR method that has been used as a viability assay to...
measure live cells only (Nocker et al. 2006) with PMA pre-treatment inhibiting the amplification of extracellular DNA and DNA from dead (membrane permeable) cells. PMA-PCR has been tested on a wide range of microorganisms including bacteria (e.g., Nocker et al. 2006; Pan & Breidt 2007; Cawthorn & Witthuhn 2008; Banihashemi et al. 2012), fungi (Vesper et al. 2008), viruses (Fittipaldi et al. 2010), and protozoa (Brescia et al. 2009). As well, studies have assessed method accuracy and sensitivity where sources of error may exist (Fittipaldi et al. 2011, 2012). However, a limited number of studies have applied the PMA-PCR method to enumerate pathogens in natural surface water (Nocker et al. 2007, 2010).

In this study, four different groups of bacterial pathogens were measured in river water using a quantitative PMA-PCR assay. Salmonella enterica, thermophilic Campylobacter, and Escherichia coli O157:H7 were selected as they are frequently detected in surface waters, are of high risk to human health, and have been used as indicator pathogens in risk assessment studies (i.e., WHO 2004). In addition, the study investigated the occurrence of Arcobacter butzleri, which has been identified in recent years as a potential foodborne and waterborne pathogen. Arcobacter was suspected to be the cause of a river water outbreak in Slovenia (Kopilovic et al. 2008) and a ground water outbreak in Idaho, USA (Rice et al. 1999). There have been limited studies on Arcobacter spp. in drinking water sources, and since A. butzleri has the highest prevalence in human infections and is most frequently isolated from water, this species was selected as a target for this work.

This study collected samples over an 8-month period from the Grand River watershed, which covers an area of approximately 7,000 km² and is located in southern Ontario, Canada. The Grand River is used as a source of drinking water by several communities, as well as for recreational purposes. The microbial quality of the Grand River water is influenced by high agricultural activities (e.g., poultry, swine, and cattle) (Dorner et al. 2007) and also by wildlife. In addition, it is highly urbanized in the central areas of the watershed and potentially affected by wastewater treatment plant discharges (Dorner et al. 2007). Pathogen concentrations were measured and compared using quantitative PCR (qPCR) with PMA pretreatment (live cells only) and qPCR without PMA pretreatment (live and dead cells). The correlation between live and total (live and dead) pathogen concentrations and the water quality indicators total E. coli and turbidity were also assessed.

**METHODS**

**Sample collection**

River water samples were collected from two locations (#1 and #2) in the Grand River located 15 km apart in an urbanized area of the watershed in Kitchener-Waterloo, Ontario (Dorner et al. 2004a, b). Thirteen samples were collected from each site over a period of 8 months (May–December 2011). As shown in Figure 1, these samples were collected more frequently in the summer (10 samples collected at intervals from May to August), followed by three samples through the fall and winter (October–December). At each location, three 1-L samples were collected in sterile polypropylene wide-mouth bottles (VWR, Mississauga, Ontario).

![Figure 1](https://iwaponline.com/aqua/article-pdf/64/2/139/400087/jws0640139.pdf)
Location #2 was closer to an upstream wastewater discharge, therefore 0.5 mL of 0.1 N sodium thiosulfate was added to the sample bottles to neutralize chlorine potentially present at this location. Samples were collected from fast-flowing areas of the river about 2–5 m away from the edge and 10–20 cm below the water surface. Samples were immediately placed on ice for transport, and then kept at 4 °C and analyzed within 24 h.

**Water quality analysis**

Temperature was recorded on site immediately after sampling. Turbidity was measured using a Hach 2100P portable turbidimeter (Loveland, CO, USA). Total *E. coli* were measured as described by Ciebin et al. (1995). Briefly, water samples were passed through 0.45 μm GN-6 membranes (47 mm; Pall Corporation, Mississauga, ON, Canada), the membranes placed on mFC basal medium (BD) containing 0.1 g L⁻¹ 5-bromo-6-chloro-3-indoxyl-β-d-glucuronide (BCIG; BioVectra, Charlottetown, PE, Canada), and incubated at 44.5 °C for 24 h. Flow rate data for the Grand River were obtained from the Water Survey of Canada (http://www.wateroffice.ec.gc.ca) from a gauging station at sample location #1.

**PMA treatment and DNA extraction**

Two liters of each river water sample were centrifuged at 17,000 × g for 40 min. The cell pellet in each bottle was resuspended with 5 mL of phosphate buffer saline (PBS, pH 7.4), aliquoted into 1.5 mL microcentrifuge tubes, and centrifuged at 12,000 × g for 5 min. The cell pellets in each tube were again resuspended in PBS, and then pooled to final volume of 800 μL for each concentrated sample. One sample from location #2 (October 26) had an unusually high solids content, and therefore the final suspension was diluted by 1:50 in PBS. Each 800 μL of concentrated sample was mixed well and divided into 400 μL subsamples, one of which was treated with PMA (to measure viable cells) and the other untreated (to measure total cells).

A 4 mM stock solution of PMA (phenanthridinium, 3-amino-8-azido-5-[3-(diethylmethylammonio) propyl]-6-phenyl dichloride; Biotium Inc., Hayward, CA, USA), was prepared in 20% (v/v) dimethyl sulfoxide, and stored in light-impermeable microcentrifuge tubes at −20 °C. PMA stock solution (1.5 μL) was added to 400 μL of each treated sample, resulting in a final PMA concentration of 15 μM. The sample was then mixed well by vortexing, followed by incubation in the dark for 5 min with constant mixing by inversion. The sample tubes were then placed on ice to avoid heating and exposed to a 500 W halogen lamp for 10 min at a distance of 20 cm with the caps open.

Both PMA-treated and untreated samples were centrifuged at 12,000 × g for 5 min, and the pellets were resuspended in 1 mL of guanidium thiocyanate (GITC) buffer [5 mol L⁻¹ GITC, 0.1 mol L⁻¹ ethylenediaminetetraacetic acid (EDTA; pH 8.0), 5 g L⁻¹ N-laurysarcosine] and transferred to a −80 °C freezer until DNA extraction. After thawing, the samples were mixed for 2 h at room temperature, followed by DNA purification using the Qiagen DNeasy tissue kit (Mississauga, ON, Canada) as described by Cheyne et al. (2010). The final elution volume was 200 μL, resulting in a DNA sample that was 5,000 × concentrated compared to the original river sample. The DNA was stored at −80 °C until analysis.

**Quantitative PCR**

qPCR assays used primer and probe sequences specific for *E. coli* O157:H7 (Ram & Shanker 2005), thermophilic *Campylobacter* (*jejuni/coli/lari*) (Van Dyke et al. 2010), *S. enterica* (Hoorfar et al. 2000), and *A. butzleri* (Brightwell et al. 2007). The specificity of the primers for the target groups was confirmed using the Basic Local Alignment Search Tool software (Madden et al. 1996). For all qPCR assays, 50 μL reaction volumes were used and contained 10 μL of DNA (equivalent to 50 mL of river water), 300 nM of each primer, 100 nM of probe, 1 × buffer, 3.5 mM MgCl₂, 1.25 U iTaq polymerase (Bio-Rad), 20 μg of bovine serum albumin (Sigma-Aldrich) and 200 μM dNTPs (Sigma-Aldrich). Amplification conditions for all assays were as follows: one cycle at 95 °C for 3 min; 50 cycles at 95 °C for 15 s, 60 °C for 30 s, 72 °C for 30 s; and one cycle at 72 °C for 10 min. Primers and probes were obtained from Sigma-Genosys (Mississauga, ON, Canada). The Bio-Rad iCycler iQ Real-Time PCR Detection System was used for qPCR analysis. Each sample was analyzed in triplicate,
and each run included duplicate standard curves and negative (no DNA) controls.

Standard curves used DNA extracted from E. coli O157: H7 ATCC 43895, S. enterica ATCC 13311, C. jejuni ATCC 33291, and A. butzleri ATCC 49616. C. jejuni cultures were grown as described by Van Dyke et al. (2010), and E. coli, S. enterica, and A. butzleri were grown in nutrient broth (BD) at 37 °C for 24 h. Cell enumeration, DNA extraction, and standard curve preparation are described by Banihashemi et al. (2012). Each qPCR assay produced standard curves with R² values of 0.99 or greater and with slopes ranging between −3.6 and −4.0 as a measure of the qPCR efficiency. DNA amplification was always observed for qPCR reactions containing 10 cells, which indicated that the detection limit was less than 10 cells per reaction. Approximately 25% of samples were checked for the presence of PCR inhibitors using an external luxB amplification method as described by Cheyne et al. (2010), and PCR inhibition was not detected in any samples (data not shown).

A control experiment to evaluate cell recovery was done by inoculating a sample from the Grand River with S. enterica to a final concentration of 4 × 10² cells mL⁻¹. The sample was concentrated by centrifugation, processed for DNA extraction, and enumerated using the Salmonella qPCR assay as described above. A second control test was performed prior to the experiments on river water samples to assess the possible effect of natural river water impurities on PMA effectiveness, and to evaluate the ability of PMA to remove the signal from dead cells in river water at the concentration used in this study (15 μM). River water was collected and concentrated by centrifugation (5,000 ×) as described previously, then divided into three 400 μL sub-samples and placed in separate microcentrifuge tubes. Two of the sub-samples were inoculated with purified C. jejuni DNA to a final concentration equivalent to 2 × 10⁶ cells mL⁻¹, one of which was treated with 15 μM PMA (to measure viable cells) and the other untreated (to measure live and dead cells). The PMA pretreatment was performed as described previously. The third sub-sample was used as a background control with no DNA inoculation or PMA treatment. The entire samples were then used for DNA purification (without centrifugation) by adding 800 μL GITC buffer to each sample, followed by mixing and column purification using the Qiagen Dneasy tissue kit. This was done so that both cell and non-cell associated (free) DNA could be measured, including the inoculated C. jejuni DNA. Columns were then eluted in 200 μL of AE buffer followed by PCR analysis as previously described.

Statistical analysis

Triplicate qPCR data were plotted as the average and confidence interval at a level of 95% (two-sided, α). Confidence intervals were used to determine the significance difference of untreated (total) compared to PMA-treated (viable) pathogen concentrations. Spearman’s rank correlation coefficient (P < 0.05 and P < 0.1; two-tailed) was used to compare viable and total pathogen concentrations in river water samples with the water quality parameters (turbidity, temperature, and total E. coli). Statistical analyses were calculated in Microsoft Excel.

RESULTS AND DISCUSSION

Water quality

The Grand River experienced higher and more variable flow rates during spring (May to June) and fall (October to December), but in summer the minimal base flow rate was dominant (Figure 1). The turbidity at location #1 ranged from 4.6 to 21.5 NTU with a median of 11.7 NTU, while at location #2 it ranged from 5.6 to 17.0 NTU with a median of 7.8 NTU, and was affected by rain events in the fall (October 26 and December 16) (Figure 2). Although the turbidity was generally higher at location #1, total E. coli were higher at location #2. Total E. coli at location #1 ranged from 2.9 × 10¹ to 1.9 × 10³ CFU 100 mL⁻¹ with a median of 7.5 × 10¹ CFU 100 mL⁻¹, and at location #2 from 6.5 × 10³ to 1.4 × 10⁵ CFU 100 mL⁻¹ with a median of 1.7 × 10³ CFU 100 mL⁻¹. Higher levels of total E. coli at location #2 may be due to relatively undiluted contributions from upstream sources including urban development and runoff, wastewater discharges, and upstream livestock and agricultural activities (Dörner et al. 2004a, b). Wildlife, including wild birds, can also influence microbial water quality in this area (Van Dyke et al. 2010). There were no temperature
differences between the two sampling locations through summer and fall (Figure 2).

**Total and viable bacterial pathogens in river water**

The method used to concentrate cells and extract DNA from river water was assessed by conducting a control experiment using river water spiked with a pure culture of *S. enterica*. This control for the centrifugation process in combination with DNA extraction and qPCR detection resulted in 63% recovery. These recovery results are similar to others that have used ultrafiltration (40–80% recovery; Holowecky et al. 2009) and centrifugation (75% recovery; Courtois et al. 2012) to recover cells from surface water.

The effectiveness of PMA in suppressing dead cells or extracellular DNA in concentrated river water samples was also tested in a separate control experiment. PMA was able to remove the signal from purified *C. jejuni* DNA below the background level (Figure 3). Because the purified DNA was added directly to the concentrated (5,000×) samples, results for this control experiment relate to values in the concentrated sample and not the original river sample. These results show that PMA effectiveness at the concentration used was not impeded by materials from the river water.

The PMA-qPCR viability method was then applied to river water samples to evaluate the levels of naturally occurring *S. enterica*, thermophilic *Campylobacter, E. coli O157: H7*, and *A. butzleri*, and compared to those using qPCR without pretreatment. Results showed that at sampling location #1, *S. enterica* concentrations were usually below the method detection limit (2 cells 100 mL−1) for both total and viable cells, except for two samples taken on June 29 and August 4, which had concentrations of 3–4 cells 100 mL−1 (Figure 4). Similarly, there were very low or non-detectable concentrations of *E. coli O157:H7* at sample location #1, which were detected twice in the summer (June 6 and August 11) and three times during the fall and early winter (15 October, 26 October, and 16 December), with a maximum value of 20 cells 100 mL−1 (Figure 4). Due to infrequent detection and low concentrations, no differences were observed between PMA-treated and untreated *S. enterica* and *E. coli O157:H7* at this location.

Thermophilic *Campylobacter (jejuni, lari, and coli)* were detected more frequently at location #1, on 10 of 13 sampling dates with a maximum value of 53 cells 100 mL−1 (Figure 4). *A. butzleri* was also frequently detected...
at location #1, in 12 of 13 samples and typically at low concentrations of below 20 cells 100 mL \(^{-1}\) (Figure 4). However, elevated levels of *A. butzleri* were observed in samples taken after heavy rains on June 6, October 15, October 25, and December 16. There were no statistically significant differences between total (no PMA) and viable (PMA-treated) samples for thermophilic *Campylobacter* and *A. butzleri*, which again may be due to the low concentrations at this sample location.

The concentrations of all four pathogen groups were higher at location #2 compared to location #1, and were detected in each sample collected from location #2 (Figure 5). The concentration of *S. enterica* ranged from below the detection level to \(1.7 \times 10^2\) cells 100 mL \(^{-1}\). PMA treatment did not affect the *S. enterica* concentration at most sampling dates, except for those taken in August (4, 11, 26), in which a signal reduction of up to 0.5 log was observed. The samples collected in August also had the highest *S. enterica* concentrations. At location #2, *E. coli* O157:H7 was detected at concentrations that ranged from 2 to \(1.9 \times 10^3\) cells 100 mL \(^{-1}\) (Figure 5). In the samples collected on August 4 and October 26, PMA treatment resulted in a statistically significant qPCR signal reduction of 0.2 log, showing that low levels of dead *E. coli* O157:H7 cells were present. *Campylobacter* concentrations ranged from 2 to \(7.0 \times 10^2\) cells 100 mL \(^{-1}\) (Figure 5). The sample collected on October 26 had the highest concentration of total (live and dead) *Campylobacter*, and dead *Campylobacter* cells were also detected in this sample (0.5 log signal reduction with PMA). A significant qPCR signal reduction (0.2 log) for PMA treated samples was also observed on June 6 and June 20, showing the presence of low concentrations of dead cells. *E. coli* O157:H7 and *Campylobacter* followed similar trends with the highest concentrations in late July and mid-late October.

Similar to location #1, *A. butzleri* in samples from location #2 was also detected at higher concentrations compared with the other pathogen groups (Figure 5). The concentration of *A. butzleri* ranged from \(3.4 \times 10^2\) to \(6.2 \times 10^3\) cells 100 mL \(^{-1}\) and it was detected on all 13 sampling dates. The total cell concentrations (without PMA treatment) were the highest during the summer and fall months. Similar to *S. enterica*, PMA treatment was able to suppress the small

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**Figure 4** | River water samples collected at location #1 over a period of 8 months and tested for *S. enterica*, *E. coli* O157:H7, thermophilic *Campylobacter*, and *A. butzleri* with PCR (●) and PMA-PCR (○) assays. Each data point shows the average of triplicate qPCR data, and error bars correspond to the range of values.
false positive signal caused by dead *A. butzleri* in samples taken in August (4 and 26).

Our results showed that for the bacterial pathogens tested, little or no difference was observed between PMA-qPCR and qPCR without PMA treatment. At location #1, the pathogens were either below or close to the detection limit, and therefore any differences between the two methods could not be measured. Even at location #2 where pathogens were detected more frequently and at higher concentrations, dead cells (or extracellular DNA) were present in only a small number of samples and the difference between total and viable cell concentrations were never more than 0.5 log 100 mL−1. An explanation for this can be that dead bacteria are a source of nutrients for microorganisms (Neilsen et al. 2007). In an environment with high microbial activity, such as the Grand River, lytic enzymes and nucleases are readily available leading to rapid degradation of cellular materials including DNA. Nuclease presence and activity have been reported in water and sediments (Bazelyan & Ayzatullin 1979). In addition, extracellular DNA release from dead cells could bind to absorbent surfaces (e.g., clays) of river sediments (Neilsen et al. 2007) and therefore be removed from surface water. Similar results were observed by Varma et al. (2009), who found little difference (less than 1 log) between PMA-qPCR and PCR enumeration of *Enterococci* in wastewater effluents. These results suggest that application of conventional qPCR alone may provide reliable data on viable cell numbers in river water and that more complicated/time-consuming methods may not always be necessary.

The results from this study showed the presence of relatively high levels of viable *A. butzleri* at both sampling locations in the river. Recent studies suggest that water may be one of the main exposure routes of *Arcobacter* diarrheal infection (Lehner et al. 2005). *Arcobacter* has been previously isolated from rivers and lakes (Collado et al. 2008, 2010; Lee et al. 2012), groundwater (Fera et al. 2008), and estuarine water (Fera et al. 2010). Collado et al. (2008) measured high concentrations of *Arcobacter* in stream water where it was detected at >10⁵ MPN 100 mL−1. Lee et al. (2012) also reported elevated levels of total *Arcobacter* in Lake Erie, in which water samples tested by qPCR at four
different beach locations had concentrations greater than $10^4$ cells 100 mL$^{-1}$. Since Grand River flows into Lake Erie, it is interesting that these studies showed similarly high *Arcobacter* concentrations.

The high concentrations of *A. butzleri* in the Grand River may be due to upstream influences including agricultural activities and wildlife. Animal livestock (cattle, poultry, and swine) have been recognized to be a significant reservoir of *Arcobacter* spp. (Kabeya et al. 2003; Van Driessche et al. 2003; Chinivasagam et al. 2007; González et al. 2007), and wild birds were shown to be reservoirs of *A. butzleri* (Pejchalova et al. 2006; Fernández et al. 2007). *Arcobacter* have also been found in municipal wastewater, and were detected in sewage or sludge (Snaird et al. 1997; Collado et al. 2008; Merga et al. 2014) and in treatment plant effluents (González et al. 2007; Rodriguez-Manzano et al. 2012). *Arcobacter* has not been previously studied in the Grand River and this is one of the few studies that have quantitatively assessed *Arcobacter* in the environment. However, until the health effects of *Arcobacter* have been quantified, the risk caused by this organism in water remains unknown.

**Correlation between bacterial pathogens and water quality parameters**

Spearman ranked correlation tests were done to assess the relationship between pathogens and water quality parameters. Correlation tests were only done using data from sample location #2, and were not performed using data at location #1 because of the low pathogen occurrence and concentrations at this site. Results in Table 1 show that both *Campylobacter* and *Arcobacter* were correlated with total *E. coli*, but not with turbidity or temperature. These results are in agreement with another study on *Arcobacter* where high fecal contamination was reported to correlate with the presence of *Arcobacter* (Collado et al. 2008). *E. coli* O157:H7 were not correlated with total *E. coli*, temperature, or turbidity. Total *E. coli* has been previously reported to be a poor indicator for *E. coli* O157:H7 due to differences in their survival in surface waters (Jenkins et al. 2011). There was also no correlation between *S. enterica* and total *E. coli*, and only total *S. enterica* was significantly correlated with turbidity and temperature ($P < 0.1$), indicating possibly higher concentrations in summer or following increased surface runoff.

### Table 1 | Correlation (Spearman’s) between pathogen concentration and turbidity, temperature, and total *E. coli* at sample location #2. Pathogens were measured by quantitative PCR (total cells) or PMA-PCR (viable cells). Only significant correlations are shown

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Turbidity</th>
<th>Temperature</th>
<th>Total <em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> O157:H7 (total)</td>
<td>−0.18</td>
<td>0.27</td>
<td>0.47</td>
</tr>
<tr>
<td><em>E. coli</em> O157:H7 (viable)</td>
<td>−0.11</td>
<td>0.19</td>
<td>0.46</td>
</tr>
<tr>
<td><em>S. enterica</em> (total)</td>
<td>−0.56*</td>
<td>0.57*</td>
<td>0.45</td>
</tr>
<tr>
<td><em>S. enterica</em> (viable)</td>
<td>0.50</td>
<td>0.50</td>
<td>0.24</td>
</tr>
<tr>
<td>Thermophilic <em>Campylobacter</em> (total)</td>
<td>−0.11</td>
<td>0.10</td>
<td>0.56*</td>
</tr>
<tr>
<td>Thermophilic <em>Campylobacter</em> (viable)</td>
<td>0.07</td>
<td>0.07</td>
<td>0.67**</td>
</tr>
<tr>
<td><em>A. butzleri</em> (total)</td>
<td>−0.18</td>
<td>0.20</td>
<td>0.54*</td>
</tr>
<tr>
<td><em>A. butzleri</em> (viable)</td>
<td>−0.03</td>
<td>−0.01</td>
<td>0.54*</td>
</tr>
</tbody>
</table>

*Significant correlation ($P < 0.1$; two-tailed). **Significant correlation ($P < 0.05$; two-tailed).

However, because of the short period of this study, we were not able to fully study the seasonal effects on bacterial concentrations. There was a slightly higher correlation value for viable (PMA-treated) compared with total (untreated) samples only for *Campylobacter* and total *E. coli*. However, since this study showed either no or very small differences between total and viable pathogen concentrations, it is expected that the viable cell method would not appreciably affect the correlation analysis with water quality indicators.

**CONCLUSIONS**

This study applied a PMA-PCR viability assay to show that dead cells or extracellular DNA were infrequently detected in water samples from the Grand River. This suggests that PCR without pretreatment may provide reliable results for bacterial pathogen detection in surface waters with little influence of false positive signal detection due to the presence of dead cells. Results showed that pathogen concentrations were different at two sample locations in the river, showing that various factors could affect microbial concentrations including localized and upstream activities. At one location, pathogen concentrations were typically close to or below the detection level, but concentrations at a second location were higher and allowed a better
evaluation of PMA efficacy. *A. butzleri* concentrations in the Grand River were higher than the other pathogen groups. This finding warrants further investigation on the pathogenicity of this organism and its occurrence in surface water.

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REFERENCES


