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# Temporal Heterogeneity of Water Quality in Rural Alabama Water Supplies

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Temporal and spatial trends for key water quality measures were evaluated in 12 rural drinking water systems within a three-county study area in Alabama. The water systems varied in size from very small (25–500 people served) to large (10,001–100,000 people served). Large-volume water samples were collected from 10 diverse locations within each system on three sampling dates. Sampling locations were assigned to one of five location categories: well, post-treatment, post-storage, in-line, and end-line. Water quality parameters (i.e., free and total chlorine, pH, turbidity, pressure, heterotrophic plate count) and microbial indicators (i.e., total coliforms, *Escherichia coli*, *Enterococci*, male-specific coliphages) were analyzed for spatial and temporal trends. Analysis of the samples from these rural water systems over nine months did not show a statistically significant association between distribution system sampling locations and water quality measures or microbial indicators. Temporal trends were consistent across sampling locations and were stronger than trends in spatial variability. However, substantial temporal heterogeneity of water quality measures was noted, potentially the result of seasonality, temperature fluctuations, and distribution system operation and maintenance practices. The study results indicate that system-level sampling efforts intended to inform microbial risk assessments must account for variability in indicators of risk over time.

**Keywords:** *drinking water distribution systems, drinking water quality, environmental health, infrastructure, large-volume water sampling, rural water systems, ultrafiltration*

A major objective of drinking water distribution systems is to provide safe drinking water to consumers. Maintaining a disinfectant residual throughout the distribution system to the point of use is a common strategy for improving microbiological safety by inhibiting the introduction, survival, and regrowth of pathogenic organisms. Despite routine monitoring and maintenance of chlorine residuals in compliance with the US Environmental Protection Agency's (USEPA's) regulatory limits, bacterial, protozoan, and viral pathogens have been detected in water distribution systems (Skraber et al. 2005, Sen & Rodgers 2004, Nichols et al. 2003, Falkinham et al. 2001, Park et al. 2001). Mechanisms that allow microorganisms to survive in the presence of chlorine include protection within biofilms, bacterial encapsulation, and growth conditions prior to chlorination (Donlan & Costerton 2002, LeChevallier et al. 1988).

Many challenges are associated with controlling microbial growth in drinking water distribution systems. Each system is unique, with complex arrays of pipes, storage tanks, and other infrastructure components. The survival of microorganisms depends on the interaction of variables such as the type and concentration of disinfectant used, pipe material, pipe surface, pipe network configuration, nutrient levels, temperature, the water's residence time in the system, and sporadic natural events such as seasonal fluctuations in precipitation and extreme temperature shifts (Lehtola et al. 2005, Ndiongue et al. 2005, Chu et al. 2003, Butterfield et al. 2002).

Controlling microbial growth and maintaining satisfactory water quality are critical issues for water suppliers, especially in small, rural distribution systems where monitoring programs lack the intensity and frequency of sampling required in larger systems. Such systems may be faced with the task of providing safe drinking water across large service areas at a reasonable cost to consumers. Small systems often function within the constraints of having limited personnel, an economically challenged customer base, aging facilities and infrastructure, and increased distribution costs resulting from low population density. These constraints create unique operation and maintenance challenges, therefore making rural systems more vulnerable to microbial infiltration and contamination, potentially increasing health risks to consumers (Reynolds et al. 2008). Problems associated with aging infrastructure in rural water systems have been linked to a disproportionate number of waterborne disease outbreaks (Sobsey 2006).

Systems that are most at risk for microbial contamination are often those subjected to the least monitoring. Sampling frequency required

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under the Total Coliform Rule varies greatly depending on system size, ranging from one to 480 samples per month. Less monitoring is required of smaller systems, creating the potential for a lag in detection of microbial threats (USEPA 2010). Because pathogens and fecal indicators are likely present at low concentrations, large-volume samples (e.g., 40–100 L) are often concentrated so that these microbes can be detected and quantified in drinking water supplies (Smith & Hill 2009). Dead-end ultrafiltration (DEUF) has been shown to be a useful method for simultaneous capture and recovery of bacteria, viruses, and protozoa from drinking water samples (Smith & Hill 2009, Kearns et al. 2008, Leskinen & Lim 2008).

Previous studies designed to estimate microbial risk in water supplies have used large-volume water samples to compare bacterial abundance and community structure at several locations within a single system (Henne et al. 2012, Sekar et al. 2012) or at several locations within single or multiple distribution systems two or three times a year (McCoy & VanBriesen 2012, Keinänen et al. 2004, Ultee et al. 2004). McCoy and VanBriesen (2012) reported predictable seasonal variability in bacterial communities that likely reflected changes in system-level disinfectant dosing.

Using these earlier studies as a starting point, the authors of the current study examined the spatial and temporal variability of microbial water quality at different locations within 12 rural Alabama water systems. Water samples were collected on three sampling dates over a nine-month period as part of an ongoing study intended to estimate microbial risks associated with rural water supplies in the region. A previous study examined point-of-use water quality at 910 households served by these 12 systems (Wedgworth et al. 2014). The main objective of the current research was to determine whether sampling location within the distribution system or date of sample collection was associated with drinking water quality measures and, potentially, microbial risk. To this end, free and total chlorine, pH, turbidity, pressure, and concentration of heterotrophic plate count (HPC) bacteria were measured at each location on each sampling date and, following DEUF, the presence of total coliforms, *Escherichia coli (E. coli)*, *Enterococci*, and male-specific coliphages in large-volume water samples was determined.

## METHODS

Twelve water systems from three rural counties participated in this research. Systems were selected on the basis of their location within one of the three counties previously defined as the study area (Wedgworth et al. 2014). Before participating water systems were recruited, institutional review board approval was obtained from the University of Alabama, Tuscaloosa (Approval No. IRB 10-OR-390-R2). Meetings were held with an operator from each system to identify sampling locations and determine an efficient sampling schedule. Water samples were collected from 10 locations within each system. The three sample collection dates were different for each system because of the logistical challenges of collecting and analyzing large volumes of water. The most efficient use of resources required five days per system for sample collection and analysis. Therefore, one round of samples was collected from all systems before the second round of samples was collected. All first-, second-, and third-round samples were collected during September 2013–January 2014, January 2014–March 2014, and March 2014–May 2014, respectively.

**Sample collection locations.** Sampling locations, determined using set parameters for estimating water quality, were selected to represent the potential for variability in water quality throughout each distribution system. Each water sample was assigned to one of five location categories. These categories included "well" samples taken directly from the groundwater well before chlorine was added. "Post-treatment" samples were taken from the well immediately after the addition of chlorine, and "post-storage" samples were collected at the storage tank outlet or at the first household along the main pipeline downstream of the tank. Samples taken from the termini of main water lines, where residence time is longest, were categorized as "end-line," and all remaining samples were designated as "in-line," making this category the most diverse. Each system had a slightly different combination of sampling locations because of the variability among system infrastructure components and the willingness of operators to cooperate and abide by the predetermined parameters. Sample collection locations included 26 wellhead pump stations, 19 fire hydrants, one cleanout (i.e., the designated entry point used for maintenance and sampling), six water tower outlets, and 58 outside faucets at public buildings and private homes.

**Operator questionnaire.** Before samples were collected, a questionnaire was administered to the operator of each water system to obtain specific information about the system and the sampling locations. This information included sample location (e.g., private home, public building, fire hydrant, pump station, water tower, cleanout), location description (e.g., well, post-treatment, post-storage, in-line, end-line), depth of well, pipe diameters and ages, and potential vulnerability of the sampling location (e.g., high- or low-pressure zone, low chlorine residual, high total coliform zone, high turbidity zone, area susceptible to line breaks, water loss).

**Sample collection.** At each sampling location, large-volume (100-L) water samples and small-volume (1-L) samples were collected. Large-volume samples were collected in five 20-L sterile vessels, and small-volume samples were collected in sterile 1-L vessels. Before sample collection, all vessels were dosed with sodium thiosulfate and sodium polyphosphate to deactivate chlorine and eliminate the formation of insoluble compounds, respectively. Any aerator, strainer, or hose that was attached to the water source was removed prior to sampling. All taps were heat-sterilized before being turned on to minimize microbial contamination on the faucet itself. Heat sterilization involved running a small propane blowtorch back and forth on the spigot for approximately 10 s to warm the spigot sufficiently to kill any microorganisms present. The spigot was allowed to cool for 3 min before sample collection (*Standard Methods* 2012). At each location, the tap was flushed for 4 to 5 min to allow the temperature and flow to stabilize prior to sampling. Once sampling had been initiated, a constant water flow was maintained to avoid dislodging any microbial growth within the faucets or pipes. Each vessel was aseptically filled to the appropriate volume, closed, and transported to laboratories at the University of Alabama for processing (NEWWA 2004).


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When samples were collected, water pressure was measured with two conforming (±5%) pressure gauges[1] on a T configuration (calibrated monthly). Turbidity was measured with a portable turbidimeter,[2] and free and total chlorine and pH were measured with a dual pocket colorimeter.[3]

**Ultrafiltration.** Immediately upon their arrival at the lab, the 100-L water samples were subjected to DEUF. This process used hollow-fiber ultrafilters (e.g., dialyzer cartridges for kidney dialysis) with pore sizes on the order of 10 nm to recover viruses and bacteria from water samples (Smith & Hill 2009). Water was pumped into the ultrafilter cartridge[4] with a peristaltic pump,[5] and filtered water was drained from the cartridge. After the 100 L of water passed through the ultrafilter cartridge, each cartridge was backwashed with 250 mL of a sterile solution containing 235 mL of phosphate-buffered saline, 2.5 mL of sodium polyphosphate, 12.25 mL of 10% nonionic detergent,[6] and 0.25 mL of 1% foam suppressor[7] to remove microorganisms within the filter fibers. The resulting ~350 mL of concentrated backwash fluid was collected in a sterile 500-mL flask.

**Recovery experiments.** Before sampling was initiated, experiments were performed to optimize the growth and enumeration of intact *E. coli* strain K12 cells from drinking water. *E. coli* was grown in nutrient broth with shaking (220 rpm) at 37°C. A growth curve was created to standardize spectrophotometric readings of the growth of colony-forming units at 600 nm. With this information taken into account, *E. coli* was grown in another flask of nutrient broth to the exponential phase, and approximately 10 million *E. coli* cells were added to 20 L of dechlorinated tap water. DEUF was conducted as described, the backwash was serially diluted in sterile water, and 100-µL portions of the dilutions were plated onto a selective medium.[8] After overnight incubation at 35°C, the number of colony-forming units were counted and percent recovery was calculated. Results indicated that the study's optimized protocol was capable of recovering approximately 70% of added stock of viable cells.

**Microbial parameters.** The 350-mL concentrated backwashes were analyzed for the presence of total coliforms, *E. coli*, *Enterococci*, and somatic coliphages. Total coliforms, *E. coli*, and *Enterococci* were detected using 1-mL, 5-mL, and 20-mL volumes of each backwash brought to a final volume of 100 mL with sterile water. Total coliforms and *E. coli* were detected by using a simultaneous detection system[9] in accordance with the manufacturer's instructions. The 97-well trays[10] were incubated at 35°C (±0.5°C) for 24 h. The samples were also analyzed for the presence of *Enterococci* by using a similar detection system[11] in accordance with the manufacturer's instructions. With this method, the 97-well trays[10] were incubated at 41°C (±0.5°C) for 24 h. After incubation, the wells that tested positive for *Enterococci* were counted for the three backwash volumes, and a most probable number (MPN) for bacterial presence was obtained using the included MPN table for total coliforms, *E. coli*, and *Enterococci*. An average MPN was calculated using the three backwash volumes.

Male-specific coliphages were detected by means of USEPA Method 1602, using the procedure involving a single agar layer. From the 350-mL backwashes, 6-mL and duplicate 1-mL, 0.1-mL, and 0.01-mL volumes were added to sterile 15-mL centrifuge tubes. If needed, tubes were brought to a total volume of 6 mL with sterile phosphate-buffered saline solution.

To each centrifuge tube, 400 µL of *E. coli* F-amp (a coliphage host strain of *E. coli* resistant to the antibiotics streptomycin and ampicillin) and 6 mL of 1.2% soy agar[12] dosed with streptomycin and ampicillin (15 µg/mL of agar) were added. Samples were thoroughly mixed and poured into sterile 100- × 15-mm Petri dishes. After the samples had solidified at room temperature, the Petri dishes were incubated for 18 to 24 h at 37°C. Circular zones of lysis (i.e., plaques) were counted on each plate, and the values for all plates from a single sample were summed. The quantity of coliphages in a sample was expressed as plaque-forming units per 8.22 mL of backwash (the sum of all volumes plated per sample).

To estimate the number of culturable heterotrophic bacteria in the water samples, the HPC membrane filtration method (Standard Method 9215D) was used. For this assay, the 1-L sample collected at each location was used. For each sample, triplicate 1-mL, 10-mL, and 100-mL volumes were individually filtered along with sterile water as a negative control. Each 0.45-mm pore size, 47-mm filter was transferred with sterile forceps to a 60- × 15-mm Petri dish containing growth medium specific to heterotrophic microorganisms[13] and incubated at 35°C for 48 h before the bacterial colonies were counted. Only plates with 20 to 200 colonies were used to determine the average colony-forming units per milliliter of water.

For all microbial measurements that used concentrated backwash, the presence of the indicator organism in 100 mL of the original 100-L water sample was calculated and reported.

**Statistical data analysis.** All data were entered into a spreadsheet[14] and transferred to a data analysis and statistical software program[15] for analysis. Distribution of the data was visually inspected using histograms and normal probability plots. The Shapiro–Wilk test was used to assess normality on all continuous water quality measures. Values for turbidity and HPC were log-transformed to achieve a more normal distribution and to make patterns in these highly skewed, abnormally distributed data more clear. To compare the continuous variables of measured water quality (free chlorine, total chlorine, pH, turbidity, pressure, and HPC) across sampling location groups and collection times, Kruskal–Wallis tests were used, and box plots were generated so that trends could be seen. Four microbial indicators (total coliforms, *E. coli*, *Enterococci*, and coliphages) were transformed into binary outcomes (presence or absence) on the basis of suggested regulatory guidelines. Pearson's chi-squared tests were used to compare the binary values of measured microbial indicators across sampling location groups and time points. Bar graphs were generated so that trends could be seen.

## RESULTS

The 12 water systems that participated in this study are located in three rural Alabama counties (listed in Table 1 as A, B, and C to preserve anonymity), and they vary in size as classified by USEPA from very small (serving 25–500 customers) to large (serving 10,001–100,000 customers). Nine of the systems are public, and three are privately owned (Table 1). Eleven of the systems use groundwater as their source water, and one uses surface water.


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**TABLE 1** Description of participating drinking water systems

| System[a] | County[b] | Population Served | System Size[c] | Source Water | Ownership |
|---|---|---|---|---|---|
| 1 | A | 729 | Small | Purchased groundwater | Public |
| 2 | A | 4,500 | Medium | Groundwater | Public |
| 3 | A | 4,200 | Medium | Groundwater | Private |
| 4 | B | 4,119 | Medium | Groundwater | Public |
| 5 | B | 1,740 | Small | Groundwater | Public |
| 6 | B | 1,440 | Small | Groundwater | Public |
| 7 | B | 369 | Very small | Groundwater Surface | Private |
| 8 | B | 2,418 | Small | water Groundwater | Private |
| 9 | B | 6,594 | Medium | Groundwater | Public |
| 10 | C | 4,440 | Medium | Groundwater | Public |
| 11 | C | 10,200 | Large | Groundwater | Public |
| 12 | C | 3,708 | Medium | | Public |

[a]Water systems are coded with numbers 1–12 for anonymity.
[b]The water systems were located in three counties coded A, B, and C for anonymity.
[c]Very small = serves 25–500 people; small = serves 501–3,300 people; medium = serves 3,301–10,000 people; large = serves 10,001–100,000 people

**TABLE 2** Associations between continuous water quality measures and sampling location categories

| | Well | Post-treatment | Post-storage | In-line | End-line |
|---|---|---|---|---|---|
| Free chlorine—*mg/L* | | | | | |
| N | 57 | 48 | 18 | 174 | 60 |
| Median | ND | 1.5 | 1.5 | 1.5 | 1.7 |
| *p* value | | | | | 0.874 |
| Total chlorine—*mg/L* | | | | | |
| N | 57 | 48 | 18 | 174 | 60 |
| Median | ND | 1.9 | 1.7 | 1.9 | 2.1 |
| *p* value | | | | | 0.869 |
| pH | | | | | |
| N | 55 | 48 | 18 | 172 | 60 |
| Median | 7.0 | 6.9 | 5.9 | 7.1 | 6.4 |
| *p* value | | | | | 0.004[a] |
| Turbidity—*ntu* | | | | | |
| N | 55 | 48 | 18 | 173 | 60 |
| Median | 0.52 | 0.69 | 0.50 | 0.65 | 0.82 |
| *p* value | | | | | 0.351 |
| Pressure—*psi* | | | | | |
| N | 54 | 48 | 18 | 165 | 53 |
| Median | 70 | 63 | 63 | 60 | 75 |
| *p* value | | | | | 0.113 |
| HPC—*cfu/100 mL* | | | | | |
| N | 57 | 48 | 18 | 174 | 60 |
| Median | 2.0 | 0.41 | 5.7 | 2.7 | 5.7 |
| *p* value | | | | | 0.002[a] |

HPC—heterotrophic plate count, N—number of samples, ND—not detected because the value was below the detection limit of the analytical instrument (0.1 mg/L)

[a]Statistically significant association ($p \leq 0.05$)

Because only one system uses surface water, preventing comparisons for this type of source water, the pretreatment data point for that system was excluded from all analyses. All participating systems use free chlorine to maintain a disinfectant residual.

To test the hypothesis that location within the system affects water quality, the authors analyzed six continuous variables (median free chlorine, total chlorine, pH, pressure, turbidity, and HPC) and four binary variables (presence or absence of total coliforms, *E. coli*, *Enterococci*, and coliphages) for potential associations across the five sampling location categories. Initially, data from the three collection dates were combined to permit evaluation of associations among sampling location categories. Significant associations were found between sample collection locations and pH and HPC (Table 2). Because no statistically significant associations were found between any of the binary water quality variables and sampling location categories, and few trends were apparent across sampling location categories (Tables 2 and 3, Figure 1 on page E412 and Figure 2 on page E413), the data were reanalyzed by collection date and location category.

Significant variability across the three collection dates was detected for all continuous variables in at least one sampling location category. As shown in Tables 4 and 5, significant associations were observed for free chlorine (in-line and end-line samples), total chlorine (post-treatment, in-line, and end-line samples), pH (well, post-treatment, in-line, and end-line samples), turbidity (end-line samples), pressure (well, in-line, and end-line samples) and HPC (post-treatment, post-storage, and in-line samples). No statistically significant associations were found between sample collection date and location and any of the binary water quality measures (Table 6). However, the authors detected significant temporal variability in the presence of total coliforms in well, in-line, and end-line samples (Table 7).

When individual collection times were examined, more trends were apparent. Post-treatment samples showed improved water quality across all sampling dates compared with well water


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**TABLE 3** Associations between binary water quality measures and sampling location categories

| | Well | Post-treatment | Post-storage | In-line | End-line |
|---|---|---|---|---|---|
| Total coliforms | | | | | |
| N | 57 | 48 | 18 | 174 | 60 |
| N (% positive) | 28 (49.1) | 22 (45.8) | 9 (50.0) | 90 (51.7) | 31 (51.7) |
| *p* value | | | | | 0.963 |
| *Escherichia coli* | | | | | |
| N | 57 | 48 | 18 | 174 | 60 |
| N (% positive) | 3 (5.26) | 1 (2.08) | 1 (5.56) | 5 (2.87) | 4 (6.67) |
| *p* value | | | | | 0.638 |
| *Enterococci* | | | | | |
| N | 57 | 48 | 18 | 174 | 60 |
| N (% positive) | 27 (47.4) | 19 (39.6) | 11 (61.1) | 87 (50.0) | 22 (36.7) |
| *p* value | | | | | 0.225 |
| Coliphages | | | | | |
| N | 57 | 48 | 18 | 174 | 60 |
| N (% positive) | 7 (12.3) | 1 (2.08) | 2 (11.1) | 16 (9.20) | 9 (15.0) |
| *p* value | | | | | 0.234 |

N—number of samples

samples for all continuous variables except turbidity. However, on the third collection date, all measures except HPC demonstrated a noticeable degradation in water quality compared with the two previous time points, which were similar (Tables 4 and 5; see Figure 3 on page E414). As expected, free and total chlorine were not detected at the well sampling locations (Table 4, Figure 3). The median value for free chlorine was highest at time point 2 across all locations except post-treatment (it was highest—2.4 mg/L—at time point 1). Free chlorine concentrations decreased from post-treatment to end-line samples for time points 1 and 3. However, at time point 2, the free chlorine concentration increased to 3.4 mg/L from 2.2 mg/L from post-treatment to end-line samples (Table 4, Figure 3). Median pH values were also higher at time point 2 at all sampling locations (Figure 3). At time points 2 and 3, median turbidity increased from well water samples to post-treatment samples but decreased at time point 1 (Table 5, Figure 3). An increase in turbidity was seen from post-treatment to end-line samples at time points 1 and 2 (Figure 3). Across all time points, median pressure was lowest in in-line samples. Pressure was highest at time point 1 at all sampling locations and lowest at time point 3 in all but the post-storage samples (Table 5, Figure 3). Growth of heterotrophic bacteria, as determined by HPC, was highest at time point 2 at all sampling locations (Figure 3).

**TABLE 4** Associations between continuous water quality measures and the time point of sample collection within each sampling location category

| Sampling Location Category | Free Chlorine *mg/L* | | | | Total Chlorine *mg/L* | | | | pH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Median | 25th | 75th | N | Median | 25th | 75th | N | Median | 25th | 75th |
| Well | | | | | | | | | | | | |
| Time point 1[a] | 19 | ND | ND | ND | 19 | ND | ND | ND | 19 | 7.2 | 6.6 | 7.8 |
| Time point 2[b] | 19 | ND | ND | ND | 19 | ND | ND | ND | 18 | 7.5 | 6.7 | 7.9 |
| Time point 3[c] | 19 | ND | ND | ND | 19 | ND | ND | ND | 19 | 5.9 | 5.9 | 6.2 |
| *p* value | | | | | | | | | 0.002[d] | | | |
| Post-treatment | | | | | | | | | | | | |
| Time point 1[a] | 16 | 2.4 | 0.70 | 3.7 | 16 | 3.5 | 1.6 | 4.8 | 16 | 7.1 | 6.6 | 7.5 |
| Time point 2[b] | 16 | 2.2 | 0.70 | 5.6 | 16 | 2.6 | 1.1 | 6.3 | 16 | 7.6 | 6.3 | 7.9 |
| Time point 3[c] | 16 | 1.2 | 0.90 | 1.6 | 16 | 1.3 | 1.1 | 1.8 | 16 | 5.9 | 5.9 | 6.2 |
| *p* value | 0.199 | | | | 0.015[d] | | | | 0.003[d] | | | |
| Post-storage | | | | | | | | | | | | |
| Time point 1[a] | 6 | 2.0 | 0.50 | 2.2 | 6 | 2.1 | 0.60 | 2.4 | 6 | 5.9 | 5.9 | 7.3 |
| Time point 2[b] | 6 | 3.0 | 1.5 | 5.6 | 6 | 3.3 | 1.7 | 6.2 | 6 | 6.4 | 5.9 | 7.4 |
| Time point 3[c] | 6 | 1.0 | 0.70 | 1.4 | 6 | 1.3 | 0.90 | 1.5 | 6 | 5.9 | 5.9 | 5.9 |
| *p* value | 0.062 | | | | 0.084 | | | | 0.781 | | | |
| In-line | | | | | | | | | | | | |
| Time point 1[a] | 58 | 2.5 | 1.5 | 3.4 | 58 | 3.1 | 1.9 | 4.2 | 58 | 7.2 | 7.0 | 7.7 |
| Time point 2[b] | 58 | 2.6 | 1.0 | 4.3 | 58 | 2.6 | 1.2 | 4.6 | 57 | 7.4 | 6.9 | 7.9 |
| Time point 3[c] | 58 | 1.2 | 0.80 | 1.4 | 58 | 1.3 | 1.1 | 1.7 | 58 | 6.1 | 5.9 | 7.1 |
| *p* value | <0.001[d] | | | | <0.001[d] | | | | <0.001[d] | | | |
| End-line | | | | | | | | | | | | |
| Time point 1[a] | 20 | 2.4 | 0.75 | 3.4 | 20 | 3.2 | 1.4 | 3.7 | 20 | 7.0 | 5.9 | 7.4 |
| Time point 2[b] | 20 | 3.4 | 1.5 | 5.1 | 20 | 4.1 | 2.0 | 5.5 | 20 | 7.3 | 7.0 | 7.7 |
| Time point 3[c] | 20 | 1.0 | 0.75 | 1.6 | 20 | 1.3 | 0.90 | 1.8 | 20 | 5.9 | 5.9 | 6.1 |
| *p* value | <0.001[d] | | | | <0.001[d] | | | | <0.001[d] | | | |

25th—25th percentile, 75th—75th percentile, N—number of samples, ND—not detected because the value was below the detection limit of the analytical instrument (0.1 mg/L)

[a]Time point 1, September 2013–January 2014, fall/winter
[b]Time point 2, January 2014–March 2014, winter
[c]Time point 3, March 2014–May 2014, spring
[d]Statistically significant associations ($p \leq 0.05$)


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Some trends were also found with the microbial water quality measures—total coliforms, *E. coli*, *Enterococci*, and coliphages (Tables 6 and 7; see Figure 4 on page E415). A noticeable but not significant degradation in water quality was observed for all microbial measures except *Enterococci* between post-treatment and end-line samples (Table 6). The presence of total coliforms was highest at time point 3 across all sample collection categories except post-treatment and lowest at time point 1 at all locations (Table 7, Figure 4). Almost 50% of all samples that tested positive for total coliforms were detected at time point 3. The percentage of samples that were positive for total coliforms decreased from well to post-treatment samples at time points 1 and 3 but increased at time point 2; however, this trend was not significant (Tables 6 and 7, Figure 4). Across all collection locations, only 14 samples were positive for *E. coli*, with 50% recorded at time point 3 (Tables 6 and 7, Figure 4). The presence of *Enterococci* decreased from well to post-treatment samples at all time points but was highest in post-storage samples (Tables 6 and 7, Figure 4); ~61% of all post-storage samples tested positive for *Enterococci*. The presence of coliphages was lowest at time point 3 across all sample collection locations and highest at time point 1 in all locations except in-line sampling sites. Coliphages appeared most prevalent in end-line samples (Tables 6 and 7, Figure 4).

## DISCUSSION

There is a growing recognition of the role of drinking water distribution systems in infectious disease outbreaks in the United States. An increasing number of disease outbreaks in community water systems has been attributed to distribution system deficiencies (Nygård et al. 2007, Liang et al. 2006, Blackburn et al. 2004, Lee et al. 2002), with the majority resulting from system-level operational events (e.g., main breaks, contaminant intrusions).

**TABLE 5** Associations between continuous water quality measures and the time point of sample collection within each sampling location category

| Sampling Location Category | Turbidity *ntu* | | | | Pressure *psi* | | | | HPC *cfu/100 mL* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | Median | 25th | 75th | N | Median | 25th | 75th | N | Median | 25th | 75th |
| Well | | | | | | | | | | | | |
| Time point 1[a] | 18 | 0.56 | 0.38 | 1.1 | 18 | 101 | 70 | 115 | 19 | 1.2 | 0.61 | 5.2 |
| Time point 2[b] | 18 | 0.46 | 0.33 | 0.86 | 18 | 73 | 45 | 100 | 19 | 12 | 0.33 | 48 |
| Time point 3[c] | 19 | 0.52 | 0.45 | 1.1 | 18 | 58 | 40 | 70 | 19 | 3.3 | 1.5 | 39 |
| *p* value | 0.558 | | | | 0.004[d] | | | | 0.170 | | | |
| Post-treatment | | | | | | | | | | | | |
| Time point 1[a] | 16 | 0.47 | 0.30 | 0.92 | 16 | 73 | 44 | 100 | 16 | 0.10 | 0.10 | 0.93 |
| Time point 2[b] | 16 | 0.92 | 0.53 | 1.5 | 16 | 60 | 53 | 90 | 16 | 1.3 | 0.35 | 36 |
| Time point 3[c] | 16 | 0.69 | 0.44 | 0.87 | 16 | 65 | 60 | 75 | 16 | 0.38 | 0.10 | 1.9 |
| *p* value | 0.080 | | | | 0.874 | | | | 0.047[d] | | | |
| Post-storage | | | | | | | | | | | | |
| Time point 1[a] | 6 | 0.46 | 0.30 | 0.60 | 6 | 75 | 60 | 100 | 6 | 0.50 | 0.10 | 1.4 |
| Time point 2[b] | 6 | 0.41 | 0.34 | 1.1 | 6 | 70 | 60 | 80 | 6 | 47 | 4.5 | 106 |
| Time point 3[c] | 6 | 1.2 | 0.47 | 1.3 | 6 | 53 | 45 | 55 | 6 | 12 | 2.3 | 47 |
| *p* value | 0.340 | | | | 0.114 | | | | 0.042[d] | | | |
| In-line | | | | | | | | | | | | |
| Time point 1[a] | 58 | 0.70 | 0.37 | 1.5 | 58 | 65 | 58 | 85 | 58 | 0.51 | 0.10 | 3.8 |
| Time point 2[b] | 57 | 0.51 | 0.32 | 0.91 | 55 | 60 | 45 | 80 | 58 | 10 | 1.1 | 96 |
| Time point 3[c] | 58 | 0.70 | 0.47 | 1.2 | 52 | 50 | 45 | 65 | 58 | 4.2 | 0.66 | 41 |
| *p* value | 0.056 | | | | <0.001[d] | | | | <0.001[d] | | | |
| End-line | | | | | | | | | | | | |
| Time point 1[a] | 20 | 1.1 | 0.47 | 2.9 | 19 | 80 | 65 | 100 | 20 | 3.9 | 0.49 | 260 |
| Time point 2[b] | 20 | 0.97 | 0.48 | 2.1 | 18 | 75 | 58 | 80 | 20 | 12 | 0.74 | 400.0 |
| Time point 3[c] | 20 | 0.53 | 0.40 | 0.84 | 16 | 58 | 50 | 75 | 20 | 3.2 | 0.71 | 41 |
| *p* value | 0.039[d] | | | | 0.033[d] | | | | 0.481 | | | |

25th—25th percentile, 75th—75th percentile, HPC—heterotrophic plate count, N—number of samples, ND—not detected because the value was below the detection limit of the analytical instrument (0.1 mg/L)

[a]Time point 1, September 2013–January 2014, fall/winter
[b]Time point 2, January 2014–March 2014, winter
[c]Time point 3, March 2014–May 2014, spring
[d]Statistically significant associations ($p \leq 0.05$)


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**TABLE 6** Associations between binary water quality measures and the time point of sample collection within each sampling location category

| Sampling Location Category | N | Total Coliforms | | *Escherichia coli* | | *Enterococci* | | Coliphages | |
|---|---|---|---|---|---|---|---|---|---|
| | | N (% Positive) | *p* value | N (% Positive) | *p* value | N (% Positive) | *p* value | N (% Positive) | *p* value |
| Well | | | 0.049[d] | | 0.348 | | 0.810 | | 0.850 |
| Time point 1[a] | 19 | 5 (26.3) | | 1 (5.26) | | 8 (42.1) | | 3 (15.8) | |
| Time point 2[b] | 19 | 11 (57.9) | | 0 (0) | | 9 (47.4) | | 2 (10.5) | |
| Time point 3[c] | 19 | 12 (63.2) | | 2 (10.5) | | 10 (52.6) | | 2 (10.5) | |
| Post-treatment | | | 0.095 | | 0.360 | | 0.543 | | 0.360 |
| Time point 1[a] | 16 | 4 (25.0) | | 1 (6.3) | | 6 (37.5) | | 1 (6.3) | |
| Time point 2[b] | 16 | 10 (62.5) | | 0 (0) | | 5 (31.25) | | 0 (0) | |
| Time point 3[c] | 16 | 8 (50.0) | | 0 (0) | | 8 (50.0) | | 0 (0) | |
| Post-storage | | | 0.513 | | 0.347 | | 0.792 | | 0.570 |
| Time point 1[a] | 6 | 3 (50.0) | | 0 (0) | | 4 (66.7) | | 1 (16.7) | |
| Time point 2[b] | 6 | 2 (33.3) | | 1 (16.7) | | 4 (66.7) | | 1 (16.7) | |
| Time point 3[c] | 6 | 4 (66.7) | | 0 (0) | | 3 (50.0) | | 0 (0) | |
| In-line | | | <0.001[d] | | 0.237 | | 0.052 | | 0.618 |
| Time point 1[a] | 58 | 20 (34.5) | | 0 (0) | | 36 (62.1) | | 5 (8.6) | |
| Time point 2[b] | 58 | 27 (46.6) | | 3 (5.17) | | 23 (39.7) | | 7 (12.1) | |
| Time point 3[c] | 58 | 43 (74.1) | | 2 (3.5) | | 28 (48.3) | | 4 (6.9) | |
| End-line | | | 0.031[d] | | 0.153 | | 0.931 | | 0.676 |
| Time point 1[a] | 20 | 7 (35.0) | | 0 (0) | | 8 (40.0) | | 3 (15.0) | |
| Time point 2[b] | 20 | 9 (45.0) | | 1 (5.0) | | 7 (35.0) | | 4 (20.0) | |
| Time point 3[c] | 20 | 15 (75.0) | | 3 (15.0) | | 7 (35.0) | | 2 (10.0) | |

N—number of samples

[a]Time point 1, September 2013–January 2014, fall/winter
[b]Time point 2, January 2014–March 2014, winter
[c]Time point 3, March 2014–May 2014, spring
[d]Statistically significant associations ($p \leq 0.05$)

**TABLE 7** Associations between binary water quality measures and location within each time point

| | N | Total Coliforms | | *Escherichia coli* | | *Enterococci* | | Coliphages | |
|---|---|---|---|---|---|---|---|---|---|
| | | N (% positive) | *p* value | N (% positive) | *p* value | N (% positive) | *p* value | N (% positive) | *p* value |
| Time point 1[a] | 119 | | 0.789 | | 0.161 | | 0.190 | | 0.648 |
| Well | 19 | 5 (26.3) | | 1 (5.26) | | 8 (42.1) | | 3 (15.8) | |
| Post-treatment | 16 | 4 (25.0) | | 1 (6.3) | | 6 (37.5) | | 1 (6.3) | |
| Post-storage | 6 | 3 (50.0) | | 0 (0) | | 4 (66.7) | | 1 (16.7) | |
| In-line | 58 | 20 (34.5) | | 0 (0) | | 36 (62.1) | | 5 (8.6) | |
| End-line | 20 | 7 (35.0) | | 0 (0) | | 8 (40.0) | | 3 (15.0) | |
| Time point 2[b] | 119 | | 0.642 | | 0.383 | | 0.586 | | 0.403 |
| Well | 19 | 11 (57.9) | | 0 (0) | | 9 (47.4) | | 2 (10.5) | |
| Post-treatment | 16 | 10 (62.5) | | 0 (0) | | 5 (31.25) | | 0 (0) | |
| Post-storage | 6 | 2 (33.3) | | 1 (16.7) | | 4 (66.7) | | 1 (16.7) | |
| In-line | 58 | 27 (46.6) | | 3 (5.17) | | 23 (39.7) | | 7 (12.1) | |
| End-line | 20 | 9 (45.0) | | 1 (5.0) | | 7 (35.0) | | 4 (20.0) | |
| Time point 3[c] | 119 | | 0.389 | | 0.201 | | 0.819 | | 0.760 |
| Well | 19 | 12 (63.2) | | 2 (10.5) | | 10 (52.6) | | 2 (10.5) | |
| Post-treatment | 16 | 8 (50.0) | | 0 (0) | | 8 (50.0) | | 0 (0) | |
| Post-storage | 6 | 4 (66.7) | | 0 (0) | | 3 (50.0) | | 0 (0) | |
| In-line | 58 | 43 (74.1) | | 2 (3.5) | | 28 (48.3) | | 4 (6.9) | |
| End-line | 20 | 15 (75.0) | | 3 (15.0) | | 7 (35.0) | | 2 (10.0) | |

N—number of samples

[a]September 2013–January 2014, fall/winter
[b]January 2014–March 2014, winter
[c]March 2014–May 2014, spring


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Microbial contamination of drinking water can put consumers at risk and potentially lead to a waterborne disease outbreak, even when concentrations of pathogenic organisms are very low (Haas et al. 1993, Rose et al. 1991, Haas 1983). After an outbreak, the goal of the investigation is detection and identification of the pathogens involved. Simultaneous concentration, detection, and quantification not only are cost-effective but also beneficial when multiple etiologic agents are suspected or when contamination has occurred but no clinical data exist to identify the pathogen (O'Reilly et al. 2007).

Although several techniques—including adsorption–elution, precipitation, and centrifugation—exist for the simultaneous concentration and detection of multiple microbes in drinking water samples (Smith & Hill 2009, Lindquist et al. 2007, Polaczyk et al. 2007, Hill et al. 2005, Payment et al. 1989), hollow-fiber UF has been shown to be among the most dependable because of its reliance on size-exclusion filtration of diverse microbes (Polaczyk et al. 2008; Hill et al. 2007, 2005; Lindquist et al. 2007; Morales-Morales et al. 2003). Tangential-flow (i.e., recirculating flow) UF is the most frequently used approach, but it must be performed quickly and requires complicated methodology, making it hard to implement in the field. DEUF, a useful alternative method for capturing and recovering diverse microbes in the field (Smith & Hill 2009, Kearns et al. 2008, Leskinen & Lim 2008), was the option selected for the current study. The DEUF technique does not allow for recirculating flow; instead, microorganisms are trapped within the ultrafilter cartridge until their removal with a backwash procedure (Hill et al. 2005). The optimized protocol used in the current study was capable of recovering approximately 70% of the added stock of viable *E. coli* cells. This level of recovery efficiency falls within previously published recovery ranges for this method (Smith & Hill 2009, Hill et al. 2005).

The results presented here show that low levels of microbial contamination were present in the 12 water systems examined, compromising the quality of the water delivered to consumers. Although it was hypothesized that sample collection location within the system (i.e., spatial variability) would be a robust indicator of the water quality measures examined, all water quality measures showed greater temporal variability than spatial variability. Consistent with these findings, two recent studies concluded that bacterial abundance and community structure in bulk water were highly similar across all sampling locations (Henne et al. 2012, Sekar et al. 2012). Henne et al. (2012) analyzed the composition of the core bacterial community in bulk water and found it was highly similar (>70%) across the entire system, consisting primarily of *Bacteroidetes* (25%), *Betaproteobacteria* (20%), *Actinobacteria* (16%), and *Alphaproteobacteria* (11%). Seker et al. (2012) assessed spatial and temporal variability in the abundance and composition of planktonic bacterial assemblages sampled from a small, looped water distribution system.

On the other hand, variability in bulk water bacterial community composition across sampling locations has also been reported (Pinto et al. 2012). Specifically, samples taken from locations within the water distribution system demonstrated more stability in the bacterial community structure than source water samples and prefiltration samples (Pinto et al. 2012). Additionally, heterogeneity in finished water samples has been extensively documented (Gale et al. 1997, Maul et al. 1990, El-Shaarawi et al. 1981). Thus, the microbiome within a water distribution system is complex and influenced by treatment practices (Pinto et al. 2014, 2012). For example, Pinto et al. (2012) demonstrated that bacteria that adhere to the filter during treatment and slough off into the effluent were unaffected by chloramination, making filter colonization a possible mechanism for bacterial survival in drinking water systems. How measures associated with microbial risk might vary in water supplies, and the extent to which variability across time and space may affect the representativeness of water quality data, are critical unknowns. Ultimately, research addressing these issues could help to safeguard potable water quality, which is fundamental to public health.

The authors of the current study hypothesized that samples collected at end-line locations would be most likely to be vulnerable to contamination because they represent the longest hydraulic residence time (i.e., the time required for the water to travel from treatment location to consumers) and would be associated with measures assumed to inform microbial risk assessments. The longer water is in the distribution system, the greater the opportunity for it to become contaminated, particularly in supplies with operation and maintenance challenges and aging infrastructure. Tinker et al. (2009) reported an increased risk of gastrointestinal illness in consumers whose water had longer residence times compared with those who consumed water with intermediate residence times. The study examined whether average water residence time in the studied zip code was related to the proportion of emergency room visits for gastrointestinal illness among residents.

Similarly, a study of distribution system water quality demonstrated that as distance from the treatment facility increased (and presumably as residence time increased), the level of bacterial contamination rose (Payment et al. 1988). Measuring the presence of total, stressed, and fecal coliforms, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Clostridium perfringens*, and *Enterococci*, Payment et al. (1988) found bacteria at all sampling sites in two distribution systems, with higher prevalence at sites distant from the treatment facility. Thus, the authors of the study reported here expected to see decreasing concentrations of free and total chlorine and increasing microbial contamination at collection locations farther from the treatment facility and toward the termini of distribution lines. In end-line samples, increased HPCs and coliphages were found at all time points. Compared with samples collected immediately post-treatment, end-line samples contained increased concentrations of total coliforms, *E. coli*, and *Enterococci* at two time points. However, none of these increases was statistically significant. Overall, the results indicated that end-line samples were no more likely to be contaminated than samples collected at any other location within the system.

Under the conditions of the current study, sample collection location was not statistically significantly associated with


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differences in water quality. This lack of association may be a result of the specific time period, settings, and conditions of the study; even so, the information could potentially provide insight into appropriate sampling designs for other studies aimed at documenting water quality in similar rural water systems.

However, seasonality indicated some interesting associations. All samples from the first round of sample collection were obtained during fall and early winter, whereas samples for the second and third collection rounds were obtained during winter and spring, respectively. Chlorine concentration and temperature have been shown to affect bacterial abundance and diversity (Poitelon 2009, Eichler et al. 2006). Because the disinfectant decay rate decreases during the cooler months (e.g., in samples from the first and second collection rounds in the current study), a lower chlorine dose is needed to maintain a consistent residual in the distribution system (Roccaro et al. 2008, Boccelli et al. 1998). Decreased bacterial abundance has been reported in winter months compared with summer months, correlating with seasonal changes in the residual chlorine concentration (McCoy & VanBriesen 2012). Other studies also demonstrated decreased bacterial loads in distribution systems during colder months (Torvinen et al. 2007, LeChevallier et al. 1991). In the current study, the median value for free chlorine was highest at time point 2, the coldest time point across all locations except post-treatment. We also observed a decrease in free and total chlorine concentrations and a statistically significant increase in total coliform concentrations at the warmest time point. This result suggests that as the temperature increased, the disinfection decay rate also increased and water quality diminished at all locations within the distribution systems.

For bacteria in aquatic environments, increased temperatures result in faster chemical and enzymatic reactions, yielding greater metabolic activity and growth. Because water temperature was not a variable measured in the current study, the authors can only hypothesize that the ambient temperature likely influenced the groundwater temperature and the temperature of the water in the distribution system.

Lengthy residence times in storage tanks can result in microbial growth, especially in water at a high temperature or with high concentrations of organic matter (Kirmeyer et al. 1999, Kerneïs et al. 1995). The disinfectant residual can be lost in stored water, enhancing the potential for microbial growth in the storage tank and downstream distribution pipes. The hydraulic configuration (e.g., storage tank design, pumping cycles) of a large city distribution system resulted in long residence times in one tank (5.6–7.9 days), which dissipated the chlorine and led to lower chlorine residuals (Gauthier et al. 2000). The results of the current study showed an increased presence of *Enterococci* and decreased free and total chlorine concentrations at post-storage locations, suggesting that increased residence times in storage tanks may have caused a decrease in chlorine residuals, enhancing microbial growth. Similar conditions have been commonly documented in urban settings, but little information is available on residence times in rural systems.

The current study area had an uncharacteristically cold winter, which may have influenced microbial growth and activity. Two winter storms brought multiple days of below-freezing temperatures and extreme winter weather to Alabama counties. During these storms, the water distribution systems studied suffered line breaks caused by frozen pipes. The negative pressure transient caused by main breaks created opportunities for the introduction of nonpotable water into the distribution systems (Funk et al. 1999), potentially resulting in contamination (Karim et al. 2003). As the ground thawed and temperatures increased, microorganisms in the soils surrounding the distribution pipes may have experienced increased mobility and been introduced into the systems via intrusion. The collection of additional samples during the summer and fall months would have allowed further testing of the assumptions that water quality was degraded because extreme cold weather increased the vulnerability of the system's infrastructure and warmer temperatures increased the mobility of pathogens.

## CONCLUSIONS

Although sampling location is likely important for assessing drinking water quality, location was not the most influential variable in the 12 rural distribution systems studied. The variability in water quality that was detected in these distribution systems reflected seasonal changes and was likely due to the varying presence and activity of microorganisms. The temporal trends identified in this study were consistent across different sample collection locations and were stronger than the trends identified for spatial variability. The results of this study demonstrate the variability of microbial indicator organisms only, whereas pathogens are the source of risk to consumers. Further analysis is required to determine whether the indicators used can predict the presence of pathogens. Nevertheless, this study highlights the need for increased longitudinal sampling for a better understanding of spatial and temporal trends in water quality in systems that serve low-density populations and have limited operational resources. Indicators of microbial quality vary over time, suggesting that longitudinal sampling may be critical for estimating microbial risks to consumers.

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## ENDNOTES

[1]Rain Bird P2A Pressure Gauge, Rain Bird Corporation, Azusa, Calif.
[2]2100Q Portable Turbidimeter, Hach Company, Loveland, Colo.
[3]Dual Pocket Colorimeter II plus pH, Hach Company, Loveland, Colo.
[4]Rexeed 25S Hemodialyzer, Asahi Kasei Medical Co., Chiyoda-ku, Tokyo, Japan
[5]Geopump™ Peristaltic Pump, Geotech Environmental Equipment, Denver, Colo.
[6]Tween 80, AMRESCO, Solon, Ohio
[7]Antifoam A, Sigma-Aldrich, St. Louis, Mo.
[8]Levine Eosine Methylene Blue Agar, Becton, Dickinson and Company, Franklin Lakes, N.J.
[9]Colilert® Quanti-Tray®/2000, IDEXX Laboratories, Westbrook, Maine
[10]Quanti-Tray®/2000, IDEXX Laboratories, Westbrook, Maine
[11]Enterolert® Quanti-Tray® /2000, IDEXX Laboratories, Westbrook, Maine
[12]1.2% Molten Tryptic Soy Agar, Becton, Dickinson and Co., Franklin Lakes, N.J.
[13]m-HPC Agar, Becton, Dickinson and Co., Franklin Lakes, N.J.
[14]Excel 2010(V14.0), Microsoft Corp., Redmond, Wash.
[15]Stata 13.1, StataCorp, College Station, Texas

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**FIGURE 1** Bar graphs illustrating the percentage of each binary water quality variable that tested positive in a 100-L sample across all sampling location categories

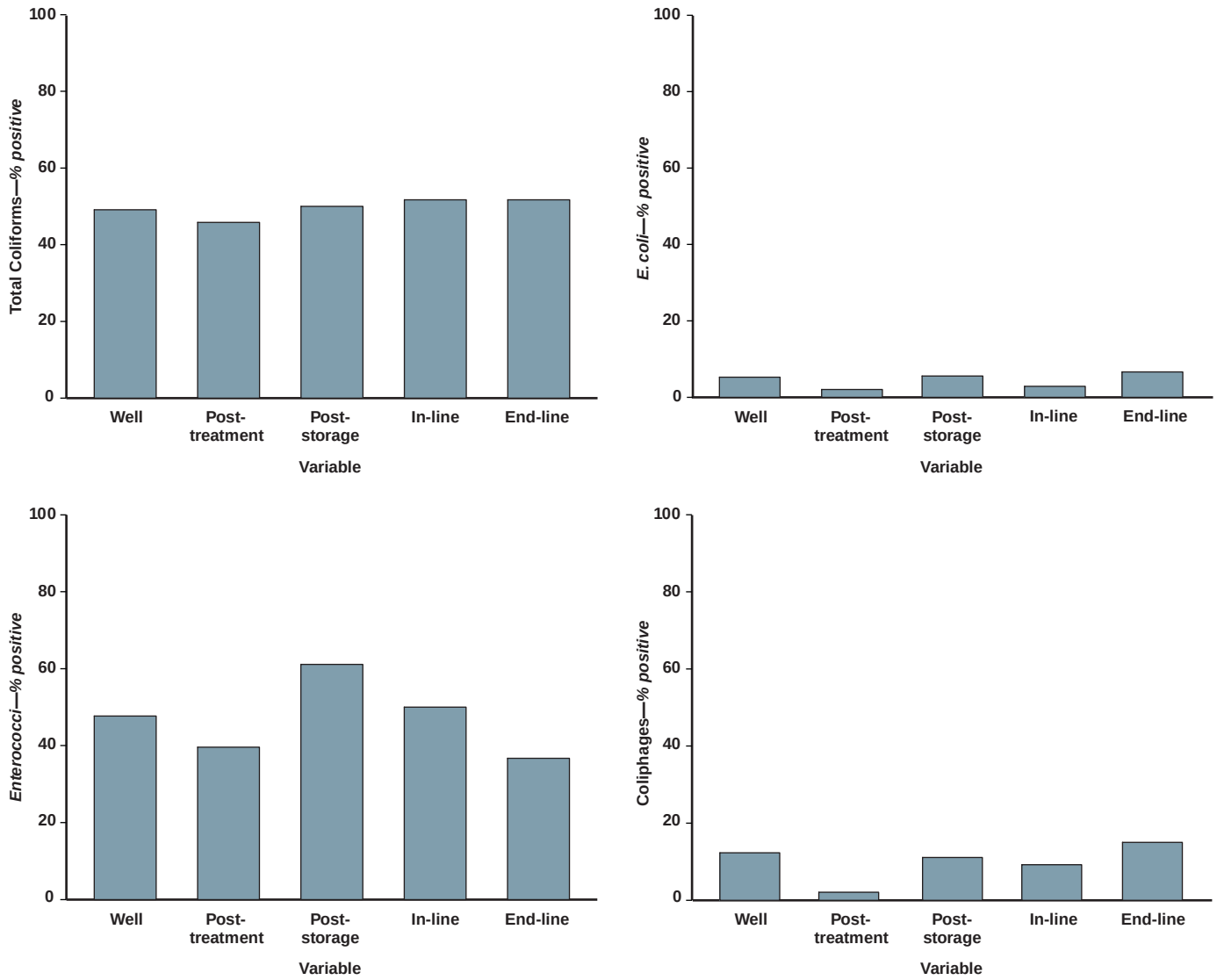



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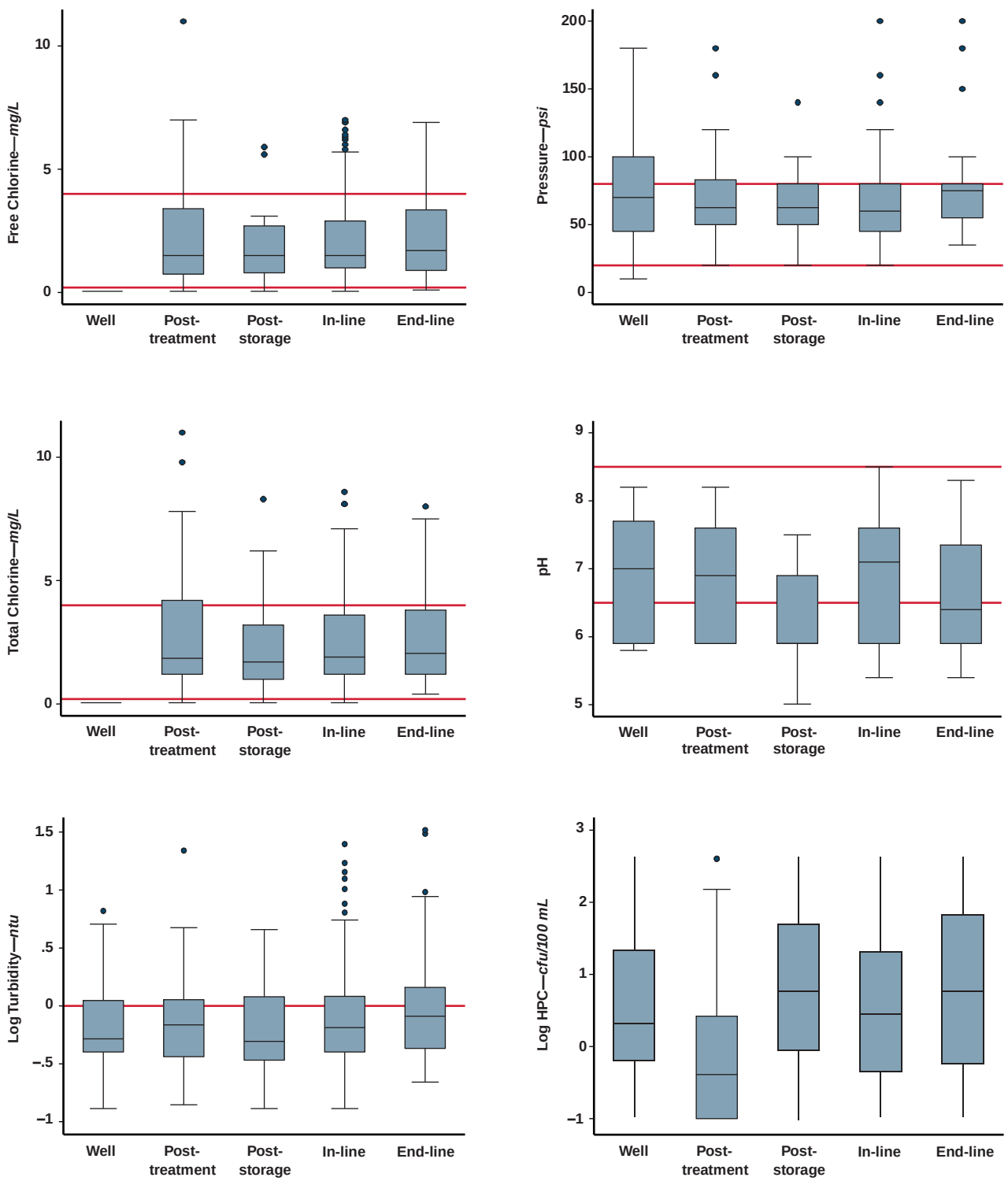
**FIGURE 2** Box-and-whisker plots of each continuous water quality measure across all sampling location categories, excluding free and total chlorine in well samples




**HPC—heterotrophic plate count, USEPA—US Environmental Protection Agency**

**The line inside each box represents the median value, the box represents the 25th and 75th quartiles, the ends of the whiskers represent the upper and lower adjacent values, and the dots represent outliers. The red horizontal lines indicate USEPA's recommended range for the respective parameter.**


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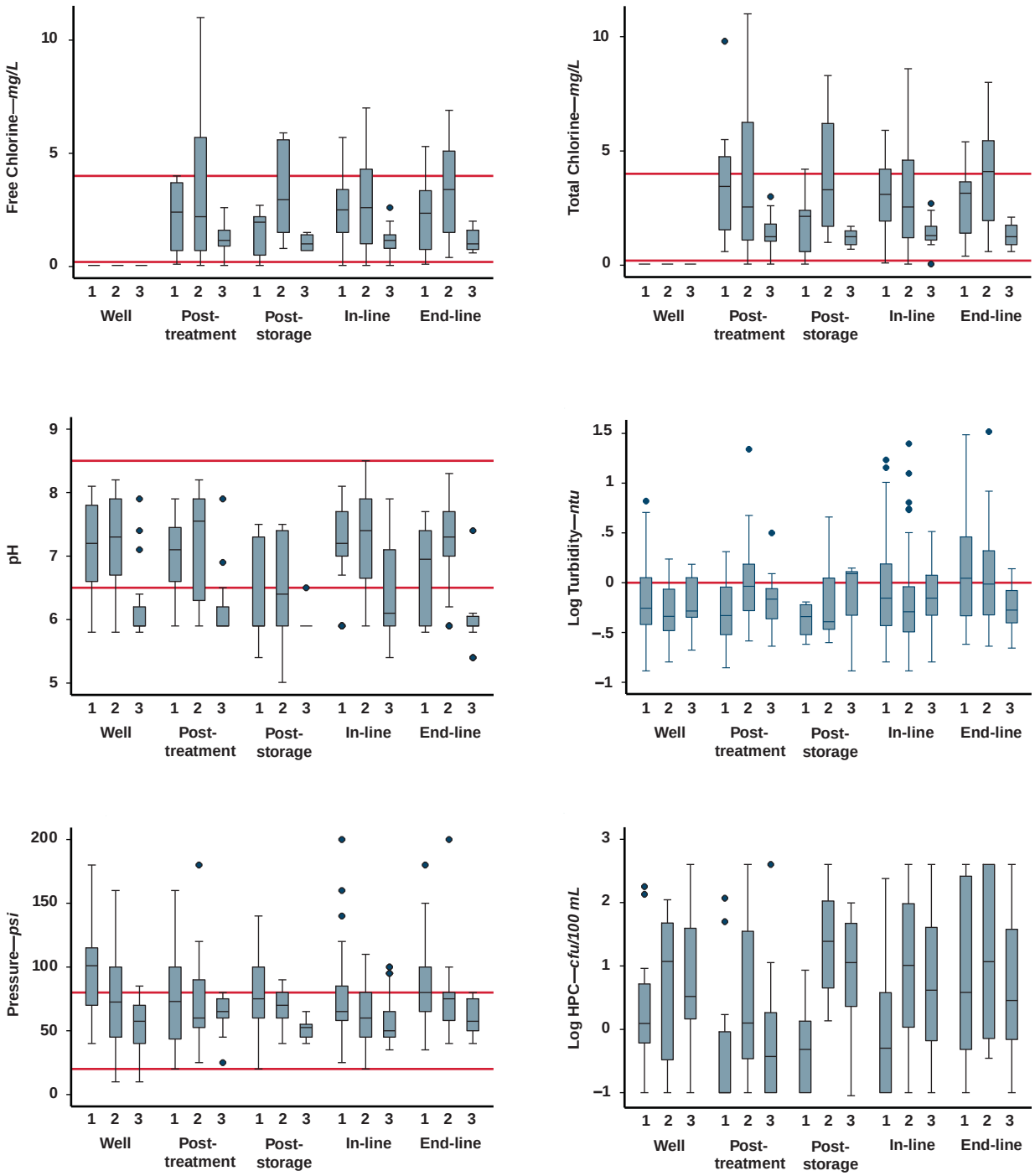
**FIGURE 3** Box-and-whisker plots of each continuous water quality measure across all sampling location categories, excluding free and total chlorine in well samples, and at each sample collection time point

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**The numbers along each plot's x-axis represent sample collection time points: time point 1, September 2013–January 2014 (fall–winter); time point 2, January 2014–March 2014 (winter); time point 3, March 2014–May 2014 (spring).**


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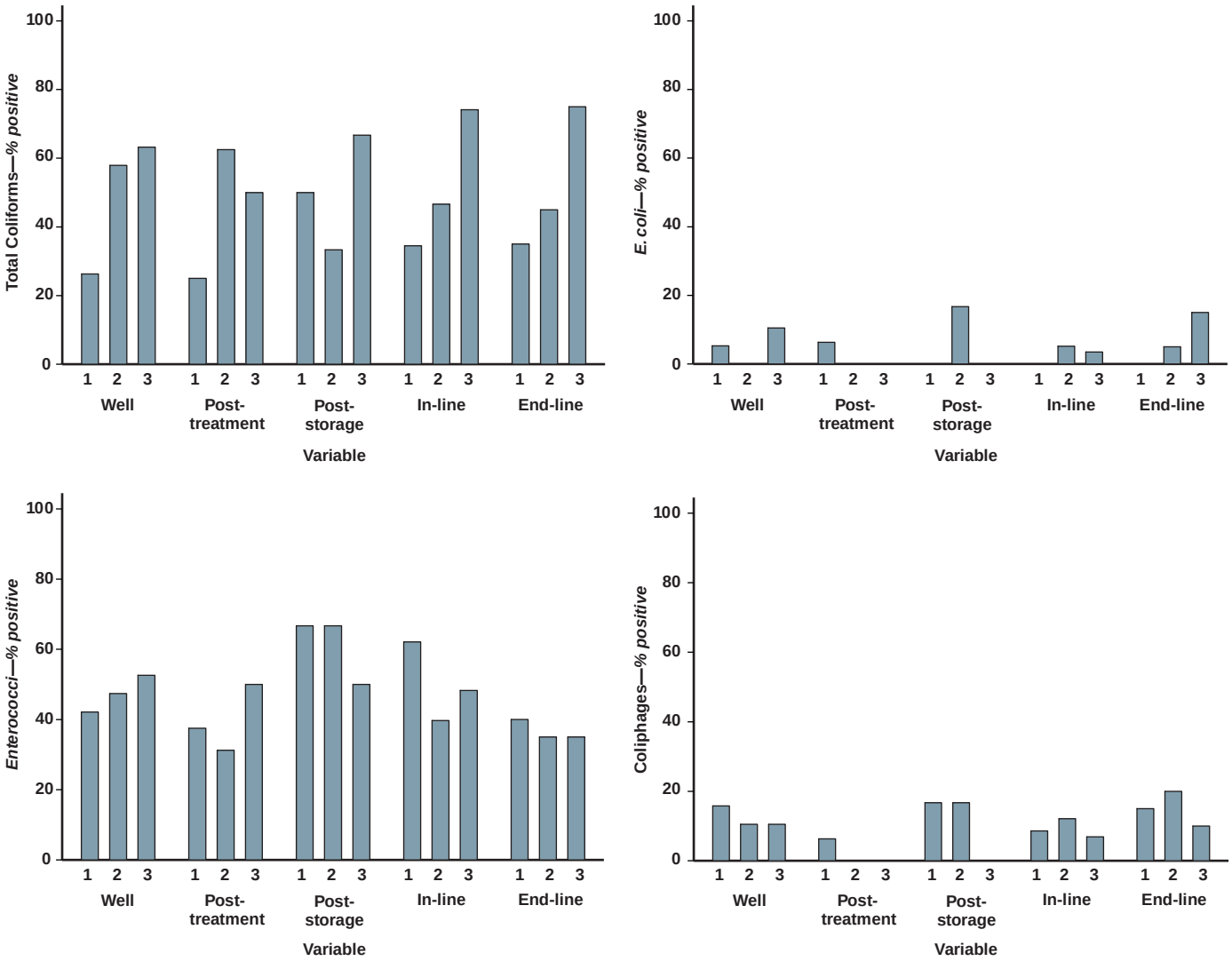
**FIGURE 4** Bar graphs illustrating the percentage of each binary water quality variable that tested positive in a 100-L sample at each sample collection time point




**The numbers along each chart's x-axis represent sample collection time points: time point 1, September 2013–January 2014 (fall–winter); time point 2, January 2014–March 2014 (winter); time point 3, March 2014–May 2014 (spring).**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