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Research Note

Temporal Stability of *Salmonella enterica* and *Listeria monocytogenes* in Surface Waters Used for Irrigation in the Mid-Atlantic United States

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ABSTRACT

Enteric bacterial pathogen levels can influence the suitability of irrigation water sources for fruits and vegetables. We hypothesize that stable spatial patterns of *Salmonella enterica* and *Listeria monocytogenes* levels may exist across surface water sources in the Mid-Atlantic U.S. Water samples were collected at four streams and two pond sites in the mid-Atlantic U.S. over 2 years, biweekly during the fruit and vegetable growing seasons, and once a month during nongrowing seasons. Two stream sites and one pond site had significantly different mean concentrations in growing and nongrowing seasons. Stable spatial patterns were determined for relative differences between the site concentrations and average concentration of both pathogens across the study area. Mean relative differences were significantly different from zero at four of the six sites for *S. enterica* and three of six sites for *L. monocytogenes*. There was a similarity between the mean relative difference distribution between sites over growing season, nongrowing season, and the entire observation period. Mean relative differences were determined for temperature, oxidation-reduction potential, specific electrical conductance, pH, dissolved oxygen, turbidity, and cumulative rainfall. A moderate-to-strong Spearman correlation ($r_s > 0.657$) was found between spatial patterns of *S. enterica* and 7-day rainfall, and between relative difference patterns of *L. monocytogenes* and temperature ($r_s = 0.885$) and dissolved oxygen ($r_s = -0.885$). Persistence in ranking sampling sites by the concentrations of the two pathogens was also observed. Finding spatially stable patterns in pathogen concentrations highlights spatiotemporal dynamics of these microorganisms across the study area can facilitate the design of an effective microbial water quality monitoring program for surface irrigation water.

The microbial quality of irrigation water recently attracted substantial attention because of the increase in foodborne diseases caused by contaminated produce. In the U.S., foodborne pathogens cause approximately 48 million cases of illness annually (Scallan, Griffin et al., 2011; Scallan, Hoekstra et al., 2011). Bacterial pathogens have been found in surface water sources potentially used to irrigate crops (Bell et al., 2015; Benjamin et al., 2013; Haymaker et al., 2019; Li et al., 2014; McEgan et al., 2014; Micallef et al., 2012; Pachepsky et al., 2011).

Escherichia coli has been proposed and used as an indicator of the microbial quality of irrigation water. Statistical threshold values and the geometric mean of *E. coli* concentrations have been proposed to characterize the microbial quality of irrigation water (U.S. Food and Administration, 2018). While some studies showed that high *E. coil* levels are related to a high possibility of detecting pathogens in irrigation water (Rajabi et al., 2011; Wilkes et al., 2009), others have demonstrated that *E. coli* levels are poor indicators of pathogen levels (Antaki et al., 2016; Benjamin et al., 2013; Pachepsky et al., 2016). The lack of a reliable bacterial indicator necessitates directly quantifying bacterial foodborne pathogens in irrigation water. Concentrations of pathogens in surface waters exhibit high spatial and temporal variability, which can make interpreting these results complicated when using them in microbial water quality assessments. Furthermore, in many cases, the quantitative recovery of the target pathogen may be

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below the limit of detection of an assay, which complicates how to incorporate those quantitative values into longitudinal datasets.

Persistent spatial patterns of *E. coli* concentrations have been observed in a specific study examining microbial water quality from specific sites (Pachepsky et al., 2018). In previous work, some water collection sites consistently contained *E. coli* concentrations greater than the average concentration across the study area whereas other sites tended to have concentrations that were lower than the average across the study area. Additionally, some sites did not deviate much from the average across the observation area. These patterns persisted during the entire period of observations during that study. The spatiotemporal alterability of environmental variables was termed temporal stability (Vachaud et al., 1985). The discovery of temporal stability helps to guide and improve the monitoring design by avoiding sites with measurements substantially different from the average of measurements across the study area (Vanderlinden et al., 2012). The presence of temporally stable patterns can often be explained by differences in the environmental or management factors among the sampling sites (Jeon et al., 2020), which promotes a greater understanding and quantification of the environmental factors and their effect on spatiotemporal variability of target microorganisms.

One approach to characterize deviations of pathogen concentrations from the average across sampling sites in a study is to use the relative differences from the average calculated across all of those sites. The persistence of the relative differences at individual locations from average value was considered the manifestation of temporal stability (Vachaud et al., 1985). This approach has been applied to spatiotemporal studies of crop yields and soil nutrients (Basso et al., 2009), along with *E. coli* levels (Jeon et al., 2020; Pachepsky et al., 2018; Stocker et al., 2021) and phytoplankton levels (Smith et al., 2021) in irrigation ponds. Until now, this method has not been applied to pathogen concentration data in irrigation water sources. Another indicator of the presence of stable spatial patterns in spatiotemporal data can be the persistence of ranks of sampling sites (Vachaud et al., 1985). The Spearman rank correlation between data from consecutive sampling dates evaluates how close are the orders of locations sorted by the concentration values.

To address water samples which may have levels of target microorganisms below the quantitative limit of detection (LOD), the Kaplan-Meier method (Kaplan and Meier, 1958) can be used to estimate the mean and standard deviation of target microorganism concentrations. When the quantitative levels in a sample are below the LOD (termed a nondetect) in a substantial number of water samples, such datasets are known as left-censored. The Kaplan-Meier method (K.M.) estimates of the population mean and standard deviation adjusted for data censoring based on the fit to the chosen type of statistical distribution of the underlying population. Because the K.M. technique is nonparametric, there is no requirement that the underlying population be normally distributed or transformable to normality. However, in adapting the technique to left-censored data (*i.e.*, samples containing nondetects), the Unified Guidance (U.S. Environmental Protection Agency., 2009) recommends that the K.M. procedure be utilized to estimate the mean and variance of a normal or normalized distribution for use in parametric statistical tests.

Investigation of spatial patterns of pathogen concentrations can improve spatial design of preventive measures and mitigation treatments related to irrigation water, which in turn can reduce the likelihood of microbial contamination of fresh produce from surface irrigation water. Using more accurate methods to estimate mean levels of target foodborne pathogens at specific sites can aid in filling quantitative data gaps to help better understand pathogen concentration variability at specific surface water irrigation sources.

The objective of this work was to identify temporal stability patterns in levels of *Salmonella enterica* and *Listeria monocytogenes* in selected ponds and rivers in the mid-Atlantic region over a 2-year sampling period.

Materials and methods

Site description, sample collection, and pathogen quantification

Samples were collected from six sites in the mid-Atlantic U.S. over 2 years, from September 2016 to October 2018, twice every month during the growing season (May-September) and once a month during the nongrowing season (October-April). Sites (and number of sampling events) included four nontidal, freshwater rivers (MA03, n = 31; MA04, n = 34; MA05, n = 32; and MA07, n = 30) and two ponds (MA10 n = 35; MA11, n = 34). The detailed descriptions of the sampling sites are summarized in Table 1 and have been previously reported (Haymaker et al., 2019; Sharma et al., 2020; Solaiman et al., 2020). Water samples were collected at these sites, and methods were previously described in the work reporting original findings (Acheamfour et al., 2021; Sharma et al., 2020). Briefly, at each site, triplicate volumes of 0.1 L, 1 L, and 10 L were collected using a Modified Moore Swab, and each sample was analyzed for the presence or absence of *S. enterica* or *L. monocytogenes.* Results were then used to calculate a modified Most Probable Number (MPN/L) value at each sampling. The limit of detection of modified MPN assay was 0.030 MPN/L. Physicochemical parameters including water temperature (℃), dissolved oxygen(mg/L), conductance(μS/cm), pH, oxygen-reduction potential (ORP), and turbidity (FNU) were measured using EXO2 or ProDSS multiparameter water quality sonde/meter (YSI, Yellow Spring, OH, USA) at each sampling event. Cumulative precipitation 1 and 7 days prior to sampling dates were accessed from a weather forecast website (https://www.wunderground.com/history/).

Temporal stability assessment

The relative differences between observations in each site and average over all sites were computed for each sampling day as each sampling site as:

$$RD_{ij} = \frac{x_{ij} - \bar{x}_j}{\bar{x}_j} \tag{1}$$

Where RD_{ij} is the relative difference at the sampling site "*i*" on the sampling day "*j*", x_{ij} is the observed concentration at the sampling site "*i*" on the sampling day "*j*", and $\bar{x}_j$ is the spatial average of x_{ij}, i.e., the average over all sites "*i*" on the sampling day "j".

The mean relative difference at the site *i* (MRD_i) is the average of RD_{ij} over all observation days, calculated by the following equation.

$$MRD_i = \frac{1}{N_t}\sum_{j=1}^{j=N_t} RD_{ij} \tag{2}$$

Table 1
Description of the six sampling sites where *Salmonella enterica* and *Listeria monocytogenes* levels were determined over 2-year period

Site	Water type	Description
MA03	Nontidal freshwater	Tributary of the Nanticoke River that flows through Delaware and Maryland into the Chesapeake Bay
MA04	Nontidal freshwater	Tributary of the Choptank River that flows through Delaware and Maryland into the Chesapeake Bay
MA05	Nontidal freshwater	Tributary of the Patuxent River along the western shore of the Chesapeake Bay
MA07	Nontidal freshwater	Tributary of the Nanticoke River
MA10	Pond water	Pond with a maximum depth of 3.4 m and a surface area of 0.26 ha; at the sampling site, the width was 20 m, and depth was 1 m; agricultural catchment area
MA11	Pond water	Pond with a maximum depth of 3 m and a surface area of 0.40 ha; at sampling site, the width was 52 m, and depth was 0.6 m; agricultural catchment area

where N_t is the total number of sampling days. Values of MRD_i were computed for both *S. enterica* and *L. monocytogenes.* Values of MRD_i greater than 0 indicate that the concentration of the pathogen at the monitoring location "i" is likely to be greater than the average. Similarly, an MRD value less than 0 means that the concentrations at the corresponding locations are likely to be lower than average. A MRD_i value close to 0 indicates that this location may provide a likely estimate of the average across sampling sites. The standard deviation (SDRD) is associated with a set of relative differences (RD_{ij}) for all sampling sites and can be calculated along with MRD as

$$SDRD_i = \sqrt{\frac{1}{N_t - 1}\sum_{j=1}^{N_t}\left(RD_{ij} - MRD_i\right)^2} \tag{3}$$

$SDRD_i$ characterizes the uncertainty associated with MRD_i values of *S. enterica* and *L. monocytogenes.* A lower SDRD value indicates a more robust temporal stability of pathogen levels. To compute MRD values, MPN/L values where no pathogen was detected (0.030 MPN/L) were replaced by one-half of the LOD value (0.015 MPN/L). MRD and SDRD values were also computed for physicochemical water quality variables.

For calculation of the Spearman rank correlation, sampling sites were ranked for each sampling day by the observed concentrations, and the rank correlations were computed to compare site ranks on two consecutive sampling days. This statistic was used to compare the similarities and differences of pathogen levels at consecutive sampling dates over all sampling sites (MA03, MA04, MA05, MA07, MA10, and MA11).

The Spearman rank correlation coefficient was defined as:

$$r_{s,j} = 1 - \frac{6\sum_{i=1}^{i=N_L}\left[rank\left(x_{ij}\right) - rank\left(x_{i,j+1}\right)\right]^2}{(N_L - 1)N_L(N_L + 1)} \tag{4}$$

where N_L is the total number of sampling sites, x_{ij} is the concentration at the specific sampling site x_i at the sampling day "j", and $x_{i,j+1}$ is the pathogen concentration at the location "i" at the next sampling day "j +1". The " If the value of $r_{s,j}$ is close to 1, the greater the level of ranking persistence between time "j" and "j+1" is observed.

Computing the descriptive statistics for data with nondetects (Kaplan-Meier calculation).

The algorithm implementing the K.M. method first constructs a partial ranking of the data, accounting for nondetects (values below the quantifiable limit of detection 0.030 MPN/L) and assigning explicit ranks to each of the detected values. No specific value is assigned to the nondetects in these calculations. The ranks are used to compute quantiles of the K.M. cumulative distribution function (CDF). Then, normal quantiles or *z*-score are computed for each value of the KM CDF. The plot of the z-scores can be fitted with a straight line. The mean and the standard deviation estimates are computed from the slope and the intercept of that line. The algorithm of the K.M. method as described in the EPA guidance report (U.S. Environmental Protection Agency., 2009) was implemented using Excel (Microsoft).

Results

Mean and prevalence values of S. enterica and L. monocytogenes

The levels of *S. enterica* and *L. monocytogenes* at each stream and pond water site over the monitoring period are presented in Supplemental File 1. In general, levels of pathogens at stream sites (MA03, MA04, MA05, and MA07) were higher than at pond sites MA10 and MA11. Average levels of both pathogens (as calculated by the Kaplan-Meier method for growing and nongrowing seasons) were significantly ($p < 0.05$) different among the sampling sites (Table 2). The overall mean values of *S. enterica* and *L. monocytogenes* were the highest at MA04 and MA05, respectively, compared with other sites (data not shown). The mean *S. enterica* concentration values were not significantly ($p < 0.05$) different between growing and nongrowing seasons at all sampling sites for both pathogens The water quality attributes are presented in the Supplementary Table S1 (Supplemental File 1) and in Supplemental File 2.

Temporal stability of pathogen levels

The mean relative differences (MRD) of *S. enterica* and *L. monocytogenes* (MPN/L) levels calculated over a 2-year period of monitoring are shown in Figure 1. The temporal stability pattern of *S. enterica* was related to the water body source. MRD values of *S. enterica* in stream water (MA03, MA04, MA05, and MA07) were all positive (>0), and the MRD values of *S. enterica* in pond water at MA10 and MA11 sites were both negative (<0). The largest mean relative difference between the specific site and average concentrations of *S. enterica* was found at the MA04 site. For *L. monocytogenes*, the largest and only

Table 2
Average concentrations and prevalence of *Salmonella enterica* and *Listeria monocytogenes* at each site as calculated by Kaplan-Meier method in produce nongrowing and growing seasons in the Mid-Atlantic U.S

		S. enterica			*L. monocytogenes*		
			Number of Samples		Number of Samples		
Site	Season	Concentration (MPN/L)[1]	Above LOD[2]	Total	Concentration (MPN/L)	Above LOD	Total
MA03	Nongrowing	1.53 ± 0.73[¶]	11	15	0.15 ± 0.04	8	13
	Growing	1.42 ± 0.68	15	16	1.86 ± 0.91	8	16
MA04	Nongrowing	1.05 ± 0.34[¶]	16	17	0.77 ± 0.62	10	17
	Growing	1.83 ± 0.83	12	17	0.31 ± 0.26	6	17
MA05	Nongrowing	0.87 ± 0.65	10	16	5.92 ± 1.28	15	16
	Growing	1.31 ± 0.69	14	15	3.63 ± 0.92	16	16
MA07	Nongrowing	0.43 ± 0.16	14	15	0.39 ± 0.29	9	13
	Growing	2.63 ± 1.09	14	15	0.28 ± 0.13	9	15
MA10	Nongrowing	0.05 ± 0.02	2	17	0.05 ± 0.02	2	18
	Growing	0.06 ± 0.02	5	18	0.63 ± 0.59	1	18
MA11	Nongrowing	0.07 ± 0.02	4	16	0.05 ± 0.02	2	16
	Growing	0.34 ± 0.24	6	18	0.04 ± 0.01	2	18

[1]The "±" symbol separates the mean and the standard error determined by the Kaplan-Meier method.
[2]Above LOD indicates that water samples at each site had a quantitative level of pathogen >0.015 MPN/L, which was the limit of detection (LOD) of the quantitative assay.
[¶] Indicates that the mean values in the growing season are significantly ($p < 0.05$) different compared to the nongrowing season at MA03 and MA04.

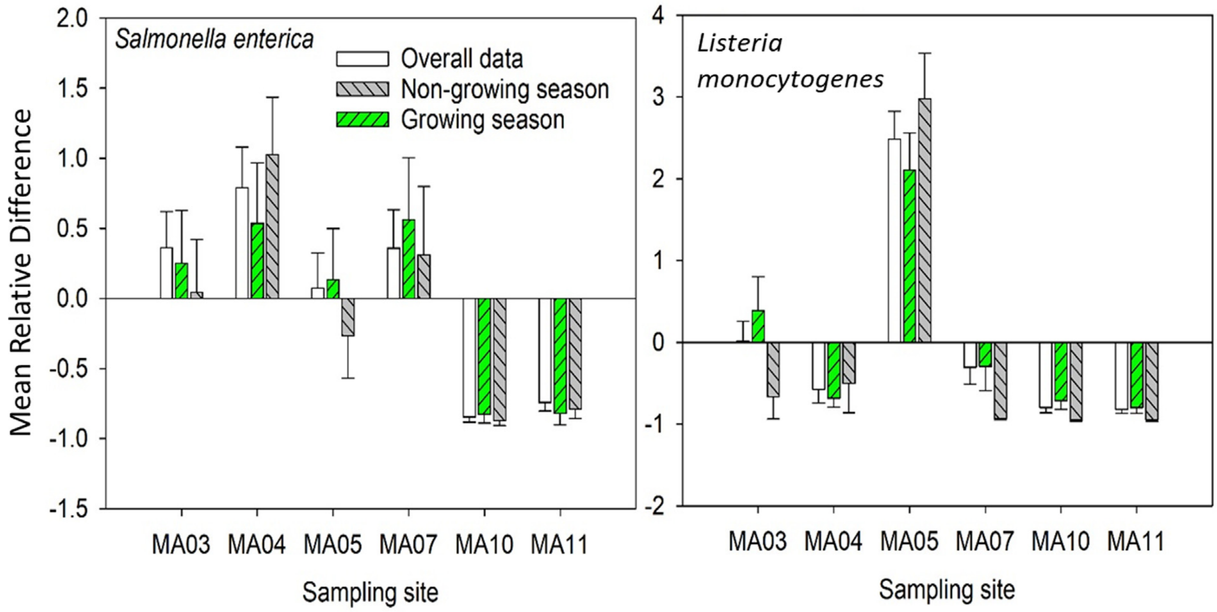

Figure 1. Mean relative difference (MRD) of *S. enterica* and *L. monocytogenes* (MPN/L) across six sampling sites. Error bars show the standard error of relative difference values.

positive MRD was found at the stream site MA05. The MRD values at the pond sites MA10 and MA 11 were negative (similar to those for *S. enterica*). The values of standard errors of MRDs are shown with error bars in Figure 1. For *S. enterica*, the MRD values for MA04, MA10, and MA11 are significantly ($P < 0.05$) different from zero , indicating that water samples from these sites, on average, will have concentrations of *S. enterica* significantly different from the average over the study area and duration (all six sites). Conversely, water samples at MA03, MA05, and MA07 on average will produce *S. enterica* levels that will not be statistically different from the average across all of the sampling sites. For *L. monocytogenes*, MRD values were significantly different from zero ($P < 0.05$) at MA04, MA05, MA10, and MA11 sites while MRD values at MA03 and MA07 were not significantly different from the average across all sites.

The MRD values of both *S. enterica* and *L. monocytogenes* in the growing (May-September) and nongrowing (October-April) seasons are also shown in Figure 1. The seasonal MRD values were similar between the seasons at each site with two exceptions (MA05 for *S. enterica*, MA03 for *L. monocytogenes*) and were not significantly different between the seasons. The sites with MRD values significantly different from zero were the same for the two seasons.

The ranking persistency, quantified with the Spearman correlation coefficients (r_s) between two consecutive monitoring dates, is shown in Figure 2. For *S. enterica*, the percentage of two consecutive sampling points (MPN/L values) which showed a strong correlation ($r_s \geq 0.7$) were 36%, while those which showed at least a moderate correlation ($r_s \geq 0.4$) was 68%. For *L. monocytogenes*, a value of $r_s \geq 0.7$ was observed in 38 % of cases, while a moderate-to-strong correlation ($r_s = 0.4 - 0.7$) was observed in 79% of cases of two consecutive sampling points. The Spearman rank correlation coefficient values for *L. monocytogenes* were mainly high or moderate except during the second nongrowing season. Based on these observations, a stronger ranking persistency was found for the *L. monocytogenes* compared to the *S. enterica* levels.

The MRD values of environmental variables are shown in Figure 3. In most cases, the MRD values for these variables did not differ significantly from zero. This indicates that the water quality measurements representative of the average across all six sites could have been made at any of the sites. Overall, the absolute values of MRDs for environmental variables were much smaller than for pathogens. However, turbidity at the MA04 site and electrical conductance at the MA05 site were notable and important exemptions to this trend. The absolute values of MRDs were substantially different from zero and values measured at other sites. Therefore, the spatial patterns were more pronounced for turbidity and electrical conductance. The MRD value for *S. enterica* appeared to be positively correlated to the MRD values for 7-day rainfall ($r_s \geq 0.657$). No significant correlative relationship was found between *L. monocytogenes* MRD levels and 7-day rainfall. MRD values of water temperature and *L. monocytogenes* correlated well ($r_s > 0.885$), with lower water temperatures correlated with greater *L. monocytogenes* levels. MRD values for *S. enterica* appeared to have a strong inverse relationship with MRD for dissolved oxygen ($r_s < -0.885$).

Discussion

Levels (MPN/L) of *S. enterica* and *L. monocytogenes* had temporally stable spatial patterns exhibited by persistent and consistent deviations of pathogen levels at individual sites from the overall average (all six sites). For *S. enterica*, the highest MRD levels were found at MA04, which also had the highest MRD value for turbidity (Fig. 3a), potentially indicating a relationship between higher turbidity and *Salmonella* levels. Higher levels of turbidity associated with sediment resuspension may be indicative of bacteria being released from sediments. Turbidity levels are related to the sediment transport process (Pandey and Soupir, 2013; Pandey et al., 2012). Xue et al. (Xue et al., 2018) observed that total coliform and *E. coli* levels were highly correlated with turbidity in groundwater and surface waters. *S. enterica* levels were positively correlated with flocculated suspended and bed sediment particles (Droppo et al., 2009). In addition to indicating movement of bacterial cells from sediments, increased turbidity and suspended solids may also shield *Salmonella spp.* from inactivation from solar radiation, increasing the longevity and persistence in irrigation water sources (Sinton et al., 2007). Finally, water with high turbidity value may represent the inflow of runoff containing animal waste that can transport *Salmonella* spp. to water sources.

The highest MRD values for *L. monocytogenes* were found at the MA05 site, which also had the highest MRD value for the specific con-

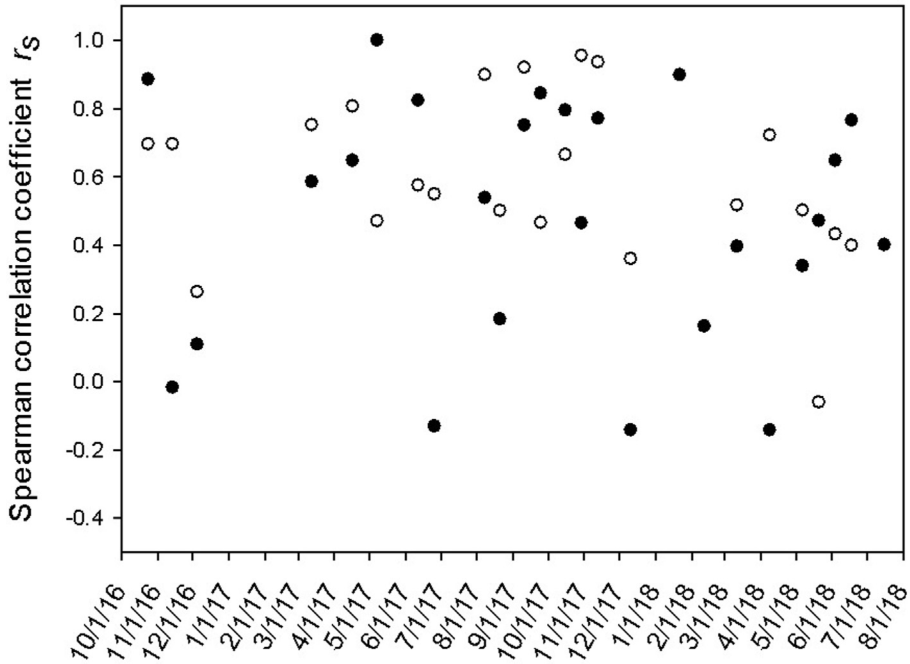

Figure 2. Spearman rank correlation (r_s) between concentrations (MPN/L) of *Salmonella enterica* (black circles) and *Listeria monocytogenes* (open circles) at specific sampling locations on consecutive sampling days over 2 years.

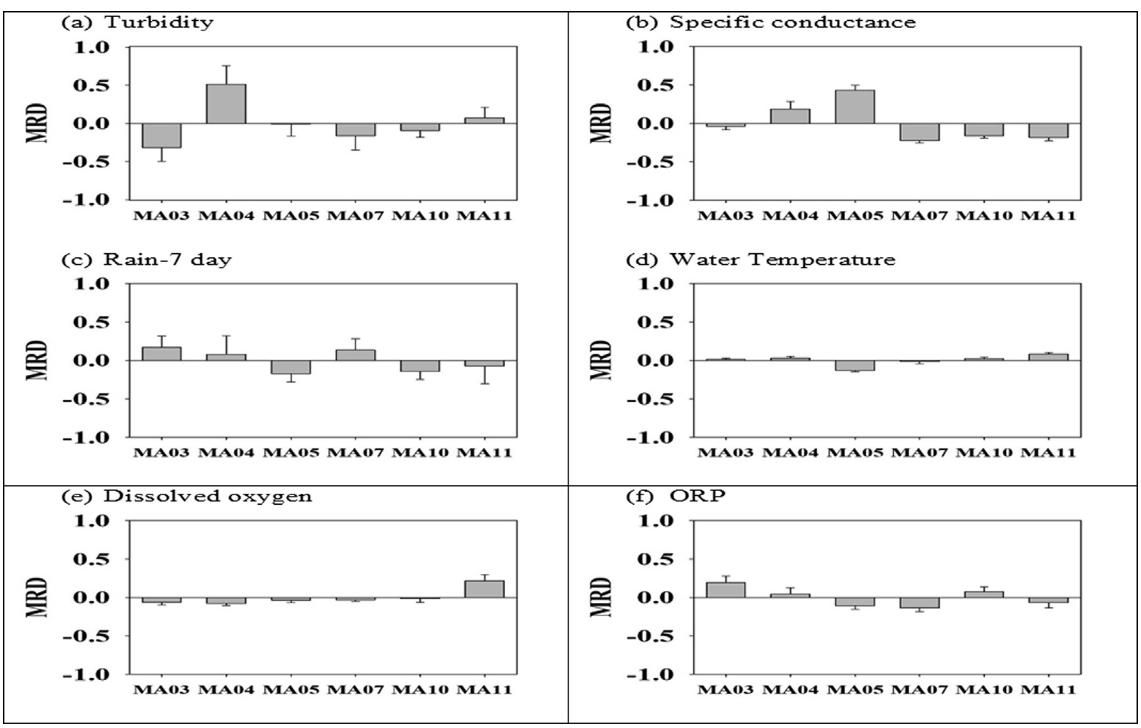

Figure 3. Mean relative difference (MRD) values of environmental variables – a) turbidity (NTU), b) specific conductance, c) Rain-7 day (inches, cumulative rainfall over 7 days preceding sampling date), d) Water temperature (°C), e) Dissolved oxygen (mg/L) and f) Oxygen Reduction Potential (mV) measured across six sampling sites (overall values). Error bars indicate standard error.

ductance (Fig. 3). Xue et al. (Xue et al., 2018) found that fecal bacteria in water samples from both surface water and groundwater were positively correlated with conductance. Higher levels of fecal indicator bacteria (and *L. monocytogenes*) may be associated with conductance due to the availability of dissolved nutrients (Mitch et al., 2010), and nitrogen and phosphorus (Monaghan et al., 2009) which these bacteria may use to extend their survival and possibly grow in these environments. However, Gu et al. (Gu et al., 2021) found that the prevalence of *L. monocytogenes* was poorly correlated with conductance in water samples from both wells and ponds. Topalcengiz et al. (Topalcengiz et al., 2017) enumerated microbial indicators (total coliform, *E. coli*, and enterococci) in 540 water samples from six ponds in west central Florida and presented that these fecal indicators did not correlate with conductance. It appears more probable that the electrical conductance in the water body is correlated with the level of nutrients, and the higher level of nutrients either supports higher *L. monocytogenes* levels or indicates that the source of these nutrients also contains *L. monocytogenes*.

S. enterica levels in ponds (MA10, MA11) were lower compared to levels in streams in Mid-Atlantic U.S (MA03, MA04, MA05, MA07). These findings are in agreement with the results of Luo et al. (2015), who monitored *S. enterica* levels in ten irrigation ponds in the upper Suwannee River watershed in the southeastern United States and reported relatively low levels compared to prior reports that examined *S. enterica* levels from stream waters in this region (Haley et al., 2009; Rajabi et al., 2011). Conditions for bacterial die-off appear to be more favorable in ponds than in streams. Irrigation ponds are usually relatively clean, shallow and typically have slow flow rates compared to flow rates in river, and these conditions may promote the inactivation of bacteria due to solar U.V. radiation (Chang et al., 1985). Recently, other authors have shown that *Salmonella* levels and prevalence may be highly associated with the high flow volumes in rivers and streams (Deaven et al., 2021).

Spearman rank correlation coefficients (r_s) proved largely moderate (0.4–0.7)-to-very strong (≥0.7) correlations (Fig. 3), indicating high levels of consistency in pathogen levels on consecutive monitoring days. Weak (≤0.4) or negative correlations were observed in 36% of cases for *S. enterica* and 20% of cases for *L. monocytogenes*. Although the overall rank correlation data point to ranking persistence, the ranking nor the r_s value consider the magnitude of differences between the values being ranked.

Interestingly, MRD values for *S. enterica* and *L. monocytogenes* over the growing and nongrowing periods at each site were similar. Although there were significant differences between the average concentrations in the two seasons for three of the six sites, the MRD values did not differ. Essentially, average values of concentrations changed from season to season, but the site-specific relative deviations from the overall seasonal mean value did not change much from one season to another. The Kaplan-Meier method was applied to obtain estimates of mean and standard deviation of *S. enterica* and *L. monocytogenes* concentrations since some water samples were below the quantitative LOD. Estimation of the Kaplan-Meier mean and standard deviation values may be slightly biased, typically with the mean on the high side and the standard deviation on the low side (U.S. Food and Administration, 2018). Larger biases are more likely whenever the detection rate is less than 50%. We realize that K.M. method assumes a specific statistical distribution type, which may affect the values of the estimated mean and standard deviation values. However, as long as the total proportion of censored measurements does not exceed roughly 70% of the values, the degree of bias will tend to be small (Tolley et al., 2016). These considerations may indicate that differences between growing and nongrowing season concentrations (Table 2) may be less accurate when the number of nondetects (values below the quantitative LOD) is high at a particular site, especially at pond sites MA10 and MA11, which contained a large number of nondetects.

The positive correlation of MRD values of *S. enterica* and 7-day rainfall amounts ($r_s \geq 0.657$) agreed with results from several previous studies of waterways in Georgia, California, and New York which showed that the *S. enterica* levels were highly affected by rainfall (Haley et al., 2009; Strawn et al., 2013; Walters et al., 2011). However, there was no significant correlation between *S. enterica* levels and rainfall in other studies in Ontario and Florida (McEgan et al., 2013; Thomas et al., 2013). The impact of rainfall on *S. enterica* concentration in water sources may depend on the surrounding land use types and water body type.

The strong negative correlation ($r_s \geq -0.885$) between MRD values of water temperature and *L. monocytogenes* MRD values agrees with previous studies that have shown that lower water temperatures promoted the prevalence or increased levels of *L. monocytogenes* (Cooley et al., 2014; Schaffter et al., 2004). Other studies also showed that the prevalence of *L. monocytogenes* was significantly higher in winter compared to spring and summer (Stea et al., 2015) when presumably, water temperatures are lower. The higher prevalence of *L. monocytogenes* in seasons or locations where the water temperature is lower cold may be attributed to the psychrotrophic nature of the pathogen, and potentially less competition from the autochthonous microflora present in the water body (Gandhi & Chikindas, 2007; McLaughlin et al., 2011).

MRD values for dissolved oxygen were lower at stream or river water sites compared to pond water sites Fig. 3. Previous work has also reported an inverse relationship between *S. enterica* levels and dissolved oxygen levels (Haley et al., 2009). One possible explanation for the negative correlation ($r_s < -0.885$) between *S. enterica* and dissolved oxygen in our current study is that the ultraviolet light-induced physiological damage to bacterial cells is promoted by higher dissolved oxygen levels, leading to bacterial cell death (Ansa et al., 2011; Ouali et al., 2013).

The MRD analysis is data-driven and that the patterns discovered with MRD analysis reflect the pathogen source, survival, and transport conditions that existed during the observation period over the observation sites. One can anticipate changes in spatial patterns of pathogen concentrations should environmental and/or management pathogen fate and transport factors drastically change. Of course, such detection assumes that the additional monitoring will be needed to establish significant differences between old and new stable spatial patterns.

Correlations between patterns of physicochemical and microbiological parameters are considered in terms of the patterns of relative deviations from average in this work. These correlations were found across observation sites and reflected situation across these sites rather then some general ecological trends. For example, the negative correlation of Lm survival pattern with temperature pattern is not equivalent to the generally known improvement of Lm survival with the decrease in water temperature. Results of this work tell only that if there is a difference in temperature between locations, better survival can be expected at location with lower temperature without reference to actual value of temperature across location.

Establishment of MRD can be beneficial in several aspects. In particular, it can indicate the occurrence of some atypical event when the RD substantially deviates from the MRD pattern. It can help to identify the potential sources of contamination considering seasonal variability. It also can indicate sites where the MRDs are close to zero and therefore data from the site may represent the average over all sites if this average is of interest. Examples of small-scale application of the temporal stability concept in microbial water quality in irrigation ponds can be found in (Pachepsky et al., 2018; Smith et al., 2021; Stocker et al., 2021). The MRD analysis appears to be efficient in processing monitoring data. Large absolute MRD values at some observation sites point at the existence of distinctive pathogen source, survival and transport conditions at these sites. The presence of such differences can be diagnosed with the MRD analysis, but a site-specific investigation is needed to find out what those differences are. The existence of the spatial patterns in deviation of concentrations from the average over the observation area points at the existence of differences in pathogen presence in waters but does not, of course, inform about the reasons for those differences.

In this work, the stable spatial patterns were found for the deviations of the average of pathogen concentrations across the study area. These deviations were characterized by the relative differences between the site value and average value across the sites. The variability of the relative differences at individual sites over the observation period was not large. Therefore, the deviations of concentrations at individual sites from the average across all the sites were quite stable.

Two years of monitoring of microbial water quality at four stream sites and two pond sites provided a dataset describing spatiotemporal *S. enterica* and *L. monocytogenes* distribution across the mid-Atlantic U. S. In general, analysis of the relative differences between the pathogen levels at individual sites and the average over the entire study area revealed that *S. enterica* and *L. monocytogenes* have stable spatial patterns that span both the produce-growing and nongrowing seasons. The persistence of ranks (the similarity in pathogen levels at two consecutive monitoring dates) revealed that pathogen levels are relatively stable. *S. enterica* and *L. monocytogenes* MRD values were correlated to different MRD values of environmental physicochemical parameters of water, which may account for the different spatial patterns observed for these two pathogens. The analysis of temporal stability patterns of pathogen concentrations can inform the choice of microbial water quality monitoring sites in a specific geographical area or watershed to represent the average concentrations across the region.

CRediT authorship contribution statement

Seongyun Kim: Writing – original draft, Methodology, Investigation, Formal analysis. **Yakov Pachepsky:** Conceptualization, Methodology, Formal analysis. **Shirley A. Micallef:** Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Rachel Rosenberg Goldstein:** Methodology, Investigation, Investigation, Project administration. **Amy R. Sapkota:** Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Fawzy Hashem:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation. **Salina Parveen:** Writing – original draft, Resources, Project administration, Methodology, Investigation, Conceptualization. **Kalmia E. Kniel:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Conceptualization. **Manan Sharma:** Writing – original draft, Methodology, Project administration, Conceptualization, Funding acquisition.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jfp.2023.100058.

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