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Persistent Patterns of *E. coli* Concentrations in Two Irrigation Ponds from 3 Years of Monitoring

Matthew D. Stocker · Yakov A. Pachepsky · Jaclyn Smith · Billie Morgan · Robert L. Hill · Moon S. Kim

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Abstract Small to medium irrigation ponds provide substantial quantities of water for irrigation in the Mid-Atlantic region of the U.S. The concentrations of the fecal indicator organism *Escherichia coli* (*E. coli*) are used to evaluate the microbial water quality of irrigation sources. Little is known about the spatiotemporal variability of *E. coli* concentrations in pond water and the possible effects on monitoring and management of the microbial quality of irrigation water from these ponds. The objective of this work was to test the hypotheses that (a) spatial patterns of *E. coli* concentrations exist that are preserved both intra- and interannually, and (b) persistent spatial patterns in water quality parameters exist and correlate with persistent patterns of *E. coli* concentrations. Sampling was conducted fortnightly during the summer months in 2016 to 2018 and consisted of taking water quality measurements at 23 and 34 locations in ponds P1 and P2, respectively. Interannual variability of *E. coli* was observed in both ponds as was substantial spatial variability of *E. coli* concentrations within each year. The mean relative difference (MRD) analysis was used to identify temporally stable patterns of *E. coli* concentrations within the ponds. These patterns found for individual years showed significant positive correlations with each other and with the overall pattern derived from the 3-year dataset. Correlation coefficients of patterns varied from 0.487 to 0.842 in P1 and from 0.467 to 0.789 in P2 ($p < 0.05$). MRD patterns of water quality parameters and of *E. coli* concentrations were also significantly correlated. Within the 3-year dataset, the highest positive correlations were observed for chlorophyll-a and turbidity while the dissolved oxygen concentrations demonstrated the greatest negative correlations. Results of the present study emphasize the advisability and feasibility of finding temporally stable spatial patterns in microbial water quality within irrigation ponds.

Keywords *E. coli* · Irrigation water quality · Mean relative difference · Temporal stability

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M. D. Stocker · J. Smith
Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA

M. D. Stocker (✉) · Y. A. Pachepsky · J. Smith · B. Morgan · M. S. Kim
USDA-ARS Environmental Microbial and Food Safety Laboratory, 10300 Baltimore Ave, Bldg. 173, Beltsville, MD 20705, USA
e-mail: Matthew.Stocker@usda.gov

M. D. Stocker · J. Smith · R. L. Hill
Department of Environmental Science and Technology, University of Maryland, College Park, MD, USA

1 Introduction

The microbial quality of water used for irrigation is a critical component of environmental quality that should be monitored to reduce the incidences of foodborne outbreaks associated with fecal contamination. The linkages between microbial water quality and public health incidents have been documented in the US and elsewhere worldwide (Pachepsky et al., 2011; Uyttendaele et al., 2015; Jongman and Korsten, 2016; Allende et al., 2018). Due to the difficulty in the detection and enumeration of pathogenic microorganisms in the environment, the levels of generic *Escherichia coli* (*E. coli*) are measured and compared to regulatory standards for microbial water quality evaluation. Currently, the Food and Drug Administration sets the allowable levels of *E. coli* in irrigation waters in the U.S. under the Food Safety Modernization Act (FSMA) (FDA, 2016) and *E. coli* is also used as a microbial water quality indicator internationally as well (Uyttendaele et al., 2015; Allende et al., 2018).

The initial guidance provided by the FDA for the evaluation of microbial water quality of irrigation source waters does not provide information regarding the selection of water sampling location(s). The dynamics of *E. coli* concentrations in surface waters have been shown to display substantial spatial and temporal variability (USEPA, 2010; Quilliam et al., 2011; Jeon et al., 2020). An et al. (2002) reported that *E. coli* densities on an inland lake displayed large spatial variation with high concentrations measured in areas with heavy recreational activity (swimming or motor boating) and where waterfowl tended to congregate. Increased levels of both *Salmonella* and *E. coli* O157 have been observed with decreased distance from rangeland in wells, ponds, drainage ditches, standing waters, and reservoirs used for irrigation (Benjamin et al., 2013). Other researchers have documented high indicator organism concentrations in areas where streams or rivers enter lakes and lower concentrations when moving outward from these locations (Brookes et al., 2004; Davis et al., 2005). Therefore, the decision on where and when to sample may have significant effects on the results of microbial water quality monitoring assessments.

Datasets resulting from microbial water quality assessments of irrigation water sources are somewhat limited and have usually focused on the temporal component over the spatial one (Draper et al., 2016; Havelaar et al., 2017; Lee et al., 2018; Topalcengiz et al., 2017). In these studies, samples are collected from as close to the irrigation intake as possible which is considered the "golden standard" (Lee et al., 2018), or from the sprinkler systems where the water is distributed (Marine et al., 2015; Pagadala et al., 2015). Many studies exist that document *E. coli* concentrations in irrigation sources such as ponds, lakes, and reservoirs, but rarely are samples taken across the water body due to limited available resources, difficulties in accessing potential sampling locations and/or due to the intended goals of the research.

Denser spatial sampling yields better information on the spatial distribution of concentrations that may be present. Lee et al. (2018) documented large spatial differences of *E. coli* and *Salmonella* concentrations among 3 locations around the perimeter of an irrigation pond measured from March to September. The authors related the spatial differences to potential microhabitats existing within the pond along with the physicochemical parameter preferences of the microorganisms. They also found the samples taken near the intake (pond interior) contained lower concentrations of *E. coli* than those sampled at the bank. Conversely, Harris et al. (2018) reported higher *E. coli* concentrations in interior sampling locations as compared to bank locations in two GA ponds. Pagadala et al. (2015) measured total aerobic bacteria, *E. coli*, and total coliforms concentrations in irrigation wells, pond water samples, and at the end of drip lines, but found no significant differences among the concentrations of all three groups. In another study, tenfold or greater differences were observed between pond water at the source and what is released at the end of drip lines or pivot sprinkler heads (Antaki et al., 2016). Another group of authors sampled 5 locations along the flow path of 6 different California reservoirs and found that the microbial water quality in downstream irrigation supplies was not statistically associated with conditions within the reservoirs, rather there was a greater similarity to water quality just upstream of the reservoirs (Partyka et al., 2018).

The need for monitoring environmental variables across spatial units with substantial internal heterogeneity is quite common across environmental disciplines. One feature helpful in monitoring design is the existence of relatively stable spatial patterns in spatiotemporal variations.

Some areas exhibit environmental variable values that tend to be lower than the average across the study area. On the contrary, other areas have values of the same variable that tend to be higher than the average. Such phenomenon is well known in hydrology and atmospheric sciences and is referred to as temporal stability (Cosh et al., 2008; Vanderlinden et al., 2012; Vereecken et al., 2016).

One approach to quantify the temporal stability is to use the mean-relative-difference (MRD) analysis which compares the relative differences between the point measurements and averages across the study area. The analysis returns information on the consistency, or stability, of a measurement taken in a given location relative to the averaged measurements in all locations within the sampling extent. In this way, locations with values consistently lower, about, and higher than the spatiotemporal average can be determined. The MRD analysis has been successfully applied in the analysis of across-the-field soil moisture contents (Vachaud et al., 1985), soil moisture, and electrical conductivity (Cosh et al., 2008; Pedrera-Parrilla et al., 2017) as well as crop yields (Huang et al., 2018). Jeon et al. (2020) used the MRD analysis to discern temporal stability of *E. coli* concentrations in a PA creek which showed dependency on the sampling season and land use. Pachepsky et al. (2018) documented temporal stability in *E. coli* concentrations over a single growing season in two irrigation ponds in Maryland. To our knowledge, no such study has documented spatiotemporal patterns of *E. coli* in irrigation water sources using multi-year data. It is important to determine if the patterns identified in a single year may be anticipated over the course of several years or if these patterns are independent

The objective of this work was to apply the MRD analysis to characterize and compare the temporal stability of *E. coli* concentrations in irrigation ponds using single- and multi-year data, and test the hypothesis that the MRD patterns of *E. coli* correlate and may be predicted using the MRD patterns of more easily measurable water quality variables.

2 Materials and Methods

2.1 Data Collection

The field sites and the data acquisition are described earlier by Stocker et al. (2019). In brief, two working irrigation ponds (Fig. 1) were sampled during the growing seasons of 2016 – 2018 at the locations indicated in the site maps. Irrigation water is withdrawn in locations 6 and 4 from ponds P1 and P2, respectively. No animal manure is applied to the surrounding lands of either property. Ponds are mostly rain- and runoff-fed. P1 receives some water from an ephemeral creek at location 23 and from another pond at location 6 when the water level is low. P2 receives water via the culvert at location 12 during rainfall events and has level-dependent outflows between locations 24 and 25. Animal interactions with the ponds are controlled by the farm managers, but P1 has been observed to occasionally shelter small flocks of geese. Location 9 at P1 is used as an entry point by the landowners for recreational activities. There is a permanent residence at the South Western part of P2 which is equipped with a septic system and domesticated dogs are free to wander the area near the pond. The two ponds are separated by a distance of roughly 130 km.

Sampling was performed approximately biweekly primarily in June, July, and August. Care was taken to avoid sampling less than 2 days after a rainfall event. In 2018, the increased occurrence of rainfall led to larger than biweekly gaps between some sampling events. Nevertheless, in the two instances indicated below, sampling occurred less than 8 h after heavy rainfall events. Weather data, including air temperature and precipitation, were collected from on-site weather stations. Sampling on all dates started at 9:00 am and ended around 10:30 am. Samples were collected from the 0 to 20 cm depth interval by hand using a multipurpose sampling platform (Kim et al., 2020), a small boat, or in the case of nearshore samples, using a 500-mL sampling rod. All samples collected were immediately placed in ice-packed insulated bags and then in a dark cooler on ice for transport. The coolers were brought to the laboratory within 2 h after sampling concluded. Concurrently with sampling, a YSI 556 MPS (Xylem, Yellow Springs, Ohio) meter was used to measure temperature (C;°C), specific conductivity (SPC; μS cm^{-1}), dissolved oxygen (DO; mg L^{-1}), and pH in 2016. In 2017 and 2018, a YSI EXO-2 sonde meter was used to measure the same set of parameters within the 0–20 cm sampling depth range but was also equipped with the sensors

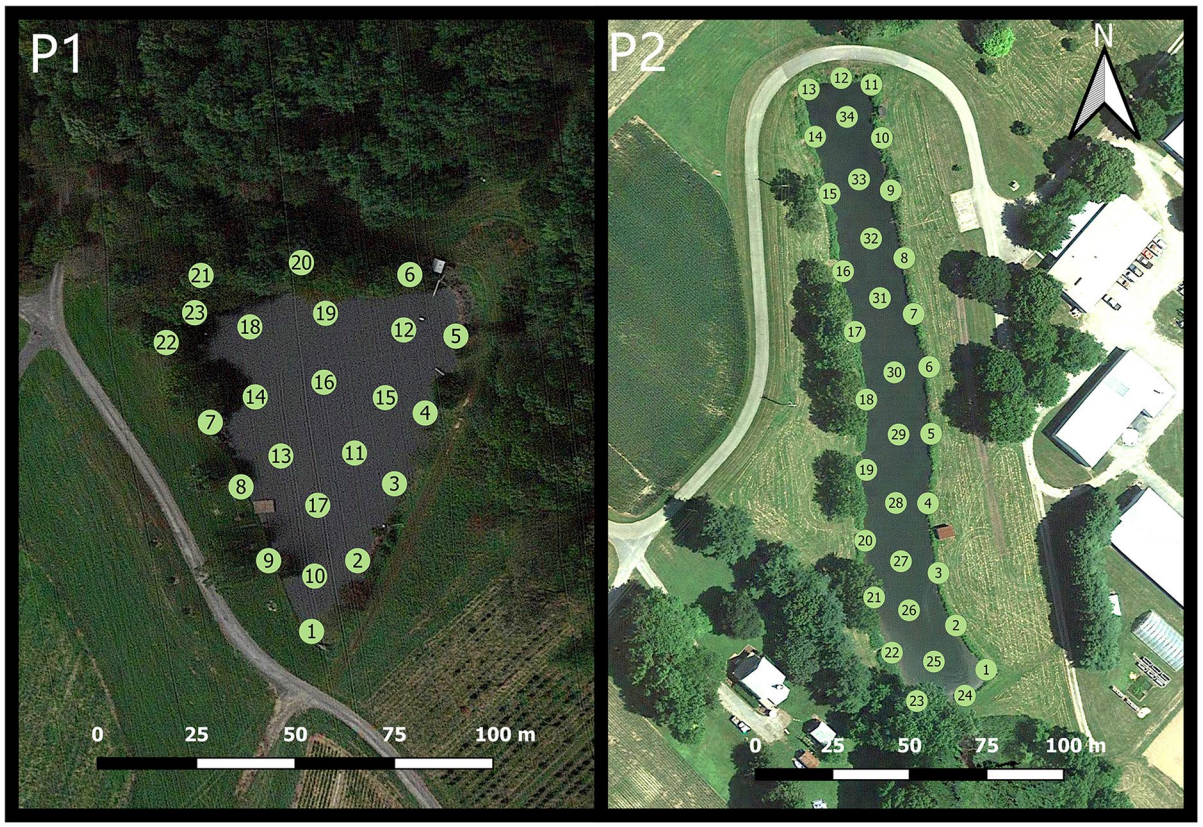

Fig. 1 Sampling locations used for the 2016–2018 observation period. P1 and P2 have 23 and 34 locations, respectively. Locations 20–23 in P1 were not sampled in 2016. In 2016, sites 16–19 in P1 were added after the 2nd sampling date. Pond P2 had locations 31–34 added after the first sampling date in 2016

to measure turbidity (NTU; NTU), fluorescent dissolved organic matter (FDOM; μg L^{-1}), phycocyanin (PC; relative fluorescent units (RFU)), and chlorophyll-a (CHL; RFU). *E. coli* was enumerated based on EPA method 1603 (USEPA, 2014) which utilizes membrane filtration. The filtered volume was 50 to 150 mL of pond water. Each sample was filtered and plated in duplicate and the CFU counts were then averaged before reporting.

2.2 Data Analysis

All concentrations of *E. coli* were $\log_{10}$-transformed prior to statistical analysis. In the cases where a non-detection (N.D.) count occurred, a minimum value of 0.5 CFU was assigned prior to transformation. The Kolmogorov – Smirnov test was used to compare distributions of *E. coli* concentrations between the pond interior and bank locations. The nonparametric Spearman correlation coefficient (r_s) was calculated to determine similarities in the ranks of locations between individual years as well as with the 3-year MRD average. Values of 1 and – 1 indicate extremes whereby there is a strong positive and negative monotonic relationship, respectively.

2.3 Temporal Stability Evaluation

The MRD analysis is a common method used to characterize temporal stability of target measurements across space and time. The relative difference RD_{ij} between x_{ij} which is the observation of variable x at location i at time j, and the spatial average of x at the same time $< x >_j$, is defined as

$$RD_{ij} = \frac{x_{ij} - \langle x \rangle_j}{\langle x \rangle_j} \quad (1)$$

The MRD for location i becomes:

$$MRD_i = \frac{1}{N_t}\sum_{j=1}^{j=N_t} RD_{ij} \quad (2)$$

where N_t is the number of observation times and i = 1,2, ….N_i, where N_i is the total number of locations.

The standard error of the relative difference $SERD_i$ of the set $RD_{i,1}$, $RD_{i,2}$….$RD_{i,\,N-t}$ of the relative differences at location i over the observation period is computed along with the MRD_i as follows:

$$SERD_i = \frac{\sqrt{\frac{1}{N_t-1}\sum_{j=1}^{N_t}(RD_{ij} - MRD_i)^2}}{N_i^{0.5}}$$

The SERD serves as a metric to evaluate the degree of temporal stability present at a specific location. If $SERD_i$ is large, this value indicates that the MRD_i in location i is not stable. Conversely, if the value is low, this result indicates a strong temporal stability of the *E. coli* concentrations.

For the calculation of the MRDs, dates with nondetection counts exceeding 15% of the sample observations for that date were excluded from the analysis. The 15% value was recommended by the EPA for the threshold value above which simple substitution of half of the reported limit is not recommended (USEPA, 2009). These dates included 5/31/2016 (33% N.D.) and 7/19/2018 (30% N.D.) at P1 and 5/31/2017 (17.5% N.D.) and 8/7/2018) (17.5% N.D.) at P2. All other dates consisted of very few to no nondetections (< 5%) and were included in the MRD analysis. Sampling dates which were associated with rainfall events (6/22/2016 and 8/8/2016 at P2) were also analyzed separately for all statistical testing.

All statistical analysis was performed using PAST software v.3.2 (Hammer et al., 2001). Site maps were created using QGIS v3.18. Figures were generated using Sigmaplot v13 (Systat Software, CA).

3 Results

3.1 Interannual Variability of E. coli Concentrations

In 2016, *E. coli* concentrations steadily increased throughout the sampling period from late May to late July in P1 (Fig. 2). The 2017 sampling season contained consistently higher concentrations of *E. coli* than what was observed for P1 during the 2016 or 2018 sampling seasons. The 2018 sampling season in P1 showed the greatest variability in concentrations and did not show any consistent trend across the

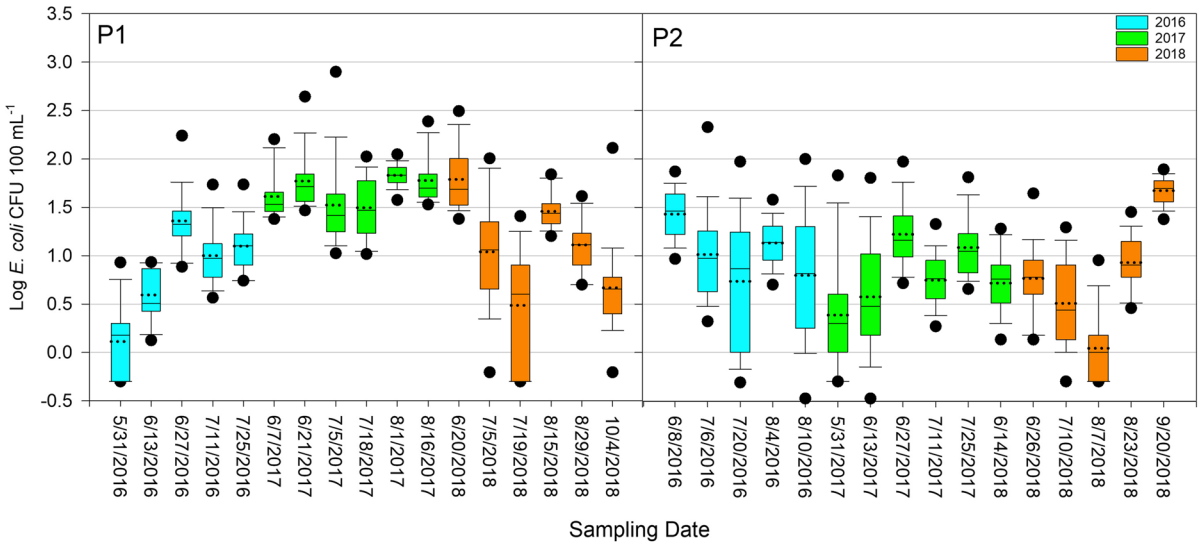

Fig. 2 Box and whisker plots showing the spread of the observed data. Upper and lower portions of boxes show the 25th to 75th percentile, respectively, (interquartile range) while whiskers show the 10th and 90th percentiles of the data, respectively. The solid and dotted lines represent the median and mean, respectively. Dots show the 5th and 95th percentiles

season. Mean logarithms of *E. coli* were 0.88 ± 0.51, 1.65 ± 0.31, and 1.09 ± 0.61 for 2016, 2017, and 2018, respectively (± separates mean and standard deviation). The Kruskal–Wallis ANOVA on ranks showed that *E. coli* concentrations, when pooled together by year, were significantly different ($P < 0.001$). Pairwise testing of any pair of years also showed significant differences ($p < 0.05$). The coefficient of variation for the log-transformed average *E. coli* concentrations at P1 was 59.1%, 18.8%, and 55.9% for 2016, 2017, and 2018, respectively.

Concentrations in P2 were similar to those values measured in P1 during the 2016 and 2018 years and displayed no consistent trend across years (Fig. 2). In P2, the across-season averages of *E. coli* were 1.01 ± 0.57, 0.80 ± 0.53, and 0.77 ± 0.59 log CFU 100 mL^{-1} for 2016, 2017, and 2018, respectively. The Kruskal–Wallis ANOVA on ranks showed that *E. coli* concentrations significantly differed between years in P2 (P = 0.001). Mann–Whitney pairwise testing showed that the concentrations measured in 2016 significantly differed ($P < 0.05$) from 2017 and 2018, but concentrations in 2017 did not significantly differ from those observed in 2018 ($P = 0.533$). In general, P2 did not show a consistent within-season trend of increase or decrease in concentrations with the progression of the sampling season. For P2, the CVs of logarithms of *E. coli* were 56.4%, 67.3%, and 77.2% for 2016, 2017, and 2018, respectively. The experiment-wide CVs for each pond when the data was pooled across years was 47.1% and 67.8% for P1 and P2, respectively.

Supplemental Fig. 1 shows the daily precipitation totals and average daily temperature at ponds P1 and P2 during the observation periods. On two dates at P2 within the 3-year observation period, samples were collected during or shortly after (< 8 h) heavy rainfall events. These dates were 6/22/2016 and 8/8/2017 and interestingly, both dates had very similar *E. coli* concentrations with very low standard errors at 3.07 ± 0.02 and 3.06 ± 0.17, respectively, indicating a high degree of mixing in the pond. Indeed, values of water quality parameters also demonstrated very low errors relative to the measured values (Supplemental Tables 1 and 2). On the 6/22/2016 rainfall event, inflow water was sampled from the culvert at location 12 and was found to have an average *E. coli* concentration of 3.92 log CFU 100 mL^{-1} which was about 7 times higher than the average value in the pond based on untransformed concentration data.

3.2 Site-Specific Variability of E. coli Concentrations

Concentrations of *E. coli* varied substantially across the two ponds during every year of the observations (Fig. 3). In accordance with Fig. 2, Fig. 3 shows that there were typically lower *E. coli* concentrations observed in P1 in 2016 and higher concentrations observed in 2017 with 2018 appearing somewhere intermediate. In general, samples from the pond interiors contained lower concentrations than those collected near the banks. On average, the concentrations of *E. coli* measured in bank and interior samples were 1.38 ± 0.16 and 1.12 ± 0.09 log CFU 100 mL^{-1}, respectively. A Kolmogorov–Smirnov test for equality of distributions showed that concentrations observed in the pond interior were significantly lower than those measured in the bank on multiple sampling dates but the number of cases of significance differed by year. In 2016, 3 of the 5 sampling dates (2nd, 4th, 5th dates) were found to contain significantly higher ($P < 0.05$) *E. coli* concentration distributions in the bank samples than the pond interior. In 2017, this significant difference occurred for 4 of 6 dates (2nd, 3rd, 5th, 6th). Conversely, in 2018, only 1 of the 6 sampling dates contained significantly different distributions (3rd date) while the other dates were found to contain more similar concentrations between the two location groups albeit always higher in the bank samples.

In P2, the inter-annual differences in *E. coli* concentrations across the sampling locations were less pronounced than in P1. This level of difference meant that despite large differences in the spatial averages observed throughout the experiment, the trends of high and low concentrations by location were preserved. Locations 8 through 13 show divergent concentrations in 2016 and 2017 whereas 2017 and 2018 were largely similar. The interior locations 25 through 33 demonstrated consistently lower concentrations than the bank samples collected across the rest of the pond. The 3-year average for the bank and interior locations was 0.97 ± 0.17 and 0.56 ± 0.11 log CFU 100 mL^{-1}, respectively. Interestingly, locations 25

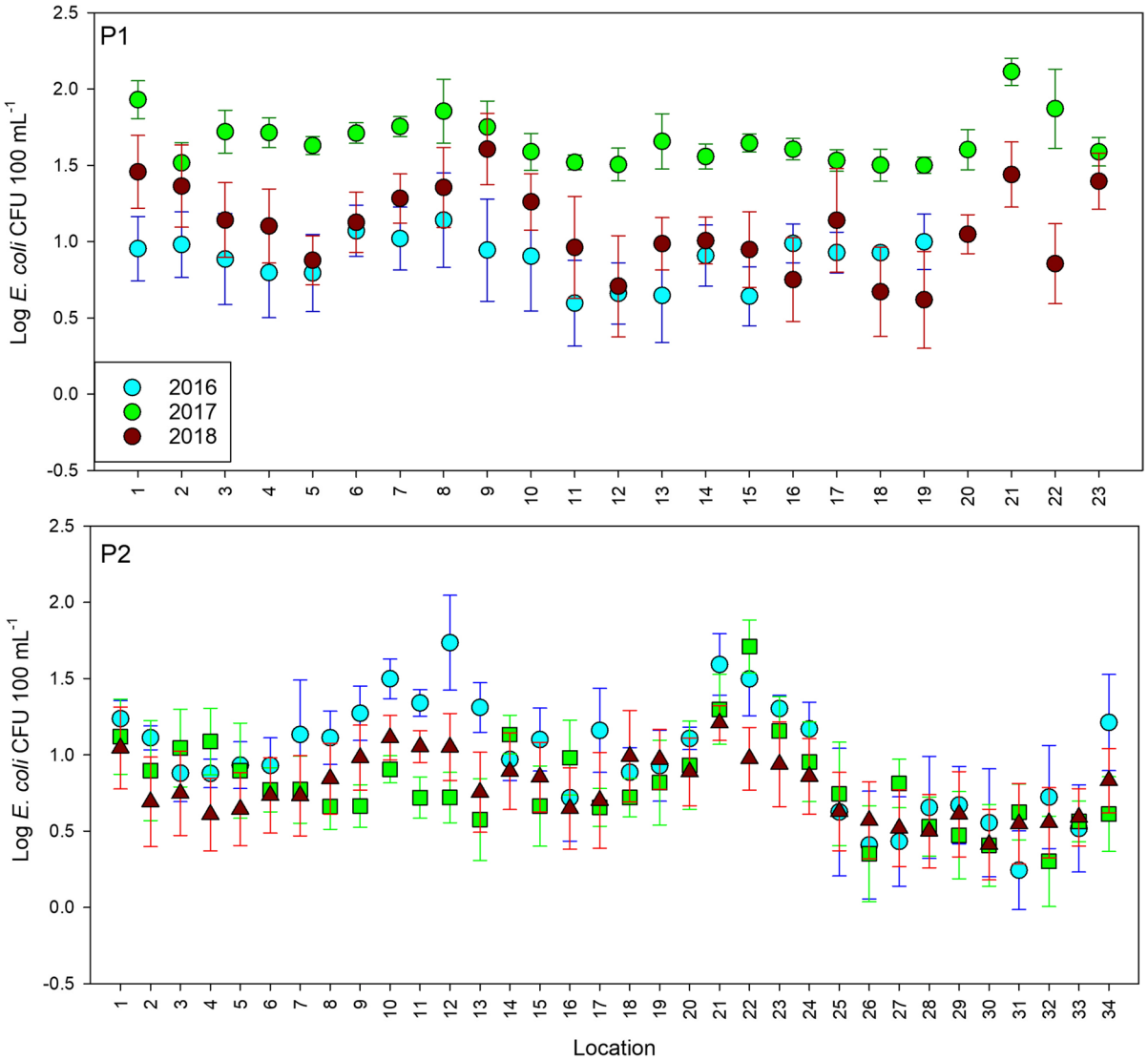

Fig. 3 Observed concentrations of *E. coli* at each location by year during the 2016–2018 monitoring period. Error bars show the standard error of the mean

and 34, which are adjacent on three sides to the bank showed concentrations more similar to bank samples than interior samples. The Kolmogorov–Smirnov test showed that in 2016 the 2nd, 3rd, and 5th sampling dates contained significantly different distributions of concentrations between the bank and interior. In 2017 only the 1st and 2nd sampling dates were found to have significantly different distributions ($P < 0.05$) and in 2018 all sampling dates but the 3rd date ($p = 0.068$) were significantly different.

3.3 Temporal Stability Assessment of E. coli Concentrations

The mean-relative-difference analysis pooled data from all 3 years for each pond and showed that some locations demonstrated temporal stability in *E. coli* concentrations with locations that were consistently lower, about, and above the spatial average on each sampling date (Fig. 4). The consistently low and consistently high locations or groups of locations may

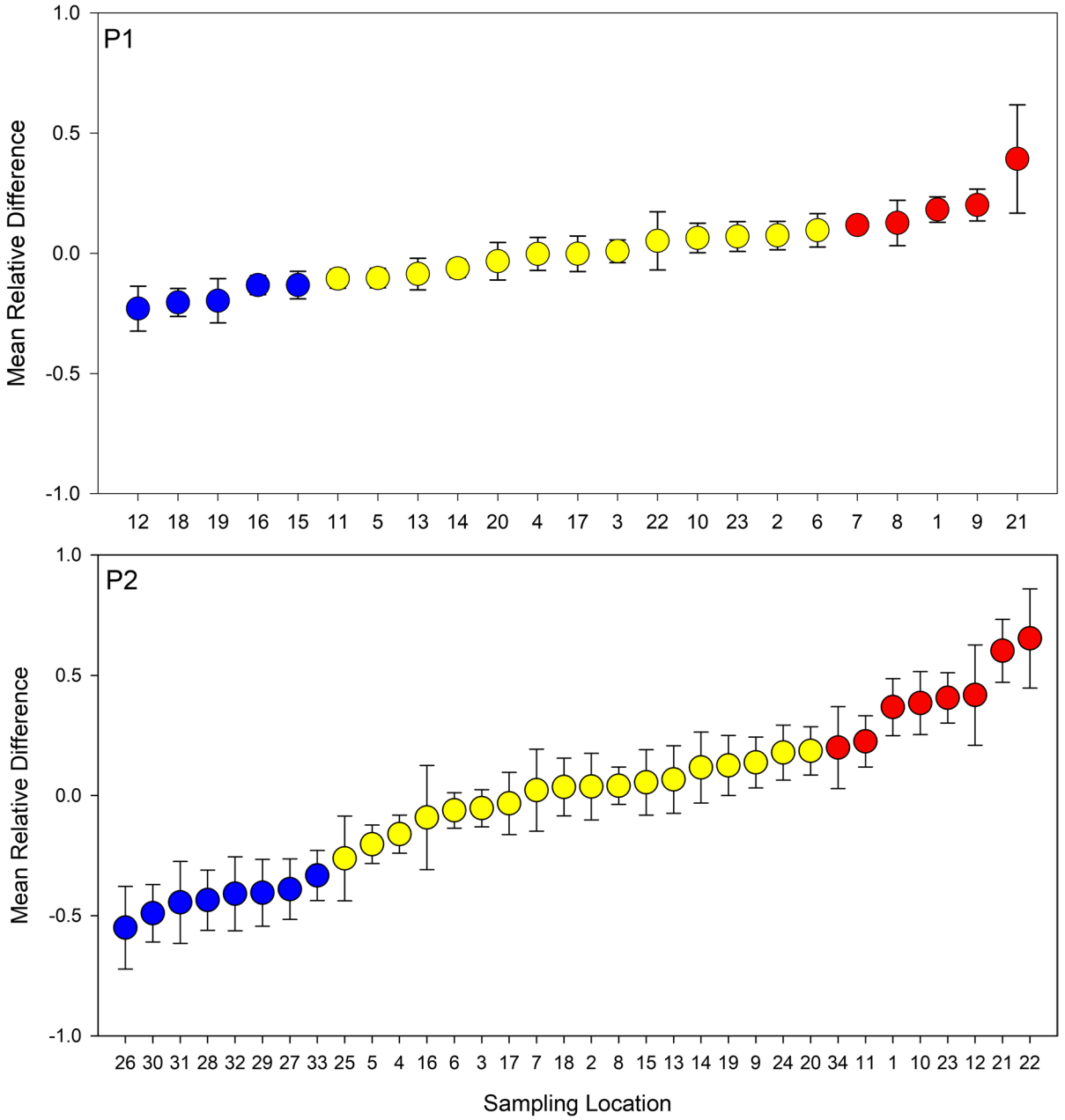

Fig. 4 Mean relative difference (MRD) values for Pond P1 (P1) and Pond P2 (P2). Symbol colors were selected based on those that are below the 25th quartile (blue), within the 25th and 75th quartiles (yellow), and above the 75th quartile (red)

be thought of as "cold" and "hot" spots or zones of *E. coli* concentrations in the pond, respectively. The degree of the temporal stability of any location presented in Fig. 4 is indicated largely by the size of the error bars present. Examination of the size of the error bars reveals locations that could potentially belong to another group of values (e.g., location 22 in P1 and location 12 in P2). However, the largest number of points is contained within the intermediate range, and, thus, these locations can be informative for determining the relative concentrations across all other locations.

The MRD graphs showed similar dynamics by location between the years although there was usually some shuffling of locations within a given group (low, medium, and high) (Supplemental Fig. 2). For instance, in P1, the hot spots in 2017 consisted of locations 3, 6, 9, 7, 8, 22, and 21 (presented in order by MRD value) and in 2018 this group consisted of locations 17, 9, and 21. Locations 9 and 21 did not change group but location 17 went from being low to being high in 2017 and 2018, respectively. Another example may be seen in the ranked order of locations in P2 within the cold and hot spots in 2017 and 2018 (Supplemental Fig. 3).

Overall, there was a moderate to high level of consistently in the ranked order of locations between years. In P1, the Spearman correlations between MRD values across years was $r_s = 0.524$ $p < 0.021$ for 2016 and 2017, $r_s = 0.842$ $p < 0.001$ for 2016 and 2018, and $r_s = 0.487$ $p = 0.018$ for 2017 and 2018. The correlation coefficients for each year's MRD and the 3-year average MRD was $r_s = 0.843$, 0.611, and 0.869 for 2016, 2017, and 2018 comparisons, respectively ($p < 0.001$ for each). P2 also showed a high degree of consistency in the ranks of locations from the MRD analysis. The Spearman correlation coefficients were 0.467 $p = 0.005$, $r_s = 0.789$ $p < 0.001$, and 0.452 $p = 0.007$ for comparisons between 2016 and 2017, 2016 and 2018, and 2017 and 2018, respectively. The strength of the correlations was generally strong between the 3-year average MRD values and each year: $r_s = 0.933$, $r_s = 0.645$, and $r_s = 0.896$ (all $p < 0.001$) for 2016, 2017, and 2018, respectively.

3.4 Correlation of MRDs of E. coli and Water Quality Variables

Average values of water quality variables are shown in Supplemental Tables 1 and 2 for P1 and P2, respectively. An MRD analysis was also performed on values of all measured variables and a correlation analysis was then performed to relate MRD values from *E. coli* concentrations with those values from the measured variables (Table 1). In P1 in 2016, the MRDs between *E. coli* and all measured water quality variables displayed significant negative relationships with similar strengths. In 2017, the direction of the correlations was similar to 2016 for that set of reported variables, however, the MRDs for pH and DO did not show significant relationships with *E. coli* MRDs. Correlations with C, SPC, turbidity, phycocyanin, and chlorophyll-a were found to be significant. FDOM showed a positive relationship with *E. coli* MRDs in both 2017 and 2018, but only in the latter year was the relationship significant. In 2018 in P1, pH, C, and DO MRDs all showed significant negative correlation with *E. coli* MRDs, while SPC, NTU, PC, CHL all showed positive relationships. Correlations of MRDs from the pooled datasets among all the years show several significant relationships. Temperature, pH, and DO MRDs demonstrated significant negative relationships, while the MRDs for NTU, PC, CHL, and FDOM showed moderate positive relationships with *E. coli* MRD values. SPC demonstrated a weak negative coefficient value when all years were pooled.

Table 1 Spearman correlation coefficients (r_s) between *E. coli* MRDs and MRDs of the listed water quality variables

	P1				P2			
	2016	2017	2018	All years	2016	2017	2018	All years
pH	**−0.681**	−0.136	**−0.489**	**−0.485**	**−0.426**	**−0.642**	**−0.490**	**−0.394**
C	**−0.646**	**−0.541**	**−0.609**	**−0.635**	**−0.419**	−0.311	**−0.351**	−0.303
DO	**−0.509**	−0.051	**−0.810**	**−0.437**	−0.312	**−0.523**	**−0.556**	**−0.45**
SPC	**−0.617**	**−0.434**	0.042	−0.299	−0.098	0.015	−0.044	−0.288
NTU	N.M	**0.53**	0.305	0.359	N.M	0.263	**0.436**	**0.607**
PC	N.M	**0.602**	**0.52**	**0.591**	N.M	0.311	**0.417**	**0.582**
CHL	N.M	**0.508**	**0.452**	**0.602**	N.M	**0.439**	0.176	**0.524**
FDOM	N.M	0.242	**0.673**	**0.669**	N.M	0.149	0.209	0.206

Values in bold indicate statistically significant ($P < 0.05$) correlations. Parameters are abbreviated as *C*, temperature; *DO*, dissolved oxygen; *SPC*, specific conductivity; *NTU* turbidity; *PC* phycocyanin; *CHL*, chlorophyll-a; *FDOM*, fluorescent dissolved organic matter; *N.M.*, stands for not measured

Like P1 in P2 during 2016, all correlations between *E. coli* MRDs and those values for water quality variables were negatively correlated, however, only the comparisons with pH and C were significant. In 2017, *E. coli* MRDs were negatively correlated with those values for pH, DO, and C with the former two showing significant relationships. SPC, NTU, PC, CHL, and FDOM all showed a positive correlation with *E. coli* MRDs, but only the CHL comparison was significant. In P2 during 2018, pH, C, and DO showed low to moderate negative significant relationships with *E. coli* MRDs, while SPC showed a very weak negative correlation. NTU, PC, CHL, and FDOM MRDs were all positively correlated with *E. coli* MRDs with the former two being significantly related. When all years of data for P2 were pooled, pH, C, DO, and SPC showed low to moderate negative relationships with *E. coli* MRDs, while NTU, PC, and CHL showed moderate and significant positive relationships. MRD values of FDOM showed a weak positive relationship with *E. coli* MRDs, which was not significant. The MRD correlations between water quality variables and *E. coli* showed the same directional relationship between ponds when all years of data were used albeit to different strengths and significances.

4 Discussion

The concentrations and concentration ranges of *E. coli* in the two studied ponds differed by year (Fig. 2). Annual variability of indicator organisms measured in the same season during consecutive years is not commonly reported, but the available literature has shown that concentrations may vary considerably. For example, Topalcengiz et al. (2017) measured *E. coli* concentrations for 3 years at six different agricultural ponds in Central Florida between 2012 and 2014. When analyzing their summer data (May and June) for 2013 and 2014, it was seen that in 5 of 6 ponds the concentrations in 2014 ranged from 3 to 13 times greater than those observed in 2013 and in 1 pond the concentration average was 118 times greater in 2014. Similarly, Jokinen et al. (2019) documented significant inter-annual differences in *E. coli* and *campylobacter* in an irrigation reservoir in Alberta, Canada. In year 1 of their study, microbial water quality exceedances were attributed to runoff from surrounding agricultural lands which occurred earlier in the season, whereas, in year 2 most of the exceedances occurred later in the season when there was little precipitation and many were attributed to wild birds and cattle. Conversely, Durham et al. (2016) reported similar concentrations of *E. coli* in the summer months for 6 lakes in Lubbock, Texas between 2011 and 2013. The largest differences in concentrations between summer seasons across their study were attributed to heavy precipitation events that are known to efficiently transport fecal material and organisms to surface waters with runoff as well as to disturb bottom sediments which seem to be a large reservoir for *E. coli* in the environment (Jenkins et al., 2015).

The present study was conducted with emphasis on avoiding measurements taken within close proximity to rainfall events to ascertain the expected concentrations during baseflow and when farmers are most likely to irrigate. For this reason, only two sampling events were performed at P2 shortly after heavy rainfall. During these sampling dates, the concentrations of *E. coli* in the pond were around 1000 CFU 100 mL^{-1} on both dates and the inflow water had concentrations roughly 10 times greater. Indeed, the USEPA has listed rainfall events as the greatest cause for temporal differences in indicator densities in surface waters (USEPA, 2010). Jenkins et al. (2015) discovered two orders of magnitude increase in fecal indicator bacteria for three ponds during stormflow events observed in their study. The authors attributed the elevated levels to runoff and sediment disturbances. Harris et al. (2018) measured the levels of *E. coli* and *Salmonella* before and after rainfall events in two irrigation ponds in GA and reported significantly higher concentrations of both organisms after precipitation events which agrees with the results of several other studies that involved measurements conducted during precipitation events (Steele et al., 2005; Chen & Chang, 2014; Kleinheinz et al., 2009). Farmers would likely need not irrigate crops for some time after heavy rainfall events, but more data is needed to determine the optimal waiting period before resuming irrigation and what factors may affect the wait time. One example comes from Jenkins et al. (2015) who reported that it took about 5 days for levels of *E. coli* and enterococci to return to pre-storm levels. Interestingly, the levels of Salmonella and *E. coli* O157 remained significantly higher 5 days after the event.

E. coli concentrations in both ponds showed a substantial amount of spatial variability in measurements taken across the ponds (Fig. 3). It was observed that on numerous dates the concentrations of *E. coli* were significantly higher in bank samples than in interior samples. Possible explanations for this observation may include decreased shading of interior locations relative to bank locations as solar radiation has been shown to increase indicator organism inactivation rates in pond water (Davies-Colley et al., 2000). Pond interior locations also had higher pH and DO levels than the banks (data not shown). Davies-Colley et al. (1999) reported that when pH values exceeded 8.5 the inactivation of *E. coli* in pond water increased, but the inactivation may also be partially related to elevated DO levels that are linked with photo-oxidative damage to fecal microorganisms in water. Bank samples are typically located along with shallower parts of ponds. The wind-driven movement of water against bank soils and sediments may cause the release of microorganisms and the settling process would be less effective near the banks as compared to the interior due to wave action. Finally, bank locations may be expected to have higher concentrations of *E. coli* because this interface is where runoff and groundwater enter, and both may be rich in fecal bacteria and nutrients depending on the conditions. It should be noted that the hot spots identified in P1 are at location 9 which coincides with a small beach where waterfowl enter the pond and location 21 where an ephemeral creek is located. In P2, location 12 is considered a hot spot as the location is adjacent to the inlet. Locations 21 and 22 may also be considered hot spots since both locations are adjacent to the residential property containing domesticated animals and a septic system.

Correlation coefficients were calculated between MRD values for *E. coli* and those of water quality variables (Table 1). In performing this analysis, we compared temporally stable patterns of variables rather than simply correlating the raw values which is the typical procedure. There was a considerable amount of consistency in the interrelationships among MRD values of *E. coli* and the values of the water quality variables. The strength of the interrelationships and the significance were the factors that varied across ponds and years. Correlation coefficients between pH and *E. coli* demonstrated a consistently negative relationship. A similar finding was reported by Draper et al. (2016) who reported significant, albeit weak, relationships between pH and aerobic plate count, total coliforms, fecal coliforms, and enterococci in irrigation waters on 39 farms in Pennsylvania over a 2-year period. Topalcengiz et al. (2017) also reported significant negative relationships between pH and *E. coli*, enterococci, and total coliforms in 6 ponds in Central Florida in a 3-year study. As previously noted, elevated pH levels (>8.5) have been linked with increased inactivation of indicator organisms in pond water (Davies-Colley et al., 1999). DO MRDs also showed consistent negative relationships with *E. coli* MRDs. The increase of pH and DO oftentimes occur simultaneously due to photosynthetic activity by planktonic organisms in water as dissolved CO_2 is converted into organic components that may serve as nutrients, and DO is released as a by-product. This process also spurs the dissociation of HCO_3 which further increases the pH (Zang et al., 2011). The positive relationship between *E. coli* MRDs and those of turbidity and dissolved organic matter may be related to the shielding effects that suspended materials provide for fecal microorganisms in water (Whitman et al., 2003). Similarly, the positive relationships with both PC and CHL may also be linked with decreased light penetration as well as the potential for cyanobacteria and algae to release dissolved organic carbon and other nutrients back into the water column which may benefit *E. coli* survival (Davis et al., 2005).

The MRD analysis was used to identify sampling locations which were consistently lower, about, or higher in *E. coli* concentrations than the average across the pond. Differences in hot and cold spots could then be related to activities on the shore or physicochemical properties of the water and the effects on fecal microorganisms in terms of changing the habitat conditions. Additional usefulness in computing MRD values for sampling locations is introduced by gaining the ability to use locations with low error, or high temporal stability, to estimate the concentrations across the rest of the pond. For example, if one were to develop an MRD distribution of *E. coli* in a pond, a location characterized by the consistent display of small errors might be used to help predict the concentrations in different locations based on the pre-established relationships between the locations in question. Similarly, the locations between the 25th and 75th percentiles of the MRD curve (indicated in yellow in Fig. 4) might be useful to help approximate

the average concentrations across the rest of the pond and would reduce the need for intensive sampling regimes. A similar type of procedure was performed by Pedrera-Parrilla et al. (2017) who were able to calculate an MRD curve for soil water contents and electrical conductivity from multiple sampling locations across a field. The authors defined a representative location from an MRD distribution as one with a low error which could be used as a constant bias to acquire the average soil water content and electrical conductivity over the observation area. One may expect a much weaker temporal stability in aquatic systems than in soil systems due to the mixing and movement of water. However, the results of the current study indicate that even with the occurrence of mixing, the temporal stability of *E. coli* concentrations was still observed. The uncertainty of the MRD curves was greater for P2 than P1 which may reflect differences in the relative degree of mixing, the fecal microbe inputs, and microbial survival conditions between the two ponds.

It must be acknowledged that the present study only studied two irrigation ponds in the Mid-Atlantic region over the course of 3-years. Indeed, many thousands of ponds exist in this area with a smaller number actively used for crop irrigation. The objective of this work was not to document *E. coli* dynamics in a large number of ponds, rather it was to demonstrate the existence of spatial and temporally stable hot and cold spots of *E. coli* concentrations which have implications for water quality sampling and the design of future monitoring programs. Pachepsky et al. (2018) showed that temporally stable patterns of *E. coli* can be detected within a single year of observations. This work aimed to assess the interannual variability of those patterns and answer the question of whether or not they are stable outside of the span of a single year. The findings presented show that within these two ponds, the temporal stability of *E. coli* concentrations can indeed be persistent. Future work should aim to determine the applicability of the MRD method on additional study sites within and outside the region examined in this work.

The existence of relatively stable spatial patterns of *E. coli* concentrations has consequences for both microbial quality monitoring and management of irrigation ponds. We note that the discovery of stable spatial patterns in this work required a high spatial density of sampling that has not been implemented in previously published research. The spatial detail of the monitoring should be increased for both detecting and accounting for temporally stable patterns. Results of this work show that sensing water quality variables may be instrumental for establishing *E. coli* MRD patterns because of the correlations between MRDs of water quality parameters and *E. coli.* Additionally, the use of drone-based imagery may be an appropriate source of data on spatial variation of major microbial habitat-affecting parameters such as chlorophyll-a, suspended solids, and dissolved organic matter concentrations (Pyo et al., 2016; Kutser et al., 2020). The existence of areas in the ponds with persistently lower *E. coli* concentrations makes these areas attractive for the placement of the irrigation water intake (Stocker et al., 2020). As irrigation progresses, water from different parts of the pond flows towards the intake and one may expect changes in microbial water quality at different times during the same irrigation event if substantial spatial heterogeneity of concentrations is observed. Future work should evaluate additional methods to increase the detection and knowledge of spatial patterns in microbial water quality studies which presents a promising research avenue.

5 Conclusions

Large interannual spatial and temporal variability of *E. coli* concentrations was documented in two irrigation ponds from biweekly grid sampling for three years during the growing seasons. Bank and interior sampling locations typically contained significantly different concentrations. In each of the ponds, the MRD analysis identified groups of sampling locations that had *E. coli* concentrations consistently below or consistently above the average concentrations across each pond. Such stable spatial patterns were related to differences in habitat conditions for *E. coli* across the ponds and pathways in which the microorganism may enter. For each pond, the individual MRDs for each year demonstrated significant positive relationships across years. The mean relative differences were also calculated for several water quality variables. The MRD patterns in dissolved oxygen, pH, and temperature showed consistently negative relationships with *E. coli* patterns in both ponds for the 3 years of the study, while the turbidity, phycocyanin, chlorophyll-a, and dissolved organic matter showed positive relationships in all cases. Future work should continue to search for efficient methods for establishing spatial

patterns of *E. coli* in surface waters. The MRD analysis shows promise to be beneficial for the improvement of monitoring and management of the microbial quality of irrigation water from ponds.

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Author Contribution MDS planned the monitoring, collected and analyzed the data, and co-wrote the manuscript. YAP conceptualized the monitoring project, acquired funding, guided the research, and co-wrote the manuscript. JES and BJM collected the data and helped plan the project, RLH provided support for the project and critically evaluated the manuscript, MK provided funding and resources for the project and critically evaluated the manuscript.

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Data Availability The datasets generated during/or analyzed the current study are not publicly available as of the submission date as the material is part of an ongoing degree program for Matthew Stocker (PhD) and Jaclyn Smith (M.Sc.). Data will become fully available after the completion of the degree programs. Requests for datasets in part or in whole can be requested from the corresponding author on reasonable request at any time.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Granted by all authors.

Conflict of Interest The authors declare no competing interest.

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