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Optimal strategies for monitoring irrigation water quality

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ABSTRACT

The quality of irrigation water drawn from surface water sources varies greatly. This is particularly true for waters that are subject to intermittent contamination events such as runoff from rainfall or direct entry of livestock upstream of use. Such pollution in irrigation systems increases the risk of food crop contamination and require adoption of best monitoring practices. Therefore, this study aimed to define optimal strategies for monitoring irrigation water quality. Following the analysis of 1357 irrigation water samples for *Escherichia coli*, total coliforms, and physical and chemical parameters, the following key irrigation water collection approaches are suggested: 1) explore up to 950 m upstream to ensure no major contamination or outfalls exists; 2) collect samples before 12:00 p.m. local time; 3) collect samples at the surface of the water at any point across the canal where safe access is available; and 4) composite five samples and perform a single *E. coli* assay. These recommendations comprehensively consider the results as well as sampling costs, personnel effort, and current scientific knowledge of water quality characterization. These strategies will help to better characterize risks from microbial pathogen contamination in irrigation waters in the Southwest United States and aid in risk reduction practices for agricultural water use in regions with similar water quality, climate, and canal construction.

1. Introduction

Historically, water quality guidelines have focused on drinking, waste, and recreational sectors, excluding waters used throughout the production of food crops. The recently developed "Standards for Growing, Harvesting, Packing, and Holding Produce for Human Consumption" establish safety guidelines for the US agriculture industry (Food and Drug Administration, 2015). Although these guidelines are scientifically based, they fail to grasp the complexity of irrigation systems and offer few suggestions for the appropriate monitoring of irrigation water safety.

The US Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) includes microbiological rules for irrigation water that are based on epidemiological studies undertaken at ocean and freshwater beaches. Little evidence exists that relates FSMA to the associated risks for fresh produce and irrigation waters. FSMA guidelines require untreated surface water used for irrigation be tested for *Escherichia coli* (*E. coli*) 20 times over 2–4 years and then <5 times annually. In water used for any purpose besides the growing of sprouts, hand washing, or direct application to food surfaces, *E. coli* concentrations cannot exceed 126 colony forming units (CFU)/100 ml, using a geometric mean of at least five samples taken over multiple days (e.g., a monthly geometric mean) or 410 CFU/100 ml in a single sample (i.e., a statistical threshold value [STV]). If the *E. coli* concentration exceeds this STV, the water can still be used to irrigate food crops if an appropriate time prior to harvest is allowed, assuming a 0.5-$\log_{10}$ die-off of *E. coli* per day. For water used in sprout irrigation, applied directly to food surfaces, or used for hand washing, *E. coli* regulations are as stringent as for drinking water (i.e., 0 CFU/100 ml). If *E. coli* concentrations exceed any of these thresholds, the water cannot be used for irrigation (Food and Drug Administration, 2015). These rules aimed at food safety fail to take under consideration the rapid spatial and temporal changes of microbial concentrations in water.

Water research undertaken in rivers, lakes, oceans, reservoirs, and irrigation canals has routinely demonstrated significant changes in microbe concentrations on short spatial and temporal scales (Boehm, 2007; Haack et al., 2004; Juahir et al., 2011; Song et al., 2012; Verhougstraete and Rose, 2014; Won et al., 2013). For instance, one study determined that *Enterococcus* concentrations at California beaches typically varied by 60% over 10 min, but could vary by as much as 700% (Boehm, 2007). Similarly, the

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FDA guidelines fail to consider the spatial variations of microbial water quality, potentially leaving the food product vulnerable to contamination.

Bacterial concentrations undergo rapid change along stream length, throughout the vertical water column (Agogué et al., 2011; Karl, 1978; Krempin and Sullivan, 1981; Llirós et al., 2010), and across stream width (Byappanahalli et al., 2003; Jones et al., 1995; Whitman et al., 2006). Thus, a single sample may not provide an adequate representation of the true microbial water quality in an irrigation water canal. Water quality scientists have also noted the implications of a single sample versus multiple samples for management actions (e.g., opening or closing of a beach) (Bertke, 2007; Reicherts and Emerson, 2010). Kinzelman et al. (2006) determined that compositing multiple lake water samples and assaying with a single test was not statistically different ($P > .02$) than analyzing multiple individual samples and reporting an average; both approaches called for similar management actions. The benefits of compositing samples include the ability to collect multiple samples from various locations (more representative of water quality) and reduced costs (by performing a single test), while still providing at least the same level of protection as collecting single or multiple samples. Considering previous research in non-irrigation systems, it is inadequate to base the safety of an entire irrigation canal on the results of a single sample.

In addition to the numerous surface water studies previously mentioned, irrigation waters have been examined for microbial contamination. Fecal indicator bacteria (e.g. total coliforms, *E. coli*, enterococci), *Salmonella* spp., *Staphylococcus aureus*, Microsporidia, *Giardia*, and *Cryptosporidium,* Noroviruses, *Campylobacter* spp., and *Clostridium perfringens* have been measured in irrigation waters throughout the world (Gerba and Choi, 2006; Ijabadeniyi et al., 2011; Kayed, 2017; Thurston-Enriquez et al., 2002). One study found irrigation water is a major risk factor for bacterial contamination of fresh lettuce due to the detection of *E. coli* and *Campylobacter*. spp. (Holvoet et al., 2014). Irrigation water and food safety concerns are further highlighted by a study that demonstrated hepatitis A virus and *Salmonella* present in water used to irrigate iceberg lettuce was associated with exceedances of the U.S. Environmental Protection Agency's acceptable annual risk level of 1:10,000 (Stine et al., 2005). Produce commonly grown using irrigation water includes corn, orchard crops, and vegetables,all of which have to potential to be consumed raw and further increasing the potential for infection from contaminated irrigation water ("USDA Economic Research Service," 2017). Together, these studies represent the diversity of microbial water quality and the importance of understanding irrigation water quality to protect fresh produce.

To help ensure adequate water safety and reduce the risks for agricultural water, microbial testing practices must be based on irrigation water-specific research, not adapted from drinking and recreational water studies. This is critically important given that during the winter months, more than 90% of all leafy greens consumed in the US are grown in the Southwest region of Yuma, AZ (http://bit.ly/2jhuwb1, accessed on 8 February 2017). In addition, Southern California produces 15% of the lettuce and leafy greens consumed by the US overall (http://bit.ly/2k4ceHK, accessed on 8 February 2017). Due to the importance of this region for fresh produce production and the current knowledge gaps in irrigation water quality science, this study aimed to better understand the spatial and temporal variations of microbial concentrations in irrigation canals, to produce a comprehensive monitoring plan, and to reduce pathogen exposure risks at the point of irrigation water application to food crops. To this end, there were four study objectives: 1) determine the most effective time of day for irrigation water monitoring; 2) define canal cross-sectional sampling locations; 3) delineate the transport of microorganisms in irrigation canals; and 4) determine the suitability of collecting single, multiple, or composite irrigation water samples for analysis.

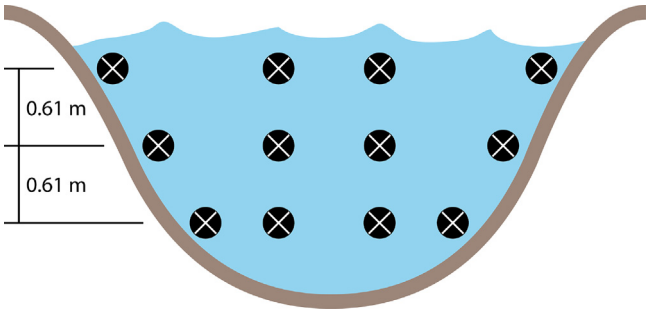

Fig. 1. Sampling schematic for defining the appropriate canal transect sample collection point.

2. Materials and methods

2.1. Site location

Sampling sites were selected following discussions with scientists from the University of Arizona and local agricultural extension centers. Samples were collected from a total of 93 unique sites among Yuma and Maricopa Counties, AZ and Imperial County, CA in the southwestern US. Sampling sites included a mixture of main, lateral, and sub-lateral canals and both cement-lined and unlined canals with varying flow dynamics. In addition, some locations were located in urban and others in rural areas.

2.2. Field analysis and sample collection

For all samples, the water temperature, air temperature, conductivity, total dissolved solids, pH, and relative humidity were measured in the field using the Multiparameter PCS Testr 35 (Oakton Instruments, Vernon Hill, IL) and the Fisherbrand Traceable Memory Hygrometer/Thermometer (Thermo Fisher Scientific, Waltham, MA). Samples were placed on ice in a cooler and transported to the laboratory for microbial processing and additional analyses (e.g., turbidity). Continuously recorded environmental variable data (wind speed and direction, barometric pressure, and antecedent precipitation) from National Weather Service stations were retrieved from the University of Utah's MesoWest interface (MesoWest, http://mesowest.utah.edu/, accessed on 10 December 2015).

To account for seasonal variations in microbial concentrations, weather variability, crop production, and water use practices, grab samples were collected between December 2014 and November 2015 using sterile 1L wide-mouth HDPE bottles (Nalgene Co., Rochester, NY). The depth below surface, the distance from the bank, the time of day, and collection location were study objective-dependent as detailed below.

To determine the most suitable time of day for irrigation water sampling, grab samples were collected 0.15 m below the water surface near the canal bank at the same site four times per day (i.e., before 09:00, 09:00–12:00, 12:00–13:00, and after 13:00). To define appropriate collection points in a canal cross-section, grab samples were collected vertically through the canal water column (at the water surface and 0.61 m and 1.22 m below the surface) and horizontally across canal transects (at both banks and ¼ of the distance of the canal width from each bank). A schematic of this sampling approach is presented in Fig. 1. To determine the best collection, processing, and results representation approach, three sampling approaches were investigated: approach A included collecting a single sample from a single collection point at 0.33 m

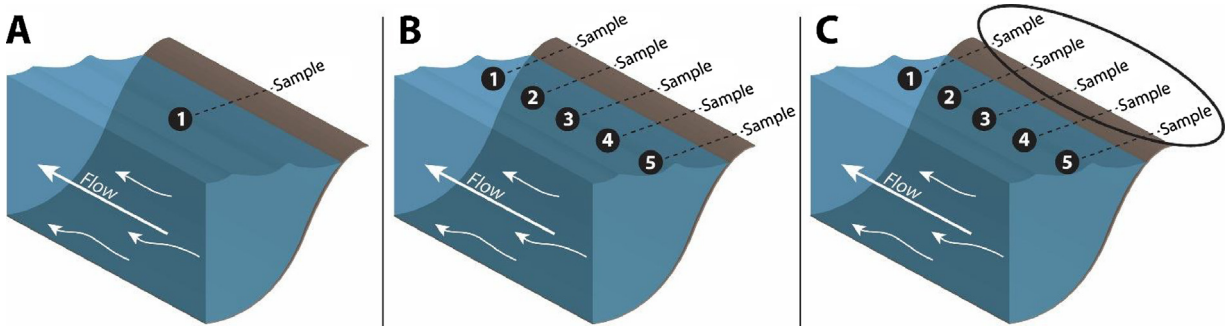

Fig. 2. Schematic for addressing the most appropriate sampling approach using A) single sample; B) multiple samples, assayed individually then geometric mean calculated; and C) multiple samples, composited into a single sample and then assayed.

from the canal bank and 0.15 m below the water surface; approach B involved collecting five samples from the same canal stretch (0.15 m below the water surface and 0.33 m from the canal bank, 2 m apart), assaying each sample individually, and calculating a geometric mean of the five samples; and approach C involved collecting five samples from the same canal stretch (0.15 m below the water surface and 0.33 m from the canal bank, 2 m apart), adding equal 20 ml volumes of well-mixed aliquots from each discrete sample to a sterile bottle to form a composite sample, then assaying in an identical manner to a single sample (100 ml total) and reporting the results as a single value. Diagrams of these sampling collection approaches are shown in Fig. 2.

To better understand the transport of microorganisms in irrigation systems, total coliforms and *E. coli* were measured following the same plug of canal water as it traveled downstream in three different canals using a flow tracking bottle. Grab samples of this same plug of water were collected using sterile 1 l HDPE wide-mouth bottles downstream at distances of 0 m, 322 m, 644 m, 966 m, 1288 m, 1609 m, 1931 m, and 2253 m from the designated starting point. The bacterial transport experiments were performed five times per canal.

Additionally, coliphage MS2 (ATCC 15597-B1; American Type Culture Collection, Manassas, VA) was used to seed the same three canals (one experiment per canal) to serve as a microbial tracer organism. This non-pathogenic virus has been used as a surrogate for many pathogens in various environments (Reynolds et al., 2015; Sinclair et al., 2009; Valdez et al., 2015). A 200 ml volume of MS2 coliphage at a concentration of approximately 1×10^{12} plaque forming units (PFU) was mixed with 20 l of canal water. This mixture was dispersed across the width of the canal and the plug of water containing the MS2 was followed downstream using a flow tracking device. Grab samples of this same plug of water were collected as before at distances of 0 m, 322 m, 644 m, 966 m, 1288 m, 1609 m, 1931 m, and 2253 m from the seeding point, in addition to 81 m, 161 m, and 241 m to limit the potential for non-detection in measurements taken further from the seeding point. The bacterial and viral tracer samples were placed on ice in a cooler and transported to the laboratory for processing and assay.

2.3. Laboratory analyses

E. coli and total coliforms were enumerated in all water samples using the Colilert Quanti-Tray® (IDEXX Laboratories, Westbrook, ME) most probable number (MPN) method following the manufacturer's instructions. Following incubation at 37 °C for 24 ± 2 h, yellow wells were recorded as positive for total coliforms and wells fluorescing "blue" under ultraviolet (UV) light were recorded as positive for *E. coli*. Positive and negative controls were used in coliform testing.

Coliphage samples were serially diluted with sterile phosphate-buffered saline (PBS; pH 7.4; Sigma-Aldrich, St. Louis, MO) to create 10, 1, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} ml duplicate subsamples. Double agar layers of tryptic soy agar (Difco, Sparks, MD) were utilized to detect MS2 coliphage on its specific host bacterium (*E. coli* ATCC 15597) following the double agar overlay method (Adams, 1959; Abbaszadegan et al., 1997). Clearings in the host lawn were counted and reported as PFU/100 ml. Sterile reagent water was used as a negative control for verification of the method integrity. MS2 coliphage background measurements were taken before seeding in the canal.

2.4. Statistical analyses

All bacterial data were log transformed prior to performing statistical analyses to minimize skewness and to ensure a normal distribution. Pearson Correlation analysis was used to identify relationships between microbial concentrations and independent variables (e.g., physical, chemical, weather, canal discharge rates). A significance level cutoff of $\alpha = 0.05$ was used for all correlative statistical tests. Stata Statistical Software (StataCorp, College Station, TX) was used for traditional statistical analyses, including Two-tailed *t*-tests, Kruskal-Wallis tests, Wilcoxon signed-rank tests, and Spearman's rank correlation tests.

3. Results and discussion

A total of 1328 samples were collected from 84 unique sites and were measured for *E. coli*, total coliforms, pH, conductivity, turbidity, total dissolved solids (TDS), air temperature, water temperature, and relative humidity. Summary results for these water quality measurements by region are provided in Table 1. Across all 1328 sites, statistically significant correlations were identified between *E. coli* and air temperature ($r = 0.13$, $P = .0003$), water temperature ($r = 0.26$, $P < .0001$), relative humidity ($r = -0.23$, $P < .0001$), pH ($r = 0.15$, $P < .0001$), conductivity ($r = -0.20$, $P < .0001$), turbidity ($r = -0.29$, $P < .0001$), and TDS ($r = -0.20$, $P < .0001$). From an additional 9 unique sites, 29 samples were measured for coliphage.

The first objective of this study was to determine the appropriate time of day for irrigation water monitoring. Samples (n = 802) were collected at the same location on the same day at four different time points: before 09:00 (n = 185), between 09:00–12:00 (n = 222), between 12:00–13:00 (n = 173), and after 13:00 (n = 222). Overall, *E. coli* ranged from 0.5 MPN/100 ml to >2419.6 MPN/100 ml [geometric mean (GM) = 20.2 MPN/100 ml] and total coliforms ranged from 77.1 MPN/100 ml to >2419.6 MPN/100 ml (GM = 821 MPN/100 ml). Results for *E. coli* and total coliforms in samples collected by time of day are shown in Table 2. While there were statistically significant differences between samples collected in the morning versus the afternoon, the most statistically significant

Table 1
Geometric mean ± the standard deviation of water quality measurements by region.

Region	n	*E. coli* (MPN/ 100 ml)	Coliforms (MPN/ 100 ml)	pH	Conduct. (μS/cm)	Turbidity (NTU)	TDS (mg/L)	Air Temp. (°C)	Water Temp. (°C)	Relative Humidity (%)
A	752	11.2 ± 133	826 ± 956	8.47 ± 0.42	1420 ±403	4.62 ± 31.6	846 ± 48.4	27.3 ± 10.2	20.9 ± 6.51	18.2 ± 20.7
B	286	33.9 ± 98.7	714 ± 667	8.63 ± 0.24	1230 ± 161	4.32 ± 31.9	869 ± 115	22.2 ± 4.58	22.1 ± 3.31	24.6 ± 25.6
C	290	9.45 ± 190	1000 ± 786	8.67 ± 0.14	1190 ± 15.7	2.72 ± 13.2	845 ± 11.2	23.3 ± 5.13	16.9 ± 1.71	29.8 ± 19.1
Total	1328	15.6 ± 143	836 ± 872	8.54 ± 0.35	1330 ± 340	4.07 ± 28.7	851 ± 69.2	25.3 ± 8.81	20.3 ± 5.56	22.1 ± 21.8

Table 2
Geometric mean ± the standard deviation for *E. coli* and total coliforms by sampling time.

Time of day	n	*E. coli* (MPN/100 ml)	Total Coliforms (MPN/100 ml)
Before 09:00	185	28.0 ± 217	935 ± 906
09:00–12:00	222	22.4 ± 98.2	826 ± 894
12:00–13:00	173	18.0 ± 115	908 ± 935
After 13:00	222	13.6 ± 119	670 ± 929

Table 3
Geometric mean ± the standard deviation for *E. coli* by sample depth and cross-section location.

Depth (m)	Cross-section Position	n	*E. coli* (MPN/100 ml)
0	Bank + Center	100	8.32 ± 172
0	Bank	49	7.81 ± 204
0	Center	51	8.85 ± 135
0.61	Bank + Center	100	8.90 ± 184
0.61	Bank	50	8.70 ± 168
0.61	Center	50	9.11 ± 201
1.22	Bank + Center	100	7.97 ± 199
1.22	Bank	49	8.49 ± 120
1.22	Center	51	7.50 ± 254
0–1.22	Bank + Center	300	8.39 ± 185
0–1.22	Bank	148	8.33 ± 166
0–1.22	Center	152	8.45 ± 201

Table 4
Geometric mean (GM) ± the standard deviation (SD) for bacteria by distance downstream from canal seeding point.

Downstream Distance (m)	*E. coli* (MPN/100 ml)		Total coliforms (MPN/100 ml)	
	n	(GM ± SD)	n	(GM ± SD)
0	15	10.5 ± 19.5	15	1810 ± 687
322	15	9.03 ± 26.2	15	1810 ± 687
644	15	7.21 ± 19.7	15	2030 ± 583
966	15	10.5 ± 32.3	15	1810 ± 687
1288	15	8.03 ± 18.1	15	2020 ± 620
1609	15	5.29 ± 16.5	15	1980 ± 631
1931	15	6.69 ± 12.7	15	2010 ± 579
2253	15	6.52 ± 12.9	15	1710 ± 756
Total	120		120	

difference between the samples collected for *E. coli* (Two-tailed *t*-test, $P < .0001$) was between those collected from 07:00–12:00 versus 12:00–16:00, with higher *E. coli* concentrations detected in the morning (GM = 24.8 ± 164 MPN/100 ml) compared to the afternoon (GM = 15.4 ± 117 MPN/100 ml). No such relationship was found with total coliforms.

Morning is thus the most conservative time to sample which supports previous studies (Lubo Liu et al., 2006; Whitman et al., 2004). In addition, the microbial water quality in the Southwest likely benefits from the high solar radiation levels previously shown to reduce bacterial concentrations. The Southwest has the highest solar radiation levels in the US (Nationa Renewalble Energy Laboratory, 2009). Therefore, the bacterial numbers would be expected to be reduced during the daytime hours with increasing exposure to the sun.

The second study objective was to determine the appropriate sample collection point within the cross-section of canals based on multiple spatial samples (Fig. 1). *E. coli* measurements were grouped in all combinations of depth and distance to the bank and the means were compared. Overall, *E. coli* ranged from <1.0 to 1550 MPN/100 ml (GM = 8.4 MPN/100 ml). *E. coli* in the top, middle, and bottom horizontal transects ranged from <1.0 to 792 MPN/100 ml (GM = 8.32 MPN/100 ml), <1.0 to 1300 MPN/100 ml (GM = 8.9 MPN/100 ml), and <1.0 to 1550 MPN/100 ml (GM = 7.97 MPN/100 ml), respectively. *E. coli* ranged from <1.0 to 1410 MPN/100 ml (GM = 8.33 MPN/100 ml) near the canal banks and from <1.0 to 1550 MPN/100 ml (GM = 8.45 MPN/100 ml) in the center of the canals. There were no statistically significant differences between depth groups (Kruskal-Wallis test, $P = .76$), transect vs. bank groups (Wilcoxon rank-sum test, $P = .73$), any depth by transect group (Wilcoxon rank-sum tests, $P > .45$), or any transect by depth group (Wilcoxon rank-sum tests, $P > .70$). Results for *E. coli* measurements at cross section points are provided in Table 3.

Results from this objective demonstrate homogenous microbial concentrations throughout the canals. No previous studies were identified that measured bacterial concentrations in irrigation canal transects. However, previous research conducted in non-irrigation water matrices demonstrated point source pollution and sediment resuspension produced rapid microbial concentration fluxes within the water column which return to a homogenous state over space and time (McDaniel et al., 2013; Pandey and Soupir, 2014; Pandey et al., 2016; Rehmann and Soupir, 2009). In the current study, the homogeneity of *E. coli* concentrations likely indicates minimal direct fecal contamination, sediment presence, or sediment resuspension in the studied canals. Confirming this will require additional research. Based on these results, we suggest irrigation monitoring samples be collected wherever safe access is achievable.

The third study objective was to address the transport of microorganisms in irrigation canals. The initial approach involved enumerating *E. coli* and total coliforms from grab samples collected in three different irrigation canals a total of five times each. However, samples (n = 40 per canal) taken from a plug of water and analyzed for *E. coli* and total coliforms did not produce a clear pattern of transport properties in these three canals (Table 4). To enhance this objective, additional samples (n = 29) were analyzed as part of a MS2 virus tracer seeding experiment using three canals (Table 5). MS2 coliphage concentrations ranged from <0.1 to 182,000 PFU/100 ml with an overall geometric mean of 608 PFU/100 ml.

The MS2 coliphage tracer highlights the amount of mixing and dilution that occurs in canals over long stretches. While these can vary greatly from canal to canal, there is a statistically significant negative relationship between distance from the seed point and coliphage concentration (Spearman's rank correlation, rho = −0.91, $P < .0001$). MS2 concentrations dropped gradually with increasing distance from the canal seeding point, as shown in Table 5. A 1-$\log_{10}$ reduction in the viral tracer concentration was observed roughly 161 m downstream of the seeding point and a 2-$\log_{10}$ reduction was observed by 966 m downstream. Differences in dilution effectiveness may be attributable to canal characteristics such as discharge

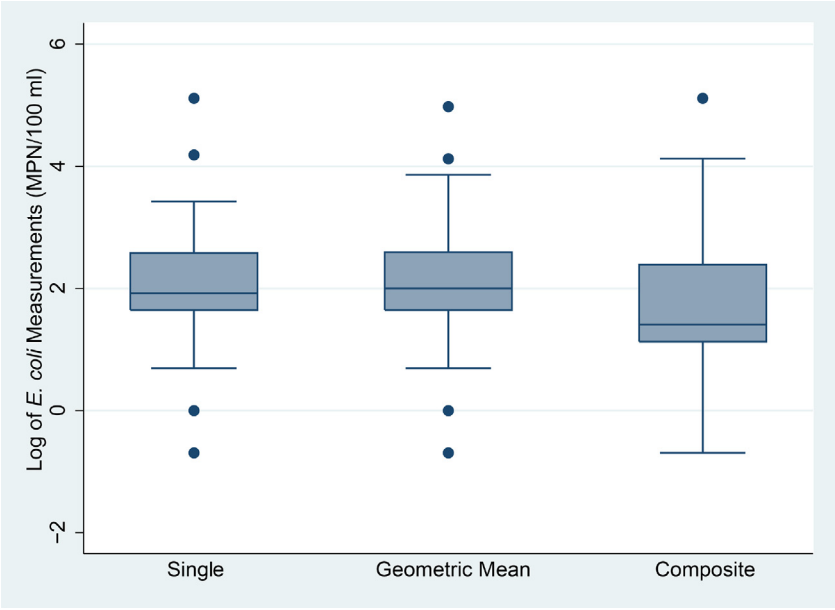

Fig. 3. *E. coli* measurements from 1) single sample, 2) geometric mean of five samples, and 3) a composite of five samples. The box represents the 25th and 75th percentiles, the horizontal line within the box represents the median concentration, the vertical lines extending from each box represent the range of *E. coli* concentrations (excluding outliers), and the dots represent outliers.

Table 5
Concentrations and geometric mean (GM) ± the standard deviation (SD) of MS2 coliphage by distance downstream from canal seeding point.

Downstream Distance (m)	MS2 Coliphage (PFU/100 ml)			
	Canal 1	Canal 2	Canal 3	(GM ± SD)
0	14,500	182,000	Not Tested	51,318 ± 118,462
81	4850	40,000	Not Tested	13,928 ± 24,855
161	3650	1440	Not Tested	2293 ± 1563
241	2710	6550	Not Tested	4213 ± 2715
322	2650	4350	4450	3716 ± 1012
644	980	2080	790	1172 ± 696
966	295	1310	301	488 ± 581
1288	157	1440	129	308 ± 749
1609	71	325	27	85.0 ± 161
1931	28.2	166	7	32.0 ± 86
2253	215	92	< 0.10	9.95 ± 108

velocity or lining type, or to atmospheric factors including wind speed or precipitation. Meanwhile, *E. coli* and total coliform concentrations showed no discernable pattern with increasing distance from the first measurement point (Table 4). Additional testing is needed to better understand the factors that promote dilution and mixing of a microbial contamination event in irrigation canals.

The fourth study objective was to determine if the best approach was to collect and report a single sample or to collect five separate samples and assay them individually (reporting a geometric mean) or as a composite (Fig. 2). The results from a composite of five samples was found to be significantly different from both a single sample ($P = 0.036$) and also from the geometric mean of the five samples tested individually ($P = 0.005$) (Wilcoxon signed-rank test). Monitoring approaches that collect a single water sample will typically fail to meet a very broad coefficient of variation; however, collecting and processing multiple samples to provide adequate representation of water quality may be cost prohibitive. A composite sample is a cost-effective alternative to testing a single or multiple samples individually. In the current study, a composite sample was the most representative sampling option of the water quality within a canal, in that it provides more information than a single sample while minimizing the effects of outlying data (high or low bacterial numbers that can lead to a misrepresentation of the water quality in a single sample or in multiple samples that are heavily influenced by one sample with outlying numbers) (Fig. 3). In addition, a composite sample requires only nominally more time and money to collect and analyze than a single sample.

Composite sampling can significantly reduce the analytical costs by reducing the number of samples while increasing the likelihood of capturing the high variation inherent to microbe concentrations in water that would likely be missed with a single sample. Previous surface water studies have shown that composite samples provide better protection of human exposure to elevated bacteria concentrations compared to a single sample (Reicherts and Emerson, 2010; Whitman and Nevers, 2004). However, even using composite samples, a sampling strategy requiring five sampling events per year will not adequately protect produce safety considering the high variability potential of bacteria in water (Boehm, 2007; Verhougstraete and Rose, 2014). In any irrigation water sampling approach, it is important that applied strategies are empirically derived from local irrigation systems.

Composite sampling does come at a cost and that cost has direct implications on identifying whether contamination exceeds a specific threshold. If *E. coli* concentrations exceed the FSMA standards, composite sampling may dilute it with "safe" water samples, resulting in an acceptable averaged result. The current project shows that water quality is homogeneous in short canal stretches and therefore, elevated bacterial concentrations would be measured using a single or composite sample. However, using a composite sample can help fulfill not only the FSMA sampling requirements but also support the overall objective of sampling: to protect food safety. Previous studies performed at recreational beaches indicate that single samples grossly over or underestimate the true *E. coli* concentrations in water (Whitman and Nevers, 2004). Using a more representative composite sampling strategy based on local irrigation water sampling will lead to greater monitoring effectiveness; produce more accurate risk assessments; and result in more accurate water quality management decisions (e.g. to use or not to use water for irrigation).

Coupling the four objectives of this study, we suggest an overall sampling strategy that produces the most relevant data for determining the risks of microbial pathogen contamination of food crop

waters. Through careful consideration of the entirety of our data as well as sampling costs, personnel effort, and the current state of scientific knowledge of irrigation water quality characterization, our study shows that microbial water quality is homogenous in short spatial scales (<161 m), but varies significantly both temporally (morning versus afternoon) and over long distances (>950 m). We therefore recommend that open canal irrigation water sampling in the Southwest region be undertaken while keeping the following guidelines in mind:

- Explore up to 950 m upstream to ensure no major contamination or outfalls exist.
- Sample before noon.
- Collect samples at any point across the canal where safe access is available.
- Composite five samples and perform a single *E. coli* assay.

This study was conducted in the Southwest United States and as such, the suggested monitoring strategy is most appropriate for the agriculture communities located in this region or in other areas that routinely experience warm air temperatures (average monthly highs of 21 °C) and low precipitation (average monthly totals of 0.97 cm) during the growing season. However, the study design offers an approach for agriculture communities anywhere to better understand the spatial and temporal variations of microbial concentrations in their irrigation canals, to produce a comprehensive monitoring plan specific for their geographical region, and to reduce pathogen exposure risks at the point of irrigation water application to the regional specific food crops. One limitation of this approach is the inability to identify specific sources of microbial contamination in water using *E. coli*, a generic bacteria found in the feces of mammals, some birds, plants, and can replicate in water outside its natural host (Mclellan et al., 2001; Whitman et al., 2005; Winfield and Groisman, 2003). Additional microbial analysis, such as molecular source tracking, would be required to identify specific sources of contamination. Despite this limitation, the current study suggests a comprehensive monitoring plan for irrigation water in the Southwest United States. This region plays a significant role in the supply of America's leafy green produce and therefore the suggested monitoring plan will reduce pathogen exposure risks at the point of irrigation water application to food crops.

4. Conclusion

The current study provides evidence-based suggestions for effective irrigation water quality monitoring. These monitoring guidelines are focused on spatial and temporal monitoring strategies specific to the Southwest United States and are aimed at practical applications considering human health risks relative to food crop contamination via irrigation waters and costs relative to sample analysis and personnel effort. More so, these suggested monitoring guidelines are based on irrigation water data, not recreational water studies, as has been the case in recent federal suggestions (e.g. FSMA). These suggested monitoring guidelines will ultimately enhance food safety, protect human health, and reduce the potential for costly food recalls stemming from produce contaminated by irrigation water.

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