CENTER for PRODUCE SAFETY

CPS 2014 RFP
FINAL PROJECT REPORT

Project Title

Optimal strategies for monitoring irrigation water quality and the development of guidelines for the irrigation of food crops

Project Period

January 1, 2015 – December 31, 2015

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Objectives

Objective 1: Determine the appropriate time of day for irrigation water monitoring based on the comparison of morning and afternoon samples collected on the same day.

Objective 2: Determine the appropriate sample collection point within the canal based on multiple spatial samples taken at cross sections of canals.

Objective 3: Address the transport of microorganisms in irrigation canals by collecting samples at equal spatial intervals (e.g., every mile) based on canal-specific discharge rates.

Objective 4: Determine if it is more appropriate to collect a single sample, multiple samples, or composite samples by comparing single, multiple, and composited multiple samples analyzed with the same method.

Objective 5: Define an overall sampling strategy to produce the most relevant data for determining the risks of microbial pathogen contamination of food crops via E. coli contaminated irrigation waters. This information will be used to develop guidelines and best management practices for growers/producers for the monitoring and use of irrigation water systems for irrigating food crops.

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FINAL REPORT

Abstract
The quality of irrigation water drawn from surface water sources can vary greatly. This is particularly true for waters that are subject to intermittent contamination events such as runoff or direct entry of livestock upstream of use. Such pollution in irrigation systems increases the risk of food crop contamination. A single sample does not adequately characterize the risk potential present in large irrigation systems often utilized in the Southwest United States. This project aimed to define optimal monitoring strategies for irrigation water quality and develop guidelines for the irrigation of food crops. Following the analysis of 1,367 samples for *Escherichia coli* and physical and environmental parameters, the following key irrigation water collection approaches are suggested: 1) Explore up to 600 m upstream to ensure no major contamination or outfalls exist; 2) Sample before noon; 3) Collect samples at any point across the canal where safe access is available; 4) Collect samples at the surface of the water; and 5) Composite five samples and perform a single *E. coli* assay. These recommendations consider the entirety of our data as well as sampling costs, personnel effort, and scientific knowledge of water quality characterization in the Southwest region. These guidelines will better characterize risks from microbial pathogen contamination in irrigation waters and aid in risk reduction practices for agricultural water.

Background
Historically, water quality guidelines have focused on drinking, waste, and recreational sectors, excluding waters used throughout the production of food crops. The agriculture industry has recently developed produce safety guidelines aimed at minimizing contamination throughout the growing, packing, shipping, and processing operations (Food and Drug Administration, 2015). Although these guidelines (Standards for Growing, Harvesting, Packing, and Holding Produce for Human Consumption) aim to establish science-based standards for agricultural processes, they fail to grasp the complexity of irrigation systems and offer few suggestions for appropriate monitoring of irrigation water safety.

The proposed U.S. Food and Drug Administration (FDA) microbiological rules for irrigation water are based on epidemiological studies undertaken at ocean and freshwater beaches. Little anecdotal evidence exists for the relationship to fresh produce and irrigation water associated risks. The proposed rules surrounding agricultural practices indicate sampling untreated surface water 20 times over 2–4 years and then 5+ times annually unless the water is from a public utility. In water used for any purpose beside sprout growing, hand washing, or direct application to food surfaces, *E. coli* concentrations cannot exceed126 CFU/100 ml using a geometric mean of at least five samples over multiple days (e.g., monthly geometric mean) or 410 CFU/100 ml as a statistical threshold value (STV). If the water exceeds this STV, it can still be used to irrigate if an appropriate number of days prior to harvest is allowed, assuming a 0.5-log die-off per day. The water rule for sprout irrigation, directly applied to food surfaces, or hand washing is as stringent as drinking water, stating that *E. coli* must not be detected (0 CFU/100 ml). If *E. coli* concentrations exceed any of these thresholds, water shall not be used for irrigation (Food and Drug Administration, 2015). These rules aimed at food safety fail to take into consideration the rapid spatial and temporal changes of bacterial concentrations in water.

Water research undertaken in rivers, lakes, and oceans has routinely demonstrated significant changes in microbe concentrations on short spatial and temporal scales (Boehm, 2007; Haack et al., 2004; Song et al., 2012). For instance, one study identified *Enterococci* concentrations typically vary by 60% over 10 minutes but varied by as much as 700% at California beaches

(Boehm, 2007). Similarly, the FDA guidelines fail to grasp the spatial variations of microbial water quality, potentially leaving the food product vulnerable to contamination. Studies undertaken in irrigation canals, rivers, lakes, and oceans have demonstrated significant changes in microbe concentrations on relatively short spatial scales (Juahir et al., 2011; Verhougstraete and Rose, 2013; Won et al., 2013).

Bacterial concentrations can undergo rapid spatial change along stream length and horizontal and vertical water profiles. Multiple studies have identified differences in microorganism concentrations throughout the vertical water column (Agogué et al., 2011; Karl, 1978; Krempin and Sullivan, 1981; Llirós et al., 2010) and horizontal water column (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 over multiple days or points in a water column. Water quality scientists have noted the implications of a single sample versus multiple samples for management actions (e.g., open or closing of a beach) (Bertke, 2007; Reicherts and Emerson, 2010). Kinzelman et al. (2006) identified that compositing multiple lake water samples and assaying a single sample was not statistically different ($P > 0.02$) to analyzing multiple individual samples and reporting an average, representing similar management actions under both approaches. The benefit of compositing samples lies with the cost reduction and ability to collect multiple spatial samples while performing a single microbial test and still providing the same level of protection. Based on previous research in non-irrigation systems, basing the safety of an entire irrigation canal off of a single sample is not adequate.

The quality of irrigation water drawn from surface water sources, such as canals, can vary greatly. This is particularly true for surface waters that are subject to intermittent, temporary contamination events such as runoff or direct entry of livestock impacts upstream of use. Such pollution in irrigation systems increases the risk of food crop contamination. A single sample does not adequately characterize the risk potential present in large irrigation systems often utilized in the Southwest U.S. since multiple studies have identified rapid spatial and temporal changes in microorganism concentrations. Practices to help ensure adequate water safety and reduce risk for agricultural water must be based on irrigation water specific research, not adapted from drinking and recreational water research. During the winter months, more than 90% of all leafy greens consumed in the U.S. are grown in the southwest region (http://www.visityuma.com/agritourism.html, accessed January 27, 2016). Due to the importance of this region for produce production and a gap in irrigation water quality science, this study aimed to understand the spatial and temporal variations of bacteria in irrigation canals, produce a comprehensive monitoring plan, and reduce pathogen exposure risks at the point of irrigation water extraction and application.

Research Methods and Results

Research Methods:

Site location

Sampling sites were selected following discussions with scientists from the University of Arizona (Tucson, AZ), Maricopa Agriculture Center (Maricopa, AZ), Yuma Agricultural Center (Yuma, AZ), and cooperating grower partners. Twelve sites were sampled in the Imperial Valley (CA), 12 in Maricopa, and 64 in Yuma (Figure 1). Sampling sites included a mixture of main, lateral, and sub-lateral canals and lined or unlined canals with varying flow dynamics.

Sample collection

Grab samples were collected between December 2014 to November 2015 to account for seasonal variations in microbial concentrations, climate variation, crop production, and water

use practices using sterilized 1L wide-mouth HDPE bottles (Nalgene Co., Rochester, NY). Depth below surface, distance from the bank, and time of day when and where samples were collected was objective dependent and detailed below. Conductivity, air and water temperature, and total dissolved solids were measured in field using handheld field probes. Samples were placed on ice in a cooler and transported to the Yuma Agriculture Extension (Imperial Valley and Yuma sites) or University of Arizona (Maricopa sites) for microbial processing and additional physical characterization.

For time of day objective specific sampling, grab samples were collected at 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, 12:00–14:00, and after 14:00). For determining specific collection points in a canal cross section, grab samples were collected vertically through the canal water column (at the water surface, 0.61 m below the surface, and 1.22 m below the surface) and horizontally across canal transects (left bank, ¼ of the distance of the canal width from the left bank, ¼ the distance from the right bank, and right bank). A schematic of this sampling approach is presented in Figure 2.

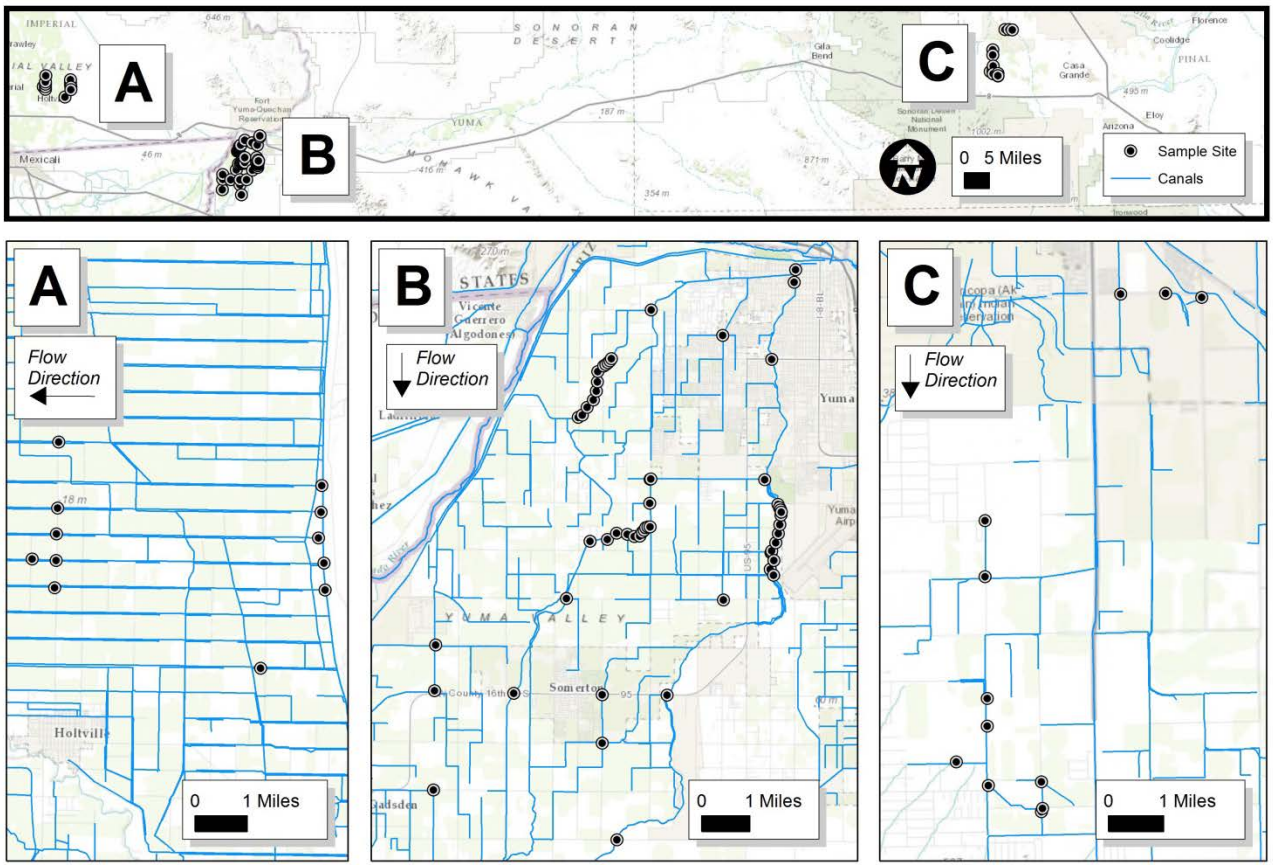

Figure 1. Map of sampling locations in (A) Imperial Valley, California; (B) Yuma, Arizona; (C) Maricopa, Arizona.

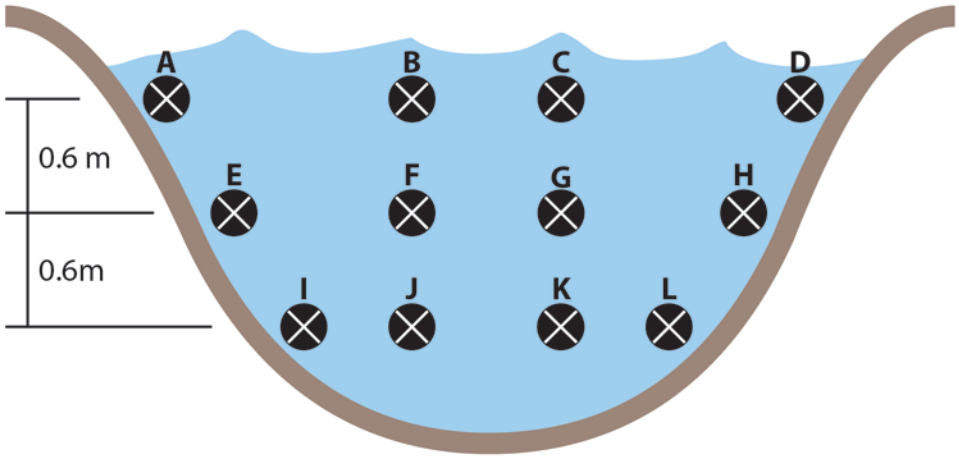

Figure 2. Sampling and labeling schematic for addressing appropriate canal transect sample collection point.

To determine the best collection, processing, and results representation approach, samples were collected in parallel to each other and perpendicular to the flow of the irrigation water. Three sample approaches were incorporated as part of this objective (Figure 3). Approach 1 was to collect a single sample from a single collection point and assay it individually in the laboratory. Approach 2 involved collecting five samples from the same canal stretch (2 m apart) and assaying each sample individually in the laboratory, and then calculating a geometric mean of the five individual samples. Approach 3 involved collecting five samples from the same canal stretch (2 m apart), and then adding equal volumes of well-mixed aliquots from each discrete sample to a sterile bottle to form a composite sample; the composite sample was then assayed identically to a single sample and reported as a single value.

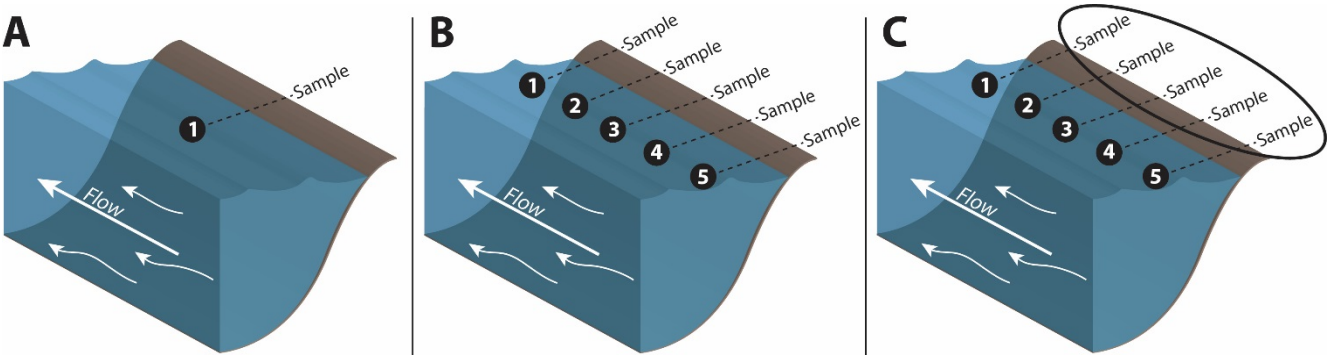

Figure 3. Sampling schematic for addressing (A) single sample, (B) multiple samples (assayed individually, then calculating geometric mean), and (C) composite samples into a single sample and assaying.

To enhance the transport study, a novel approach was added which involved the addition of MS2 coliphage to irrigation canals. This non-human, plant or animal 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 10^8–10^{10} plague forming units (PFU)/mL was mixed with 20 L of canal water. This mixture was dispersed across the canal and the plug of water was followed downstream using flow tracking devices (e.g., Planktos HydroSphere or a tracking bottle). Grab samples were collected downstream at 0, 80, 160, 240, 320, 643, 966, 1287, 1609, 1931, and 2253 m. As with bacterial samples, the

viral tracer samples were placed on ice in a cooler and transported to the Yuma Agriculture Extension (Imperial Valley and Yuma sites) or University of Arizona (Maricopa sites) for processing.

Laboratory analysis
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 manufacturer instructions. Following incubation at 37°C for 24 ± 2 hours, yellow wells were recorded as positive for total coliforms and wells fluorescing "blue" under UV light were recorded as positive for *E. coli*. All water samples were tested for turbidity, salinity, and pH in the laboratory. Data on environmental variables (air temperature, wind speed and direction, relative humidity, barometric pressure, and antecedent precipitation) are collected continuously at automated weather stations managed as part of The University of Arizona Meteorological Network (AZMET, http://ag.arizona.edu/azmet/, accessed December 10, 2015). Data for Yuma/Imperial Valley and Maricopa sites were retrieved from stations Yuma Valley and Maricopa, respectively.

Coliphage samples were serially diluted with sterile PBS to create 10, 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} mL subsamples. Double agar layers were utilized to detect a coliphage strain on selected hosts (*E. coli* ATCC 15597) following EPA method 1601 (United States Environmental Protection Agency, 2001). Clearings in the host lawn were counted and reported as PFU/ml. ΦX-174 coliphage was used as a positive control for verification of media integrity. Sterile reagent water was used as a negative control for verification of method integrity.

Statistical analysis
All bacterial data were log transformed to minimize skewness. Pearson correlation analysis was used to identify relationships between microbial concentrations and independent variables (e.g., physical, chemical, weather, and 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-sample *t*-tests and analysis of variance (ANOVA) and Kruskal-Wallis tests.

Classification and regression tree (CART) analysis was used to identify associations between *E. coli* (dependent variable) and the independent hydrological, physical, and environmental variables. Following methods presented by Martin et al. (2011) and Verhougstraete and Rose (2013), CART recursively split dependent variables into homogeneous groups based on analysis of all independent variables using a partitioning algorithm, a 10-fold cross validation criterion, and a minimum stopping criterion of five observations per subgroup (Martin et al., 2011). CART outputs were trimmed using the one-standard error rule (De'ath and Fabricius 2000). All CART analyses were performed using the R software system (R Foundation for Statistical Computing, Vienna, Austria).

Research Results:
A total of 1,367 samples were collected and measured for *E. coli*, total coliforms, pH, conductivity, turbidity, dissolved organic solids, and water temperature. Summary results for these water quality measurements are provided in Table 1. Overall, there were 88 unique sites with an average of 15 samples per site. With respect to the latest Food Safety Modernization Act (FSMA), two sites had *E. coli* geometric means >126 MPN/ 100ml. When a statistical threshold value (STV) of 410 *E. coli* MPN/100 ml was applied to all sites, one site exhibited a 31% violation rate (12/38 samples). Across all sites, statistically significant correlations ($p < 0.001$), although weak, were identified between *E. coli* and air temperature ($r = 0.1414$), water

temperature (r = 0.2472), relative humidity (r = -0.1799), pH (r = 0.1521), and dissolved organic solids (r = -0.1888).

While CART analyses were performed for each objective, the results did not provide useful insight into the development of irrigation water monitoring guidelines. Future efforts will include further exploration and analysis of the dataset using CART.

Table 1. Summary statistics of water quality measurements

Region	*E. coli* (MPN/100ml)			Total coliforms (MPN/100ml)			pH	Conductivity	Turbidity	DOS	Temp. air	Temp. water	% relative humidity
	Min.	Mean	Max.	Min.	Mean	Max.	Mean (min-max)						
Imperial Valley	<1.0	47.9	1553.1	210.5	1254.7	2419.6	8.67 (8.28-9.16)	1190 (1145-1232)	4.37 (0.43-213)	845 (813-873)	23.9 (12.3-32.1)	17.0 (12.7-22.2)	34.8 (8.80-87.3)
Maricopa	<1.0	79.1	1203.3	102.2	912.3	2419.6	8.63 (8.0-10.9)	1235.6 (810-1720)	10.3 (0.17-405)	876 (580-1220)	22.7 (13.1-31.2)	22.4 (15.6-29.4)	31.9 (7.10-94.5)
Yuma	<1.0	44.6	2419.6	77.1	1255.9	2419.6	8.48 (5.28-8.89)	1468 (1099-2260)	7.85 (0.34-817)	847 (561-1440)	29.3 (6.98-47.2)	21.9 (10.8-36.6)	26.8 (2.10-86.3)
TOTAL	<1.0	52.6	2419.6	77.1	1183.2	2419.6	8.55 (5.28-10.9)	1360 (810-2260)	7.63 (0.17-817)	854 (561-1440)	26.7 (6.98-47.2)	20.9 (10.8-36.6)	29.6 (2.10-94.5)

The first objective of this project was to determine the appropriate time of day for irrigation water monitoring. Samples (n=802) were collected at the same location and day at four different time points: before 09:00 (n=185), 09:00–12:00 (n=222), 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 (mean = 64.3 MPN/100 ml) and total coliforms ranged from 77.1 MPN/100 ml to >2419.6 MPN/100 ml (mean=1199.7 MPN/100 ml). Descriptive statistics for *E. coli* and total coliforms in samples collected for this objective are shown in Table 2. The only statistically significant difference in time of day sampling identified was between morning (07:00–12:00) and afternoon (12:00–16:00) (Two-sample *t*-test: $p < 0.0001$).

Table 2. Time of day statistics

Time of day	*E. coli* (MPN/100 ml)				Total coliforms (MPN/100 ml)		
	Min.	Arithmetic mean	Max.		Min.	Arithmetic mean	Max.
Before 09:00	<1.0	93.6	>2419.6		137.4	1299.6	>2419.6
09:00-12:00	<1.0	61.6	770.1		102.2	1176.9	>2419.6
12:00-13:00	<1.0	56.3	770.0		77.1	1297.6	>2419.6
After 13:00	<1.0	48.9	1203.3		77.1	1063.1	>2419.6

The second objective was to determine the appropriate sample collection point within the canal based on multiple spatial samples taken at cross sections of the canal. *E. coli* measures were grouped in all combinations of depth and distance to bank, and means were compared. Overall, *E. coli* ranged from <1.0 to 1553.1 MPN/100 ml (geometric mean = 8.08 MPN/100 ml). *E. coli* in the top, middle, and bottom horizontal transects ranged from <1.0 to 1553.1 MPN/100 ml (geometric mean = 18.7 MPN/100 ml), <1.0 to 161.6 MPN/100 ml (geometric mean = 7.67 MPN/100 ml), and <1.0 to 77.6 MPN/100 ml (geometric mean = 3.72 MPN/100 ml), respectively. *E. coli* ranged from <1.0 to 1553.1 MPN/100 ml (geometric mean = 12.8 MPN/100 ml) near the canal banks and from <1.0 to 77.6 MPN/100 ml (geometric mean = 5.30 MPN/100 ml) in the center of the canals. There were no statistically significant differences between any groups (Kruskal-Wallis and ANOVA tests). Descriptive statistics for *E. coli* measurements at cross section points are provided in Table 3.

Table 3. Descriptive statistics for *E. coli* (MPN/100 ml) at each spatial point in canal cross sections

ID	N	Minimum	Mean	Maximum	Std. Dev.	Median
A	24	3	429.14	1553	519.872	128.95
B	25	1	10.64	36	9.430	7.50
C	25	1	15.31	47	15.767	16.90
D	25	16	73.10	152	43.817	48.80
E	25	5	65.78	162	43.527	52.00
F	35	2	6.97	15	3.123	6.29
G	25	1	8.36	17	4.436	8.60
H	25	1	3.48	14	3.554	2.00
I	25	1	4.23	11	2.307	4.10
J	26	1	4.63	16	4.302	4.77
K	25	1	9.71	78	14.592	7.40
L	24	1	5.74	13	4.628	4.10
Total	**309**	**1**	**50.38**	**1553**	**182.133**	**7.33**

*Refer to Figure 2 for canal point ID location.

The third objective was to address the transport of microorganisms in irrigation canals. The initial approach involved enumerating *E. coli* and total coliforms from grab samples. However, samples (n=131) taken from a slug of water and analyzed for *E. coli* and total coliforms did not produce a clear picture of transport properties in irrigation canals (Figure 4 and 5). Descriptive statistics for *E. coli* and total coliforms from this objective are provided in Table 4.

Table 4. Descriptive statistics for objective 3, identifying the transport of microorganisms in irrigation canals

Downstream distance (m)	*E. coli* concentration (mean ± std. dev.)	Total coliforms concentration (mean ± std. dev.)	Coliphage concentration (mean ± std. dev.)
0	19.1 ± 19.5	1950.1 ± 687.2	39994.0 ± 79605.3
80*	-	-	14950.0 ± 21829.0
160*	-	-	1696.7 ± 1838.4
240*	-	-	3086.7 ± 3291.2
320	21.5 ± 26.2	1950.1 ± 687.2	2790.0 ± 1494.9
643	16.4 ± 19.7	2137.9 ± 583.1	4223.3 ± 4872.4
966	25.1 ± 32.3	1950.1 ± 687.2	588.3 ± 624.0
1287	16.7 ± 18.1	2189.2 ± 619.8	345.1 ± 616.3
1609	12.9 ± 16.5	2116.6 ± 631.2	137.2 ± 166.2
1931	11.8 ± 12.7	2109.0 ± 578.9	65.4 ± 88.1
2253	12.6 ± 12.9	1911.9 ± 755.8	102.2 ± 107.6

** No E. coli or total coliform samples collected at these points.*

In addition to the initial 131 microbial samples collected to address this objective, 40 samples were collected as part of a virus seeding experiment to enhance the objective. This effort was performed following the low *E. coli* and total coliform concentrations routinely measured in irrigation canals of the study region. Coliphage concentrations ranged from 0.016 to 18,200 PFU/100 ml (geometric mean = 67.4 PFU/100ml). Results from this preliminary approach demonstrate a lower concentration of the viral tracer roughly 600 m downstream of the release point, albeit not statistically significant ($p > 0.05$). A distance of 1,000 and 1,600 m was required

for the average coliphage virus concentrations to drop below 410 PFU/100 ml and 100 PFU/100 ml, respectively. Descriptive statistics for coliphage viral tracer are provided in Table 4.

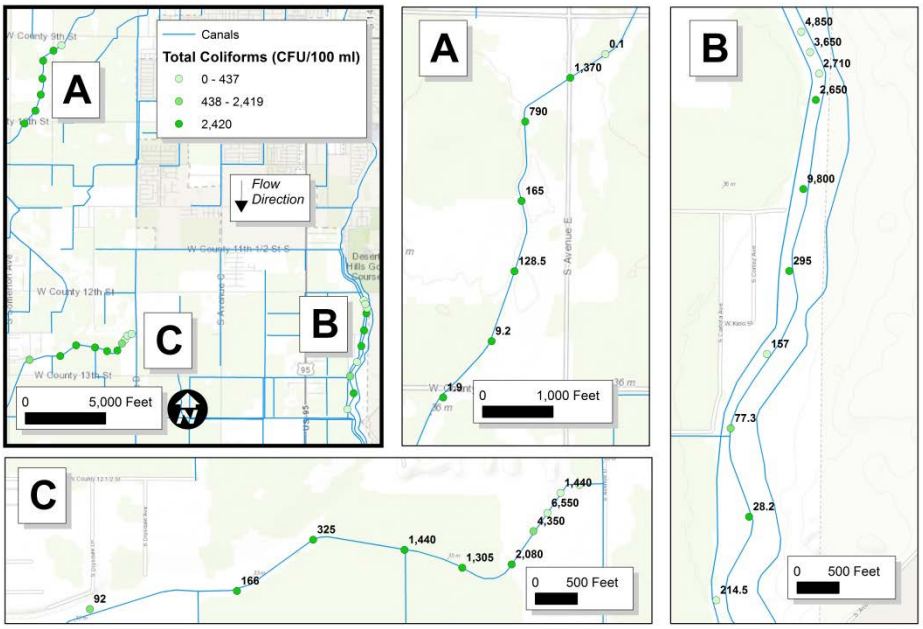

Figure 4. Measured concentrations of total coliforms throughout irrigation canals.

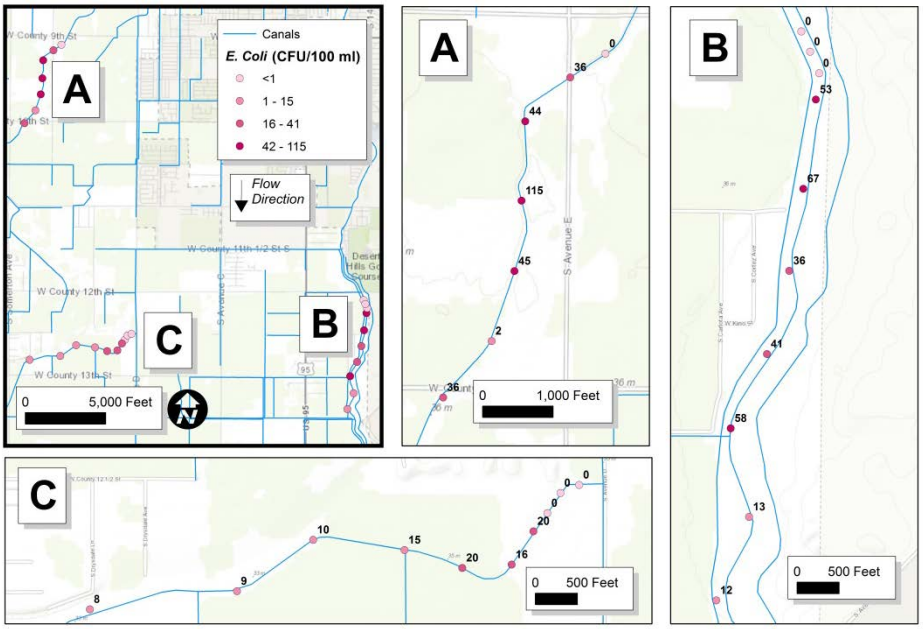

Figure 5. Measured concentrations of *E. coli* throughout irrigation canals.

The fourth objective aimed to determine the best sample analysis approach (e.g. a single sample, the geometric mean of five samples, or a composite of five samples). There was no statistically significant difference between the three types of samples (i.e., a single sample, the geometric mean of five samples, or a composite sample). However, when comparing individual groups, statistical differences were identified between a single sample and a composite sample ($p = 0.036$) and the geometric mean of five samples and a composite sample ($p = 0.005$) as

shown in the box and whisker plot in Figure 6. Monitoring approaches that collect a single water sample typically will fail to meet a very broad coefficient of variation. However, collecting and processing multiple individual samples to provide adequate representation of water quality may be cost prohibitive. A composite sample is an alternative to a single or multiple sample approach. In the current study, a composite sample appears to be more representative of the water quality within a canal than either a single sample or multiple samples in that it provided 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). Also, a composite sample requires only nominally more time and money than a single sample.

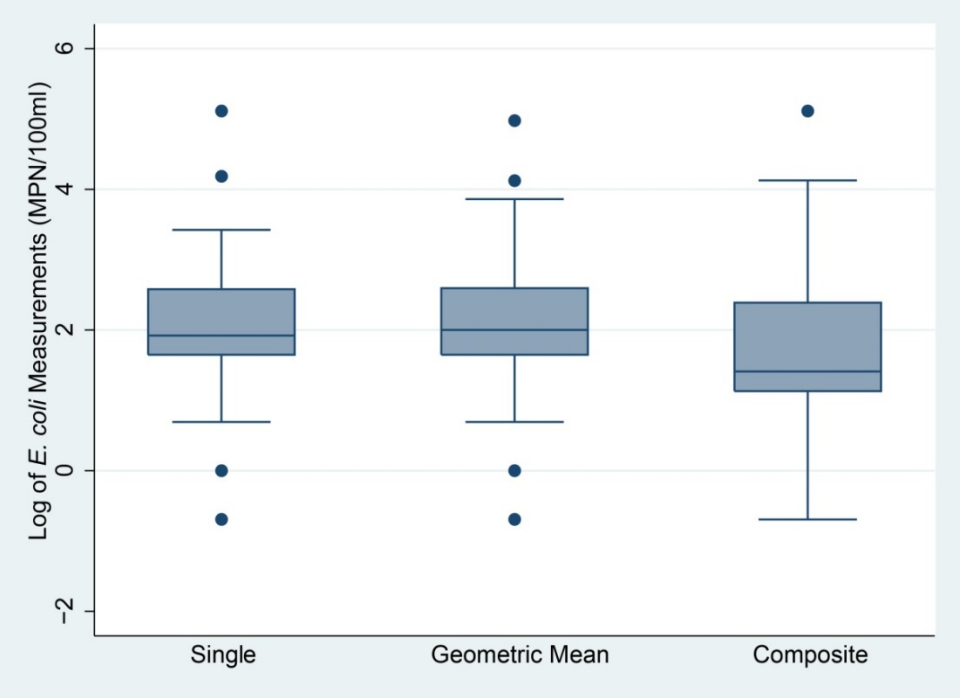

Figure 6. Box and whisker plot of *E. coli* measurements from 1) single sample, 2) geometric mean of five samples, and 3) a composite of five samples. The box represents the 25th and 75th percentiles, the horizontal line within the box represents the median concentration, the vertical lines extending from each box represent the range of *E. coli* concentrations (excluding outliers), and the dots represent outliers.

The final objective of this project was to define an overall sampling strategy that produces the most relevant data for determining the risks of microbial pathogen contamination of food crop waters by taking a comprehensive examination of the data gathered in objectives 1 through 4. Cumulatively, our data suggest microbial water quality is homogenous in short spatial scales (<600 m), but varies temporally (morning and afternoon) and over long distances (>600 m).

Outcomes and Accomplishments

During this project, we collected and processed 1,367 samples for *E. coli*; more than double the number of samples we originally proposed (n=670). The increased sample size provided additional scientific support to the recommended guidelines.

In addition to the 1,367 samples, 40 samples were collected as part of a virus seeding experiment to enhance objective 3. The original proposal aimed to measure only *E. coli* to define the microorganism transport abilities in irrigation canals. However, *E. coli* concentrations

were providing unexceptional information and in an attempt to enhance the meaning of this project, a non-pathogenic viral tracer was added to an irrigation canal and measured spatially downstream. This experiment and dataset will serve as pilot data for future projects aimed at better understanding transport studies in canal systems and into crop fields.

Additionally, the results and suggested guidelines would have the greatest impact on growers and farmers if they were made readily available in a simple format. Thus, we drafted an English language pamphlet to distribute to growers and farmers, which clearly informs them of our proposed best monitoring strategies for irrigation water use.

Summary of Findings and Recommendations

Through careful consideration of the entirety of our data as well as sampling costs, personnel effort, and scientific knowledge of water quality characterization, we suggest all open canal irrigation water sampling in the Southwest region be undertaken with the following measures:

- Explore up to 600 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
- Collect samples at the surface of the water
- Composite five samples and perform a single *E. coli* assay

These recommendations have been summarized in a 1-page flyer draft (Appendix 1). The current funding for this project did not allow for this flyer to be wholly developed. Future support should be used to finalize this flyer under professional guidance and include a Spanish version on the back. These flyers should be distributed to growers and farmers in the Southwest region of the United States.

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APPENDICES

Publications and Presentations

Preliminary results from this research were presented at the 2015 CPS annual meeting and the final results will be presented at the 2016 meeting. A peer-reviewed manuscript of this work is in preparation and will be submitted in 2016 to the Journal of the Total Environment.

Budget Summary

A detailed concurrence invoice was submitted to the Center for Produce Safety on January 19, 2016, by The University of Arizona Sponsored Projects Services. In brief, the budget summary included salary support for students and Jonathan Sexton for sample collection, salary support for Nate Lothrop for analysis and final report/manuscript development, materials for sampling and laboratory supplies, and a HydroSphere used to track the transport of microorganisms through water.

Suggestions to CPS

It has been a pleasure working with the Center for Produce Safety on this project and we look forward to a long collaboration addressing food safety. We have two suggestions/requests for the Center for Produce Safety:

The first is a direct request to apply additional funds to finalize the guideline flyer as a two-sided, bilingual guideline flyer (draft presented below) to be distributed to growers and farmers in the Southwest U.S.

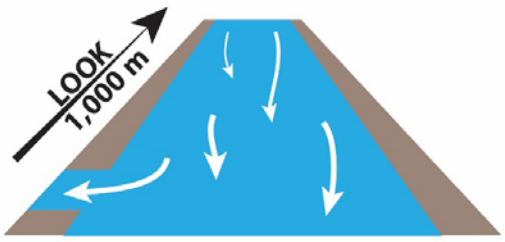
The second request is that CPS funds be allowed to support primary investigator salaries if they can demonstrate no or minimal state/university financial support. The research CPS supports is critical but when investigators rely solely on external grants, it becomes difficult to justify continuing to propose the much needed research if the investigator receives no salary support from the projects.

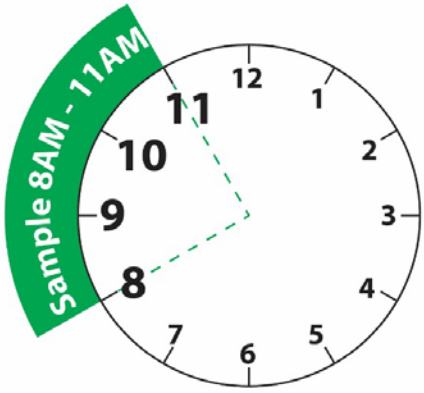
Appendix 1. **DRAFT GUIDELINE FLYER**

Irrigation Water Sampling: *E. Coli*
Best Practices

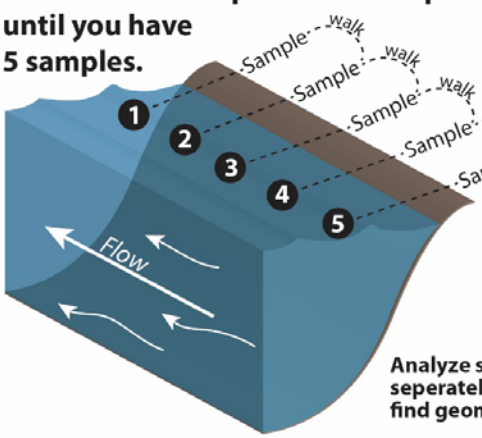
1. Look for any potential sources of contamination 1,000 m upstream of water withdraw.
2. Sample between 8 - 11 AM.
3. Safely take water samples anywhere in the canal stream.
4. Take a water sample and walk upstream until you have 5 samples.
5. If you have $ to analyze 5 samples, analyze each separately and calculate the geometric mean of the *E. Coli* concentration.
6. If you have $ to analyze <5 samples, mix the 5 samples and analyze the mixture.

Look upstream 1,000 m for contamination from withdraw canal.





Take a water sample and walk upstream until you have 5 samples.



Have $ to analyze:

5 samples → Analyze samples seperately and find geometric mean

<5 samples → Mix samples and analyze mixture