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Multistate Evaluation of Microbial Water and Sediment Quality from Agricultural Recovery Basins

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Abstract

Agricultural recovery basins are an important conservation practice designed to provide temporary storage of sediment and water on farms before low-volume discharge. However, food safety concerns have been raised regarding redistribution of captured sediment and water to fields used for human food production. The purpose of this study was to examine the potential microbiological risk that recovery basins may contribute to nearby produce fields and to evaluate characteristics that may influence or mitigate those risks. Water and sediment samples were collected from participating farms in three states and evaluated for bacterial indicators and pathogens over several months. Overall, 45% (n = 48) of water samples and less than 15% (n = 13) of sediment samples were positive for *Salmonella* spp. In water samples, the occurrence of *Salmonella* was positively associated with the use of surface water as a source of irrigation compared with groundwater as well as log-scale increases in *Escherichia coli* concentration. In sediment samples, *Salmonella* was associated with basin location (region) and basin fill levels. Sediment exposed to drying during dewatering had lower concentrations of indicator *E. coli* and a lower proportion of *Salmonella* positives than submerged sediment from the same pond. Surrounding landscape characteristics, including vegetative coverage, proximity to livestock operations, and evidence of wildlife, were not correlated with pathogen occurrence in either sediment or water samples, suggesting that although habitat surrounding ponds may be an attractant to wildlife, those features may not contribute to increased pathogen occurrence in agricultural recovery basins.

Core Ideas

- Pathogen occurrence in on-farm recovery basins varies by region and irrigation water source.
- Wildlife, domestic animals, and vegetation were not correlated with pathogen occurrence.
- Allowing captured sediments to dry may greatly reduce microbial load before reapplication.

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Agricultural croplands in the United States cover over 400 million acres and account for 18% of total land use (Nickerson, 2011). Discharge from agricultural fields, also called nonpoint-source pollution, can significantly reduce water quality of nearby surface waters while increasing rates of erosion and loss of valuable top soil (Long et al., 2010; Naramngam and Tong, 2013; Pimentel et al., 1995). Nationwide issues of water quality and resource scarcity have led resource agencies to rethink management of runoff and nonpoint-source pollution by the implementation of national-, regional-, and watershed-scale conservation programs. Programs that help to mitigate loss of soil and water resources are important to sustain the future of US food production. Many of these programs are managed by the Natural Resource Conservation Service (NRCS) within the USDA. The NRCS has a long history of working with landowners through conservation planning and through financial and technical assistance to establish conservation practices (CPs). These CPs are intended to benefit the soil, water, air, plants, and animals that promote productive lands and healthy ecosystems, including the reduction of environmental impacts from agricultural lands to terrestrial and aquatic environments.

Reducing the loss of topsoil and reusable water through runoff from agricultural fields is one way of decreasing erosion and improving local water quality. Recovery basins are an important CP designed to remove, collect, and provide temporary storage of sediment and water on farms before low-volume discharge. Recovery basins go by many names depending on the region and manner in which they are installed; water and sediment control basins, tail-water recovery ponds, or sediment basins are a few examples, but all generally serve the same purpose. Sediment basins on irrigated agricultural fields are constructed primarily for the reduction of sedimentation into local waterways and to retain valuable topsoil. Growers and landowners periodically excavate the basins to maintain their capacity and effectiveness. Tail-water recovery ponds, alternatively, are primarily used to capture and retain water capable of being reused for irrigation and are rarely re-excavated.

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Abbreviations: CC, Central Coast, California; CFU, colony-forming unit; CP, conservation practices; GEE, generalized estimation equations; IV, Imperial Valley, California; NaPP, sodium polyphosphate; NCV, Northern Central Valley, California; NRCS, Natural Resource Conservation Service; QIC, quasilikelihood under the independence model criterion; TSB, tryptic soy broth.

Although sediment basins have proven to be effective at capturing topsoil, questions have been raised about the food safety implications of redistributing these captured sediments to fields used for human food production. It is well established that sediments associated with standing water are microbiologically rich and capable of harboring bacteria far in excess of the overlying water column (Bai and Lung, 2005). According to recent studies, sediment-laden water can be a favorable environment for bacterial persistence (Benjamin et al., 2013), and bacteria survival rates are higher in sediments when compared with the overlying water (Haller et al., 2009).

Similarly, tail-water recovery ponds are valuable tools for capturing reusable irrigation water. However, use of these water supplies may have regulatory consequences, particularly since the adoption of the produce safety requirements under the 2011 Food Safety Modernization Act (FDA, 2011). Bacterial counts generally fluctuate in tail-water recovery ponds depending on the source of water used for irrigation. For example, Pahl et al. (2013) showed that surface water sources contributed significantly higher concentrations of fecal indicator bacteria to ponds than groundwater sources.

The purpose of this study was to examine the potential microbiological risk a properly installed sediment basin or tail-water recovery pond may contribute to nearby produce fields with respect to redistribution of basin sediments and reuse of captured water. More specifically, we sought to determine whether pathogenic bacteria could be found in the waters and sediments associated with sediment basins and tail-water recovery ponds, and, if so, whether detection probability decreased after previously submerged sediment was exposed during basin dewatering. Environmental conditions, field characteristics, and sources of water were evaluated as contributing factors to the microbial water and sediment quality of basins/ponds. These data will be used to create guidance documentation for the implementation and management of these CPs in the future.

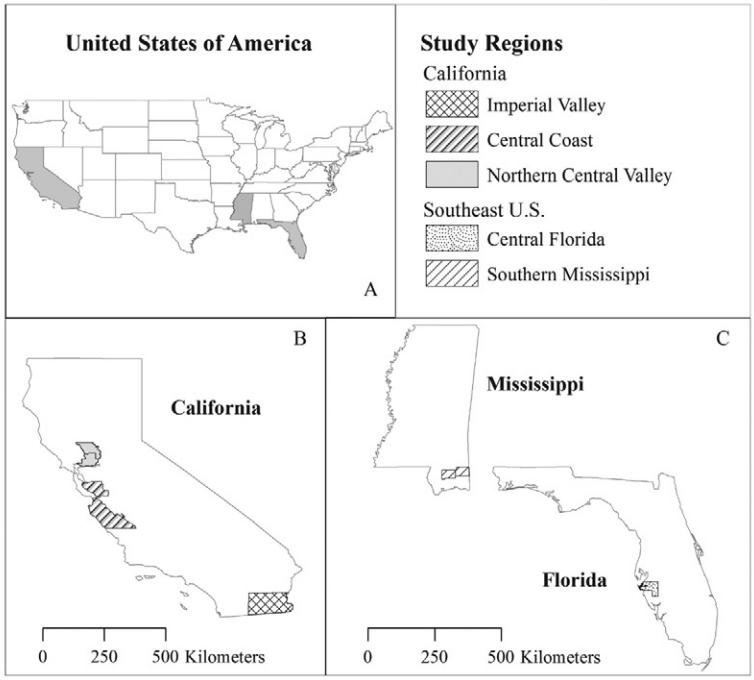

Fig. 1. Map of study areas. (A) Overview of the United States, with states included in the study shaded gray. Insets B and C include shaded generalized subregions within each state where sampling occurred. Detailed locations were omitted for the purpose of confidentiality.

Materials and Methods

Site Selection

Sample collection was conducted in produce-growing regions of California, Mississippi, and Florida. In California, we chose sediment basins from three subregions distinct for their climatological differences and large agricultural production areas: the Imperial Valley (IV), the Central Coast (CC), and the Northern Central Valley (NCV) (Fig. 1). Sites in the southeastern United States (Central Florida and Southern Mississippi) were chosen, with the aid of NRCS personnel, to identify basins that were installed using NRCS guidelines. Although some of the California sites were constructed with NRCS guidance, others were constructed to decrease the amount of sedimentation into local waterways in accordance with Regional Water Quality Control Board discharge requirements. Before the onset of sample collection, a confidentiality agreement was established between the individual growers and UC Davis researchers. All identifying information, such as location and name of the farm or lot, were blinded from laboratory staff.

Sample and Environmental Data Collection

Water samples were taken at the same point of entry at each basin on a monthly basis for a minimum of 3 mo during the growing season (California: May 2011–February 2012; Florida: November 2011–February 2012; Mississippi: November 2011–March 2012). High-volume water sampling was performed using a peristaltic pump (Solinst Canada Ltd.) with approximately 2.7 m of sterile tubing attached to a telescoping pole and a secondary tube attached to a hose barb on the top of a sterile 20-L carboy (Nalgene, Thermo Fisher Scientific Corp.). Once the carboy was filled, the tubing attached to the carboy was removed to fill an additional 1-L sterile container (Nalgene, Thermo Fisher Scientific Corp.). Samples were placed in coolers and maintained at ~4°C until laboratory analysis. Physical (temperature and turbidity) and chemical (dissolved oxygen, pH, conductivity [$\mu s\ cm^{-1}$], and nitrates [$mg\ L^{-1}$]) parameters were measured with a YSI Professional Plus handheld multiparameter meter (YSI Inc.) and a portable turbidity meter (Lamott Co.). The meters were calibrated in accordance with the manufacturer's recommendations before each sampling event. In addition to the physical water quality parameters, meteorological data were collected using the California Irrigation Management Information System (CIMIS), administered by the California Department of Water Resources, and the Florida Automated Weather Network (FAWN), administered by the University of Florida Institute of Food and Agricultural Sciences. At the time of this study the State of Mississippi did not have a network of weather stations with data accessible by the public.

Sediment grab samples were collected using a PONAR bottom dredge (AMS Inc.). The dredge was deployed from a floating platform or using an extension arm at a distance of 1 to 3 m from water's edge. A 200-g composite sediment sample was aseptically taken from the top 5 cm of sediment inside the dredge using a sterile scoop and placed in a 710-mL Whirl-Pak bag (Nasco). The dredge was dipped to remove residual sediment, wiped and cleaned with 70% ethanol between samples. For sediment basins with little to no water, the top 2.5 cm of sediment was removed and discarded, and a 200-g composite sample was taken from the newly exposed area and placed into a 710-mL Whirl-Pak bag. Sediment samples were placed on ice (~4°C) and transported to the laboratory for analysis.

Observational data were also collected during each sampling event, including bank vegetation (% coverage), irrigation method (flood, drip, or overhead) and source (ground or surface), use of chemical treatment, presence of wildlife (mammalian, avian, reptilian, amphibian or fish), animal agriculture within 0.5 km of the basin (dairy, poultry, cattle, sheep, or horses), aquatic vegetation within the basin (% coverage), presence of overhanging trees (% coverage), and fill-level of basin as a percentage of maximum capacity. Transects (1 × 100 m) were conducted along the upper bank of each basin for the presence of animal feces (wild or domestic) and rodent burrows. Fecal deposits were subdivided into three size classes: (i) bird and small rodents; (ii) rabbits, raccoons, and reptiles; and (iii) canines, deer, and horses.

Sample Analysis

Indicator *Escherichia coli*

Sample water (up to 100 mL) was processed using standard membrane filtration techniques (American Public Health Association, 2012). Each filter was placed onto CHROMagar EC medium (CHROMagar Microbiology) and incubated for 2 to 3 h at 37°C for resuscitation and transferred to 44.5°C for an additional 18 to 20 h. Results (blue colonies) were counted and reported as colony forming units (CFU) per 100 mL. Two to three presumptive positive colonies from each sample group were confirmed using biochemical confirmation.

For sediment, 10 g of composite sample was added to 150 mL of a mild surfactant shaking solution (0.0001% Tween 80, 0.001% [w/v] sodium polyphosphate (NaPP), and 0.00001% Antifoam A) in a 200-mL conical tube (Corning). The resulting volume was noted to calculate concentration of bacteria per gram of sample. The mixture was then shaken using a mechanical wrist action shaker (Burrell Scientific) for 5 min and centrifuged at 1000 rpm for an additional 5 min. A sample of the resulting supernatant (10–20 mL) was filtered via membrane filtration and plated on selective media as described previously for water samples.

E. coli O157:H7/*Salmonella*

Concentration of the 20-L water sample was accomplished using a highly portable, battery-operated ultrafiltration apparatus modified from Hill et al. (2005). Before ultrafiltration, a single-use ultrafilter (filter) (Fresenius Medical Care) was blocked using 0.01% NaPP solution. Once the water sample was concentrated (~40×), the filter was flushed for approximately 2 min using a 500-mL elution solution consisting of 0.001% Tween 80, 0.01% NaPP, and 0.0001% Antifoam A. The resulting elution volume was added to the concentrated sample, and this retentate (500–800 mL) was processed for *E. coli* O157:H7 and *Salmonella.* Pathogen processing followed a protocol described by Atwill and Carabez (2011) with the following modifications: dry irradiated Tryptic Soy Broth (TSB) (EMD Millipore) medium was added directly into the retentate at a proportion of 30 g L^{-1} (TSB/retentate). The enriched sample was incubated for 2 h at 25°C, shaken at 100 rpm, incubated further for 8 h at 42°C at 100 rpm, and held at 4°C until processed using immunomagnetic separation as instructed by the manufacturer (Life Technologies).

For sediment, two separate 50-g aliquots of the composite sample were enriched independently using 150 mL of prepared TSB (EMD Millipore) per aliquot, incubated for 2 h at 25°C and 100 rpm, incubated further for 8 h at 42°C and 100 rpm, and held at 4°C until processed with immunomagnetic separation as instructed by the manufacturer (Life Technologies).

To determine pathogen assay performance, environmental water/sediment samples were spiked with *E. coli* O157:H7 and *Salmonella* at specific concentrations and processed as described above. Concentrations of the stock suspension of *E. coli* O157:H7 and *Salmonella* were estimated using an optical density–based growth curve and confirmed by spread plating a series of 10-fold serial dilutions. The assay was able to detect as low as 1.31 CFU *E. coli* O157:H7 and 0.95 CFU *Salmonella* in 10 g of sediment. In water, the assay successfully detected <1 CFU 100 mL^{-1} of both *E. coli* O157:H7 and *Salmonella*.

Statistical Analysis

Descriptive statistics for *E. coli* concentrations and frequency of *Salmonella* occurrences were calculated using Stata 12.1 software (StataCorp LP). Water and sediment characteristics were presented as arithmetic means and standard deviations for normally distributed outcomes (log-transformed indicator *E. coli*) and as frequency (%) for categorical outcomes (*Salmonella* and *E. coli* O157:H7). To allow for log transformation of the small number of *E. coli* nondetects, a marginal value (0.0001) was added to each result before transformation. Data were described according to state (Florida, Mississippi, and California) for water samples and according to California subregion (IV, CC, NCV) for sediment samples. Differences in concentration of log-transformed *E. coli* and pathogen frequency among and between groups (states and subregions) were evaluated using ANOVA followed by Tukey's post hoc evaluations. Trends in the occurrence of pathogens were determined for different regions (southeastern United States and California), basin fill levels (>50%, <50%), irrigation sources (ground or surface), bank vegetation (>50%, <50%), and the presence of wildlife at the time of sampling (mammals, amphibians, birds, and fish). The statistical significance of trends in indicator *E. coli* concentration and the occurrence of *Salmonella* were examined using logistic regression models. Independent models were created for water and sediment samples. Multiple generalized estimation equations (GEEs) were used to address the issue of repeated measures within basins.

Variables with univariate significance $p \leq 0.10$ were assessed for inclusion in the final model. The quasilikelihood under the independence model criterion (QIC) was used to select the appropriate working correlation structure for GEE analyses as well as which subsets of covariates produce the best model fit

(Cui, 2007). The model with the lowest QIC value was considered the most appropriate model.

Results

In all, 13 growers throughout California, Florida, and Mississippi agreed to participate in this study, resulting in samples collected from 28 tail-water recovery/sediment basin systems on 20 separate farms.

Water

Of the 107 water samples collected during the course of this study, nearly 45% (n = 48) were positive for *Salmonella*, whereas none (0%) tested positive for *E. coli* O157:H7 (Table 1). *Salmonella* occurrence in Florida water samples was 79%, whereas all water samples (100%) from Mississippi tested positive. Overall, approximately 70% of samples that exceeded the industry standard of water quality (indicator *E. coli* >235 CFU 100 mL^{-1}) were positive for *Salmonella* (9/13); however, ~81% of all *Salmonella* positives occurred when *E. coli* concentrations were lower than 235 CFU 100 mL^{-1} (39/48).

In California, only 23.9% of water samples were positive for *Salmonella* (Table 1); however, over one third of the enrolled basins (n = 7 of 18) were regularly treated with copper sulfate (bluestone), and all of these treated basins were in a single subregion (CC). Although bluestone is typically used for algae inhibition, it is also an effective antimicrobial agent (Nies, 1999); the average indicator *E. coli* counts in water samples were nearly 20 times lower in samples from bluestone-treated basins (n = 21; X = 16.55; SD, 53.90) than in nontreated basins (n = 86; X = 305.65; SD, 1073.7). Water quality parameters, including temperature, conductivity, turbidity, and pH, did not correlate with the occurrence or concentration of pathogens or indicator bacteria (p > 0.10) and were not evaluated further. Environmental data from weather monitoring stations (CIMIS and FAWN) were also excluded due to lack of data for the Mississippi basins.

A graphical evaluation of predicted results and raw occurrence data using the logit function revealed that the logit model was poorly fitting (Fig. 2) and that a complementary log–log (CLL) function with a nonsymmetric increase in response could be more appropriate for modeling these systems. Evaluation of the correlation structure between and within sampling basins

Table 1. Water sample results by state.

State	Total	Visits†	Indicator *E. coli*‡	*E. coli* O157:H7 positives	*Salmonella* positives§
	n	no.	100 mL^{-1}	no.	no.
California	71	5	341.5 (1178.2)	0	17 (23.9)
Florida	24	4	68.1 (122.8)	0	19 (79.2)
Mississippi	12	3	62.8 (78.7)	0	12 (100.0)
Total	107	12	248.9	0	48 (44.8)

† Number of repeated monthly sampling events.

‡ Average *E. coli* colony forming units with SD in parentheses.

§ Number of positive samples with percent of total samples in parentheses.

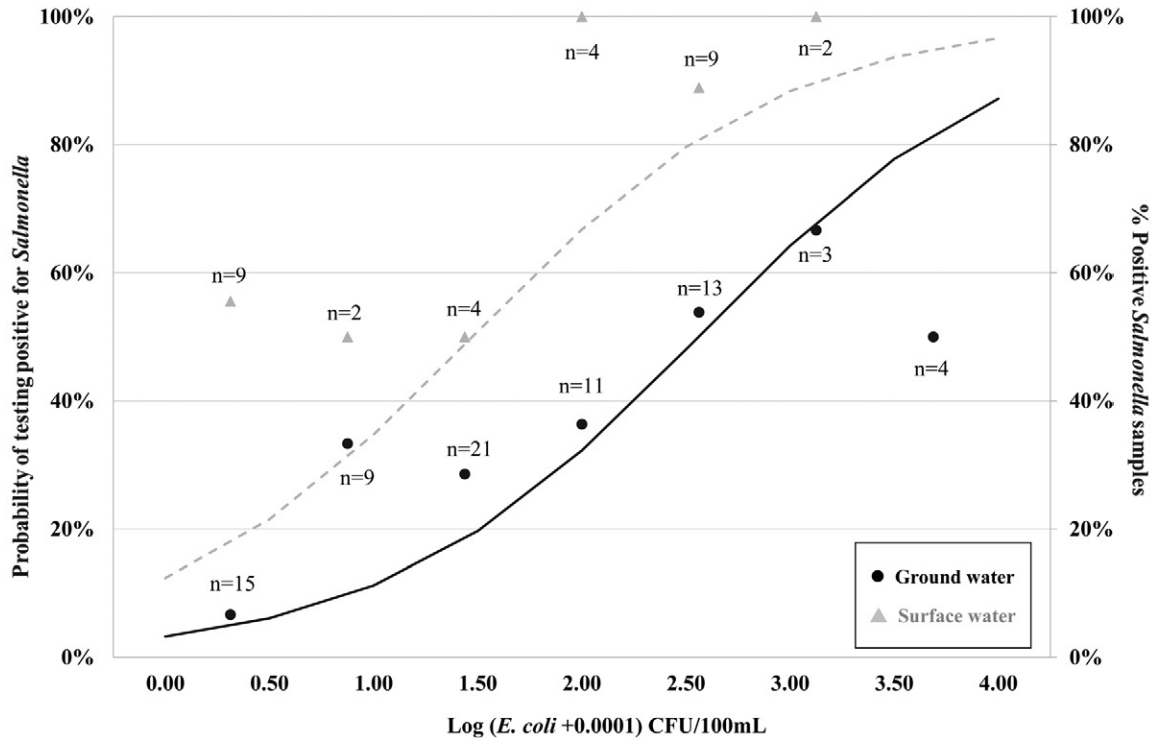

Fig. 2. Modeled probability (logit function) of detecting *Salmonella* in water samples as a function of irrigation source water (black, groundwater; gray, surface water) and log-transformed *Escherichia coli* concentrations. Raw data for the percentage of *Salmonella* positive samples at binned values of *E. coli* are plotted with frequency of samples/bin listed as reference. CFU, colony forming units.

using GEE suggested independence was the most appropriate correlation structure (i.e., lowest QIC value). There was marginal evidence that an autoregressive structure with a lag of two might be appropriate. However, some basins were only sampled on three occasions. Results of the CLL regression model indicate that region (southeastern United States or California) and log-transformed indicator bacteria counts [log (*E. coli* CFU + 0.0001)] were significant predictors of *Salmonella* in water samples ($p < 0.05$) (Table 2; Fig. 2, 3). For every log increase in indicator *E. coli*, the model predicts a greater than twofold increase in the odds of detecting *Salmonella*.

The use of surface water as an irrigation source (reservoir or canal) was a marginally significant predictor of *Salmonella* ($p = 0.058$) and was retained in the model because it significantly improved the model's fit (lowest QIC). Basins on farms that used surface water (reservoir or canal) to irrigate were 2.4 times more likely to test positive for *Salmonella* than those using groundwater (Table 2). The use of bluestone as a potential negative predictor was evaluated but was not significantly associated ($p > 0.05$) with pathogen occurrence and did not improve the overall model fit and so was excluded from further analysis. Interestingly, the presence of mammalian wildlife was significantly and negatively associated with *Salmonella* presence; the basins where mammals were observed were nearly 80% less likely to have *Salmonella* in water samples than basins where mammalian wildlife was not observed. Neither the presence of other wildlife (avian, reptilian, amphibian, or fish) nor the presence of animal agricultural operations contributed to the likelihood of a sample testing positive for *Salmonella*.

Sediment

In Florida and Mississippi, tail-water recovery ponds were not allowed to dewater and were used primarily as reservoirs of recirculated irrigation water. Owing to the difference in the purpose of tail-water recovery ponds and sediment basins, sediment samples were only collected in sediment basins, all of which were found in California ($n = 19$), IV ($n = 3$), CC ($n = 9$), and NCV ($n = 7$), for a total of 89 samples (Table 3). Less than 15% ($n =$

Table 2. Population averaged complementary log-log regression model for the association of *Salmonella* occurrence in water with irrigation source, presence of mammalian wildlife, and the log transformed concentration of indicator *Escherichia coli*, grouped by basin.

	Coefficient	OR†	*p* value	95% CI‡
Intercept	−0.641	0.527	0.139	0.225–1.232
Irrigation source water				
Ground§	0.000	1.000		
Surface	0.856	2.354	0.058	0.971–5.709
Region				
Southeast§	0.000	1.000		
California	−2.222	0.108	0.000	0.042–0.277
Mammalian wildlife				
Absent§	0.000	1.000		
Present	−1.484	0.227	0.002	0.088–0.582
Log (*E. coli* CFU 100 mL^{-1} + 0.0001)	0.971	2.640	0.000	

† Odds ratio.

‡ Confidence interval of the odds ratio.

§ Category is referent; therefore, coefficient is 0.0 and OR is 1.0.

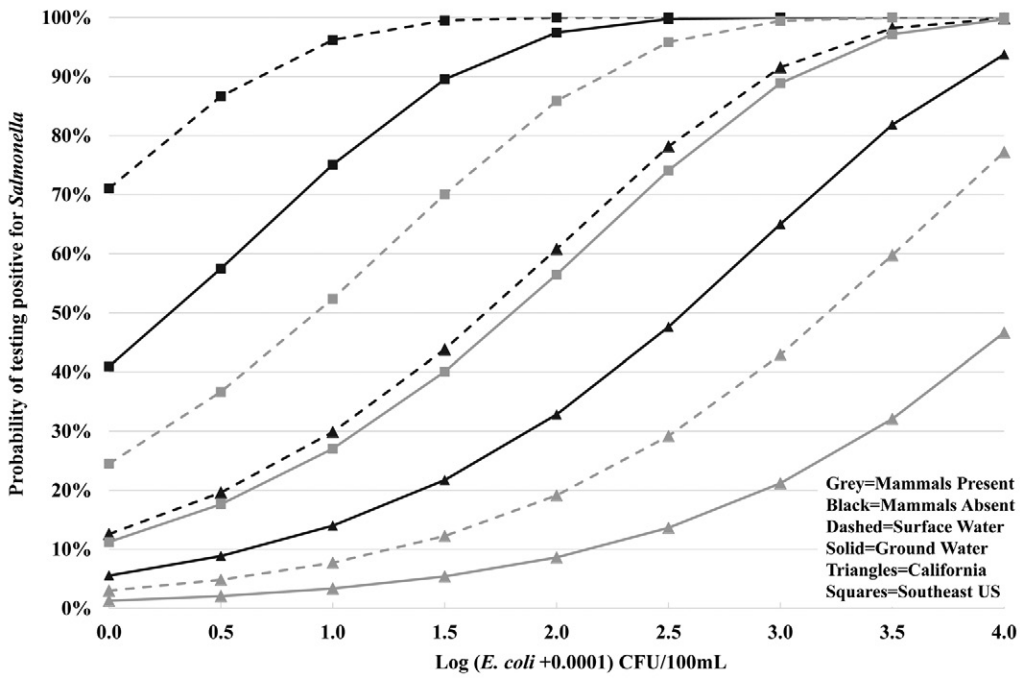

Fig. 3. Modeled probability (complementary log–log function) of detecting *Salmonella* in water samples as a function of log-transformed *Escherichia coli* concentrations and several site-specific criteria. Gray lines indicate the presence of mammalian wildlife; black lines indicate no mammals. Solid lines indicate the use of groundwater for irrigation; dashed lines indicate the use of surface water. Square markers represent samples from the southeastern United States; triangles represent samples from California.

Table 3. Sediment sample results by study region within California.

Region	Total	Indicator *E. coli*†	*E. coli* O157:H7 positives	*Salmonella* positives‡
	n	1 g^{-1}	no.	no.
Imperial Valley	9	396.1 (1024.3)	0	5 (55.6)
Central Coast	42	160.0 (718.6)	0	1 (2.4)
Northern Central Valley	38	86.0 (321.6)	0	7 (18.4)
Total	89	152.3	0	13 (14.6)

† Average *E. coli* colony forming units with SD in parentheses.

‡ Number of positive samples with percent of total samples in parentheses.

13) of sediment samples tested positive for *Salmonella* (Table 3), whereas 0% ($n = 0$) samples tested positive for *E. coli* O157:H7.

Produce growing subregion was a significant predictor of *Salmonella* occurrence in the final CLL model for sediment samples (Table 4). Postestimation comparisons of inter-subregional differences indicate the occurrence of *Salmonella* positives was significantly higher in IV samples than in either CC ($p = 0.004$) or NCV ($p = 0.0125$) samples. Sediment samples from the NCV were not significantly different from CC samples ($p > 0.05$). Statistical comparison of log-transformed indicator bacteria counts [log (*E. coli* CFU + 0.0001)] did not reveal a statistical difference ($p > 0.05$) between growing subregions NCV and IV from CC; however, *E. coli* counts from the IV samples were significantly higher than those from the NCV ($p = 0.040$).

According to the results of the CLL regression model (Table 4), neither the presence of bank vegetation coverage >50%, the presence of aquatic vegetation, nor the presence of overhanging trees contributed to an increased odds of *Salmonella* occurrence in sediment samples ($p > 0.05$) and were excluded from the final model. Additionally, as with the water models, the use of bluestone treatment did not contribute significantly to the odds of detecting pathogens and was excluded from the final model. Growing subregions CC and NCV, basin fill level (>50%), and density of fecal deposits were all negatively associated with *Salmonella* presence in samples. Density of fecal deposits in 1 × 100 m transects varied greatly by classification; type 1 feces (e.g., bird) was the most common contribution, making up more than 68% of all feces detected and averaging 16 deposits per transect (mean, 16.0; SD, 31.1).

Sediment taken from basins that were more than half filled to capacity were over 80% less likely to test positive for *Salmonella* than submerged sediment from basins that were beginning to dewater based on observed capacity levels. Because basins were allowed to dewater, a greater proportion of sediment was exposed to drying; however, the sediment remaining underwater frequently had elevated levels of bacteria. Of the 89 sediment samples collected, 19 were taken from exposed, but not necessarily dry, sediments. Indicator *E. coli* counts were lower, on average, in exposed sediments (mean, 45.7; SD, 70.9) than in unexposed sediments (mean, 181.2; SD, 699.2); however, this difference was not statistically significant ($p > 0.05$). Exposure status was not significantly associated with a decrease in likelihood of testing positive for *Salmonella*. Although more than one third (39%) of sampled sediment basins were allowed to dewater more than 50% of capacity, only one basin was allowed to go completely dry.

Discussion

Worldwide agricultural production accounts for more than 80% of freshwater consumption (Pimentel et al., 1997). In the United States, agriculturally derived contaminants have been considered the single most consistent source of poor water quality to surface and groundwater supplies (Osborne and Kovacic, 1993). The use of reduced-quality water sources for agricultural applications has been accelerated recently due to limited availability and degradation of clean water sources used for irrigating crops (Pimentel et al., 1997). Tail-water recovery ponds and sediment basins are two CPs that are effective at detaining sediment-laden agricultural runoff, thereby capturing valuable topsoil and potentially reusable water. However, concerns have been raised regarding the use of these captured resources in human food production systems.

This study sought to evaluate whether sediment basin and tail-water recovery ponds act as reservoirs of sediment associated and waterborne pathogens and to determine the impact of

Table 4. Complementary log-log regression model for the occurrence of *Salmonella* in sediment samples in California as a function of region, basin fill level, and concentrations of fecal deposits within 1 × 100 m transects, with location as a random effect.

	Coefficient	OR†	*p* value	95% CI‡
Intercept	1.182	3.260	0.085	0.849–12.519
Region§				
IV	0.000	1.000		
CC	−3.320	0.036	0.004	0.004–0.344
NCV	−1.752	0.173	0.013	0.044–0.686
Water level, %				
0–50	0.000	1.000		
50–100	−1.662	0.190	0.035	0.041–0.888
Fecal deposits	−0.153	0.879	0.074	0.763–1.013

† Odds ratio.

‡ Confidence interval of the odds ratio.

§ CC, Central Coast, California; IV, Imperial Valley, California; NCV, Northern Central Valley, California.

dewatering on pathogen persistence. The Florida and Mississippi tail-water recovery ponds that we sampled were not allowed to dewater and were used primarily as reservoirs of recirculated irrigation water. These structures were installed to maintain a 20-yr sediment capacity, and consequently these ponds contained more wildlife (reptiles, amphibians, and fish) than seen in basins where sediments were periodically removed.

Occurrence of Pathogens in Water

During normal environmental conditions, waterborne pathogens are often in flux and are difficult to quantify (Jenkins et al., 2008; Micallef et al., 2012). To account for this, we utilized an ultrafiltration technique (Hill et al., 2005) that enabled us to detect pathogen occurrence even at low levels by using high volumes of water (20 L), which is orders of magnitude greater than recommended in standard procedures (American Public Health Association, 2012). In this study, we found *Salmonella* in approximately 45% (48/107) of water samples taken across all three states, which is nearly six times the occurrence found in other studies (Micallef et al., 2012). Although *Salmonella* spp. have been shown to be more stable than indicator *E. coli* in environmental waters (Rhodes and Kator, 1988), it is possible that some of the negative detections, particularly of *E. coli* O157:H7, may have been the result of die-off during transport.

Results of a complementary log–log regression model suggest that the use of surface water (canal or reservoir) as a source of irrigation greatly increased the probability of detecting *Salmonella* in basin water samples. These results are supported by previous studies that found increased bacterial loading in surface water sources of irrigation water (Leskinen et al., 2012; Steele and Odumeru, 2004). For instance, a study conducted in 2011 on the occurrence of indicator *E. coli* in irrigation water supplies found that on-farm reservoirs were nearly three times more likely to exceed recommended microbial standards than groundwater taken from the same property (Atwill et al., 2011).

Although multiple studies have shown that the relationship between the occurrence of pathogens correlates poorly with concentrations of indicator organisms (Edge et al., 2012; Field and Samadpour, 2007), this study did find a significant relationship between *Salmonella* occurrence and log-scale increases in the concentration of *E. coli* in water samples; for every 10-fold increase in indicator bacteria, the odds of detecting *Salmonella* increased over 2.5 times (odds ratio, 2.64; 95% confidence interval, 1.57–4.43). This seeming contradiction in results is likely the result of a nonlinear relationship between indicators and pathogens, with correlations more likely to occur once a threshold value has been exceeded, and a result of our study design. We tested large volumes of water (20 L) for pathogens, increasing the likelihood of detecting *Salmonella* and quite possibly of finding a correlation with *E. coli*. However, the majority (39/48, 85%) of *Salmonella* positives occurred when *E. coli* concentrations were below the industry standard of 235 CFU 100 mL^{-1}.

According to NRCS engineering requirements, the construction of approved CPs, both sediment basins and tail-water recovery ponds, requires the inclusion of bank vegetation to reduce erosion. The presence of aquatic vegetation, although not required, may provide additional benefit by using excess nutrients and improving water clarity (Jansson et al., 1994; Williams et al., 2010). In this study, neither the presence of bank vegetation nor aquatic vegetation was significantly associated with an increased odds of detecting *Salmonella* in water or sediment samples. This suggests that the perception of introduced risk associated with vegetated banks and ponds may be unwarranted, although the benefit they provide has been documented.

In some areas of the United States, the reuse of recovered irrigation water is encouraged to avoid overtaxing valuable groundwater supplies and is seen as a sustainable practice. Studies of irrigation water have found that surface sources of irrigation water are likely to contain pathogens at some point in time (Pachepsky et al., 2011), although prevalences vary widely by region (Cooley et al., 2007; Duffy et al., 2005). Our data indicate that samples from tail-water recovery ponds in the southeastern United States are almost 10 times more likely (odds ratio, 9.22; 95% confidence interval, 3.61–23.58) to test positive for *Salmonella* than samples taken from sediment basins in California. In order for growers to make use of this valuable water supply, it may be necessary for water to be treated with a disinfecting step before reapplication on commodities grown for human consumption.

We found that the presence of mammals at sediment basins was negatively associated with the odds of detecting *Salmonella* in water. These results were somewhat surprising, and further evaluation of the association was considered, including whether the presence of mammals was a proxy for location. Although mammals were recorded more frequently at California basins (15%) than in the southeastern United States (11%), this difference was not significant. Further, the retention of both region and mammal presence in the model, grouped by basin, suggests that it is not a location effect. Mammalian presence was not associated with a particular source of water, presence of vegetation, concentration of rodent burrows, or fecal deposits.

Occurrence of Pathogens in Sediment

As in water samples, pathogens in sediment are in flux and can be difficult to detect using standard culturing techniques, particularly when using low sample volumes and weights. We used a two-step process of enrichment and detection via immunomagnetic separation that enabled us to use a larger amount of sediment (100 g) than has been typically collected in similar studies (Badgley et al., 2010; Benjamin et al., 2013).

It is well established that sediment may have bacterial loads orders of magnitude higher than in the overlying water column (Sherer et al., 1992); consequently, resuspension of accumulated sediments can significantly affect the quality of the surrounding water column (Coffey et al., 2013; Lewis et al., 1986). Further, sedimentation of particles or resuspension of particulate matter with associated microorganisms are principal factors in the survival of bacterial pathogens in tail-water recovery ponds (Stenstrom and Carlander, 2001). It is outside of the scope of this study to discuss particle attachment, settling rates, and biofilm formation, but it suffices to say that bacterial pathogens have an affinity for submerged sediments and may persist there longer than in the water column (Droppo et al., 2009). However, despite the availability of organic material, sediments are limited in nutrition and energy for bacterial maturation (Pommepuy et al., 1992) and may be particularly hostile when exposed to drying and UV radiation (Atwill et al., 2012). At this time, little

information is available on the persistence of pathogenic bacteria in agricultural sediments, particularly in CPs like sediment basins and tail-water recovery ponds.

A recent study concluded that irrigation pond water and sediment, as well as the water and sediment from the ditches leading to these ponds, have an increased occurrence of *Salmonella*, although these sediments were not allowed any drying time (Micallef et al., 2012). Similar studies have also found that in exposed soils other environmental factors, such as solar radiation, temperature, and dryness, greatly diminished pathogen populations (Oliveira et al., 2012). We found a precipitous decrease in the presence of *Salmonella* in sediment samples once basins had been exposed to drying through dewatering, which seems to support the assertions of the aforementioned studies. However, we did not find any significant correlations between pathogen occurrence or *E. coli* concentration and solar radiation, relative humidity, or air temperature. Although this relationship was nonsignificant, this is likely due to the small sample size of exposed sediments (21%) and inadequate power to detect a difference. It does appear, however, that allowing sediment basins to dewater and the sediments to dry is likely to reduce the probability of *Salmonella* detection.

Results of CLL regression models indicate that fecal deposit concentrations along the basin bank were negatively associated with *Salmonella* occurrence in sediments. Although this association was not significant ($p > 0.05$), its inclusion in the model significantly increased the model fit. Similarly to the association between mammal presence and reduced detection of *Salmonella* in water samples, this relationship was somewhat surprising. Further investigation revealed that there were higher concentrations of feces recorded at basins that also had <50% vegetation coverage ($n = 35$; mean, 33.4; SD, 61.5) compared with >50% coverage ($n = 54$; mean, 16.8; SD, 37.3), and the highest proportions of low-vegetation basins (<50% coverage) were found in the CC subregion (65% of low-vegetation basins), which also had low *Salmonella* occurrence (7.7% of total positives). Although this might suggest a detection bias toward nonvegetated basins or that fecal concentrations are a proxy for subregion, the fact that fecal concentration was retained in the model along with subregion may suggest otherwise. The impact of animal presence within and around basins as it relates to pathogen occurrence needs to be more closely evaluated.

Conservation tools have been increasingly useful for water quality programs across the United States. However, after recent foodborne outbreaks associated with wildlife intrusion, many of these programs have been abandoned because of a perception of increased risk to the consumer (Crohn and Bianchi, 2008). Safe use of the water, soil, and sediment within studied CPs requires a better understanding of the microbial behavior in sediments and the overlying water column and environmental conditions that affect the removal or inactivation of pathogens (i.e., solar radiation and drying). Studies indicate that the time necessary for 100% die-off of pathogens depends on initial loading, specific die-off rate, and certain environmental conditions (Atwill et al., 2012; Ma et al., 2012); therefore, CPs that both increase the die-off rate and reduce pathogen loading would greatly decrease pathogen persistence in water and sediment samples.

Conclusions

We found no indication that the presence of vegetation or wildlife surrounding sediment basins and tail-water recovery ponds had any influence on the likelihood of testing positive for pathogens in water or sediment samples. Although the reuse of water for irrigation may require a disinfecting step, the reapplication of captured sediments may offer reduced risk if sediments are allowed to dry completely. In general, providing growers with the incentive to conserve water and soil resources and make more efficient use of the dwindling ground and surface water supplies can be achieved by CPs like sediment and tail-water recovery ponds.

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