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BACTERIA MONITORING IN THE UPPER ILLINOIS RIVER WATERSHED

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Bacteria Monitoring in the Upper Illinois River Watershed

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The Federal Clean Water Act requires that states identify impaired water bodies that do not meet the applicable water quality standard (WQS) and do not support assigned designated use(s). Pathogens are often cited as the cause of streams and rivers being added to the 303(d) list of impaired water bodies. The Arkansas Department of Environmental Quality assesses water-quality data against defined WQS to ensure public health and safety, especially for primary and secondary contact uses.

The Illinois River Watershed (IRW) in northwest Arkansas had seven streams on the 2008 303(d) list of impaired water bodies due to elevated levels of *E. coli*. In the current study, water samples were collected during the primary contact season (May through September) in calendar years 2012, 2013 and 2014 at 29 sites across the seven streams on the 303(d) list in the IRW. Water samples were analyzed for *E. coli* numbers, which were evaluated against the applicable WQS as defined in the Arkansas Pollution Control and Ecology Commission Regulation 2.

Violations of the applicable WQS were variable from year to year and between stream reaches and individual sites. One reach (IR028) on the Illinois River violated the applicable WQS for *E. coli* numbers in 2014, but only two of the four sites where water samples were collected along this reach actually violated the WQS. The three sites where water samples were collected on Little Osage Creek frequently violated the applicable WQS. For example, *E. coli* numbers exceeded 410 colonies (col)/100 mL for over 50% of the water samples collected at all three sites during the 2012 and 2014 primary contact seasons. A threshold relationship existed between elevated levels of *E. coli* and the amount of pasture land within the riparian zone, where the only sites that violated the applicable WQS for *E. coli* had more than 50% pasture land in the riparian zone.

Results from this study suggest that elevated *E. coli* numbers are a localized issue. Land use within the riparian zone, and especially direct animal access to the streams, likely drove the observed increases in *E. coli*. However, this study showed that the majority of the reaches and sites sampled were within the regulatory limits for *E. coli* during the primary contact season.

Introduction

The Federal Clean Water Act requires that all states submit to the U.S. Environmental Protection Agency (USEPA) a list of impaired water bodies which fail to meet water-quality standards (WQS) based on their designated use(s). Nation-wide, pathogens are listed as the most common cause of impairment resulting in water bodies being added to the 303(d) list (USEPA, 2015a). *Escherichia coli (E. coli)* is an indicator organism for fecal contamination of streams and rivers used by the USEPA and other regulatory agencies. Major sources of fecal contamination include livestock, wildlife, leaky septic systems and runoff from urban and agricultural landscapes.

In Arkansas, the Department of Environmental Quality (ADEQ) utilizes water-quality data from various sources to compare against applicable WQS defined in the Arkansas Pollution Control and Ecology Commission Regulation 2 (APCEC, 2014). Approximately 390 km of streams and rivers in Arkansas are impaired because they exceeded the WQS for *E. coli* (USEPA, 2015b). The WQS for *E. coli* varies in assessment technique in Arkansas, including comparisons to geometric means based on several samples in a short period as well as percent of samples exceeding an applicable value for a stream over the recreational season. The intent is to protect human health during primary and secondary contact recreation in streams, and the stream ends up on the 303(d) list when *E. coli* numbers exceed assessment criteria.

Water-quality impairment due to pathogens is a concern in the Illinois River Watershed (IRW) in northwest Arkansas, where seven streams or rivers were on the 303(d) list of impaired water bodies in 2008 (ADEQ, 2008). In a 2009 study, *E. coli* numbers across the IRW were highly variable both within and across almost 30 sites (Haggard et al., 2010). In that study, the geometric mean of *E. coli* numbers ranged from 44 to 1962 colonies (col)/100 mL, while maximum values reached from 200 to 12000 col/100 mL across sites sampled during seasonal base flow. The objectives of the current study were (1) to evaluate *E. coli* numbers at different sites along each of the reaches in the IRW on the 303(d) list of impaired water bodies for pathogens and (2) to compare the data against the applicable WQS (APCEC, 2014). Water samples were collected during the primary contact seasons (May through September) of calendar years 2012, 2013 and 2014 at a total of 29 sites in seven streams – the Illinois River, Baron Fork, Muddy Fork, Osage Creek, Little Osage Creek, Spring Creek and Clear Creek.

Study Area

This project focuses on the Upper Illinois River Watershed (UIRW; HUC 11110103), which is within the Boston Mountains and Ozark Highlands ecoregions in northwest Arkansas. Headwaters of the Illinois River originate near Hogeye, Arkansas and flow north through Savoy, then west into Oklahoma near Watts. The UIRW drains an area of 1952 km^2, of which 50.3% is pasture and grassland, 35.9% is forest, 8.8% is urban and suburban, 4.3% is transitional and 0.3% is water (Arkansaswater.org, 2015). Land use throughout the watershed is also changing, with increases in residential, commercial and industrial development. The IRW has been designated a priority watershed for the Arkansas Natural Resources Commission (ANRC) 319 Nonpoint Source Program.

A total of 29 sites were sampled across 10 reaches, as designated by ADEQ, including sites on the Illinois River and its major tributaries (Figure 1; Table 1). The main tributaries to the Illinois River that were sampled in this study include Clear Creek, Osage Creek, Little Osage Creek, Spring Creek, Muddy Fork and Baron Fork. All study reaches were on the 2008 303(d) list of

impaired waterbodies for pathogens, with the source of impairment unknown (ADEQ, 2008).

Methods

Bacterial sampling and laboratory analysis

Water samples were collected every 1 to 3 weeks for a total of 8 or 9 times between May 1 and September 30 (primary contact season as defined in APCEC Regulation 2) each calendar year during 2012, 2013 and 2014. Water samples were collected from the thalweg in sterile containers (VRW International; Gallaway, TN) which remain sealed in the packaging until sample collection. After collecting the water sample, containers were sealed and transported on ice to the laboratory and then processed immediately. Water samples were analyzed for *E. coli* at the Arkansas Water Resources Center Water Quality Laboratory, certified for bacterial analysis, using the IDEXX Colilert Total Coliform and *E. coli* method (APHA 9223 B). The most probable number (MPN) of colonies (col)/100 mL was evaluated and reported.

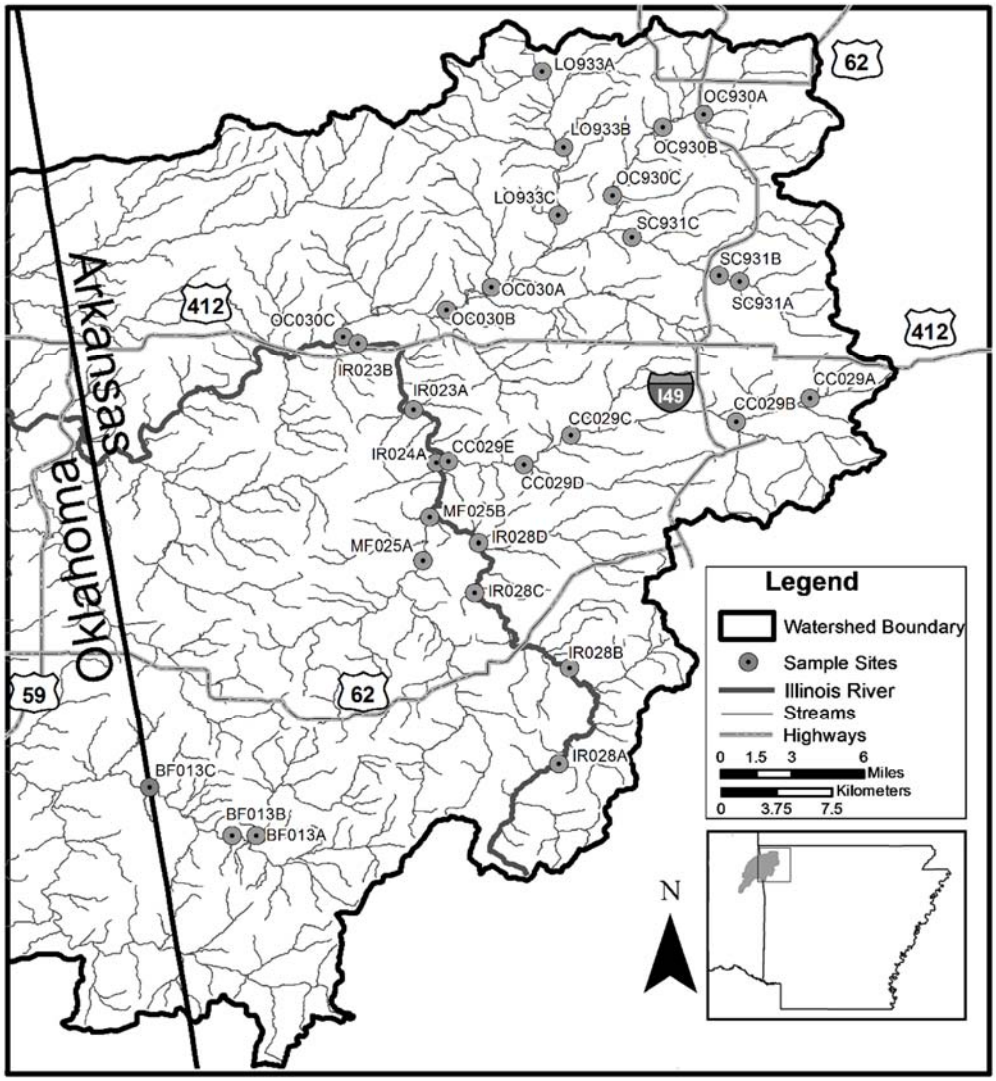

Figure 1. Map of the 29 sites sampled to monitor pathogens in the Illinois River Watershed.

Land use land cover calculations for the riparian zone

ArcMap (ESRI, 2011) was used to plot sample locations, delineate riparian land area and calculate riparian zone land use land cover (LULC) for each site (WGS1984 coordinate system; U.S. Geological Survey 2011 National Land Use Land Cover). Riparian zones for each site included the area of land approximately 45 m on each side of the stream channel; this distance (45 m) is on the upper end of the recommended distance for riparian buffers adjacent to the stream channel (NRCS, 2011; USDA, 2015). Delineated riparian zones extended 2 km upstream from each sample site. When there was a tributary confluence within the 2 km distance upstream from the sample point, riparian area for that tributary was included for a total distance of 2 km from the sample point. Only direct tributaries to each study stream were included. This means that the total land area evaluated could be different for each study site, depending on how many direct tributaries entered the stream within 2 km upstream from the sample site.

Data analysis

The APCEC Regulation 2 lists the following water quality standard for *E. coli*:

"For assessment of ambient waters as impaired by bacteria, the below listed applicable values for E. coli shall not be exceeded in more than 25% of the samples in no less than eight (8) samples taken during the primary contact season [May 1 through September 31]."

The applicable value for *E. coli* in streams and rivers is 410 col/100 mL, and applies to all the streams sampled in the current study, with the exception of the Illinois River. The Illinois River is designated as an ecologically sensitive waterbody (ESW) due to the presence of the Neosho Mucket mussel, and as such, the applicable value for *E. coli* is 298 col/100 mL as defined in the regulation.

The percentage of *E. coli* measurements exceeding 298 col/100 mL (for the Illinois River) or 410 col/100 mL (for all other streams sampled) were evaluated by site and reach for each primary contact season in 2012, 2013 and 2014. Statistix Analytical Software (Tallahassee, FL) was used to calculate descriptive statistics, including maximum, minimum and quartiles, for *E. coli* numbers for each reach and individual sites. Non-parametric change point analysis (NCPA; R Software) was used to relate riparian LULC to the percent of water samples with *E. coli* numbers exceeding the applicable value. NCPA is a non-linear, threshold based approach to evaluate the relationship between a predictor variable (e.g. percent pasture) and a response variable (e.g. percent exceedance for *E. coli* numbers).

Table 1. Reaches (as defined by ADEQ) in a given stream or river, site ID and qualifier, coordinates and HUC12 descriptions for study sites for pathogens monitoring in the Upper Illinois River Watershed.

Reach	Stream Name	Site ID	Site Qualifier	Latitude	Longitude	HUC12
023	Illinois River	IR023A	at Robinson Road	36°08'06.29"N	94°21'29.48"W	111101030103
	Illinois River	IR023B	at Kincheloe Road	36°10'32.68"N	94°23'30.72"W	111101030103
024	Illinois River	IR024A	at Highway 16	36°06'10.08"N	94°20'39.03"W	111101030103
028	Illinois River	IR028A	at Hogeye Road	35°55'13.59"N	94°16'14.00"W	111101030101
	Illinois River	IR028B	at Illinois Chapel Road	35°58'43.21"N	94°15'50.37"W	111101030102
	Illinois River	IR028C	at Bethel Blacktop Road	36°01'28.83"N	94°19'17.46"W	111101030102
	Illinois River	IR028D	at Goose Creek Road	36°03'16.22"N	94°19'07.11"W	111101030103
013	Baron Fork	BF013A	at Bush Valley Road	35°52'35.08"N	94°27'13.02"W	111101030701
	Baron Fork	BF013B	at Highway 45	35°52'36.02"N	94°28'05.41"W	111101030701
	Baron Fork	BF013C	at Salem Springs North Road	35°54'21.72"N	94°31'05.84"W	111101030701
025	Muddy Fork	MF025A	at Mateer Road	36°02'39.18"N	94°21'10.33"W	111101030403
	Muddy Fork	MF025B	at Viney Grove Road	36°04'11.41"N	94°20'54.71"W	111101030403
030	Osage Creek	OC030A	at Rocky Comfort Road	36°12'37.14"N	94°18'40.99"W	111101030305
	Osage Creek	OC030B	at Logan Cave Road	36°11'46.53"N	94°20'18.04"W	111101030305
	Osage Creek	OC030C	at Old Highway 68	36°10'48.56"N	94°24'03.04"W	111101030305
930	Osage Creek	OC930A	at New Hope Road	36°18'53.00"N	94°10'55.08"W	111101030301
	Osage Creek	OC930B	at South Rainbow Road	36°18'23.54"N	94°12'26.51"W	111101030301
	Osage Creek	OC930C	at Healing Springs Road	36°15'55.09"N	94°14'15.05"W	111101030301
933	Little Osage Creek	LO933A	at Brookside Road	36°20'25.01"N	94°16'49.01"W	111101030303
	Little Osage Creek	LO933B	at Mill Dam Road	36°17'39.58"N	94°16'02.88"W	111101030303
	Little Osage Creek	LO933C	at Highway 264	36°15'13.73"N	94°16'14.92"W	111101030303
931	Spring Creek	SC931A	at Silent Grove Road	36°12'49.96"N	94°09'41.13"W	111101030302
	Spring Creek	SC931B	at North 40th Street	36°12'59.72"N	94°10'23.12"W	111101030302
	Spring Creek	SC931C	at Wagon Wheel Road	36°14'25.04"N	94°13'33.06"W	111101030302
029	Clear Creek	CC029A	at North Crossover Road	36°08'31.07"N	94°07'04.08"W	111101030201
	Clear Creek	CC029B	at Wilkerson Rd	36°07'40.02"N	94°09'46.06"W	111101030201
	Clear Creek	CC029C	at Wheeler Road	36°07'12.29"N	94°15'46.66"W	111101030204
	Clear Creek	CC029D	at Harmon Road	36°06'07.56"N	94°17'29.18"W	111101030204
	Clear Creek	CC029E	at West U of A Beef Farm Road	36°06'13.77"N	94°20'12.89"W	111101030103

Results

Illinois River, ADEQ Stream Segments 023, 024 and 028

In the Illinois River, there were three separate reaches that were sampled during the primary contact season (May through September). The most downstream reach (IR023) had two sampling sites, the middle reach (IR024) had only one sampling site, and the upstream reach had four sampling sites (Figure 1). Each reach and individual site was evaluated against the applicable water quality standard (WQS) for *E. coli* numbers. The Illinois River is an ecologically sensitive waterbody (ESW) due to the presence of the Neosho Mucket mussel, and thus the applicable standard states that *E. coli* numbers shall not exceed 298 col/100 mL in more than 25% of the water samples collected in no less than 8 events during the primary contact season (APCEC, 2014).

In reach IR023 (downstream reach), *E. coli* numbers ranged from 11 to 1120 col/100 mL across the two sites (IR023A and IR023B) and over the three seasons sampled (2012, 2013 and 2014; Table 2). The percent of the *E. coli* numbers across the reach that exceeded 298 col/100 mL was 12.5% or less in all three seasons. When looking at the sites individually, *E. coli* numbers did not violate the applicable WQS at either site nor during any individual primary contact season.

In reach IR024 (which contained only one sampling site), *E. coli* numbers ranged from 18 to 1203 col/100 mL across all three primary contact seasons (Table 2). *E. coli* numbers did not exceed 298 col/100 mL in any of the water samples collected in 2012 or 2013, where the maximum number observed was 172 col/100 mL. During the 2014 season, *E. coli* numbers exceeded 298 col/100 ml in 11% of the water samples collected. Since the percent exceedance was never greater than 25%, there were no violations of the WQS for *E. coli* at this site during the study period.

In reach IR028 (upstream reach), the *E. coli* numbers ranged from 2 to 1300 col/100 mL across all four sampling sites and over the three seasons sampled (Table 2). This reach and select individual sites showed some instances of violating the WQS. For example, *E. coli* numbers exceeded 298 col/100 mL in more than 27% of the water samples collected across the reach in 2014. However, *E. coli* numbers exceeded the limit of 298 col/100 mL for 25% or less of the water samples collected in 2012 and 2013.

In the IR028 reach, the percent exceedance of the WQS was variable spatially or between individual sites. The *E. coli* numbers at the most upstream site (IR028A) only violated the WQS during the 2014 sampling season (33.3% exceedance; Table 2). The *E. coli* numbers at the next two downstream sites (IR028B and IR028C) did not violate the WQS in any of the three years sampled. However, the *E. coli* numbers at the most downstream site (IR028D) exceeded 298 col/100 mL in at least 50% of the water samples collected in each year.

Table 2. Summary statistics for *E. coli* numbers for each reach and individual sites on the Illinois River for each primary contact season (May through September) during 2012, 2013 and 2014. The table includes the number of samples collected (N), the geomean (Geo.), minimum (Min.), median (Med.), and maximum (Max.) *E. coli* as the most probable number (MPN) of colonies (col)/100 mL. The percentage of *E. coli* measurements exceeding the limit of 298 col/100 mL (% Exc.) is also shown. Bold values for % Exc. represent a reach or stream that violated the applicable WQS in a given year (*E. coli* numbers exceeded 298 col/100 mL for more than 25% of the samples collected during the primary contact season; APCEC Regulation 2).

Site ID	Year	N	Geo.	Min.	25th	Med.	75th	Max.	% Exc.
Reach IR023	2012	16	40	18	27	32	60	166	0
	2013	16	77	24	39	54	101	1120	12.5
	2014	18	71	11	39	63	90	921	11.1
Reach IR024	2012	8	44	22	30	45	67	71	0
	2013	8	42	20	23	33	106	172	0
	2014	9	49	18	25	37	50	1203	11.1
Reach IR028	2012	32	104	2	50	80	308	1300	25.0
	2013	32	105	7	52	120	261	921	15.6
	2014	36	143	7	67	187	370	921	**27.8**
IR023A	2012	8	53	22	31	56	70	166	0
	2013	8	73	26	35	48	131	866	12.5
	2014	9	85	32	39	78	113	921	11.1
IR023B	2012	8	31	18	26	30	33	75	0
	2013	8	81	24	40	67	101	1120	12.5
	2014	9	59	11	34	54	83	649	11.1
IR024A	2012	8	44	22	30	45	67	71	0
	2013	8	42	20	23	33	106	172	0
	2014	9	49	18	25	37	50	1203	11.1
IR028A	2012	8	97	44	52	85	116	687	12.5
	2013	8	109	24	56	120	240	410	12.5
	2014	9	271	119	160	238	507	921	**33.3**
IR028B	2012	8	23	2	13	32	53	68	0
	2013	8	40	7	14	46	114	285	0
	2014	9	60	11	21	58	185	345	11.1
IR028C	2012	8	111	42	73	80	225	345	12.5
	2013	8	77	36	58	66	128	154	0
	2014	9	111	17	65	118	194	866	11.1
IR028D	2012	8	465	120	269	446	1001	1300	**75.0**
	2013	8	355	151	216	361	541	921	**50.0**
	2014	9	230	7	199	378	495	649	**55.5**

Baron Fork, ADEQ Stream Segment 013

In Baron Fork, one reach (BF013) was sampled at three different sites (BF013A, BF013B and BF013C) during the primary contact season (May through September) of 2012, 2013 and 2014 (Figure 1). The reach and the individual sample sites were evaluated against the applicable WQS. For Baron Fork, which is not an ESW, an extraordinary resource waterbody (ERW) or a natural and scenic waterway (NSW), the WQS states that *E. coli* numbers shall not exceed 410 col/100 mL in more than 25% of the water samples collected in no less than 8 events during the primary contact season (APCEC, 2014).

In this reach (BF013), *E. coli* numbers ranged from 1 to 2420 col/100 mL across all three sites and study years (Table 3). There were some instances where water samples had *E. coli* numbers that exceeded 410 col/100 mL, but this happened for less than 25% of the water samples collected in each year for the entire reach. Thus, Baron Fork did not violate the WQS when aggregating data at the reach level during the study period.

E. coli numbers did not exceeded 410 col/100 mL for more than 25% of the samples collected at sites BF013A and BF013C in all three study years, nor at site BF013B in 2013 or 2014. Only site BF013B violated the applicable WQS during the primary contact season in 2012, where *E. coli* numbers exceeded 410 col/100 mL for 38% of the water samples collected. None of the individual sites have violated the WQS in the last two primary contact seasons (2013 and 2014).

Table 3. Summary statistics for *E. coli* numbers for the reach and individual sites on Baron Fork for each primary contact season (May through September) during 2012, 2013 and 2014. The table includes the number of samples collected (N), the geomean (Geo.), minimum (Min.), median (Med.), and maximum (Max.) *E. coli* as the most probable number (MPN) of colonies (col)/100 mL. The percentage of *E. coli* measurements exceeding the limit of 410 col/100 mL (% Exc.) is also shown. Bold values for % Exc. represent a reach or stream that violated the applicable WQS in a given year (*E. coli* numbers exceeded 410 col/100 mL for more than 25% of the samples collected during the primary contact season; APCEC Regulation 2).

Site ID	Year	N	Geo.	Min.	25th	Med.	75th	Max.	% Exc.
Reach BF013	2012	24	60	6.3	13	46	220	1300	16.7
	2013	24	30	2	13	39	56	2420	4.2
	2014	27	35	1	12	39	93	1553	11.1
BF013A	2012	8	66	12	20	61	163	816	12.5
	2013	8	46	13	22	41	121	172	0
	2014	9	75	15	45	55	182	548	11.1
BF013B	2012	8	202	13	51	259	1057	1300	**37.5**
	2013	8	69	6	26	51	139	2420	12.5
	2014	9	78	12	17	61	414	1553	22.2
BF013C	2012	8	16	6	9	16	28	51	0
	2013	8	9	2	4	9	24	39	0
	2014	9	7	1	3	4	27	141	0

Muddy Fork, ADEQ Stream Segment 025

In Muddy Fork, one reach (MF025) was sampled at two different sites (MF025A and MF025B) during the primary contact season during 2012, 2013 and 2014 (Figure 1). The downstream site (MF025B) is located just upstream from the confluence with the Illinois River, between sites IR028D and IR024A. Muddy Fork is not classified as an ESW, ERW or a NSW, so the WQS that this reach and the two sites were evaluated against is that *E. coli* numbers shall not exceed 410 col/100 mL in more than 25% of the water samples collected in no less than 8 events during the primary contact season (APCEC, 2014).

In reach MF025, *E. coli* numbers ranged from 9 to 3730 col/100 mL across the two sites and over the three seasons sampled (2012, 2013 and 2014; Table 4). The numbers of *E. coli* exceeded 410 col/100 mL in zero, 12.5 and 16.7% of the water samples collected during 2012, 2013 and 2014, respectively. The Muddy Fork reach never violated the WQS for *E. coli*, when aggregating data at the reach level.

Similarly, when looking at the sites individually, *E. coli* numbers exceeded 410 col/100 mL for less than 25% of the water samples collected during the primary contact seasons in each study year. There were no violations of the applicable WQS at sites MF024A and MF024B during the study period.

Table 4. Summary statistics for *E. coli* numbers for the reach and individual sites on Muddy Fork for each primary contact season (May through September) during 2012, 2013 and 2014. The table includes the number of samples collected (N), the geomean (Geo.), minimum (Min.), median (Med.), and maximum (Max.) *E. coli* as the most probable number (MPN) of colonies (col)/100 mL. The percentage of *E. coli* measurements exceeding the limit of 410 col/100 mL (% Exc.) is also shown. Bold values for % Exc. represent a reach or stream that violated the applicable WQS in a given year (*E. coli* numbers exceeded 410 col/100 mL for more than 25% of the samples collected during the primary contact season; APCEC Regulation 2).

Site ID	Year	N	Geo.	Min.	25th	Med.	75th	Max.	% Exc.
Reach MF025	2012	16	54	19	29	56	77	326	0
	2013	16	124	26	52	117	223	980	12.5
	2014	18	130	9	50	98	335	3730	16.7
MF025A	2012	8	47	19	22	44	68	326	0
	2013	8	84	26	44	61	178	579	12.5
	2014	9	71	9	25	54	135	3730	11.1
MF025B	2012	8	62	28	39	70	98	108	0
	2013	8	183	51	116	145	348	980	12.5
	2014	9	239	70	84	219	643	1553	22.2

Osage Creek, ADEQ Stream Segments 030 and 930

In Osage Creek, two separate reaches were sampled during the primary contact season (May through September) in 2012, 2013 and 2014 (Figure 1). Reaches OC930 (upstream reach) and OC030 (downstream reach) each have three sites that were sampled, with site OC030C being the most downstream site, located just above the confluence with the Illinois River, downstream from site IR023B. Osage Creek is not classified as an ESW, ERW or a NSW, so the WQS that this reach and the two sites were evaluated against is that *E. coli* numbers shall not exceed 410 col/100 mL in more than 25% of the water samples collected in no less than 8 events during the primary contact season (APCEC, 2014).

In reach OC030 (downstream reach), *E. coli* numbers ranged from 11 to 2750 col/100 mL across the two sites and three study years (Table 5). The percent of water samples that exceeded 410 col/100 mL was 4.2% or less in all three years, and *E. coli* numbers only exceeded 410 col/100 mL in one water sample collected in 2013. Thus, the applicable WQS for *E. coli* was never violated in this reach, when aggregating data at the reach level.

E. coli numbers at site OC030A exceeded 410 col/100 mL in only one water sample collected (12.5%) in 2013 and in zero water samples collected in 2012 and 2014. Sites OC030B and OC030C never exceeded 410 col/100 mL during any study year, where the maximum number observed was 326 col/100 mL. The applicable WQS for *E. coli* was never violated at any site for any study year in reach OC030.

In reach OC930 (upstream reach), *E. coli* numbers ranged from 5 to 2130 col/100 mL across the three sites and three primary contact seasons that were sampled (Table 5). *E. coli* numbers exceeded 410 col/100 mL in only two water samples (8.3%) in 2012 and zero times in 2013 and 2014. The applicable WQS for *E. coli* was never violated for reach OC930.

E. coli numbers at sites OC930A and OC930B exceeded 410 col/100 mL in only one water sample collected in 2012 and in none of the water samples collected in 2013 or 2014. *E. coli* numbers never exceeded 410 col/100 mL at site OC930C during any study year, where the maximum number of *E. coli* observed was 308 col/100 mL across all three years. The applicable WQS for *E. coli* was never violated at any site for any study year in reach OC930.

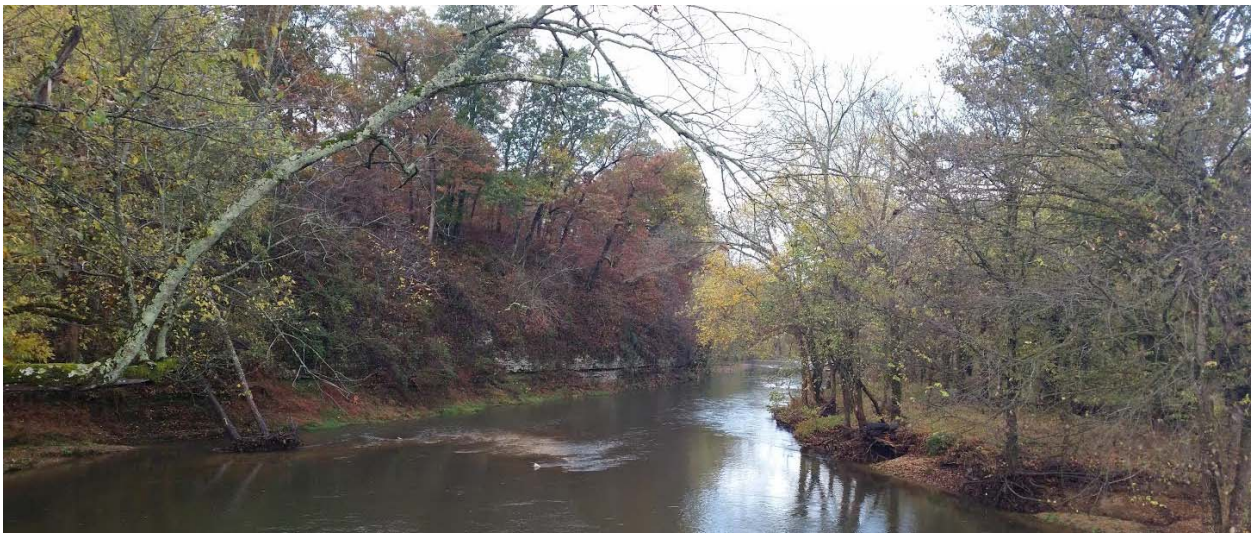

Table 5. Summary statistics for *E. coli* numbers for each reach and individual sites on Osage Creek for each primary contact season (May through September) during 2012, 2013 and 2014. The table includes the number of samples collected (N), the geomean (Geo.), minimum (Min.), median (Med.), and maximum (Max.) *E. coli* as the most probable number (MPN) of colonies (col)/100 mL. The percentage of *E. coli* measurements exceeding the limit of 410 col/100 mL (% Exc.) is also shown. Bold values for % Exc. represent a reach or stream that violated the applicable WQS in a given year (*E. coli* numbers exceeded 410 col/100 mL for more than 25% of the samples collected during the primary contact season; APCEC Regulation 2).

Site ID	Year	N	Geo.	Min.	25th	Med.	75th	Max.	% Exc.
Reach OC030	2012	24	51	13	31	60	86	161	0
	2013	24	66	15	30	47	137	2750	4.2
	2014	27	50	11	36	50	73	158	0
Reach OC930	2012	24	71	5	30	75	156	2130	8.3
	2013	24	65	11	41	60	110	308	0
	2014	27	94	26	57	105	172	387	0
OC030A	2012	8	45	20	26	43	85	104	0
	2013	8	82	17	27	44	209	2750	12.5
	2014	9	58	25	34	55	101	121	0
OC030B	2012	8	82	36	45	86	139	161	0
	2013	8	60	15	35	47	129	326	0
	2014	9	57	29	42	50	76	158	0
OC030C	2012	8	36	13	14	51	68	88	0
	2013	8	57	23	27	44	133	291	0
	2014	9	37	11	28	40	52	104	0
OC930A	2012	8	37	5	7	36	66	2130	12.5
	2013	8	53	11	38	49	91	206	0
	2014	9	152	50	109	172	204	387	0
OC930B	2012	8	87	23	46	65	185	770	12.5
	2013	8	42	18	31	43	63	68	0
	2014	9	55	26	32	57	88	199	0
OC930C	2012	8	111	18	97	133	190	236	0
	2013	8	124	55	106	115	171	308	0
	2014	9	100	51	73	105	128	210	0

Little Osage Creek, ADEQ Stream Segment 933

In Little Osage Creek, one reach (LO933) was sampled at three different sites during the primary contact season (May through September) of 2012, 2013 and 2014 (Figure 1). Little Osage Creek flows into Osage Creek in between the two study reaches of Osage Creek. The reach and the individual sample sites were evaluated against the applicable WQS. For Little Osage Creek, which is not an ESW, ERW or a NSW, the WQS states that *E. coli* numbers shall not exceed 410 col/100 mL in more than 25% of the water samples collected in no less than 8 events during the primary contact season (APCEC, 2014).

In reach LO933, *E. coli* numbers in collected water samples ranged from 15 to 11780 col/100 mL across the three study sites and primary contact seasons (Table 6). This reach violated the WQS in 2012 and 2014, where *E. coli* numbers exceeded 410 col/100 mL in 58.3 and 66.7% of the water samples collected. In 2013, *E. coli* numbers exceeded 410 col/100 mL in only 12.5% of the samples collected, and thus the applicable WQS was not violated during this study year, when assessed at the reach level.

In all three sites (LO933A, LO933B and LO933C), *E. coli* numbers exceeded 410 col/100 mL in at least 50% of the samples collected during the 2012 and 2014 sampling seasons, thus violating the applicable WQS. The percent of water samples collected where *E. coli* numbers exceeded 410 col/100 mL was 25% or less in all sites during the 2013 primary contact season. Therefore, the applicable WQS was not violated at any site during 2013.

Table 6. Summary statistics for *E. coli* numbers for the reach and individual sites on Little Osage Creek for each primary contact season (May through September) during 2012, 2013 and 2014. The table includes the number of samples collected (N), the geomean (Geo.), minimum (Min.), median (Med.), and maximum (Max.) *E. coli* as the most probable number (MPN) of colonies (col)/100 mL. The percentage of *E. coli* measurements exceeding the limit of 410 col/100 mL (% Exc.) is also shown. Bold values for % Exc. represent a reach or stream that violated the applicable WQS in a given year (*E. coli* numbers exceeded 410 col/100 mL for more than 25% of the samples collected during the primary contact season; APCEC Regulation 2).

Site ID	Year	N	Geo.	Min.	25th	Med.	75th	Max.	% Exc.
Reach LO933	2012	24	352	15	220	488	1015	2420	**58.3**
	2013	24	195	34	109	200	287	2280	12.5
	2014	27	548	108	326	488	816	11780	**66.7**
LO933A	2012	8	436	15	242	793	1015	2420	**62.5**
	2013	8	329	59	198	235	1336	2280	25.0
	2014	9	958	108	438	980	1986	11780	**77.8**
LO933B	2012	8	322	21	229	462	557	1553	**62.5**
	2013	8	216	70	134	263	377	411	12.5
	2014	9	410	179	208	461	749	816	**66.7**
LO933C	2012	8	312	28	83	428	1183	1733	**50.0**
	2013	8	105	34	76	111	137	291	0
	2014	9	419	219	308	435	532	816	**55.6**

Spring Creek, ADEQ Stream Segment 913

In Spring Creek, one reach (SC913) was sampled at three different sites during the primary contact season (May through September) of 2012, 2013 and 2014 (Figure 1). Spring Creek also flows into Osage Creek in between the two study reaches of Osage Creek, upstream from where Little Osage Creek enters Osage Creek. Spring Creek is not classified as an ESW, ERW or a NSW, so the WQS that this reach and the three sites were evaluated against is that *E. coli* numbers shall not exceed 410 col/100 mL in more than 25% of the water samples collected in no less than 8 events during the primary contact season (APCEC, 2014).

In reach SC913, *E. coli* numbers in collected water samples ranged from 4 to 435 col/100 mL across all three sites and study years (Table 7). The percent of water samples collected where *E. coli* numbers exceeded 410 col/100 mL was 4.2% or less in all three years. The applicable WQS for *E. coli* was never violated when aggregating the data at the reach level.

E. coli numbers at site SC913A exceeded 410 col/100 mL in only one water sample (12.5%) collected in 2013 and in zero water samples collected in 2012 and 2014. *E. coli* numbers at sites SC913B and SC913C never exceeded 410 col/100 mL in any samples collected during the primary contact seasons, where the maximum number observed was 172 col/100 mL. The applicable WQS for *E. coli* was never violated at any site for any study year in reach OC030.

Table 7. Summary statistics for *E. coli* numbers for the reach and individual sites on Spring Creek for each primary contact season (May through September) during 2012, 2013 and 2014. The table includes the number of samples collected (N), the geomean (Geo.), minimum (Min.), median (Med.), and maximum (Max.) *E. coli* as the most probable number (MPN) of colonies (col)/100 mL. The percentage of *E. coli* measurements exceeding the limit of 410 col/100 mL (% Exc.) is also shown. Bold values for % Exc. represent a reach or stream that violated the applicable WQS in a given year (*E. coli* numbers exceeded 410 col/100 mL for more than 25% of the samples collected during the primary contact season; APCEC Regulation 2).

Site ID	Year	N	Geo.	Min.	25th	Med.	75th	Max.	% Exc.
Reach SC913	2012	24	39	4	23	43	82	179	0
	2013	24	62	22	32	52	127	435	4.2
	2014	27	54	19	36	53	79	238	0
SC913A	2012	8	30	4	10	35	101	179	0
	2013	8	81	30	36	57	168	435	12.5
	2014	9	68	20	37	79	112	238	0
SC931B	2012	8	40	13	29	44	60	91	0
	2013	8	54	22	24	46	127	172	0
	2014	9	52	19	36	50	76	137	0
SC931C	2012	8	49	21	23	58	90	119	0
	2013	8	54	22	37	50	82	138	0
	2014	9	44	20	34	48	61	67	0

Clear Creek, ADEQ Stream Segment 029

In Clear Creek, one reach (CC029) was sampled at five different sites during the primary contact season (May through September) of 2012, 2013 and 2014 (Figure 1). Clear Creek flows into the Illinois River just downstream of site IR024A. The reach and the individual sample sites were evaluated against the applicable WQS. For Clear Creek, which is not an ESW, ERW or a NSW, the WQS states that *E. coli* numbers shall not exceed 410 col/100 mL in more than 25% of the water samples collected in no less than 8 samples taken during the primary contact season (APCEC, 2014).

In reach CC029, *E. coli* numbers ranged from 3 to 2420 col/100 mL across all five sites and three seasons sampled (Table 8). Across the reach, *E. coli* numbers exceeded 410 col/100 mL in 5% or less of the water samples collected during each of the primary contact seasons in 2012, 2013 and 2014. The applicable WQS was never violated when aggregating the data at the reach level.

E. coli numbers at sites CC029A and CC029B exceeded 410 col/100 mL in only one water sample (12.5%) collected in 2012 and not in any water samples collected in 2013 or 2014. At sites CC029C and CC029D, *E. coli* numbers did not exceed 410 col/100 mL in any of the water samples collected during the primary contact season in any study year, where maximum *E. coli* numbers observed was 387 col/100 mL. At CC029E, 22.2% of the water samples collected had *E. coli* numbers that exceeded 410 col/100 mL. There were no violations of the applicable WQS for *E. coli* at this site during the study period.

Table 8. Summary statistics for *E. coli* numbers for the reach and individual sites on Clear Creek for each primary contact season (May through September) during 2012, 2013 and 2014. The table includes the number of samples collected (N), the geomean (Geo.), minimum (Min.), median (Med.), and maximum (Max.) *E. coli* as the most probable number (MPN) of colonies (col)/100 mL. The percentage of *E. coli* measurements exceeding the limit of 410 col/100 mL (% Exc.) is also shown. Bold values for % Exc. represent a reach or stream that violated the applicable WQS in a given year (*E. coli* numbers exceeded 410 col/100 mL for more than 25% of the samples collected during the primary contact season; APCEC Regulation 2).

Site ID	Year	N	Geo.	Min.	25th	Med.	75th	Max.	% Exc.
Reach CC029	2012	40	75	3	31	82	205	921	5.0
	2013	40	51	3	34	82	86	921	0
	2014	45	88	19	37	89	164	2420	4.4
CC029A	2012	8	33	3	9	39	133	411	12.5
	2013	8	87	33	57	87	160	172	0
	2014	9	80	26	34	66	208	308	0
CC029B	2012	8	187	45	116	184	297	921	12.5
	2013	8	67	36	41	73	92	148	0
	2014	9	116	48	68	99	243	378	0
CC029C	2012	8	80	21	29	93	210	222	0
	2013	8	30	12	24	30	39	75	0
	2014	9	47	19	32	37	64	167	0
CC029D	2012	8	125	32	68	142	241	387	0
	2013	8	51	34	36	49	74	87	0
	2014	9	80	20	29	113	170	206	0
CC029E	2012	8	38	19	19	27	53	345	0
	2013	8	38	10	16	41	100	178	0
	2014	9	151	20	63	101	543	2420	22.2

Relationship between E. coli and Spatial and Land Cover Characteristics

The occurrence of *E. coli* numbers exceeding the applicable WQS appears to be a localized issue (Figure 2). Specifically, there appeared to be no connection between a site with high numbers of *E. coli* and the next site downstream. For example, maximum *E. coli* numbers in water samples collected at the middle site on Baron Fork (BF013B, Figure 2) exceeded 1000 col/100 mL in all three years (Table 5); however, the next site downstream (BF013C) on Baron Fork never exceeded 410 col/100 mL in any of the water samples collected during any of the sampling seasons (Table 5). This type of observation was consistent across the reaches sampled within the Illinois River Watershed, except Little Osage Creek where all sites sampled had high *E. coli* numbers in water samples relative to the WQS and most other sites sampled in the other reaches.

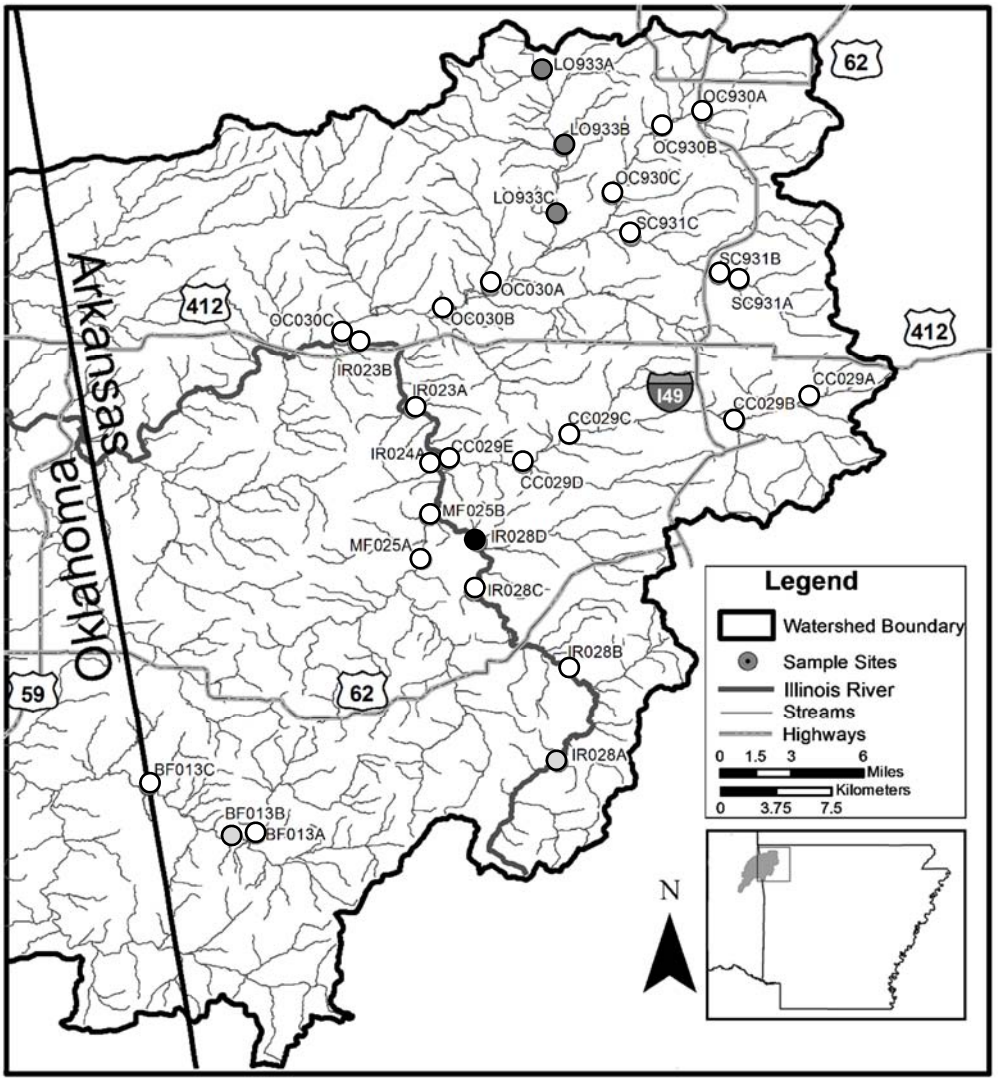

Figure 2. Monitoring sites in the Illinois River Watershed. The shading of the site symbols represents the incidence of *E. coli* exceeding the standard of 298 CFU/100 mL more than 25% of the time of sampling during the primary contact season (May 1 through September 30) of each year (APCEC Regulation 2). White, light gray, dark gray and black symbols represent sites with 0, 1, 2 or 3 years of violations of *E. coli* standards.

The sources of bacteria (including *E. coli*) are external and highly variable from one stream and watershed to the next. The *E. coli* may come from direct deposition into the water by pets, domestic animals, and even wildlife (Bradford et al. 2013, Wilkes et al. 2013), or these bacteria may be washed from the landscape into the stream and rivers during rainfall events that produce runoff (USEPA 2001, Bradford et al. 2013). The numbers of bacteria in streams and rivers generally increase with increasing streamflow, especially during the high flows associated with storm events (Christensen et al. 2002, Crowther et al. 2002). However, studies have also shown that bacteria such as *E. coli* can survive in sediments at the bottom of the stream (Garzio-Hadzick et al. 2010) and that these bacteria are resuspended during the high flow events contributing to the elevated bacteria numbers (Muirhead et al. 2004, Garzio-Hadzick et al. 2010). However, the current study targeted base flow conditions during sampling where the primary source of *E. coli* is likely direct access and deposition by domestic grazing animals and wildlife.

Because *E. coli* numbers were localized, the percent land use and land cover (LULC) for urban, forest, grassland, pasture and woody wetlands were calculated for the riparian zone within 2 km upstream of each sampling site. Table 9 gives LULC percentages in the riparian zone across all sites, showing large variation across sites and categories. A significant threshold was present in the relationship between the percent of pasture land within the riparian zone and the percentage of *E. coli* numbers in water samples that exceeded the applicable limit (298 col/100 mL for the Illinois River and 410 col/100 mL for all other streams; Figure 3).

Sites that had less than 50% pasture land within the riparian zone had 25% or less of the water samples with *E. coli* numbers exceeding either 298 col/100 mL (applicable for the Illinois River) or 410 col/100 mL (applicable for all study streams except for the Illinois River). The sites with greater than 50% pasture land within the riparian zone had percent exceedances that varied from zero to 78%. The only sites that violated the applicable WQS for *E. coli* had more than 50% pasture land in the riparian zone. The connection likely relates to livestock access to the streams in relative close proximity to the sites where water samples were collected. Other studies have shown a positive relationship between pasture land cover and pathogen detection (Crowther et al. 2002) and have reported increased bacteria numbers when cattle have direct access to the stream channel (Davies-Colley 2004, Wilkes et al. 2011).

Summary and Considerations

This report contains information that can be used to help prioritize and guide watershed management activities to address water quality impairments caused by *E. coli*. For example, high *E. coli* numbers were significantly related to the amount of pasture land in the riparian zone within 2 km upstream of the sampling site. The only sites that violated the applicable WQS had greater than 50% pasture in the riparian zone upstream. Streams or individual stream reaches in the IRW that have more than 50% pasture land use in the riparian buffer zone should be targeted for best management practices and education outreach to landowners.

While the percent pasture in the riparian buffer zone was related to violations of the applicable WQS for *E. coli*, not all sites with greater than 50% pasture had elevated numbers of *E. coli*. Future studies should investigate other possible variables associated with high levels of *E. coli*. For example, it might be useful to quantify how often and how many cattle have access to streams that flow through pasture land.

Livestock access to streams means there is potential for direct deposition of fecal matter into the flowing waters, which is a known source of *E. coli* in streams (Wolfson and Harrigan 2010). Additionally, studies should aim to identify the sources of *E. coli* to better understand the relative contribution of livestock, wildlife, and other vectors within the IRW.

A total of approximately 165 km across ten reaches and seven streams in the IRW were listed as impaired by pathogens (ADEQ 2008). However, *E. coli* numbers appear to be a localized issue where violations of the applicable WQS may happen at some sites in a reach, but not others and not even the next sampling site downstream. For example, in the Illinois River reach 028 (IR028), the most downstream site (IR028D) violated the applicable WQS for *E. coli* during all three primary contact seasons sampled, and the most upstream site (IR028A) violated the WQS only during the 2014 primary contact season. But, the middle two sites (IR028B and IR028C) never violated the applicable WQS for *E. coli*. Long reaches could be separated into smaller reaches, allowing for portions of the stream to be removed from the 303(d) list.

Table 9. Land use land cover (LULC) for the riparian area (see methods for description of riparian area) for each study site. Includes the area and the percent of the total area for urban and suburban, forest, pasture and grassland, and woody wetlands LULC, and the total riparian area in square meters.

Reach	Site ID	% Urban	% Forest	% Grassland	% Pasture	% Woody Wetlands	Total Area (m^2)
023	IR023A	0.1	38.2	2.2	43.2	16.3	293,600
	IR023B	9.3	14.4	0.0	25.6	50.6	182,500
024	IR024A	2.4	34.1	10.3	44.7	8.5	432,900
028	IR028A	2.2	23.0	0.0	74.8	0.0	252,300
	IR028B	0.2	22.3	0.0	63.7	13.8	182,600
	IR028C	8.4	36.7	2.8	36.1	15.9	346,500
	IR028D	0.2	18.2	0.0	50.7	30.9	314,900
013	BF013A	4.1	59.5	5.6	26.2	4.6	320,700
	BF013B	8.8	20.2	0.0	65.5	5.5	384,700
	BF013C	1.3	36.4	0.8	59.0	2.5	230,800
025	MF025A	2.2	59.6	3.4	34.8	0.0	264,400
	MF025B	1.5	29.7	0.0	66.7	0.0	310,200
030	OC030A	0.5	36.7	2.3	50.9	9.5	379,100
	OC030B	11.1	30.5	1.3	28.6	28.5	336,600
	OC030C	3.9	22.6	2.7	20.2	50.7	222,300
930	OC930A	53.5	15.0	0.0	31.5	0.0	437,800
	OC930B	11.6	27.7	0.0	57.6	3.0	395,500
	OC930C	13.2	45.2	2.0	39.7	0.0	473,500
933	LO933A	15.1	4.9	0.0	67.3	1.2	179,900
	LO933B	5.9	36.3	0.1	57.7	0.0	408,400
	LO933C	11.2	33.1	0.0	55.7	0.0	524,100
931	SC931A	27.5	44.4	4.0	20.4	1.7	429,400
	SC931B	35.5	49.8	1.5	8.0	2.4	303,800
	SC931C	2.5	41.9	0.0	54.8	0.8	273,100
029	CC029A	22.7	17.1	0.0	58.9	1.4	404,100
	CC029B	11.1	69.2	2.3	17.4	0.0	183,200
	CC029C	4.7	25.8	0.7	67.6	0.0	353,400
	CC029D	5.6	43.3	16.7	34.4	0.0	460,500
	CC029E	9.6	36.8	5.2	33.9	9.7	324,700

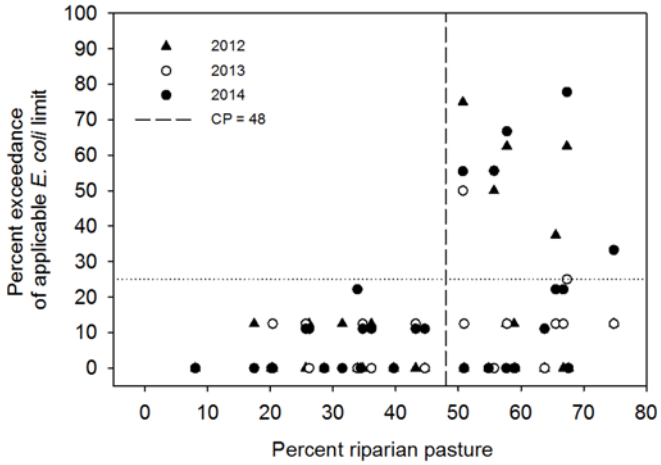

Figure 3. Scatter plot of the percent of exceedances of the *E. coli* limit (298 CFU/100 mL) for all sites by year in response to the percent riparian pasture land cover. The horizontal dotted line represents the 25th percent line, which if *E. coli* concentrations exceed 298 CFU/100 mL more than 25% of the time during the primary contact season (May through September), the regulation would be violated. The vertical solid line is drawn at represents the change point at which increases in pasture land cover relates to a significant shift in the percent exceedance for *E. coli*. The vertical dashed line represent the 95th percent confidence interval for the change point (the 5% confidence interval is equal to the change point). The black triangles, white circles, and black circles represent samples collected during the 2012, 2013 and 2014 primary contact seasons, respectively.

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