

Monitoring of Total Coliform and *Escherichia coli* Levels in a Second Order Stream in West-Central Oklahoma

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Total coliform and *Escherichia coli* (fecal coliform) bacteria levels were monitored monthly from April to September 2006 in Little Deep Creek, Custer County, Oklahoma. Little Deep Creek is a second to second order stream that receives runoff from urban (Weatherford, OK), agricultural, and grazing land and empties into Deer Creek. Over the sampling period, stream levels of total coliform bacteria and *E. coli* were $12,901 \pm 10,817$ cfu /100 mL and 226 ± 309 cfu /100 mL (mean \pm standard deviation), respectively. Samples collected on May 5, 2006 following a heavy rain event showed significant elevations in both total coliform ($23,690 \pm 12,972$ cfu /100 mL) and *E. coli* (825 ± 310 cfu /100 mL). *E. coli* levels on the May 5 date exceeded the Oklahoma Water Resource Board standard (406 cfu /100mL) at all four sample sites. Total coliforms were also elevated on July 27, 2006 but *E. coli* levels did not peak at this time. Significant differences in bacterial levels were not apparent between the sample sites over the study period. However, the two downstream sites appeared to have higher levels than the upstream sites during the heavy runoff event of May 5, 2006. Our data suggest that non-point sources determine fecal coliform levels in Little Deep Creek, Custer County, Oklahoma. © 2007 Oklahoma Academy of Science

Introduction

Streams in the United States must meet water quality standards for maximum levels of total coliform and *Escherichia coli* (fecal coliform) bacteria to assure safe use. These bacteria are not necessarily harmful but indicate the possible presence of parasites and pathogens that could be harmful to livestock and humans (USEPA, 1997). Most species classified as total coliforms are wide-spread in the environment being found in soils, surface waters, surfaces of plants, the intestinal tracts of mammals, birds, fish, mollusks, and insects (Leclerc et al. 2001). *E. coli* and fecal coliforms are subsets of the total coliform group and flourish in the intestinal tracts of mammals, including humans. Fecal coliform bacteria can enter a stream through runoff that contacts fecal material from wildlife, domesticated animals, and humans in the watershed. The standard in Oklahoma for recreational water use (primary body contact) is a maximum 126 cfu /100 mL *E.*

coli bacteria (cfu = colony forming units), measured as monthly geometric means with no sample exceeding 406 cfu /100 mL (OWRB, 2006).

We undertook this study based on preliminary measurements that indicated high total coliform counts in Little Deep Creek, a second order stream in western Oklahoma (Figure 1). The purpose of this study was to confirm the elevated bacterial levels and evaluate differences in total coliform and *E. coli* counts according to location, date, and weather conditions. We hoped to identify any point sources for the coliform contamination and factors that influence coliform load in the stream.

Materials and Methods

Little Deep Creek is located in Custer Co. in west-central Oklahoma. The stream is approximately 18 Km in length and empties into Deer Creek which in turn feeds into the Canadian River. The study area lies on the

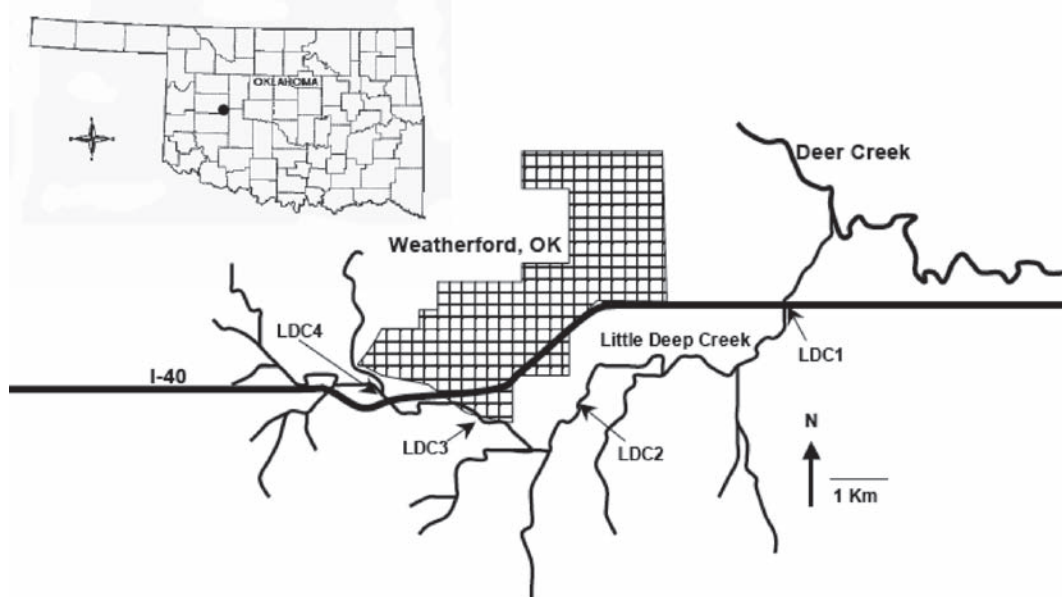


Figure 1. Map indicating the location of Little Deep Creek and sample sites along the stream channel.

border between the Cross Timbers Transition and the Rolling Red Hills Ecoregions of Oklahoma (Woods et al. 2005). Soils in the area are dominated by silt loam (Cialella 1996). Land use in the watershed includes urban (City of Weatherford), cropland, rangeland-open grass, and mixed bottomland (Cialella 1996). Precipitation and other weather data during the study period were obtained from the Oklahoma Climatological Survey (OCS 2006) monthly summaries. We selected four sites along Little Deep Creek (Figure 1) that were sampled 6 times at ~one month intervals from April to September 2006. Site LDC1 was located 11.75 Km downstream from site LDC 4, the most upstream site. Sites LDC3 and LDC4 are located 0.5 Km and 3.5 Km respectively upstream of the Weatherford, OK sewage treatment plant. Sites LDC1 and LDC2 are located 8.25 Km and 3 Km respectively downstream of the plant.

We collected a water sample for bacterial analysis at each site using sterile Whirl-Pak® bags attached to a gimbaled, telescoping rod. In addition, a field blank of sterile buffer was processed at the stream on each date. On May 5, 2006 additional water samples were collected at the release point

for treated effluent from the Weatherford sewage treatment plant. Samples were collected approximately 50 m downstream of the effluent discharge and directly from the effluent discharge. We measured *in situ* stream conditions at the four sites including water temperature/dissolved oxygen (YSI Model 55 dissolved oxygen system), pH (Corning pH-20 meter), conductivity (Oakton conductivity meter WD-35607-30), turbidity (LaMotte 2020e turbidimeter), and flow velocity (Marsh-McBirney model 201D flowmeter). Meters were calibrated against traceable standards at the study sites. Air temperature and relative humidity were measured with a Thermo-Hygro pen-type meter.

Water samples were returned to the laboratory and immediately processed for bacterial analysis. The water samples were diluted with sterile phosphate buffer to 10, 1, or 0.1mL of a 100mL sample and then filtered using Nalgene® sterile funnels with (0.45 μ) membrane filters. The filters were cultured with m-ColiBlue24® medium at 37°C for 24 hours (Hach Company, 1999). After incubation, we took digital photographs of the plates and used ImageJ® software (NIH, 2006) to count colonies of

total coliform bacteria. The *E. coli* colonies were counted manually from color prints of the photographs. Colony forming units (cfu) per 100 mL stream water for *E. coli* and total coliforms were calculated based on the dilution of the water samples. A database was constructed using StatView® (Abacus Concepts, 1992) and differences between sample dates and sites were analyzed using ANOVA and the Fisher PLSD post-hoc test ($P = 0.05$).

Results

Stream Conditions

Sample sites on Little Deep Creek were similar for most of the physical and chemical parameters monitored (Table 1). Sites

1 & 2 (downstream sites) exhibited significantly ($P < 0.05$) higher flow velocities, pH levels, and lower conductivity compared to the upstream sites (LCD 3 & LDC 4). Water temperature tended to increase at the downstream sites but the difference was not statistically significant ($P = 0.07$). Turbidity did not differ significantly between sites but was more variable between dates at the downstream sites. Turbidity levels increased following two significant rain events that occurred on May 5 & August 30, 2006 (Table 2).

Bacterial Analysis

Levels of total coliform bacteria and *E. coli* (fecal coliform) in Little Deep Creek averaged $12,901 \pm 10,817$ cfu / 100 mL and 226

Table 1. Physical/chemical parameters at the Little Deep Creek study sites. Values are means \pm SD, n = 6.

Means for the sites were calculated based on measurements taken on 6 sample dates from April 21 to September 22, 2006.

Sample Site	Air		Water		Dissolved		Current	
	Temperature (C)	Relative Humidity %	Temperature (C)	Oxygen (mg/L)	Conductivity (μ S)	pH	Turbidity (NTU)	Velocity (m/s)
LDC1	28.3 \pm 5.7	43.8 \pm 11.8	25.6 \pm 4.6	9.46 \pm 2.01	857 \pm 424	7.99 \pm 0.31	8.5 \pm 9.9	0.38 \pm 0.1
LDC2	27.0 \pm 3.9	50.8 \pm 14.2	24.3 \pm 3.7	8.81 \pm 1.27	1172 \pm 104	7.78 \pm 0.31	9.4 \pm 15.1	0.42 \pm 0.1
LDC3	27.2 \pm 3.1	51.2 \pm 15.8	22.3 \pm 3.3	10.06 \pm 2.42	1713 \pm 148	7.56 \pm 0.21	5.9 \pm 4.9	0.29 \pm 0.1
LDC4	27.1 \pm 2.6	54.7 \pm 16.2	21.4 \pm 4.0	8.39 \pm 1.36	1684 \pm 253	7.41 \pm 0.21	11.3 \pm 6.8	0.23 \pm 0.1

Table 2. Physical/chemical parameters in Little Deep Creek on the dates sampled. Values are means \pm SD, n = 4.

Means for a date were calculated based on measurements taken at the 4 sample sites sample on that day.

Sample Site	Air		Water		Dissolved		Current	
	Temperature (C)	Relative Humidity %	Temperature (C)	Oxygen (mg/L)	Conductivity (μ S)	pH	Turbidity (NTU)	Velocity (m/s)
4-21-06	26.9 \pm 2.0	27.5 \pm 1.3	21.4 \pm 2.5	11.51 \pm 1.73	1407 \pm 415	7.79 \pm 0.32	3.1 \pm 1.8	0.30 \pm 0.1
5-05-06	21.6 \pm 0.7	59.5 \pm 3.9	18.7 \pm 0.9	8.35 \pm 0.49	1430 \pm 271	7.38 \pm 0.19	23.0 \pm 11.6	0.28 \pm 0.1
7-06-06	31.4 \pm 1.9	51.0 \pm 10.2	28.0 \pm 2.9	10.36 \pm 1.35	1424 \pm 396	8.05 \pm 0.37	4.2 \pm 2.7	0.33 \pm 0.1
7-27-06	31.1 \pm 3.6	65.0 \pm 8.3	27.2 \pm 1.3	7.77 \pm 0.97	1154 \pm 242	7.83 \pm 0.25	3.1 \pm 1.6	0.28 \pm 0.1
8-30-06	27.1 \pm 0.5	58.3 \pm 3.5	24.5 \pm 2.6	7.18 \pm 0.03	1448 \pm 397	7.56 \pm 0.28	14.6 \pm 8.7	0.38 \pm 0.2
9-22-06	26.3 \pm 0.5	36.5 \pm 3.3	20.6 \pm 2.3	9.90 \pm 0.91	1275 \pm 887	7.51 \pm 0.18	4.8 \pm 4.6	0.41 \pm 0.1

± 309 cfu / 100 mL (mean \pm sd.), respectively over the course of the study. Mean total coliform bacteria levels were not significantly different at the four sites ($P > 0.40$). We did detect significantly elevated levels of total coliforms ($23,690 \pm 12,972$ cfu / 100 mL) and *E. coli* (825 ± 310 cfu / 100 mL) on May 5, 2006 (Figures 2 and 3). This sample date followed a significant rainfall that increased runoff into the stream as indicated by a significant turbidity increase (Table 2). Levels of both total coliform and *E. coli* appeared highest at the downstream sites (LDC1 & LDC2) compared to the upstream sites (Figures 2 and 3). Levels of *E. coli* on May 5, 2006 exceeded water quality standards at all four sites. A second significant increase in total coliforms ($24,950 \pm 10,881$ cfu / 100mL) was detected on July 27 (Figure 2) but did not coincide with an elevation in *E. coli* levels (Figure 3). A rain event was not associated with the July 27, 2006 peak as confirmed by low turbidity readings (3.1 ± 1.6 NTU). Interestingly, total coliform levels during the July 27 peak were lowest at the downstream site LDC1 compared to the sites farther upstream, especially LDC 3 and LDC4 that are located above the sewage treatment plant (Figure 2). In contrast to the May 5, 2006 sample date, the rainfall associated with the August 30,

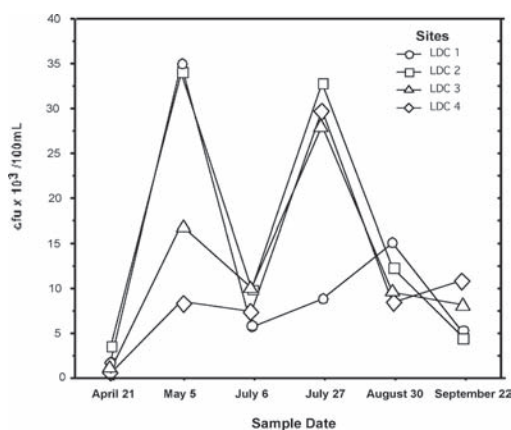


Figure 2. Total coliform levels (colony forming units / 100 mL) in Little Deep Creek in relation to sample date and sample site. See Figure 1 for locations of sample sites along the stream channel.

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2006 sample date did not produce significant increases in either total coliforms or *E. coli* in the stream (Figures 2 and 3).

Samples taken on May 5, 2006 from the stream above the sewage treatment plant effluent release, below the effluent release and the effluent itself indicated that levels of total coliform in the effluent release (10 cfu / 100mL) are less than 0.1% of the levels present in the stream water above the release (17,172 cfu / 100mL). No *E. coli* was detected in the effluent itself. Also on May 5, 2006, total coliform and *E. coli* levels immediately downstream of the effluent release were 19,300 cfu / 100mL and 1200 / 100mL, respectively.

Discussion

Levels of total coliform and *E. coli* bacteria in Little Deep Creek were highly variable both between sites and sample dates (Figures 2 and 3). This variability did not seem to be related to stream conditions which were relatively consistent between sites and dates sampled (Tables 1 and 2). However, the May 5 peak in coliforms (Figures 2 and 3) appeared to be related to a significant rain event. The Weatherford area received 4.4 cm of rain on April 28 and an additional 2.4 cm of rain on May 5 (OCS 2006). These

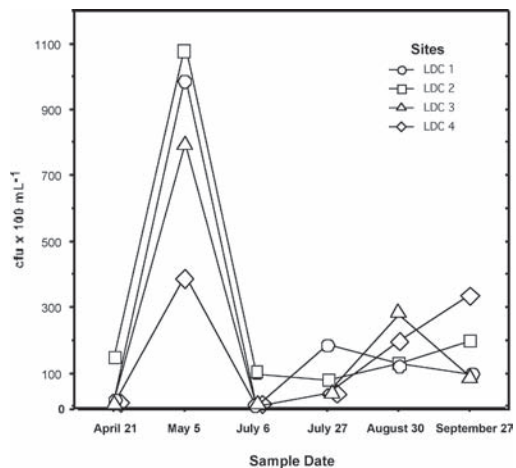


Figure 3. *Escherichia coli* levels (colony forming units / 100mL) in Little Deep Creek in relation to sample date and sample site. See Figure 1 for locations of sample sites along the stream channel.

heavy rains increased turbidity in the stream suggesting erosion in the watershed (Table 2). The soil erosion and runoff likely carried bacteria into Little Deep Creek as indicated by other studies. Kistemann et al. (2002) found that fecal bacteria increased 10 to 100 times during extreme rainfall and runoff events in tributaries of reservoirs located in North Rhine-Westphalia, Germany. In the Tillamook Bay watershed in Oregon, the highest levels of fecal bacteria coincided with fall storm events (Sullivan et al. 2005).

Land use in the watershed likely contributed to the patterns of fecal bacteria in the stream. Sullivan et al. (2005) found that fecal coliform bacteria were highest in streams whose watersheds contained high proportions of agricultural land, dairy cattle, confined feedlot operations and human population. Mehaffey et al. (2005) found that fecal coliform bacteria levels in streams were positively correlated with percent erodible soils, urban development, and percent agricultural land in the watershed. Urban development (city of Weatherford) and agriculture/cattle grazing dominate land use in the Little Deep Creek watershed (Cialella 1996). This suggests the potential for high fecal bacteria in the stream.

The Weatherford, OK sewage treatment plant is a potential source of fecal coliform bacteria for Little Deep Creek. The plant releases approximately 0.8×10^6 gallons of chlorinated effluent into the stream each day (Mr. Jack Olsen, personal communication). However, our analysis of the effluent on May 5, 2006 appears to eliminate the Weatherford sewage treatment plant as a point source of fecal coliform contamination.

Cattle grazing within the watershed is a likely contributor of *E. coli* to Little Deep Creek. Bacterial levels were greatest at the downstream sites (Figures 2 and 3) on May 5. Tributaries to Little Deep Creek located downstream of site LDC 3 (Figure 1) drain land that is primarily used for agriculture and pasture. Heavy rain events would wash any fecal material from the pastures into the

stream. Runoff from areas containing fecal material of cattle origin has been shown to contain elevated fecal coliform levels (Soupir et al. 2006). Studies by Gagliardi and Karns (2000) and Byappanahalli et al. (2006) indicated that *E. coli* can survive and replicate in soils for extended periods of time. Furthermore, *E. coli* was found to leach at a more consistent rate and for a longer period of time in sandy loam soils (Gagliardi and Karns 2000) such as those found in the Little Deep Creek watershed. Therefore, fecal material may have accumulated on pastureland in the Little Deep Creek watershed during the dry spring period and provided an abundant bacterial source for the May 5, 2006 peak in total coliforms and *E. coli*.

Somewhat puzzling is the absence of a bacterial peak associated with the rain event that preceded the August 30, 2006 sample date. Weather records indicate that the Weatherford area received 4.7 cm of rain on August 26 (OCS 2006). Rain was abundant for the entire month of August ranging from 5 to 7.5 cm above normal for the area. This extended period of precipitation may have washed fecal material from the watershed prior to the August sample date. Another possibility is that the lower than normal precipitation and higher than normal temperatures during June and July may have not been conducive for survival of fecal coliform bacteria in the soils of the watershed. Similarly, Sullivan et al. (2005) reported that fall storm events in the Tillamook, Trask, and Wilson Rivers in Oregon produced more dramatic increases in fecal coliform bacterial levels than did more intense storms in winter and spring. They speculated that moisture conditions or length of the dry period preceding a storm may have a significant effect on the release of fecal coliform bacteria from the watershed.

A rain event was not associated with the elevated levels of total coliform bacteria detected on July 27, 2006 (Figure 2). This peak in total coliforms was most pronounced at

the three upstream sites (Figure 2) and was not accompanied by a spike in *E. coli* (Figure 3). Stream conditions on this date appeared to be strongly influenced by the preceding two months of hot and dry conditions in the area. For example at site LDC4 on July 27, water temperature was high (27 °C), dissolved oxygen was low (6.6 mg / L), and foam was observed floating on the water near the sampling site. A distinct odor typical of stagnant conditions was also noted at this time. The total coliform peak on July 27, 2006 may have been due to growth of environmental coliforms that are classified as psychrotrophic, growing optimally at temperatures around 32 °C (Leclerc et al. 2001). We speculate that environmental coliforms may have bloomed in the upper reaches of Little Deep Creek at this time due to favorable conditions for their growth.

Our study confirms that Little Deep Creek in Custer County, Oklahoma experiences periods of elevated total coliform bacteria and *E. coli* that can exceed water quality standards. The association of elevated *E. coli* at downstream sites with increased runoff (May 5, 2006) indicates that contamination is from non-point sources in the watershed. Land use and drainage patterns suggest pastureland used to graze cattle is a likely source. Testing of effluent from the Weatherford sewage treatment plant does not implicate the effluent as a point source for bacterial contamination. Our data are consistent with previous studies that indicate that rain events play a significant role in determining fecal coliform levels in streams. Monitoring practices for fecal coliforms should take into account rain events especially in areas like western Oklahoma where precipitation can be episodic and intense.

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