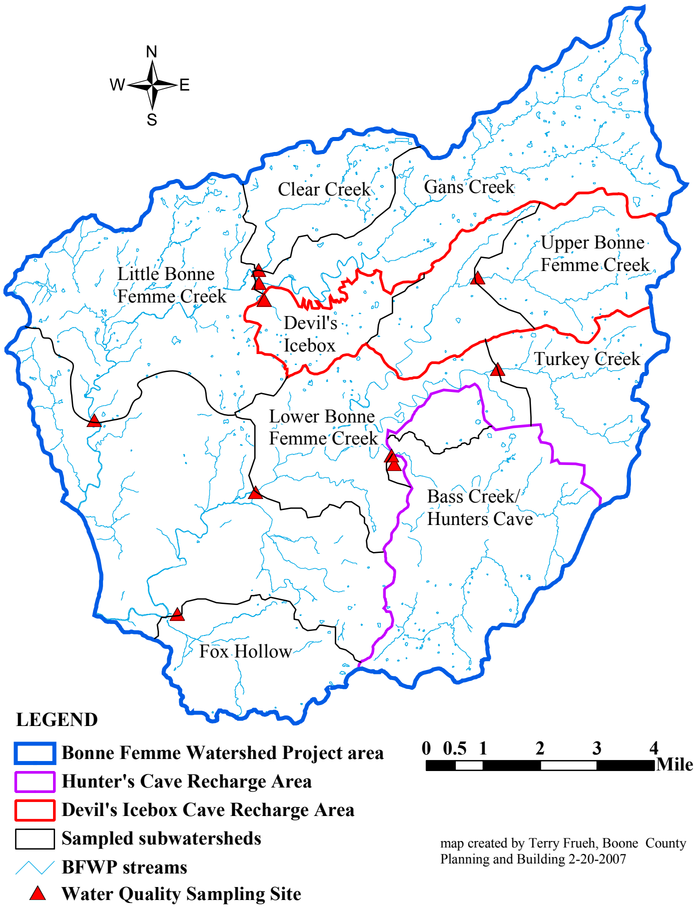
Water Quality Monitoring Final Report

Water quality monitoring in the Bonne Femme watershed has been ongoing since 1999, when studies were initiated at Hunters and Devil's Icebox Spring Branches (Lerch et al., 2001; Lerch et al., 2005). In 2001, the monitoring was expanded to include six surface sub-watersheds in addition to the two caves, and with the initiation of the Bonne Femme 319 project in 2003, an additional two surface sites were added bringing the total number of monitoring sites to ten (Figure 1). The current monitoring program includes eight surface sub-watersheds (Clear Creek, Gans Creek, Upper Bonne Femme (at US 63), Turkey Creek, Bass Creek, Lower Bonne Femme (at Nashville Church Rd.), Little Bonne Femme Creek, and Fox Hollow) and the two karst recharge areas (Devil's Icebox and Hunters spring branches). This monitoring scheme covers about 80% of the entire watershed. Samples were collected once per quarter, since 4th quarter 2003, for nutrients, turbidity, pH, dissolved oxygen, specific conductivity, and temperature at all sites. Sampling for fecal bacteria was conducted for 4 weeks each quarter, with samples collected at weekly intervals. Bacterial analyses included fecal coliforms (FC), generic E. Coli (EC), and qualitative analyses for specific pathogenic bacteria – E. Coli O157:H7, Salmonella, and Shigella. FC analyses have been conducted at eight of ten sites since 2001; EC analyses have been conducted since 4th quarter 2004; and pathogen specific analyses have been conducted since 4th quarter 2005. If there was no stream flow, samples were not collected from stagnant pools.



map created by Terry Frueh, Boone County Planning and Building 2-20-2007

Figure 1 Bonne Femme watershed monitoring sites.

All laboratory methods and the sampling scheme were detailed in the Quality Assurance Project Plan (Lerch, 2004).

General Stream Water Properties

The general water quality properties included temperature, specific conductivity (how many ions are in solution), dissolved oxygen, pH, and turbidity. These parameters were measured once each quarter and coincided with the collection of samples for nutrients, herbicides (2nd quarter only), and one of the weekly pathogen samples within a quarter. The dissolved oxygen data are expressed as absolute concentration (mg/L) and relative concentration (% saturation). Percent saturation is the measured dissolved oxygen as a percentage of the oxygen solubility in water for a given water temperature.

The general parameters were typically not statistically different over sites when the data were averaged over all ten quarters (Table 1). Only pH was statistically different, with the Upper Bonne Femme Creek site having significantly lower pH than all but two sites. The Upper Bonne Femme Creek sub-watershed has the highest intensity of row crops (67% of the sub-watershed), and the lower pH may reflect the impact of NH_4-based fertilizer usage. Overall, the slightly alkaline pH and moderately high specific conductivity reflected the influence of the limestone bedrock on the water chemistry. Limestone bedrock will create slightly alkaline

Table 1 General stream water properties by site.

Site	Temperature	pH	Specific Conductance	Dissolved Oxygen	Dissolved Oxygen	Turbidity
	^{o}C*		µS/cm	mg/L	% Saturation	NTU***
Clear Creek.	13.1	7.88	525	11.84	111.2	3.6
Gans Creek.	11.7	7.76	397	11.57	105.2	17.5
Devils Icebox	11.6	7.53	424	11.05	101.7	22.9
Upper Bonne Femme	13.6	7.22	478	9.79	95.7	28.3
Turkey Creek.	13.8	7.49	586	12.04	117.1	22.7
Hunters Cave	11.5	7.73	409	11.37	103.7	11.9
Bass Creek.	13.7	7.80	455	14.39	140.3	12.6
Lower Bonne Femme	12.8	7.47	408	11.39	108.6	12.1
Little Bonne Femme	12.6	7.63	446	11.06	99.4	19.4
Fox Hollow	14.6	7.60	520	10.92	107.0	3.3
Average across sites	12.9	7.61	465	11.54	109.0	15.4
LSD**	NS	0.28	NS	NS	NS	NS

* ^{o}C= Celsius. Fahrenheit = (9/5 ^{o}C) + 32

**LSD = least significant difference. This value is the minimum difference between sites to be considered statistically different. NS = not significantly different across sites. Data are averaged over 10 quarters (3rd quarter 2004 – 4th quarter 2006).

***NTU = Nephelometric Turbidity Units.

conditions as the limestone is dissolved by the groundwater which recharges the streams. The soluble nature of limestone, compared to most other bedrock, results in fairly high dissolved ion levels in the water, and this is reflected in the specific conductivity data. In addition, Upper Bonne Femme Creek and Turkey Creek occasionally had very high specific conductance (>700 µS/cm) due to the use of salt on US 63 in the winter months. Eight of ten sites had average dissolved oxygen levels that were at or near 100% saturation. The lowest observed dissolved oxygen levels occurred in the third quarter of each year when the stream water temperature was highest. The lowest dissolved oxygen level observed was 5.11 mg/L (62.6% saturation); therefore, no site was under the state standard level of 5.0 mg/L. The much >100% saturation levels observed at Turkey and Bass Creeks reflected the persistent nuisance algal growth conditions at these sites. Turbidity measures the clarity of the water, and thus, both suspended sediment and algae can contribute to lower clarity and higher turbidity. Highest turbidity was observed under runoff conditions when the suspended sediment content of the water is high. Turbidity levels were occasionally elevated under low flow conditions, suggesting that algal growth was negatively impacting water clarity, especially in the 2nd and 3rd quarters of the year.

Dissolved oxygen and turbidity levels showed that eutrophication was not a problem in these streams, but nuisance algal growth was a common condition (see additional discussion in the Nutrient section). Eutrophication is a condition marked by excessive algal growth which occurs because of high nitrogen and phosphorus concentrations in the streams. The algal bloom phase begins as water temperature rises in the spring, and dissolved oxygen levels may greatly exceed 100% saturation because algae are photosynthetic organisms and photosynthesis generates oxygen. The algal bloom phase is then followed by death and decay of the algae during the late summer to early fall, resulting in very low dissolved oxygen levels that are harmful to fish and other aquatic life. Although the 3rd quarter dissolved oxygen data were the lowest of any quarter, this was mainly an effect of water temperature rather than algal decay.

Nutrients

Five separate nutrient analyses were conducted: total Nitrogen (TN); total Phosphorous (TP); dissolved nitrate-N (NO_3-N); dissolved ammonium-N (NH_4-N); and dissolved orthophosphate-P (PO_4-P). Average nutrient concentrations by site are summarized in Table 2. Statistical analyses (analysis of variance) were conducted to determine if significant differences in average concentration existed between sites.

In general, nutrient concentrations in the Bonne Femme sub-watersheds were similar to or lower than other agricultural watersheds in northern Missouri (Blanchard and Lerch, 2000; Goolsby et al., 1999). This is partially due to the lower row crop intensity of the Bonne Femme watershed compared to most northern Missouri watersheds. In addition, soils in the most intensively cropped sub-watersheds (Upper Bonne Femme Creek, Turkey Creek, Bass Creek, and Gans Creek) are predominantly claypan soils of the Mexico-Leonard Association, and these soils, although runoff prone, tend to have lower nutrient concentrations than the more well-drained soils of north-central and especially northwestern Missouri. Perhaps a better way to put these data into perspective, however, is to compare nutrient concentrations of the Bonne Femme sub-watersheds to the recommended nutrient criteria established by the U.S. Environmental Protection Agency (EPA) (USEPA, 2000). EPA established these nutrient criteria to maintain aquatic invertebrate diversity and to prevent nuisance algal growth and eutrophication (excessive algal growth leading to low dissolved oxygen conditions). Based on the nitrogen criteria, all sub-watersheds suffer some degree of impairment, and this is consistent with field observations and the EPT (stream bug) data. The criteria for TP and PO_4-P would suggest that some streams are eutrophic, but this has not been observed as indicated above by the dissolved oxygen data. Instead, nuisance algal growth conditions and some loss of invertebrate diversity appear to be the predominant conditions throughout the watershed.

Significant differences were observed only for TN and NO_3-N across sites. For both TN and NO_3-N, the Devil's Icebox Spring Branch had the highest concentrations while Clear Creek had the lowest concentrations. TN concentrations in the Devil's Icebox Spring Branch were significantly higher than all sites except Bass Creek, and they were, on average, more

Table 2 Average nutrient concentrations by site*.

Site	Total N	NO_3-N	NH_4-N	Total P	PO_4-P
	----------------------------------mg/L----------------------------------				
Clear Creek.	0.33	0.14	0.028	0.068	0.053
Gans Creek.	0.68	0.23	0.046	0.163	0.059
Devils Icebox	2.11	1.71	0.032	0.159	0.102
Upper Bonne Femme Creek.	1.26	1.03	0.079	0.205	0.094
Turkey Creek.	1.24	0.97	0.048	0.155	0.076
Hunters Cave	0.65	0.24	0.019	0.102	0.039
Bass Creek.	1.48	1.09	0.033	0.092	0.055
Lower Bonne Femme Creek.	0.61	0.45	0.039	0.104	0.049
Little Bonne Femme Creek.	0.87	0.46	0.049	0.091	0.034
Fox Hollow	0.58	0.27	0.044	0.087	0.049
Average across sites	0.98	0.66	0.042	0.123	0.061
LSD**	0.72	0.75	NS	NS	NS
EPA Nutrient Criteria***	0.28-1.50	0.03-1.0^		0.01-0.09	0.003-0.06

*Average of all samples from 4th quarter 2003 to 3rd quarter 2006 (no. of samples = 11-13).

**LSD = least significant difference. This value is the minimum difference between sites to be considered statistically different. NS = not significantly different across sites.

***Lower end of the concentration range may cause decreased invertebrate diversity and nuisance algal growth while higher concentrations cause eutrophication.

^Combination of NO_3-N and NH_4-N.

than twice the concentration of six of the sites. For NO_3-N, the Devil's Icebox Spring Branch had significantly greater concentrations than six of the other nine sites. Averaged across sites, NO_3-N accounted for about 67% of the TN, but those sites with the highest NO_3-N concentrations had >70% of their TN as NO_3-N, suggesting that nitrogen sources such as fertilizers, on-site sewers, and animal manures were impacting these sites. Comparisons of water quality between the two cave streams and their primary losing streams showed opposite trends for TN and NO_3-N. For the Devil's Icebox Spring Branch, the concentrations of TN and NO_3-N were much higher than its primary source of water, which is the Upper Bonne Femme Creek. The Pierpont sinkhole plain is the only land area that lies between the Upper Bonne Femme Creek and the resurgence of the Devil's Icebox spring, leading to the conclusion that the increased TN and NO_3-N were derived from the sinkhole plain. Land uses within the sinkhole plain are mainly pasture land and some residential development. Since pastures generally receive little or no fertilizer inputs, the likely sources of nitrogen were cattle and on-site sewers. The primary source of water for Hunters Cave is Bass Creek. Here the comparison between the cave stream and its water source showed the TN and NO_3-N concentrations were significantly lower in the cave stream compared to its surface water source. Apparently, the other sources of water to Hunters Cave (two tributaries of Turkey Creek) had lower TN and NO_3-N concentrations which diluted the more contaminated Bass Creek water.

Although TP and PO_4-P concentrations were not significantly different across sites, there was a considerable range in the data. TP concentrations varied from a low of 0.068 mg/L at Clear Creek to a high of 0.205 mg/L at Upper Bonne Femme Creek. PO_4-P concentrations varied from a low of 0.034 mg/L at Little Bonne Femme Creek to a high of 0.102 mg/L at the Devil's Icebox Spring Branch. Three of the fours sites with the highest TN concentrations also had the some of the highest TP concentrations, but there was generally not a good correlation between TN and TP concentrations or between NO_3-N and PO_4-P concentrations. For instance, Gans Creek had low TN concentrations, but it had the second highest TP concentration. Bass Creek had the second highest NO_3-N concentration, but it was in the lower half of the sites for its PO_4-P concentration.

Herbicides

One or more herbicides were detected at every site for the four sets of samples collected in the 2nd quarter of the year (Table 3). There were no statistical differences in average herbicide levels across sites for any of the herbicides measured, indicating widespread transport of these chemicals from agricultural production, but it also reflected the generally low levels of the herbicides detected. Herbicide levels in row crop watersheds typically peak during the 2nd quarter of the year since this is when most of the herbicides are applied in the Midwest (Blanchard and Lerch, 2000; Lerch and Blanchard, 2003). However, average concentrations by site were lower than concentrations measured in streams of northern Missouri and southern Iowa (Lerch and

Blanchard, 2003). Overall, atrazine and its metabolites were detected at higher levels compared to the acetanilide herbicides (i.e., metolachlor, alachlor, and acetochlor), reflecting the common usage of atrazine and its high propensity to be transported by surface runoff. Concentrations of atrazine, DEA, DIA, metolachlor, and acetochlor generally were related to the amount of row crops in each sub-watershed. For example Upper Bonne Femme and Turkey Creeks have the highest proportion of land area in row crops among the ten sites, and they also had the overall highest herbicide levels. Metribuzin and alachlor usage were apparently very low as these two herbicides were generally not detected. Low usage of these compounds also reflects state wide trends. It should be noted that the sampling scheme used in this study was too infrequent to adequately characterize herbicide concentrations. Peak herbicide concentrations were most likely much higher than those reflected in this report. However, previous research at Hunters Cave and Devil's Icebox Spring Branch showed that herbicide transport was not the primary water quality problem in the Bonne Femme watershed (Lerch et al., 2001).

<u>Fecal Bacteria</u>

Table 3 Average herbicide concentrations by site*.

Site	Atrazine	DEA**	DIA**	Metribuzin	Metolachlor	Acetochlor	Alachlor
	---------------------------			µg/L***			---------------------------
Clear Creek.	0.050	0.032	<0.010	0.011	0.004	<0.006	<0.005
Gans Creek.	0.770	0.314	0.129	<0.010	0.033	0.107	<0.005
Devils Icebox Spring	1.81	1.23	0.551	<0.010	0.177	0.225	<0.005
Upper Bonne Femme	4.23	1.94	0.824	<0.010	0.476	0.360	<0.005
Turkey Creek.	2.07	1.38	0.663	<0.010	0.221	0.468	<0.005
Hunters Cave	0.536	0.242	0.054	0.010	0.003	<0.006	<0.005
Bass Creek.	1.92	0.591	0.203	<0.010	0.004	0.094	0.183
Lower Bonne Femme	1.53	0.732	0.313	<0.010	0.082	0.250	0.121
Little Bonne Femme	1.60	0.641	0.304	<0.010	0.133	0.135	0.005
Fox Hollow	0.359	0.127	0.043	<0.010	0.051	0.076	<0.005
Average across sites	1.49	0.723	0.308	<0.010	0.118	0.172	0.031

*Average of samples collected in the 2nd quarter of 2004, 2005, and 2006 (no. of samples = 3 or 4).

**Atrazine metabolites. DEA = deethylatrazine; DIA = deisopropylatrazine.

***µg/L = parts per billion.

Two indicator groups of water-borne pathogens were monitored in the streams, fecal coliform and E. Coli. Both groups are considered indicator organisms associated with improper waste management. Fecal coliforms represent a broad array of bacterial species present in mammal feces while E. Coli is a single bacterial species that is also present in mammal feces. E. Coli is also a subset of the fecal coliforms, thus E. Coli levels for a given sample will be less than the fecal coliform concentrations. These indicator bacteria generally do not survive long in soils or water; thus, their consistent detection in water over time indicates one or more sources of continual input. Neither of these groups represents direct measurement of disease-causing (i.e., pathogenic) organisms, but pathogens are likely to be present when the levels of these indicator bacteria in water are high. The reason for monitoring both indicator groups was related to the differences in State and Federal water quality standards. In Missouri, the water quality standard for swimming or other whole body contact is 200 colony forming units (cfu)/100 mL of water based on fecal coliform concentrations while the Federal standard is 126 cfu/100 mL based on E. Coli concentrations. Note that the whole body contact standards are distinctly different from the maximum contaminant levels allowed in finished drinking water. The U.S. EPA maximum contaminant level for drinking water for either fecal coliform or E. Coli is zero cfu/100 mL, which is routinely achieved with disinfection techniques used by drinking water treatment plants.

Over the course of this study, fecal coliform and E. Coli data ranged from <10 cfu/100 mL to >5000 cfu/100 mL at all sites. Because of the wide range in the data, statistical analyses were performed on the $\log_{10}$ transformed data. The log-transformed data varies over a narrower range than the raw data and this allows for better discrimination in the statistical analyses. Average log transformed fecal coliform and E. Coli data by site are given in Table 4. Fecal coliform data ranged from 1.72 $\log_{10}$(cfu/100 mL) at Clear Creek to 2.49 $\log_{10}$(cfu/100 mL) at Fox Hollow. The two sites with the highest fecal coliform concentrations, Turkey Creek and

Table 4 Average fecal coliform and *E. Coli* concentrations by site.

Site	Fecal Coliform	*E. Coli*
	--------$\log_{10}$(cfu/100 mL)*-----------	
Clear Creek.	1.72	1.54
Gans Creek.	2.07	1.91
Devils Icebox Spring Br.	2.30	2.06
Upper Bonne Femme Creek.	2.17	1.95
Turkey Creek.	2.46	2.38
Hunters Cave	1.93	1.73
Bass Creek.	2.00	1.84
Lower Bonne Femme Creek.	1.97	1.86
Little Bonne Femme Creek.	2.14	1.94
Fox Hollow	2.49	2.26
Average across sites	2.13	1.95
LSD**	0.35	0.35

*Statistical analysis was performed on log transformed data.

**LSD = least significant difference. This value is the minimum difference between sites to be considered statistically different.

Fox Hollow, had statistically greater concentrations than the five sites with the lowest concentrations (Clear Creek, Gans Creek, Bass Creek, Hunters Cave, and Lower Bonne Femme Creek). Based on statistical differences among sites, the average fecal coliform concentrations fell into three categories: high – Fox Hollow, Turkey Creek, and Devil's Icebox Spring Branch; medium – Upper Bonne Femme Creek, Little Bonne Femme Creek, and Gans Creek; and low – Bass Creek, Lower Bonne Femme Creek, Hunters Cave, and Clear Creek. Average fecal coliform concentrations of the high category sites were equal to or greater than the whole body contact standard (2.30 $\log_{10}$(cfu/100 mL) = 200 cfu/100 mL).

Average E. Coli data varied from a low of 1.54 $\log_{10}$(cfu/100 mL) at Clear Creek to a high of 2.38 at $\log_{10}$(cfu/100 mL) at Turkey Creek. On average, E. Coli concentrations were about 9% lower than fecal coliform concentrations. The two sites with the highest average E. Coli concentrations, Turkey Creek and Fox Hollow, had significantly greater concentrations than every site except the Devil's Icebox Spring Branch (Table 4). Average E. Coli concentrations at the two highest sites also exceeded the Federal whole body contact standard (2.1 $\log_{10}$(cfu/100 mL) = 126 cfu/100 mL). Categorizing the sites based on statistical differences between sites resulted in the following: high – Turkey Creek and Fox Hollow; medium – Devil's Icebox Spring Branch, Upper Bonne Femme Creek, Little Bonne Femme Creek, and Gans Creek; low – Lower Bonne Femme Creek, Bass Creek, Hunters Cave, and Clear Creek. Thus, both sets of indicator bacteria resulted in very similar categories based on statistical differences across sites. The three sub-watersheds with the highest levels of bacterial contamination (Turkey Creek, Fox Hollow, and Devil's Icebox Spring Branch) have consistently greater inputs of fecal bacteria compared to the other sites. Although these data do not indicate the source of the fecal bacteria, there are three likely sources in the Bonne Femme watershed – on-site sewers, livestock, and wildlife.

The U.S. EPA recommends that five approximately equally spaced samples be collected over 30 days when monitoring for compliance with the fecal bacterial whole body contact standards.

Since our scheme was very similar to the recommended scheme (four samples collected at weekly intervals over 28 days), the data were used to assess compliance of the Bonne Femme watershed streams with the State and Federal water quality standards. Another requirement for comparing data against the whole body contact standards is that the geometric mean of a sample set is computed and compared against the standard rather than the arithmetic mean. The geometric mean is computed as $(x_1Xx_2Xx_3\ldots Xx_n)^{1/n}$, where x_1 equals the bacterial concentration of the 1st sample in a set, with up to n samples collected. For our sampling scheme, n equals 4. The geometric mean for data covering a wide range will be less skewed than an arithmetic mean, and therefore, very high or very low bacterial concentrations will not have an undue impact on the geometric mean. This method was used to compute the fecal coliform and E. Coli geometric means for each quarterly sample set for the Bonne Femme watershed streams. The data were then grouped by site and the percentage of quarters exceeding the whole body contact standards were graphed (Figure 2). All sites exceeded the State and Federal standards at least 10% of the time and the three sites with the highest bacterial contamination exceeded both standards >60% of the time. Even Clear Creek, which receives much of its base flow from groundwater pumped from the USGS Environmental Research Center Laboratory, exceeded the standards in a few quarters. Overall, the results showed that the fecal coliform standard (200 cfu/100 mL) used by the State of Missouri was exceeded in 40% of the quarters at seven of the ten sites. However, the Federal standard was shown to be more stringent. The Federal whole body contact standard for E. Coli (126 cfu/100 mL) was exceeded in 50% of the quarters at eight of ten sites.

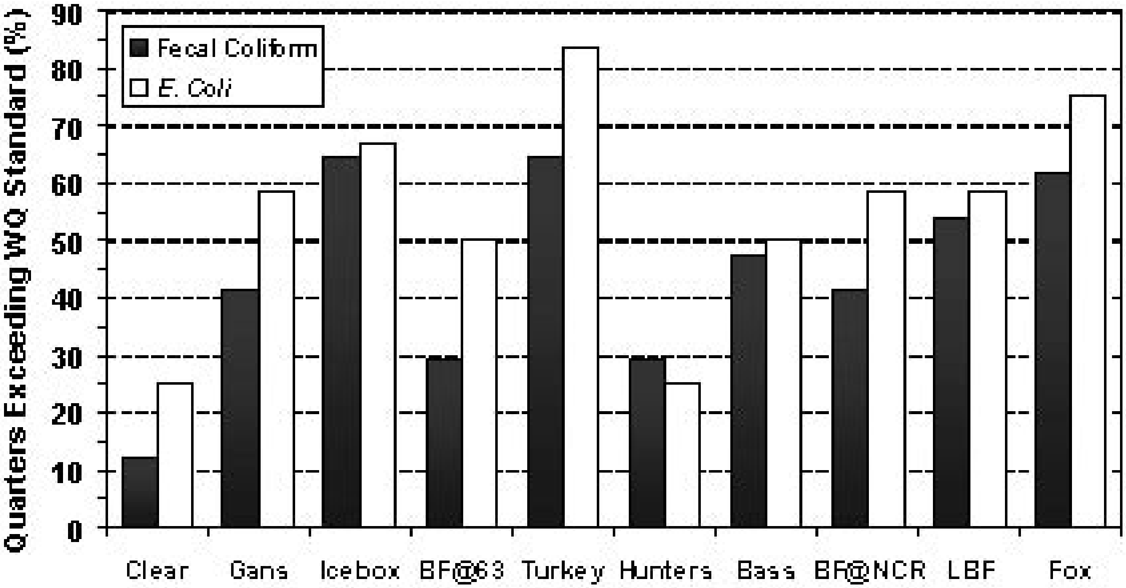


Figure 2 Percentage of quarters in which state and federal water quality standards for whole body contact were exceeded.

Data are based on computation of geometric mean of 4 samples/quarter and compared against state and federal water quality standards. Federal Whole Body Contact Standard = 126 cfu/100 mL based on *E. Coli*. Missouri Whole Body Contact Standard = 200 cfu/100 mL based on Fecal Coliforms. Fecal coliform data were based on 17 quarters (1st Q 2001 to 3rd Q 2006); *E. Coli* data were based on 12 quarters (4th Q 2003 to 3rd Q 2006).

Specific Water-Borne Pathogens

Beginning with the 3rd quarter of 2005, additional analyses were conducted by the USDA-Agricultural Research Service for the detection of three specific water-borne pathogens: E. Coli O157:H7, Salmonella, and Shigella. The methods used were based on DNA extraction from water samples collected at each site, followed by addition of a DNA primer that binds to one or more specific gene sequences that are indicative of a particular organism.

In the case of E. Coli O157:H7, three separate genes were required for positive identification (Fratamico et al., 1995) whereas a single gene was used to identify Salmonella (Aabo et al., 1993) and Shigella (Hartman et al., 1990). These methods are qualitative, meaning that they are limited to indicating the presence or absence of the pathogens. These three organisms are known human pathogens capable of causing food-borne gastrointestinal illnesses, but they are also associated with feces and therefore may contaminant streams and lakes, causing disease through oral contact or ingestion of contaminated water (Wikipedia, 2006). Salmonella and Shigella are genus classifications that can be further categorized into several species, with each species having multiple serotypes (or strains). E. Coli O157:H7 is one of hundreds of serotypes of the species E. Coli, and it is a common food contaminant associated with the guts of grain-fed cattle. The Centers for Disease Control and Prevention (http://www.cdc.gov/ncidod/dpd/healthywater /factsheets/ ecoli.htm) states that, "E. Coli O157:H7 is most commonly found on a small number of cattle farms where the bacteria can live in the intestines of healthy cattle." In addition, E. Coli O157:H7 has also been detected in the guts of swine and deer, which may also serve as carriers for the disease. Like fecal coliforms and generic E. Coli, these disease causing bacteria can enter surface waters through sewage overflows, polluted storm water runoff, and polluted agricultural runoff.

Each of the three pathogens was detected at most of the ten sites monitored (Figure 3), and at least one pathogen was detected at every site. Shigella was detected at eight of ten sites, but generally at lower frequency than Salmonella or E. Coli O157:H7. Salmonella was the most commonly detected pathogen at four of the ten sites, with 33% of the samples collected

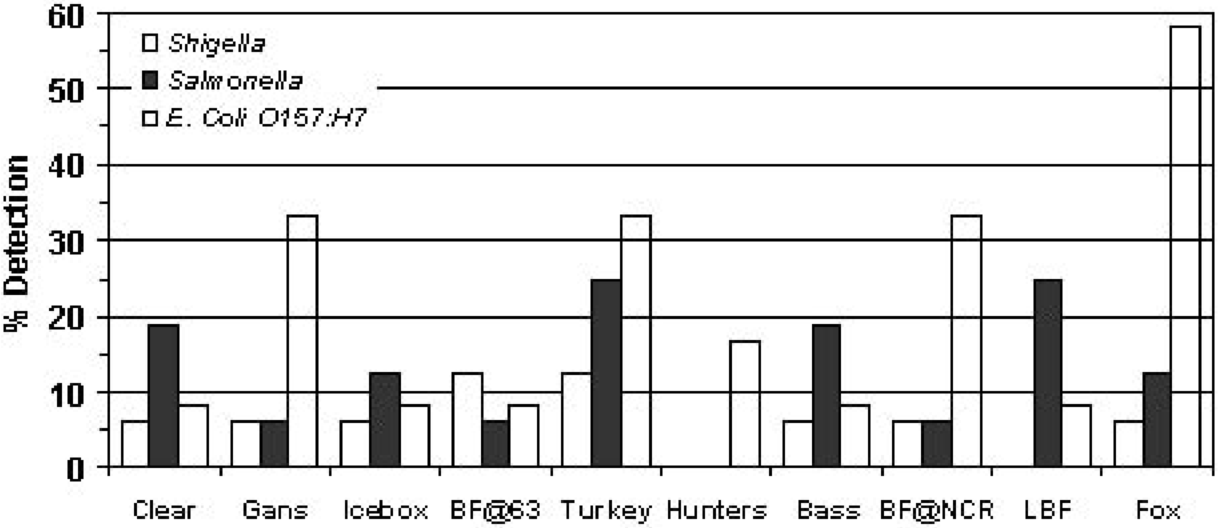


Figure 3 Detection frequency of specific waterborne pathogens in Bonne Femme watershed.

Data for *Salmonella* and *Shigella* are based on 16 samples per site (3rd quarter 2005 to 2nd quarter 2006); data for *E. Coli* O157:H7 are based on 12 samples per site (4th quarter 2005 to 2nd quarter 2006).

from Turkey and Little Bonne Femme Creeks testing positive for Salmonella. E. Coli O157:H7 was the most commonly detected of the pathogens, with at least one detection at every site. Five of the ten sites had multiple detections of E. Coli O157:H7. Three sites (Gans Creek, Turkey Creek, and Lower Bonne Femme Creek) had E. Coli O157:H7 detected in 33% of their samples, and Fox Hollow had E. Coli O157:H7 detected in 58% of it's samples. These data do not definitively indicate source, but they do point to cattle as a probable source of E. Coli O157:H7 at those sites with frequent detections. Of the common carriers of E. Coli O157:H7 (cattle, swine, and deer), swine can be eliminated as there are no sizable swine operations within the Bonne Femme watershed. Deer are likely responsible for the widespread nature of the detections, explaining the presence of E. Coli O157:H7 at sites with otherwise low fecal contamination, such as Clear Creek and Hunters Cave (Table 4). Although data on specific numbers of cattle by sub-watershed cannot be reliably compiled, there are major cattle operations in the four watersheds with the highest detection frequency of E. Coli O157:H7. Furthermore, the Fox Hollow sampling site is immediately downstream from a large cattle grazing operation (see additional discussion below).

Fecal Bacteria Contamination in Relation to Season, Land Cover, and Stream Properties

The data collected from the monitoring of the Bonne Femme watershed streams showed that fecal bacterial contamination of streams varied significantly across sites and over time. In an effort to explain these differences, several factors were considered to explain the observed variation, including season, land cover (Figure 4), and general stream water properties (based on data from Table 1). Statistical analyses were performed to determine if these factors were related to fecal bacterial contamination.

Statistical analysis of fecal bacterial contamination over time (i.e. quarters of the year) showed significant differences based on the season in which the sample was collected (Table 5). For both fecal coliforms and E. Coli, the 2nd and 3rd quarters of the year had significantly greater levels of fecal bacteria than the 1st and 4th quarters of the year. Given that the input sources (human, cattle, wildlife) do not vary considerably with the season of the year in this watershed, the data indicates that fecal bacterial contamination of the streams was strongly weather related. In the 1st and 4th quarters of the year, colder air and soil temperatures likely resulted in faster die-off of fecal bacteria released to the environment, and therefore, there were fewer bacteria available for transport during fall and winter compared to spring and summer. Additionally, precipitation events in spring and summer are more frequent and more likely to generate runoff than in fall and winter. Thus, the 2nd and 3rd quarters apparently had greater populations of fecal bacteria surviving in the soil environment combined with a greater probability of runoff events capable of transporting fecal bacteria to the streams.

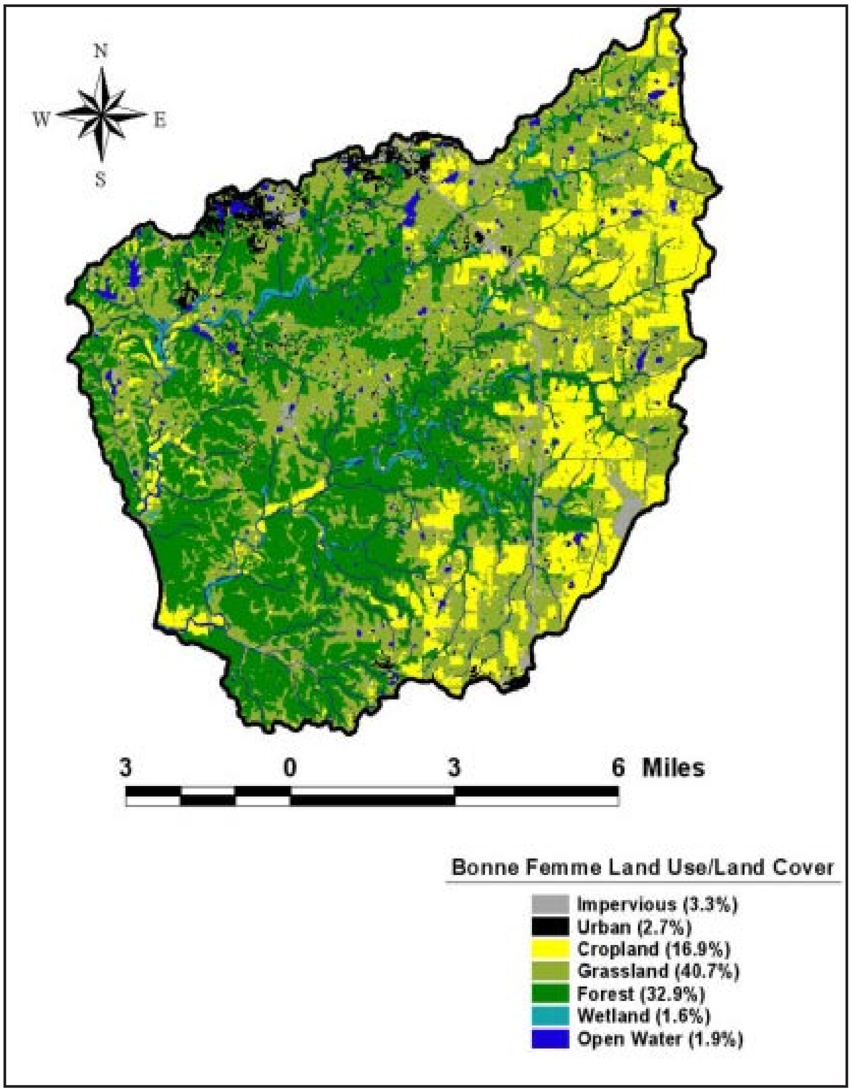


Figure 4 Land use/land cover for the Bonne Femme watershed. Data were obtained from 30-m resolution LANDSAT data collected from 2000-2004.

Of the stream water properties measured (temperature, pH, specific conductivity, dissolved oxygen, and turbidity, Table 1), there were no significant correlations of these parameters to fecal coliform or E. Coli concentrations in the streams. However, a much larger data set exists at the two cave sites for the stream water properties and fecal coliform concentrations, with data collected as far back as 1999 and at much greater frequency than was conducted for this project (Lerch et al, 2001). Of the general stream water properties measured at the two caves, only turbidity was shown to significantly correlate to the fecal coliform concentrations. At Hunters Cave, 72% of the variation in fecal coliform concentrations could be explained by the turbidity levels of the water. The correlation between turbidity and fecal coliform concentrations at the Devil's Icebox Spring Branch was much lower, but still significant, because high bacterial concentrations were observed even when turbidity was low. Other researchers have reported a significant relationship between fecal bacterial concentrations and turbidity (Rasmussen and Ziegler, 2003) in surface streams, and it is probable that with a more intensive monitoring regime such a relationship also exists for the surface streams in the Bonne Femme watershed. The only other physical parameter that significantly correlated to fecal bacterial concentrations was stream discharge, but this data only exists at the two cave sites. Although both fecal coliform and E. Coli concentrations significantly correlated to stream discharge at the caves, E. Coli showed a much stronger correlation to discharge than fecal coliforms. The correlations of fecal bacterial concentrations to turbidity and stream discharge indicated that fecal bacterial concentrations, in general, will be greatest for runoff events with high turbidity. These events have enough energy to induce soil erosion, resulting in transport of sediment-bound fecal bacteria to the streams.

Table 5 Average fecal coliform and *E. Coli* concentrations by quarter of the year.

Quarter	Fecal Coliform	*E. Coli*
	log10(cfu/100 mL)	
1st	1.53	1.24
2nd	2.50	2.28
3rd	2.47	2.34
4th	1.95	1.86
LSD	0.22	0.21

None of the major land cover classes (impervious, urban, row crops, grasslands, or forest, Figure 4) was significantly correlated to either fecal coliform or E. Coli concentrations (Table 4) in the streams. This result suggests multiple sources or fairly uniformly distributed non-point sources of fecal bacteria exist across the sub-watersheds. Given the wide variation in land cover and human population across sub-watersheds (Figure 4), multiple but different sources apparently exist. Multiple sources seemed to be the cause of contamination in most sub-watersheds (e.g., Turkey Creek, Little Bonne Femme Creek, Upper Bonne Femme Creek, and Gans Creek) while site specific sources of fecal bacteria appear to be responsible for the high levels observed at two sites (Devil's Icebox Spring Branch and Fox Hollow).

The site specific sources in the Devil's Icebox Spring Branch appear to be from private residences within the Pierpont sinkhole plain where on-site sewers discharge to the cave via transport through the sinkholes. Evidence for this is two-fold: 1) the consistently higher levels of fecal bacteria in the Devil's Icebox Spring Branch compared to Upper Bonne Femme Creek, the main source of water to the Devil's Icebox Spring Branch; and 2) frequently observed high concentrations under low-flow conditions. Regarding the first point, the increase in fecal bacterial concentrations between Upper Bonne Femme Creek and the Devil's Icebox Spring Branch (Table 4) indicates that additional sources are entering the cave between the losing stream reach in Upper Bonne Femme Creek and the cave stream resurgence. The only land area between these points is the sinkhole plain. Moreover, the distance between the losing reach of Upper Bonne Femme Creek and the Devil's Icebox Spring Branch resurgence is at least four miles, and it would be expected that some die-off of the fecal bacteria or dilution from other tributaries to the cave stream would occur along this lengthy flow path if there were no other bacterial inputs. For example, comparison of fecal bacterial concentrations in Hunters Cave to

Bass Creek, the main water source to Hunters Cave, showed that the levels in Hunters Cave were consistently lower than Bass Creek (Table 4). Thus, dilution or die-off occurred along the sub-surface flow path, yet this flow path is much shorter than that of the Devil's Icebox Spring Branch. With regards to the second point, under low flow conditions the Devil's Icebox Spring Branch had 18 of 41 samples with fecal coliform concentrations >200 cfu/ 100 mL compared to only 10 of 39 samples >200 cfu/100 mL at Upper Bonne Femme Creek. For the E. Coli data, Devil's Icebox Spring Branch had 21 of 41 samples with concentrations > 126 cfu/100 mL while Upper Bonne Femme Creek had only 9 of 40 samples >126 cfu/100 mL. Since high bacterial inputs were apparent under low flow conditions, this precludes surface runoff from livestock grazing lands or wildlife as the source, and thus, implicates on-site sewers as the probable source of this additional input to the cave. As discussed above, similar trends for TN and NO_3-N were also observed between Upper Bonne Femme Creek and the Devil's Icebox Spring Branch, providing further evidence that on-site sewers in the sinkhole plain have contributed to water quality degradation in the Devil's Icebox Cave Branch.

The other monitoring site with site-specific causes of contamination is Fox Hollow. The monitoring site is immediately downstream of a sizable cattle operation. The cattle have unrestricted stream access (and were frequently observed in the stream) and manure is stored in the open within 100 feet of the stream. In addition, the pasture land adjacent to the stream is overgrazed and there are no riparian management practices employed to stabilize the stream banks or to mitigate fecal bacterial transport. Not coincidentally, this site had the highest fecal coliform levels, 2nd highest E. Coli levels, and the highest occurrence of E. Coli O157:H7 of the sites monitored.

Significant fecal bacterial contamination occurred at several sites for which no site specific sources of bacteria were apparent, and therefore, multiple sources appeared to be the cause of contamination. This was the case for Turkey Creek, Upper Bonne Femme Creek, Little Bonne Femme Creek, and Gans Creek. For example, Turkey Creek had the highest fecal coliform, E. Coli, and occurrence of specific pathogens as any site except for Fox Hollow. Turkey Creek has a very low human population, but 43% of this sub-watershed's area is grasslands with several sizable cattle operations. As was the case in Fox Hollow, many of the grassland areas are overgrazed, cattle have unrestricted access to the streams, and there is little or no riparian management, especially in the upper portions of the sub-watershed. Apparently, multiple cattle operations were the cause of contamination in Turkey Creek. Sub-watersheds with substantial human populations and considerable agricultural land uses, such as Little Bonne Femme Creek and Gans Creek, likely have a combination of human sewage and cattle inputs as the sources of fecal contamination. Sites with the lowest contamination, such as Clear Creek and Hunters Cave, may largely represent background inputs from wildlife with only limited contributions from cattle or on-site sewers.

Conclusions

The following general conclusions can be reached from the monitoring study:

- General stream water properties indicate no acute contamination, with all five properties measured falling within typical ranges for carbonate bedrock streams, and dissolved oxygen levels above the State minimum standard of 5 mg/L;
- Nutrient levels were similar to or less than streams in other agricultural watersheds of northern Missouri. There was no evidence of acute contamination at any site;
- The combination of dissolved oxygen, turbidity, nutrient levels, and field observations indicated that all sites have some level of nuisance algal growth and presumed loss of macroinvertebrate diversity, but eutrophication conditions have not occurred at any site;
- At least one herbicide or metabolite was detected in every sample at all sites, but typically at low levels. Atrazine and its metabolites had the highest average concentrations at all sites;
- Fecal bacterial contamination was widespread with significant differences observed across sites and over seasons. Concentrations of fecal bacteria were highest in spring and summer;
- Whole body contact standards for fecal bacteria were commonly exceeded. Seven of ten sites exceeded the State fecal coliform standard 40% of the time. Eight of ten sites exceeded the Federal E. Coli standard 50% of the time;
- Frequency of detection of specific pathogens was in the following order: E. Coli O157:H7 > Salmonella> Shigella. The pattern of E. Coli O157:H7 detections indicated that cattle were the probable source;
- Of the general stream water properties measured, concentrations of fecal bacteria were significantly correlated only to turbidity and stream discharge (based only on the two cave sites);
- Land cover classes did not significantly correlate to the concentrations of fecal bacteria;
- Multiple sources apparently were the cause of contamination in most sub-watersheds while site specific sources of fecal bacteria appear to be responsible for the high levels observed at the Devil’s Icebox Spring Branch (most likely from on-site sewage) and Fox Hollow (most likely from cattle).

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