



**Missouri  
Department of  
Natural Resources**

**Biological Assessment Report**

**Mulberry Creek  
Bates County**

**March 29, 2001**

Prepared for:

Missouri Department of Natural Resources  
Water Protection and Soil Conservation Division  
Water Pollution Control Program

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## **1.0 Introduction**

At the request of the Water Pollution Control Program (WPCP), the Environmental Services Program (ESP) Water Quality Monitoring Section (WQMS) conducted a biological assessment of Mulberry Creek, northeast of Amoret in western Missouri. Mulberry Creek was added to the 303(d) list of impaired waters in 1998 due to a suspected elevation in sulfate levels. Much of the watershed supplying this creek consists of abandoned mine land (AML) that was formerly strip mined for coal.

Miami Creek, an adjoining drainage free of AML influence which is located northwest of Butler, was selected as a control site to compare with Mulberry Creek. This comparison was to determine whether a biological impairment exists in a system with elevated sulfate levels. Sampling at Mulberry and Miami creeks was conducted on March 29 and April 3, 2001, respectively, to provide data to the WPCP for use in evaluating and comparing the biological integrity of the two streams. Dave Michaelson, Cecilia Campbell, and others of the Environmental Services Program, Air and Land Protection Division conducted the sampling.

On March 22, 2001 a study plan was submitted to the WPCP (Appendix A). Because standard statistical methods were proposed in the study plan, a null hypothesis of no difference between macroinvertebrate communities of a high sulfate stream and a control was stated. Six contiguous stations were sampled at each study site.

## **2.0 Study Area**

Mulberry Creek originates near the Missouri-Kansas state line in the Amsterdam area and flows southeast through an upper watershed dominated by grasslands (see Table 1) with extensive areas of abandoned mine land. The stream reach assessed is class "C" with beneficial use designations of "livestock and wildlife watering" and "warm water aquatic life protection, human health/fish consumption."

Miami Creek originates near the Missouri-Kansas state line in the Merwin area and flows southeast through a watershed dominated by grasslands (see Table 1). The stream reach assessed is class "C" with beneficial use designations of "livestock and wildlife watering" and "warm water aquatic life protection, human health/fish consumption." This stream was chosen as a control in the study due to several factors: its close proximity to the study stream within the same Ecological Drainage Unit (EDU); a watershed of comparable size; and a lack of observable AML within the watershed.

Mulberry and Miami creeks are located within the Plains/Osage EDU. An EDU is a region in which biological communities and habitat conditions can be expected to be similar. Please see Appendix B for maps of the EDUs and the 14 digit Hydrologic Units (HU), #10290102100001 and #10290102120001, that contain the sampling reaches for Mulberry and Miami creeks, respectively. See Table 1 for a comparison of land use for

the 14 digit HU. Land cover data were derived from the Thematic Mapper satellite data from 1991-1993, and interpreted by the Missouri Resource Assessment Partnership (MoRAP).

Table 1  
Percent Land Cover

	Urban	Crops	Grassland	Forest	Swamp
EDU	0.2	23.0	54.9	17.9	0.3
Miami HU	0.0	27.9	61.0	9.2	0.0
Mulberry HU	0.0	16.3	72.4	8.3	0.0

### 3.0 Site Descriptions

Mulberry Creek Stations #1 through #6 (N ½ sec. 22, T. 40 N., R. 33 W. & S ½ sec. 15, T. 40 N., R. 33 W.) were located both upstream and downstream from an unnamed county road one mile north of Highway 52 northeast of Amoret. Average stream width downstream of the bridge was 32 feet; the total length of stream sampled downstream of the bridge was approximately 1920 feet. Geographic coordinates at the downstream terminus of Station #1 are Lat. 38.26553°, Long. -94.56002°. Discharge was measured at 6.58 cubic feet/second (cfs). Average stream width upstream of the bridge was 26.5 feet; the total length of stream sampled upstream of the bridge was approximately 1590 feet. Geographic coordinates at the upstream terminus of Station #6 are Lat. 38.26553°, Long. -94.56233°. Discharge was measured at 4.14 cfs. The watershed at the most downstream segment was approximately 32.5 mi<sup>2</sup>.

Miami Creek Stations #1 through #6 (W ½ sec. 33, T. 41 N., R. 32 W. & E½ sec. 32, T. 41 N., R. 32 W.) were located both upstream and downstream from an unnamed county road 0.5 mi. south of State Road F west of Passaic. Average stream width downstream of the bridge was 36 feet; the total length of stream sampled downstream of the bridge was approximately 2880 feet. Geographic coordinates at the downstream terminus of Station #1 are Lat. 38.31617°, Long. -94.46983°. Discharge was measured at 7.12 cfs. Average stream width upstream of the bridge was 40 feet; the total length of stream sampled upstream of the bridge was approximately 1600 feet. Geographic coordinates at the upstream terminus of Station #6 are Lat. 38.31894°, Long. -94.47849°. Discharge was measured at 7.48 cfs. The watershed at the most downstream segment was approximately 46.3 mi<sup>2</sup>.

### 4.0 Methods

#### 4.1 Macroinvertebrate Collection

A single standard habitat (non-flowing water with depositional substrate) was sampled at each of the stations as described in the Semi-quantitative Macroinvertebrate Stream

Bioassessment Project Procedure (SMSBPP). Other habitats available during the spring sample season (coarse substrate, large woody debris, and rootmat) were not sampled due to their potential ephemeral nature at these sites. It was decided that only the depositional areas (i.e., pools) were likely to have been hydrated during the previous months and that sampling this habitat would provide the best assessment of the macroinvertebrate community. A standardized sample collection procedure was followed as described in the SMSBPP.

#### **4.2 Discharge Measurements**

Stream velocity was measured using a Marsh-McBirney Flo-Mate Model 2000. Discharge was calculated per the methods in the Standard Operating Procedure MDNR-FSS-113 (Flow Measurement in Open Channels).

#### **4.3 Macroinvertebrate Analysis**

A standardized sample analysis procedure was followed as described in the SMSBPP. One deviation was made to the metrics that are calculated in the project procedure. The Biotic Index was not calculated because it is a general indicator of organic pollution and this study was specific to sulfate.

#### **4.4 Quality Assurance/Quality Control (QA/QC)**

QA/QC procedures were followed as described in the SMSBPP.

#### **4.5 Stream Habitat Assessment**

A standardized assessment analysis procedure was followed as described for Glide/Pool Habitat in the Stream Habitat Assessment Project Procedure.

#### **4.6 Statistical Methods**

Macroinvertebrate biological indices (Total Richness, EPT Index, and Shannon Diversity Index) and Habitat Assessment scores were compared among sites using a two-tailed t-test at the  $\alpha = 0.05$  level. A two-tailed test is used for comparing the means from two populations when the variance is unknown and the data are collected in pairs (i.e., for each sample collected at Mulberry Creek one was also collected at Miami Creek). This study was designed for data to be collected in pairs in an effort to obtain observations that were alike in all respects except for the biological indices we were comparing. Upon completion of the two-tailed test, the null hypothesis (equal means) among sites was either accepted or rejected. In cases where the null hypothesis was rejected, means of the various biological indices were compared to determine which site, the control or the test, was elevated in diversity.

Because the t-test is a parametric method, each data set was tested for normality before proceeding. All statistical interpretations were conducted using SigmaStat<sup>®</sup> (version 2.03, Jandel Scientific, San Rafael, California) software. An *a priori* p-value of <0.05 was selected to determine statistically significant differences among data sets.

## **5.0 Observations**

Stream stage at Mulberry and Miami creeks appeared to be at base flow, with all available habitat types submerged by flowing water. There was no evidence of recent high water events and the creeks exhibited characteristics consistent with a glide/pool regime.

## **6.0 Data Results and Analysis**

### **6.1 Water Chemistry**

The results of eight Mulberry Creek water chemistry samples collected from 1998 to 2001 were provided by the WPCP. All water quality samples were collected at or within 1.5 river miles downstream of the study site. Of those samples, none exhibited sulfate concentrations in excess of 1000 mg/L, the upper limit for state water quality standards. The mean concentration of sulfate in samples was 295 mg/L, ranging from 139-428 mg/L.

In addition, the results of four Miami Creek water chemistry samples collected in April and June 2001 were provided by the WPCP. All samples were collected at the study site with the exception of one, which was collected approximately three river miles downstream. The mean concentration of sulfate in samples was 49 mg/L, ranging from 20-63 mg/L.

### **6.2 Habitat Assessment**

Following macroinvertebrate collection, habitat assessment scores (see Table 2) were recorded for each sample reach at both sites according to methods described in the Stream Habitat Assessment Project Procedure. According to the project procedure, for a study site to fully support a biological community, the total score from the physical habitat assessment should be 75% to 100% similar to the total score of the reference site. The mean habitat score of Mulberry Creek was 92% of the mean habitat score of Miami Creek.

Additionally, a two-tailed t-test used to compare habitat assessment scores between sites showed that the mean habitat score of 100.67 (of a possible 200) for six reaches sampled on Mulberry Creek was not significantly different than the Miami Creek mean score of 109 ( $p = 0.069$ ).

Although neither comparison is meant to provide an absolute distinction between sites, it does provide a method for determining whether substantial differences exist between a study site and its reference. In this study, both the project procedure and the statistical analysis lead to the conclusion that the study site should support a comparable biological community.

Table 2  
Habitat Scores

Stream	Station #1	Station #2	Station #3	Station #4	Station #5	Station #6	Mean
Mulberry	100	91	106	94	103	110	100.67
Miami	100	111	103	107	115	118	109.00

### 6.3 Biological Assessment

Macroinvertebrate metrics, with the exception of the EPT Taxa, were higher at Mulberry Creek (see Table 3). There was no statistically significant difference in the EPT Taxa Index among sites ( $p = 0.515$ ). For the EPT Taxa the null hypothesis of no difference would be accepted.

The Mulberry Creek mean Taxa Richness of 34.17 was significantly higher than the mean of 23.67 at Miami Creek ( $p < 0.001$ ). The mean Shannon Diversity Index of 2.393 at Mulberry Creek was also significantly higher than the mean of 2.027 at Miami Creek ( $p = 0.010$ ). For Taxa Richness and the Shannon Diversity Index the null hypothesis would be rejected. Although Taxa Richness and the Shannon Diversity Index are significantly different at Mulberry and Miami creeks, the difference could have been in either a positive or negative direction for the study stream as compared to the control. In this case the study stream is elevated in diversity over the control stream.

Chironomidae taxa made up the majority of the total count at both sites, comprising an average of 60% of individuals at both sites. Chironomidae taxa made up 26% of Taxa Richness at Mulberry Creek and 25% at Miami Creek.

The highest number of taxa found at a single sample station was 40 at Mulberry Creek; at Miami Creek, the highest number of taxa was 28. As is common in prairie streams, few EPT taxa were found. No more than five species of EPT taxa were found at either location and generally very few individuals of each taxon were present in samples.

Table 3  
 Macroinvertebrate Metrics

Stream/metric	Station #1	Station #2	Station #3	Station #4	Station #5	Station #6	Mean
Mulberry Taxa Richness	33	34	40	32	33	33	34.17
Miami Taxa Richness	28	26	22	25	18	23	23.67
Mulberry EPT Taxa	2	1	3	2	2	2	2
Miami EPT Taxa	2	2	2	3	1	0	1.67
Mulberry Shannon Index	2.36	2.21	2.66	2.27	2.72	2.14	2.39
Miami Shannon Index	2.27	2.05	2.06	2.04	1.87	1.87	2.03

## 7.0 Discussion

Although sulfate concentrations in Mulberry Creek water samples were higher than the control site, it did not appear to have impaired the macroinvertebrate community during spring 2001. Taxa Richness and the Shannon Diversity Index both favored Mulberry Creek over Miami Creek by showing a significant increase in diversity. Due to the relatively low abundance of EPT taxa, no significant difference among sites could be observed with that metric.

In summary, despite its smaller watershed size, lower flow rate, and higher sulfate levels compared to Miami Creek, Mulberry Creek displayed higher taxa richness and Shannon Diversity Index. Overall, habitat and land use were very similar at each site with both exhibiting steep, incised banks and cattle access along much of both sample reaches. In addition, the macroinvertebrate community from the permanent pools in the 303(d) listed stream were at least as diverse as the control stream as measured by three of the four metrics used for biological criteria in Missouri streams.

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## **Appendix A**

Proposed Bioassessment Study Plan  
Mulberry Creek  
March 22, 2001

**Missouri Department of Natural Resources**  
**Proposed Bioassessment Study Plan**  
**West Tebo Creek, Mulberry Creek, and 2nd Nicholson Creek**  
**March 29, 2001**

Objective

Compare macroinvertebrate communities between 303(d) streams listed for sulfate and non-sulfate impaired control streams.

Hypothesis

Null = No difference between macroinvertebrate communities of high sulfate streams and controls.

Background

All high sulfate streams have relatively small watersheds and are considered either temporary or intermittent. Because of this situation any biological assessment must be accomplished when the streams are well hydrated. The time period that coincides with established MDNR macroinvertebrate bioassessments is mid-March through mid-April. All streams will be sampled only one year, one season, unless environmental conditions dictate further sampling.

Study Design

**General:** Although over eight years of work has been invested in biological criteria development in wadeable and perennial streams by MDNR, the streams in question are in a different classification and require adapting standard procedures. Ecoregional reference streams for this size class are not currently available, thus a paired watershed approach is proposed. Considerable caution was taken in selecting controls that are of comparable size and uninfluenced by coal mining. West Tebo Creek (watershed area 19 sq. mi.) is paired with East Fork of Honey Creek (watershed area 17 sq. mi.); Mulberry Creek (watershed area 33 sq. mi.) with upper Miami Creek (watershed area 47 sq. mi.); and 2<sup>nd</sup> Nicholson Creek (watershed area 14 sq. mi.) with Little Drywood Creek (watershed area 10 sq. mi.).

Sampling units will be at the reach scale. Each stream will be sampled in six reaches. Each reach will be determined as twenty average stream widths, which is consistent with the MDNR Semi-quantitative Macroinvertebrate Stream Bioassessment Project Procedure (SMSBPP). Stream reaches will be selected within one segment of stream in which conditions can be expected to be approximately the same. To account for potential influences that would affect some segments but not all segments, discharge, water chemistry, and habitat measures will be taken during sampling.

**Biological Sampling Methods:** The MDNR SMSBPP will be modified to fit the smaller stream size of this study. Only one of the three standard habitats normally collected in larger streams will be sampled. This habitat, non-flowing water over depositional substrate, will standardize samples between reaches. Each reach sample will be a composite of six, approximately 1 square meter, sub-samples from various pools within the reach. The advantage of taking a composite sample in small intermittent streams that dry to varying degrees is the ability to minimize the problems associated with the past hydration of each individual pool.

**Physical and Habitat Sampling Methods:** The MDNR Stream Habitat Assessment Project Procedure (SHAPP) will be utilized at all locations. The habitat score will, in part, allow a measure of the variability of factors that might influence macroinvertebrate communities between reaches. Discharge will also be measured at the most upstream and most downstream locations. Because no major tributaries are to be present between the most upstream and downstream reaches, flow conditions and water quality are expected to be similar between all reaches.

**Chemical Sampling Methods:** All streams will be sampled for sulfate, conductivity, chloride, pH, and dissolved oxygen on a quarterly basis to establish the background and range of conditions. Control streams are expected to have sulfate levels well below state standards.

**Laboratory Methods:** Macroinvertebrates collected at all sampling locations will be processed and identified as stated in the MDNR SMSBPP and the MDNR Standard Operating Procedure MDNR-FSS-209, Taxonomic Levels for Macroinvertebrate Identification.

**Data Recording and Analysis:** Data recording will be done in a Microsoft Access database according to the MDNR Standard Operating Procedure MDNR-WQMS-214, Quality Control Procedures for Data Processing. Data analysis is automated within the Access database. Four standard metrics are calculated according to the SMSBPP. One of these, the Biotic Index, is designed to respond to organic enrichment and will not be utilized in this study. The remaining metrics of Total Taxa (TT), Ephemeroptera, Plecoptera, Trichoptera Taxa (EPTT), and the Shannon Index (SI) will be calculated for each reach. The six reach samples for each stream will be used for mean comparison analysis (unpaired t-test) if data is normally distributed or Mann-Whitney Rank Sum Test if data cannot fit the normality distribution. The probability level for concluding a significant difference will be  $p < 0.05$ . Analysis will be done on a paired watershed approach and, since all sites are within the same ecological region, the possibility exists of attempting to pool all control data if variability allows. This possibility would also allow for analyses consistent with biological criteria as developed for wadeable/perennial streams.

**Data Reporting:** Results of the study will be written in report format.

**Quality Control:** As stated in the various MDNR Project Procedures and Standard Operating Procedures.

**Attachments**

Standard Operating Procedures

Taxonomic Levels for Macroinvertebrate Identification (MDNR-FSS-209)  
Quality Control Procedures for Data Processing (MDNR-WQMS-214)

Project Procedures

Semi-quantitative Macroinvertebrate Stream Bioassessment Project Procedure  
Stream Habitat Assessment Project Procedure



## **Appendix B**

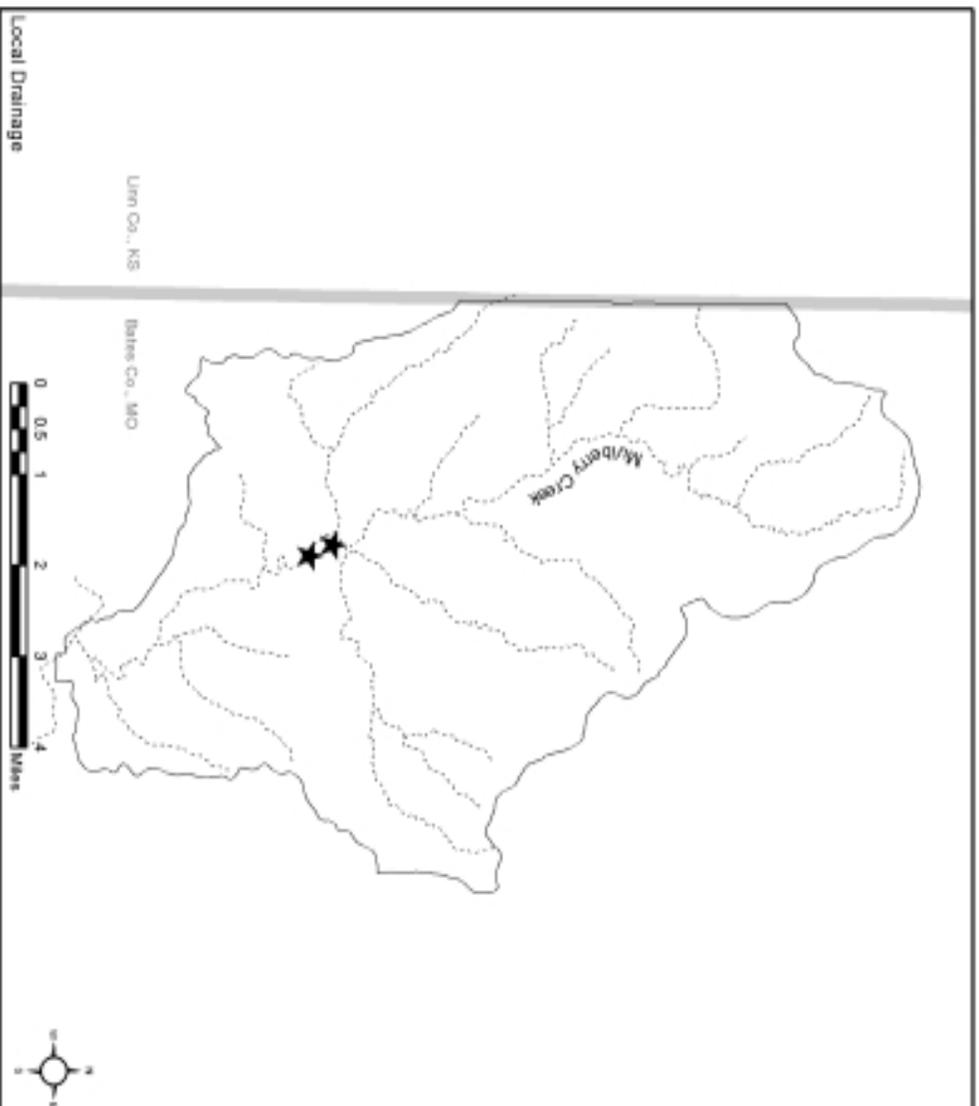
Maps

Mulberry Creek  
Prairie/Osage EDU

&

Miami Creek  
Prairie/Osage EDU

## Mulberry Creek Study Site



- ★ Sampling Location
- ▭ County Boundary
- ▭ Local Drainage
- Stream/River

### Local Drainage and Biologic Sampling Site Location

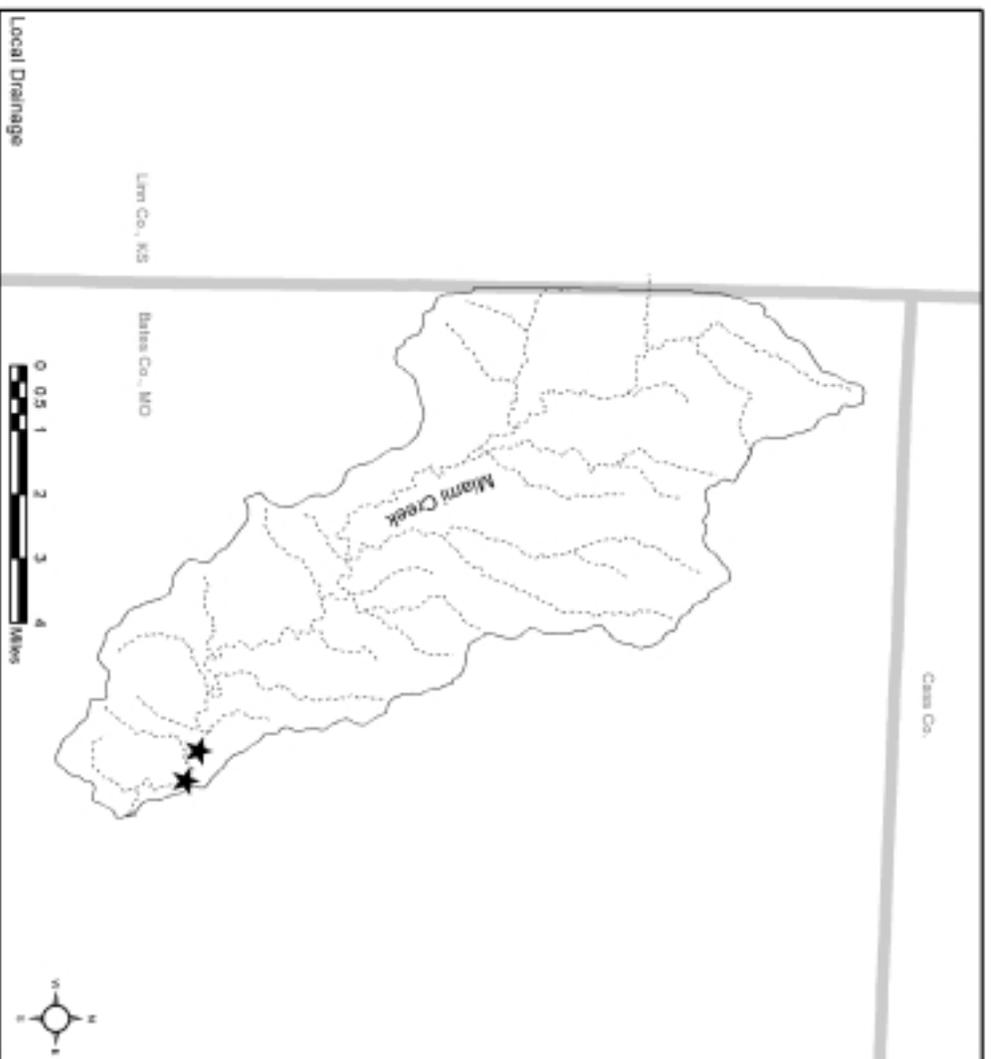
**Ecological Drainage Unit (EDU)** - An EDU is an area that contains a unique combination of habitats and organisms. Missouri is divided into 19 EDUs as shown in the inset map below. This site is located in the highlighted EDU.

**Local Drainage** - The local drainage area, also known as a 14 Digit Hydrologic Unit, is shown in the main map at left. This area is a portion of the local watershed. Missouri is split into over 1500 such units.



Ecological Drainage Unit

## Miami Creek Study Site



- ★ Sampling Location
- ▭ County Boundary
- ▭ Local Drainage
- Stream/River

Local Drainage and Biologic Sampling Site Location

Ecological Drainage Unit (EDU) - An EDU is an area that contains a unique combination of habitats and organisms. Missouri is divided into 19 EDUs as shown in the inset map below. This site is located in the highlighted EDU.

Local Drainage - The local drainage area, also known as a 14 Digit Hydrologic Unit, is shown in the main map set left. This area is a portion of the local watershed. Missouri is split into over 1500 such units.



Ecological Drainage Unit