Madison County Mines Natural Resource Damage Assessment
Study Plan
Updated March 2023

QUANTITATIVE SURVEYS OF FRESHWATER MUSSELS IN THE LITTLE ST. FRANCIS RIVER, MISSOURI

INTRODUCTION

The Little St. Francis River (LSFR) is the primary drainage system for the Madison County Mines National Priorities List Site (MCM) located near Fredericktown, Missouri at the southern portion of the Old Lead Belt. Heavy metal mining occurred within the MCM from the mid 1700's through the 1960's, and was recently reinitiated at the former Anschutz site within the MCM. Pre-environmental regulation and inefficient mining operations resulted in soil, sediment, and surface water resources contaminated with elevated levels of heavy metals including cadmium, lead, zinc, copper and nickel, which are continuing to release in the environment and exposing natural resources. The Natural Resource Trustees (US Fish and Wildlife Service on behalf of the U.S. Department of the Interior and the Missouri Department of Natural Resources on behalf of the State of Missouri) initiated a natural resource damage assessment and restoration (NRDAR) process in 2007, including finalization of a Damage Assessment Plan in 2015. This study plan is intended to complement qualitative mussel surveys conducted by the U.S. Fish and Wildlife Service (Service) in July 2022, and describes methodologies to obtain quantitative measures of freshwater mussel density, richness, relative abundance and associated sediment metals concentrations in the LSFR. Further study of metals concentration in the LSFR and its tributaries, as well as mussels is consistent with the assessment activities identified in the Trustees' Damage Assessment Plan.

The LSFR is a large tributary to the St. Francis River, an important stream for mussel diversity which supports 46 species including the federally listed Pink Mucket (*Lampsilis abrupta),* Rabbitsfoot (*Theliderma cylindrica*), Snuffbox (*Epioblasma triquetra)*, and Western Fanshell (*Cyprogenia aberti*) whose status is currently proposed as threatened. The Trustees conducted qualitative mussel surveys in summer 2022 to identify mussel presence/absence and determine species richness within the LSFR, however, quantitative data to determine baseline density and recruitment estimates have not been collected.

Due to the lack of comprehensive freshwater mussel data within the LSFR and the proximity of federally listed mussel species to the extensive heavy metal contamination present throughout the LSFR, the Trustees, led by the Service, will evaluate the density, species richness, relative abundance, and recruitment at up to 13 sites on the LSFR and 1-2 reference sites on the St. Francis River to help establish baseline population data. Previously surveyed mussel beds, occupied sites identified during the July 2022 qualitative surveys, and areas of suitable habitat with unknown mussel presence/absence will be targeted for this study. The reference site will be selected from known mussel communities on the St. Francis River and will be based on similar stream order and underlying geology to the LSFR. The study objectives are to:

1) Determine freshwater mussel richness, relative abundance, density, and recruitment data from known mussel beds and suitable habitat with unknown mussel occupancy;
2) Collect instream sediment from mussel beds and adjacent gravel bars to provide data on the concentrations of heavy metals present within suitable mussel habitat.

Additionally, through the Comprehensive Environmental Response, Compensation, and Liability Act Superfund program, the Environmental Protection Agency (EPA) is performing ongoing response actions within the MCM Site and throughout the LSFR. As part of the remedial investigation, EPA collected data that showed both surface water and sediment in the LSFR and its tributaries had heavy metal concentrations (including Cd, Cu, Ni, Pb, and Zn) that could adversely impact aquatic life communities. Some concentrations exceeded Probable Effects Concentrations (PEC; MacDonald et al 2000) for heavy metals. A more recent EPA sampling effort (2020-21) characterized 42 miles of the mainstem of LFSR and 60 miles of tributaries. However, stream bank and sediment metals concentrations collected as part of this EPA effort focused on public access points and areas of high potential human health exposure. These locations may not adequately represent heavy metal concentrations in all instream habitat types, and may not fully account for heavy metal exposure experienced by freshwater mussels.

The results of this investigation will be summarized in a final NRDAR report and the mussel species data will be included within the state of Missouri's freshwater mussel database (MDC 2017).

METHODS

Mussel Bed Delineation and Sampling

Quantitative sampling in the form of quadrats will be conducted at 15 total sites including 1-2 reference sites on the St. Francis River, to estimate mussel density, relative abundance, species diversity, and document recruitment (Strayer and Smith 2003). Prior to conducting surveys at known occupied mussel beds, the boundary of each mussel bed will be delineated to establish the sampling area. This allows quantitative surveys to be focused on the portion of the channel occupied by mussels, and minimizes site variance to provide more accurate population estimates (Strayer and Smith 2003). Visual and tactile searches will be used to determine the linear and lateral extent of the area occupied by mussels. Searches will be conducted systematically in a zig-zag pattern across the channel and in an upstream or downstream direction. Tactile search methods involve disturbing the top layer of substrate by hand to increase detection of mussels at or just below the substrate surface. The boundary will be marked with a Trimble® GeoXT™ where mussel densities drop to less than 1 individual/m^2 as estimated by the diver. Sites will be surveyed by at least 2 experienced biologists familiar with the regional fauna. Searches will be conducted during periods of low-flow to increase access and reduce sampling of unsuitable habitat. For sites with unknown mussel occupancy that represent suitable habitat, biologists will use the previously described methods to delineate the extent of suitable habitat, and the boundary will be marked where a noticeable chance in habitat type or substrate stability occurs.

Following mussel bed or suitable habitat delineation, 45 0.25m^2 quadrats will be evenly spaced within the mussel bed boundary using a systematic sampling approach with 3 random starts (Smith *et al.* 2001, Strayer and Smith 2003, Roberts *et al.* 2016).

Quadrats will be positioned on the stream bottom at each identified sampling point and all visible mussels will be collected. Following initial visual surveys, large cobble and flat rocks will be removed and remaining substrates will be excavated to a depth of approximately 10cm. Samples will be sieved through a floating 7mm screen and sorted for mussels. Length and age (counting external growth lines) will be estimated, and species recorded for each individual mussel prior to returning them to their original quadrat location. Any dead species not represented by live individuals will also be noted.

Any dead shells collected will be classified as fresh-dead, dead, or subfossil. Fresh-dead shells represent individuals in which the soft anatomy has not fully decomposed, and indicate the individual has recently perished. Dead shells have some luster to the nacre (innermost layer of the shell) and have a relatively intact periostracum (outermost layer of the shell). Subfossil shells have chalky and lusterless nacre and are missing considerable amounts of the periostracum (Buchanan 1980). The rate at which shell material decomposes following the death of a mussel depends on a variety of factors, including whether the shell was above or below the substrate, whether the shell was in the water or immersed, species, and shell thickness. In general, dead shells represent mussels that have been dead for less than a year and subfossil shells represent mussels that have been dead for more than a year.

Sediment Sampling

The goal of sampling sediment is to determine concentrations of heavy metals in stream sediments in occupied or suitable mussel habitats. Sediment sampling will be conducted at each site where live mussel data are collected. Before the substrate sample is taken and the site is disturbed, underwater photos will be taken of the substrate at each of the subsample points to document the make-up of the top layer of substrate. Approximately 7 to 15 kilograms of sediment will be collected at each location and GPS readings will be recorded.

Sediment sampling methods are based on those used by Roberts et al. 2016, and are similar to sediment sampling and analysis used by EcoAnalysts and CERC on the Spring River (EcoAnalysts 2018). Two composite substrate samples will be taken at each site, one within the mussel or identified suitable habitat and one from an adjacent gravel bar. Samples will consist of five subsamples or aliquots taken from random points. Sediments will be collected from relatively slow-moving water near physically adequate mussel habitat consisting of riffle/run complexes with relatively stable gravel sized particles and from adjacent depositional gravel bar areas. Each composite sample from mussel habitat will be collected from water less than 15 cm (6 inches) deep. The five aliquots will be placed in a high density polyethylene (HDPE) mixing vessel using a plastic scoop, homogenized, and then spooned into a Ziploc® brand 1 gallon size freezer bag. Samples will be labeled and placed in a cooler for transfer to the US Fish and Wildlife Service Columbia, Missouri office (USFWS office) for drying and XRF analysis and then transferred to the U.S. Geological Survey Columbia Environmental Research Center (CERC) for further chemical analysis. Used HDPE vessels and collecting scoops will then be

placed in a storage bag for decontamination including a nitric acid rinse for later reuse. Sample labels will include a unique sample identifier, site name, date, and initials of collector. At sites where visible mine waste is present, additional composite samples may be collected for heavy metal and grain size analyses.

Duplicate sediment material will be collected at certain sampling locations for the purpose of quality control/verification of metals analysis. Duplicate samples will be selected to reflect a relative range of metal concentrations: high, medium, and low, based on heavy metal concentration in EPA's recent (2020-21) sampling data. One quality control (QC) sample will be analyzed for every tenth sample, or one QC sample will be collected by each team per day, whichever number is greater. Two separate bags should be collected with alternating spoonfuls of sample placed in each bag.

Sediment samples will be recorded in a log book and a chain of custody form. The chain of custody form will be maintained with the samples and will accompany the samples to the USFWS office and CERC laboratory. The samples and chain of custody will be signed over to the sample custodian at the USFWS office (if different from the collector) and at CERC.

Metals Analysis

Sediment samples will initially be screened for metals (Pb, Zn, Cd, Ni, Cu, and Co) concentrations using an XRF meter followed by Inductively Coupled Plasma or Atomic Adsorption at the CERC laboratory. The XRF analysis will be completed using a 2007 Thermo Niton Xl3t 600 XRF (Thermo Scientific, Billerica, MA) following EPA method 6200 (EPA 2007). Samples will be allowed to air dry for seven days or until less than 20% moisture has been achieved. A portion of each sediment sample will be sieved to less than 2 mm. Both the less than 2 mm and the bulk sample will be analyzed by XRF. Samples will be thoroughly mixed within the Ziploc® bag by shaking and/or hand manipulation. Each sample will then be analyzed for 90 seconds by placing the instrument directly against the bag with the sediment in full contact with the portion of the bag in contact with the XRF window. An arithmetic mean will be calculated from three separate readings for each sample, with the sample fully mixed and shaken between each reading and used as the best representation of the sample metals concentrations. The use of the XRF to analyze ex-situ samples according to the above methods has been used in other Southeast Missouri NRDAR assessment studies (Roberts et al. 2009 and 2016) and the data have shown correlation with ICP/MS. Data generated through XRF analysis will be compared to ICP/MS data to determine whether results of metals concentrations between the methods are comparable.

A suite of calibration verification check samples will be used to check the accuracy of the XRF instrument and to assess the stability and consistency of the analysis for the analytes of interest. Check samples will be analyzed at the beginning of each working day, during active sample analyses, and at the end of each working day. The measured value for each target analyte should be within ±20 percent (%D) of the true value for the calibration verification check to be acceptable. If a measured value falls outside this range, then the check sample should be reanalyzed. If the value continues to fall outside the acceptance range, the instrument should be

recalibrated, and the batch of samples analyzed before the unacceptable calibration verification check will be reanalyzed (USEPA 1998).

Following XRF analyses, samples will be submitted to CERC for analysis of total Pb, Zn, Cd, Cu and Co using Inductively Coupled Plasma or Atomic Adsorption following EPA method 3050b "Acid Digestions of Sediment, Sludges, and Soils".
A summary of the analytical parameters and methods are provided below:

Table 1. Analytical Parameters

Sample Type	Analytical Method	Analyte	Fraction analyzed	Estimated Number of samples
Contamination characterization	Office/laboratory XRF and ICP or AA EPA 3050b	Pb, Zn, Cd, Ni, Cu, Co	Bulk and <2mm	30
QC samples	Office/laboratory XRF and ICP or AA EPA 3050b	Pb, Zn, Cd, Ni, Cu, Co	Bulk and <2mm	6

Table 2. Budget

Study Component	Quantity	Costs
Personnel	~600 hours field work (5 biologists); 160 hours field prep, data analysis, and report writing	$43,520
Travel	Lodging, M&IE, and vehicle costs for 5 biologists for 3 weeks	$10,680
Metals analyses	36 samples analyzed by CERC	$12,600
TOTAL		$66,800

Table 3. Timeline

	Jan-2023	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan 2024	Feb	Mar
Field Work Planning															
Field Surveys															
Data Analysis															
Report Writing															

Data Management

The Service will retain all data and associated metadata related to the mussel surveys which is anticipated to include:

- qualitative mussel data (species lists, presence absence data, numbers of individuals)
- bank survey data (dead shell classification)
- habitat descriptions
- substrate classification
- water quality data
- quantitative mussel data (species lists, numbers of individuals, density, length, age)
- Further analyses of collected data
- XRF analysis of sediment

A final data release will be made publicly available by the Service consistent with applicable law and regulations.

Upon completion of sediment lab analyses, CERC's Supervisory Research Chemist will retain the data and associated metadata. A final data release will be made publicly available by CERC consistent with applicable law and regulations.

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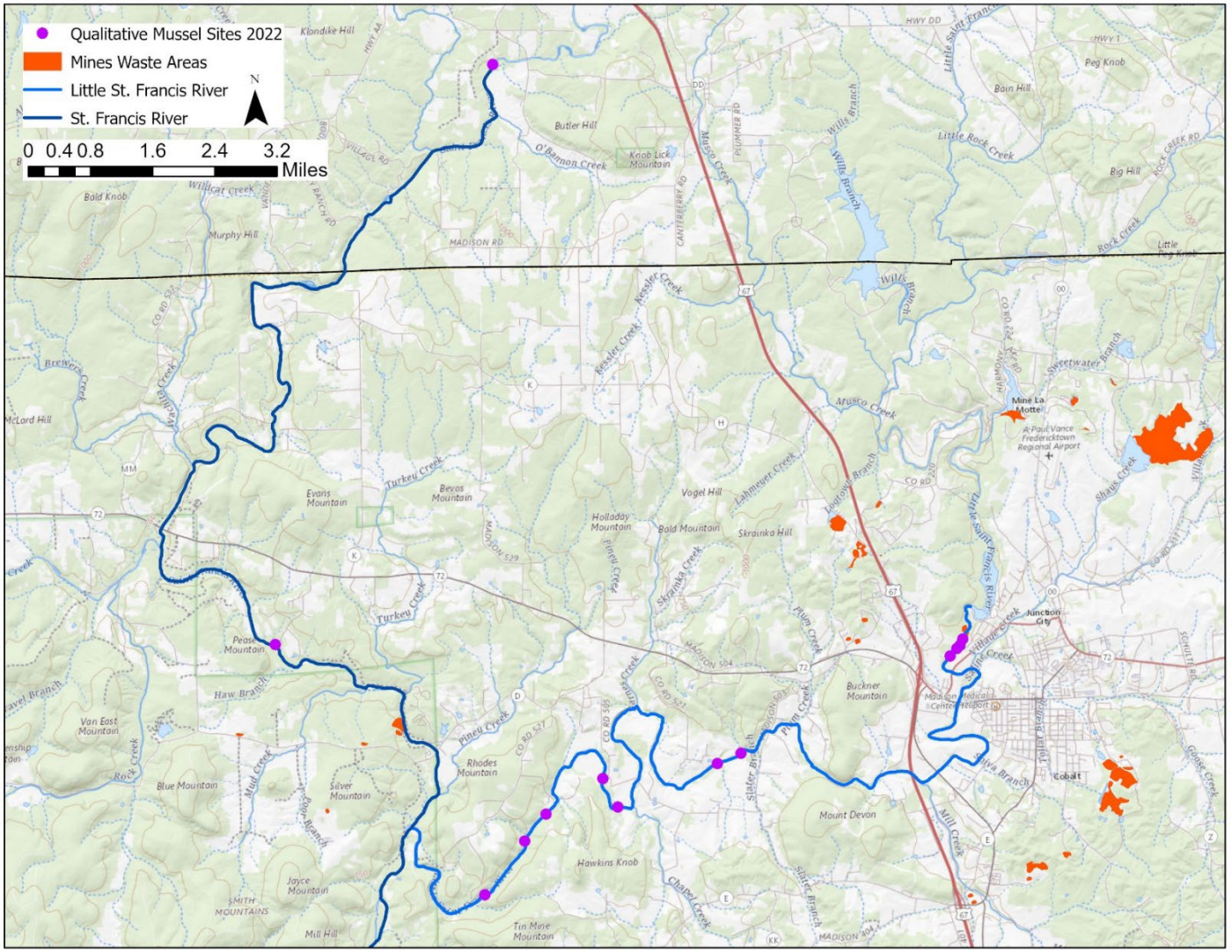

Figure 1 Little St. Francis River Project Location and qualitative sample sites from 2022.