

**Long-Term Monitoring of Freshwater Mussel Populations and Heavy
Metal Sediment Contamination in the Lower Big River, Missouri**

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July 8, 2010

Introduction

The Big River is part of the Meramec River system, which consists of clear, gravel-bottomed streams of the Ozark region in east-central Missouri. The Big River originates in northern Iron County, Missouri and flows 225 km (140 mi) north to its confluence with the lower Meramec River in St. Louis County, Missouri. The Big River watershed drains approximately 1537 km² (955 mi²) of the upper Mississippi River Basin in portions of 6 Missouri counties. The main tributaries of the Big River include Mineral Fork and Terre Bleue and Cedar creeks. The Big River drains the “Old Lead Belt”, which is an historic mining subdistrict within the current Southeast Missouri Lead Mining District (district).

Recent studies have shown that Big River sediments are contaminated with high levels of heavy metals (e.g. lead, zinc, and cadmium) from mineral mining in the upper portion of the watershed (Roberts et al. 2010, Besser et al. 2010). A study conducted by the U.S. Fish and Wildlife (USFWS) suggests that these contaminated sediments have degraded freshwater mussel communities in the Big River, resulting in fewer mussels, fewer species, and a more limited distribution (Roberts et al. 2010). Accordingly, more than 98.6 miles of the Big River, that contains mining-contaminated sediments, are associated with degraded mussel communities (Roberts et al. 2010).

The lowermost portions of the Big River, specifically, the 15 miles prior the confluence with the Meramec River, contain extant mussel populations. Furthermore, the lowermost 10 miles of the Big River support federally endangered scaleshell (*Leptodea leptodon*) and pink mucket (*Lampsilis abrupta*) mussels (MDC Mussel Database 2009, Roberts et al. 2010). Unfortunately, geologic sampling within the Big River suggests that sediments containing heavy metals are migrating downstream with time (Pavlowsky, 2010). For this reason, monitoring extant mussel populations is a pressing and vital need. If contaminated sediments continue to migrate downstream, these populations may be impacted, or may, in time, be extirpated. Subsequently, the USFWS proposes to monitor freshwater mussel populations and sediment contamination in the lower Big River.

Monitoring of freshwater mussel populations

The goal of long-term mussel sampling is to monitor species richness, relative abundance, density, and recruitment of mussel populations; and to monitor the presence of the federally endangered scaleshell and pink mucket mussels. Up to three monitoring sites will be established and sampled once every three years, from 2010 until 2013; additional monitoring will depend upon monitoring results and future funding. Sampling sites will be selected for monitoring based on the presence of suitable mussel habitat and previous reports of high mussel abundance (Buchanan 1979, Roberts and Bruenderman 2000, MDC Unpubl. Mussel Database 2008, Roberts et al. 2010). At the outset of sampling, the USFWS has selected the known mussel beds identified as “Highway W, Below Byrne’s Mill Dam, and House Springs” in Roberts et al., 2010. New sampling sites may be surveyed, as deemed necessary, to gain a better understanding of present conditions. Both qualitative and quantitative sampling methods will be required to meet these goals and are described below.

Timed qualitative searches will be used to assess species richness and relative abundance of freshwater mussels. Timed searches are useful in determining species richness and relative abundance at a given location; and in detecting rare species (Strayer *et al.* 1997, Vaughn *et al.* 1997, Obermeyer 1998, Strayer and Smith 2003). Qualitative searches will result in a list of species present (species richness); the number of mussels collected per unit of time (Catch Per Unit Effort, CPUE); and the relative abundance of each species (the number of individuals of each species out of the total caught).

Timed searches will involve visual and tactile searches for live mussels while snorkeling, or wading if water is too shallow to snorkel in. Visual searches include disturbing and fanning gravel substrates by hand, and moving cobble and large flat rocks. These techniques increase efficiency of mussel searching and the likelihood of locating juveniles, smaller specimens, and individuals burrowed into the substrate. Mussels will be identified and recorded as they are found. On-shore searches of dead shell material will also be conducted on gravel bars and in raccoon/muskrat middens. All dead shells collected during timed searches that are not represented by living species, will be retained as voucher specimens. All sampling locations will be searched until at least 1.5 person-hours of search time fail to increase the number of mussel species present. All sites will be surveyed by at least 2 biologists that are experienced with mussel sampling and familiar with the regional fauna. Searches will be conducted during periods of low flow when aquatic habitats are accessible for visual searches.

Dead specimens of mussel species not represented by live individuals will be classified as either fresh dead, dead, or subfossil. Fresh dead shells represent individuals in which the soft anatomy has not fully decomposed, and indicate that the individual has recently perished. Dead shells retain a lustrous nacre (on the inside of the shell) and have a relatively intact periostracum (or “skin-like” covering on the outside of the shell). Subfossil shells have a chalky and lusterless nacre and the periostracum has peeled off considerably (Buchanan 1979 and 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; the 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.

At each survey reach the sampling methods, total sampling effort, the number of living specimens of each species found, and the species represented by shell material only will be recorded. Subjective descriptions will be made of the habitat in which each mussel species are found and of the surrounding stream habitat conditions. The approximate dimensions, location, and general water depth of the site will be described.

Physical habitat will be evaluated at each mussel survey site using the habitat assessment protocol described by Barbour *et al.* (1999). From this method a numerical score is generated representing habitat quality by rating the various stream parameters on a scale of 0 to 20 with the habitat quality increasing with number. The following stream habitat

parameters will be evaluated: epifaunal substrate/cover, embeddedness, velocity/depth regime, sediment deposition, channel flow status, channel alteration, frequency of riffles, bank stability, bank vegetation, and riparian zone. Ratings for each parameter will be determined by averaging the values independently assigned by two surveyors familiar with the regional stream conditions following visual inspection of the targeted stream reach. The final physical habitat score is the sum of the averaged ratings for each of the habitat parameters (theoretical maximum = 200). Together with reach-specific environmental chemistry data from sediment samples, these scores provide a general basis for distinguishing between contaminant-limited and physical habitat-limited mussel populations.

Quantitative mussel sampling will be conducted at each of the sampling stations to provide estimates of mussel densities (individuals/m²). Sites will be delineated such that only the portion of the channel with suitable, occupied mussel habitat will be sampled. The sampling area will be measured and gridded by anchoring a tape measure parallel with the stream channel. Quadrat coordinates will be determined successively from a list of random numbers and located in the stream by using a second tape measure and a large T-square to measure 90 degrees off the anchored tape. A 0.25-m² quadrat, as is standard, (Strayer and Smith 2003), will be positioned on the stream bottom and all visible mussels will be collected. Following this initial search, cobble and flat rocks will be removed by hand, and the substrate will be searched by mixing and fanning by hand until no mussels remain. Mussels will be identified, enumerated, and repositioned into the substrate within the quadrat location. The lengths of mussels from every other quadrat will also be measured.

Sediment Sampling:

The goal of sampling sediment is to monitor the amount of lead, cadmium, and zinc in stream sediments, in habitat occupied by mussels, over time. Composite sediment samples will be collected from each long-term mussel sampling station in the Big River. Multiple sediment samples may be collected to characterize changes affecting sedimentation within a sampling station (i.e. above and below mill dams, at low water crossings, or within tributaries).

Sediments will be collected from relatively slow-moving water near mussel sampling areas described in the above section. Each composite sample will contain no less than 5 aliquots collected within an approximately 100-m² area, from water less than 15 cm (6 inches) deep. Collected aliquots will be deposited into a high density polyethylene (HDPE) mixing vessel using a plastic scoop, homogenized, and then spooned into a Ziploc® brand 1-gallon freezer bag. Samples will be labeled and placed on ice for temporary storage until transferred to the laboratory for further preparation and analysis. Used HDPE vessels and collecting scoops will then be placed in a storage bag for cleaning and nitric acid rinse for later reuse.

Approximately 0.5-1.0 kg of sediment will be collected at each sampling station. Additional sediment material will be collected at certain sampling locations for the

purpose of quality control/quality assurance. One quality control (QC) sample will be collected for every tenth sample, or one QC sample will be collected by each team per day, whichever number is greater. For these samples approximately 1.5- 2.0 kg will be required: 2 separate bags will be prepared with alternating scoops of homogenized sediments placed in each bag.

Sediment samples will be analyzed by an X-ray fluorescence (XRF) meter. Sediment samples for XRF will be analyzed using a 2007 Thermo Niton XI3t 600 XRF (Thermo Scientific, Billerica, MA). Samples analyzed by XRF will be allowed to air dry for at least 1 week in the laboratory until totally dry. Samples will be thoroughly mixed within the Ziploc® bag by shaking and/or by hand manipulation. Samples will then be dry sieved to less than 2 mm in diameter. Materials remaining on the sieve will be discarded and materials passing through the sieve will be placed in new plastic bags. Each sample will then be analyzed for 90 s by placing the sample bag directly against the XRF analytical aperture in Thermo Niton's "Portable Test Stand" (Thermo Scientific, Billerica, MA), a fully shielded device that allows for computer controlled hands-free operation of the meter. 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.

A suite of calibration verification check samples will be used to check the accuracy of the XRF and to assess the stability and consistency of the analysis for the analytes of interest. Thermo Niton XRFs are internally calibrated prior to each use employing Compton normalization. Check samples will be analyzed at the beginning of each working day, during active sample analyses, and at the end of each working day. For the calibration verification check to be acceptable, the measured value for each target analyte must be within ± 10 percent (%D) of the true value. If a measured value falls outside this range, then the check sample will be reanalyzed (USEPA 1998). If the measured check value again falls outside of the acceptable range, then the instrument will be internally calibrated again until the check sample falls within the acceptable error range.

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