APPENDIX V

Protocol for Long-Term Monitoring of Freshwater Mussel Populations in the Lower Osage River, Missouri

--Revised (April 2021)--

Lower Osage River Protection and Enhancement Program
Project 08-06

Protocol for Long-Term Monitoring of Freshwater Mussel Populations in the Lower Osage River, Missouri

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Lower Osage River Protection and Enhancement Program
Project 08-6

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INTRODUCTION

Background

The lower Osage River (LOR) includes the final 129.2 kilometers (80.27 river miles) of the Osage River between Bagnell Dam and its confluence with the Missouri River in central Missouri. At least 39 species of freshwater mussels, including 3 federally endangered species, have been reported in the LOR (Table 1) (Grace and Buchanan 1981, Ecological Specialists, Inc. 2003). Bagnell Dam is a hydropower facility that was completed in 1931, creating the Lake of the Ozarks. It is owned by Ameren Missouri, who received a renewed 40-year operating license issued by the Federal Energy Regulatory Commission (FERC) in 2007. The new license includes enhancements to benefit aquatic resources and reduce impacts to federally listed species in the LOR. These changes include operational amendments to help improve dissolved oxygen levels and flow regime and the establishment of the Lower Osage River Protection and Enhancement Program (LORPEP). The LORPEP is a restoration program that allows the implementation of adaptive management actions to help protect and mitigate impacts to aquatic resources in the LOR. The main elements of this program include instream habitat improvements, freshwater mussel propagation, and monitoring for program effectiveness. Long-term mussel monitoring under the LORPEP meets the requirements of the license (Article 412 of the March 30, 2007, Osage Project license, FERC No. 459), and the U.S. Fish and Wildlife Service's (USFWS) September 19, 2006, Biological Opinion. Other specific monitoring efforts may be developed and conducted prior to and/or following future LOR restoration actions. Such efforts may follow the described approach, but depending on species, habitats, and conditions, a variation of techniques may be necessary that would be described in separate annual and progress reporting.

Long-term monitoring can provide data needed to indicate species demographics and can be used to help conserve mussel populations and implement LORPEP actions in the future. Mussels are known to be good indicator species and thus can help reflect water quality and the health of the aquatic ecosystem (U.S. Department of Agriculture 2007, Van Hassel and Farris 2007). Therefore, regular monitoring and long-term data sets are vital tools to provide an assessment of mussel population trends, changes in overall mussel community, and status of species of conservation concern (SOCC), including federally protected species. The LORPEP monitoring will also be used to help us evaluate the effectiveness of the operational enhancements implemented at Bagnell Dam and identify environmental conditions that may contribute to mussel recruitment events. Knowledge of local mussel population trends can help inform the planning and evaluation of LORPEP projects, particularly those that involve habitat enhancements. Monitoring will also facilitate mussel restoration efforts by informing management decisions, and helping to identify locations of brood stock for propagation, release locations, and suitable evaluation of cultured mussels released in the LOR.

This monitoring protocol supersedes the previous version filed with FERC in 2015 (Simmons *et al.* 2015). This update improves the monitoring description and was deemed necessary following initial sampling efforts as field experiences revealed the need for some minor changes in methodology to improve crew safety, data collection accuracy, and study repeatability.

Modifications include site names, adjustment to site delineations, and the sampling schedule. Additionally, coordinates of sampling locations are included for future reference.

Goals and Objectives

The goal of the long-term monitoring program is to track population demographics of freshwater mussels in the LOR until the expiration of the current FERC license in 2047. Because most riverine mussel species are sedentary and share similar habitat requirements, they are often concentrated in localized areas of suitable habitat called “mussel beds”. If conditions remain suitable, mussels can persist at one location for decades or longer. However, one difficulty with assessing mussels at the community-level is identifying potential differences between species life history characteristics, such as growth and recruitment rates, reproductive strategies, maximum size and longevity, and possible habitat associations. Haag (2012) categorized mussels into three categories according to life history strategies: opportunistic, periodic, and equilibrium. The opportunistic-strategy species have traits that are thought to be good adaptations to persist in disturbed and unstable but productive environments. The equilibrium-strategy species have traits that are associated with stable, productive habitats. The periodic-strategy species have traits that allow persistence in unproductive habitats that are subject to large-scale, cyclical environmental variation or stress. Because the goal of the protocol is to assess the mussel community as a whole and over a long period of time, knowing parameters surrounding species variation is important to help determine whether any species are declining or at risk of imperilment.

To help achieve the goal of the study, known mussel beds in the LOR were initially assessed for monitoring suitability and potential habitat for priority mussel species (i.e., imperiled, threatened, or endangered species). Sites selected were delineated and designated for monitoring. Each site is monitored within a sampling schedule (Table 2) to track changes in overall population density (all species combined), relative abundance of species, recruitment rates, and species richness. Sampling efforts are expected to also provide data to evaluate the success of cultured and tagged mussels released within long-term monitoring sites in the future. The recapture of tagged mussels during future monitoring efforts will provide important information on habitat requirements, survival, age and growth, and longevity of released animals.

A secondary goal of the mussel monitoring is to provide limited population data on the invasive Zebra Mussel (*Dreissena polymorpha*). This non-native species has been known to spread prolifically in a variety of habitats and negatively affect native mussel populations by attaching to their shells and surrounding habitat (Nalepa and Schloesser 1993). They differ from native mussels in that they have direct development and a pelagic life stage (i.e., veliger) that allows them to drift and colonize areas downstream by attaching to hard surfaces such as rocks, woody debris, shells of native mussels, and other submerged objects. Zebra Mussel were first detected in the lower portions of the Lake of the Ozarks in 2006. Since that time, living Zebra Mussel have been observed in the LOR attached to rocks and native mussels. Tracking Zebra Mussel populations over time will help assess their impact on native mussel populations in the LOR.

Study Area

The Osage River is a large tributary of the Missouri River and supports diverse freshwater mussel populations in its un-impounded reaches. Its watershed drains an area of east-central Kansas and a large portion of west-central Missouri. The river generally flows eastward through Harry S. Truman Reservoir and Lake of the Ozarks. The LOR study area begins in central Missouri at the Bagnell Dam tailwater below Lake of the Ozarks and flows northeastward for 129.2 kilometers (80.27 river miles) to its confluence with the Missouri River (Figure 1). This segment is a lower-gradient, moderately clear, gravel-bottom river with at least 39 species of freshwater mussels reported (Table 1). This mussel community includes 3 federally endangered and 4 state endangered species [Missouri Department of Conservation (MDC 2021)]. The federally listed species are Pink Mucket (*Lampsilis abrupta*), Scaleshell (*Leptodea leptodon*), and Spectaclecase (*Margaritifera monodonta*).

Mussel populations have not been systematically monitored in the LOR, but several faunal surveys have been performed. Utterback (1916 and 1917) provided the first species accounts for the Osage River in the early 1900s. This effort was followed by limited faunal surveys in the 1970s and 1980s (Grace and Buchanan 1981, Oesch 1995). Ecological Specialists, Inc. (ESI) completed a thorough survey in the LOR in 2000 and 2001. The ESI surveys were completed specifically to help define resource concerns in an environmental assessment for the relicensing of Bagnell Dam (ESI 2003). In addition to intensive surveys of select sites, ESI identified suitable habitat while traversing the LOR and completed reconnaissance-level surveys at all known and newly discovered mussel sites during a period of low-flow (ESI 2001). Between 2009 and 2013, the LOR was again searched extensively between river kilometer (RK) 20 (upstream of Lock and Dam #1) and RK 123.3 (downstream of Bagnell Dam) for suitable mussel habitat to identify and evaluate potential long-term monitoring sites (Roberts *et al.* 2014). During these efforts, 18 mussel beds were identified and their boundaries delineated for monitoring (Table 2).

METHODS

Site Selection and Delineation

The initial 18 monitoring sites in the current monitoring program represent mussel beds known to occur in the LOR in 2013 with a density > 1 individual per square meter (m^2) and an area > 2000 m^2 (Figure 1). Sites selected for monitoring include areas throughout the LOR found in previous studies (Grace and Buchanan 1981, ESI 2001 and 2003, Roberts *et al.* 2014). Sites found by Grace and Buchanan (1981) and ESI (2001, 2003) traditionally were labeled by river mile as determined by older topographical maps. This numbering system was continued in Roberts *et al.* (2014), the previous version of this protocol (Simmons *et al.* 2015), and in annual LOR mussel monitoring reports from 2014 to 2019. However, this river mile system was based on a river length of 81.7 miles from Bagnell Dam to the confluence with the Missouri River. Using recent aerial imagery/maps and precise geospatial measuring, we determined the length of the LOR to be 80.27 miles. Therefore, the site names are updated according to this recent information and river miles (formally used for site names) are converted to river kilometers to improve the site descriptions (Table 2, Figure 1). All site names are the river kilometer (rounded to the nearest tenth) as measured from the center of the delineated monitoring site. These new site names will be used in future reporting of mussel monitoring in the LOR.

In preparation for monitoring, each site boundary was delineated for all 18 monitoring sites by completing visual searches while snorkeling or diving. Visual searches were performed by individuals experienced in identifying suitable mussel habitat and mussel identification. Work sometimes involved disturbing the top layer of sediment and rocks by hand (e.g., raking or fanning) to help reveal mussels. The site boundary was marked with a Global Positioning System (Garmin® GPSMAP® 60CSx (GPS)) once the mussel density became < 1 individual per m^2 as estimated by the diver. Additional time was spent searching beyond the boundary until the area was uninhabited by mussels. Polygons were created for each site from the marked points using Esri ®ArcMap™ 10 (Roberts *et al.* 2014). Following bed delineation, preliminary quantitative sampling was conducted to confirm the area contained an overall average density of ≥ 1 mussel/m^2. The GPS points for all monitoring site polygons and site dimensions are listed in Appendix A. Site information and GIS Shapefiles for these points (along with the river mile, river kilometer, river line, and mussel bed polygons) are housed at the USFWS's Missouri Ecological Services Field Office.

The purpose of mussel bed delineation is to focus future sampling efforts in the portion of river channel that mussels occupy. This minimizes the variance of population estimates and generates higher confidence intervals surrounding the true mussel densities (Strayer and Smith 2003). Additional sites may also be selected and delineated for future LOR restoration opportunities or in the event fieldwork leads to locating other suitable habitat, additional mussel beds, or unique habitats (e.g., side channels, training structures, rocky shorelines, bluff pools, backwaters, perennial wetlands, etc.). Additionally, the need may arise for species-specific studies that may improve the understanding of the LOR mussel community and their habitats. Monitoring of unique habitats may require different methods, which will be described in future revisions (i.e., Spectaclecase studies are mentioned below).

<u>Site Re-delineation</u>

River systems are dynamic and mussel beds may migrate, shift, or expand beyond the defined boundary of current bed delineations. Therefore, it may be necessary to re-delineate monitoring sites periodically if environmental or population changes are observed in those areas (e.g., rapid population declines at a monitoring site). Also, any sites that no longer meet the criteria of a mussel bed may be removed from the regular sampling schedule and assigned a more periodic sampling regime.

<u>Sampling Schedule</u>

The sampling schedule of mussel monitoring sites in the LOR described by Simmons *et al.* (2015) is updated as follows. Each monitoring site will be sampled regularly over time until

2047. The 18 current sites are separated into 3 sections representing the upper, middle, and lower reaches of the LOR (Table 2). One site (i.e., primary site) was selected from each of these sections where more frequent monitoring will occur than the remaining sites (once every 3 years) to help reduce statistical variation. These sites are chosen to best represent their respective section in terms of species richness and density based on preliminary data (Roberts *et al.* 2014). The rationale for more frequent sampling is to have at least 1 site from each reach where data represent a more frequent time frame between samples to help track potential acute changes or notable observations. The remaining sites are scheduled for sampling once every 5 years with 1 site randomly chosen and sampled from each of the 3 sections annually for the first 5 years to establish the future sample rotation. After a site is sampled, it is removed from the list. An initial round of sampling has been completed, and therefore, the future sampling order has been established (Table 2). This sampling regime will help promote more frequent observations of river-wide changes in mussel population parameters, streamline monitoring of future conservation and restoration activities at those locations, and possibly help correlate notable population changes with environmental events (such as drought and flooding or other river management activities).

Mussel monitoring will be scheduled during low-flow conditions with adequate water visibility to maximize sampling efficiency and safety. Power generation, environmental variables (i.e., high-flows and severe weather), and employee scheduling might limit the actual number of sites completed annually. Two sampling protocols are implemented simultaneously while working at each site to maximize limited crew, time, and field opportunities as discussed below.

Overall Sampling Methods

Two different sampling techniques are required to achieve the goals of this program. The first method is referred to as “quantitative sampling” and is used to monitor overall density (all species combined), relative abundance, and recruitment of mussel populations in the LOR. The second sampling technique involves timed searches to track species richness (i.e., total number of species present). Both sampling techniques will be implemented within defined areas that are randomly distributed within the monitoring sites. Employing both sampling techniques increases the detection probability of rare species and provides a more accurate species richness estimate within each monitoring site. Figure 2 illustrates the sampling layout for both methods as described below.

Quantitative Sampling

Quantitative sampling involves counting live mussels within a defined area (i.e., quadrat) randomly located within each monitoring site. This sampling requires effective removal, sorting, and sieving of sediments from within the quadrat to improve the detection of all mussels, including small individuals. Data generated by this method provides estimates of mussel density (mussels/m^2), relative abundance of each species (percent of total mussels collected), and helps to document recruitment events. With sufficient samples and time, these data will serve to reflect species distribution and population trends within and between sites as well as help indicate correlations between habitat and/or environmental conditions and recruitment events or population declines. These results would also serve to help determine the overall status of the mussel community in the LOR.

The number of quantitative samples evaluated annually may be standardized among sites with the goal of achieving the minimum number of samples necessary to estimate density within 35% of the mean with 95% confidence (Dunn 2000, ESI 2001). Fifty samples are a reasonable starting point, given the typical field opportunities in the LOR and the number of sites to evaluate yearly. However, more samples may be required at some sites if those do not achieve the desired level of precision. If the sample size is not logistically achievable, the site may not be included in quantitative sampling, and may only be sampled using timed searches or removed from the list of monitoring sites. For quantitative sampling, 50 random points will be generated for each monitoring site (within the delineated area of the mussel bed) using the "Create Random Points" feature, with a 5 m buffer distance, within the Data Management Toolbox in Esri ®ArcMap™ 10.7. The buffer is necessary to avoid overlapping sample locations. The coordinates of the random points are then uploaded to a precision GPS (Humminbird® 1198C SI Combo™ and Trimble GeoXT™ with Trimble® Tornado™ Antenna).

The randomized points are located within each site using the GPS equipment. At each random point location, a 0.25 m^2 sampling quadrat with an attached 3 mm mesh bag (Figure 3) is positioned on the river bottom (adjacent to the boat anchor) and the substrate excavated to a depth of approximately 10 cm or to solid bedrock and placed directly into the mesh bag. The bed materials are transferred to the surface and sieved through a 7 mm screen and sorted (Figure 4) to increase the probability that all mussels within the sample are found. Mussels within the sample are identified, counted, measured (posterior to anterior length), and external growth lines counted for individuals that are ≤ 5 years old and older individuals exhibiting clear growth lines. After processing, all live mussels are returned to the river near their point of origin.

<u>Timed, Semi-quantitative Searches</u>

The second sampling technique employs a more qualitative approach to track species richness (i.e., total number of species present) over time at a specific location using timed searches. This involves tactile searches (e.g., disturbing, raking, and fanning gravel substrates by hand and moving cobble and large rocks) while visually searching for mussels. Timed searches are often used to determine species presence and detect rare species. Qualitative methods produce a more complete list of species at a given location when compared to quantitative sampling alone (Strayer *et al.* 1997, Vaughn *et al.* 1997, Obermeyer 1998, Strayer and Smith 2003). However, qualitative data have limitations when comparing results from different sampling periods. Many variables may affect species detection and sample efficiency, including abundance, site and sampling conditions, shell size, behavior, sampling methods, and experience. To make the data more comparable over time, the amount of search area, time, sampling conditions, and methods can be controlled and standardized.

Timed, semi-quantitative searches are also conducted at each site using the same 50 random points generated for quantitative sampling to improve efficiency and possibly provide additional comparisons between quantitative and semi-quantitative samples. Once the quantitative sample is obtained and being processed by the surface crew, the diver conducts a 5-minute search beyond the quantitative sampling point within a circular, 12.56 m^2 area or "Timed Search Unit" (TSU) (Figure 2). The TSU boundary is defined by a 2 m cord attached to the boat anchor and held by the diver. During the search, the diver can freely move throughout the TSU area holding the cord to ensure the search is confined within the TSU. Search methods include equal efforts of visual and tactile methods. All mussel species encountered are quickly identified and recorded on a dive slate by the diver. Only species presence is recorded for each TSU. No length or age data are collected for specimens found during timed searches with exception to tagged individuals or a SOCC). The dive slate has a species list in permanent print so that only a check mark is required to record the species present within each TSU. Any questionable species are collected and brought to the surface and either verified by other malacologists, photographed and returned to the river, and/or vouchered. Dead shells representing species not collected live at the site are also recorded and vouchered as described below.

Random sampling locations (quadrats/TSUs) that are deemed hazardous to the diver or crew are avoided and replaced with additional randomized sample locations. Shallow locations not requiring diving equipment employ the same survey tactics with portable floating sieves and anchors.

Dead Shell Material

Shells of dead mussels can persist on the bottom of the river for long periods of time and serve as a record of past species presence. Dead shell material found during monitoring not represented by live individuals are noted and classified as fresh-dead (FD), weathered-dead (WD), or subfossil (SF). Fresh-dead shells represent individuals in which the soft anatomy has not fully decomposed and indicate a recently perished individual. Weathered-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 an individual depends on a variety of factors including the species, shell thickness, whether the shell was above or below the substrate, and whether the shell was in the water. In general, weathered-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.

Spectaclecase

Spectaclecase is federally Endangered and state Endangered in Missouri and considered Vulnerable throughout its range (S3/G3) (MDC 2021). While, the largest fraction of the Spectaclecase population may occur in Missouri, numbers appear to be declining. Spectaclecase is typically associated with a slightly different habitat from other riverine species. Most riverine mussel species in the LOR co-occur in discrete areas, forming a mussel bed in suitable habitat conditions. In general, these areas are stable shoals, runs, and glides that receive constant flow throughout the year and are protected from sheer stress (i.e., the scouring effects of high-flow events). Spectaclecase is often found in deeper areas, in clusters around boulders, or under flat rocks where few or no other species are present (Baird 2000). In the LOR, it has been found in small numbers in the described habitat along the base of training structures and rocky shorelines (Roberts *et al.* 2014). This species may also be present elsewhere in the LOR around other training structures or in deep pools along bluffs as reported in other Missouri streams (Roberts and Bruenderman 2000, Bruenderman *et al.* 2001). Similar but separate methodology would be required to monitor Spectaclecase given its unique habitat and clustered distribution. More information is needed on the distribution of Spectaclecase in the LOR to determine if additional sampling is warranted or if the protocol could be tailored to include this species. Therefore, during the planned monitoring period, areas containing potential habitat may be opportunistically sampled to better understand the species distribution and known locations may be visited periodically to confirm its persistence through time. Also, monitoring would likely follow the "adaptive sampling" methods described in Baird (2000). These are quantitative methods used to help account for high densities of individuals occurring in isolated areas (i.e., non-even distribution) which is the tendency described for the species and associated habitat. The quantitative sample methods also vary from this protocol in that when Spectaclecase are encountered at random locations, additional quantitative samples are performed adjacent to the original sample point and all subsequent adjacent quadrats until the species is no longer present. Since all other species found would also be recorded, the TSU samples would not be necessary.

<u>Zebra Mussel</u>

As part of this monitoring protocol, Zebra Mussel presence will be documented for individuals found attached to native mussels within quantitative samples. The number of individual Zebra Mussel attached to mussel shells will be counted if there are ≤ 10 individuals present. If > 10 Zebra Mussel individuals are attached, estimated numbers will be categorized as > 10, >50, or >100 and noted. If the Zebra Mussel population increases in the LOR to an extent that counting individuals on native shells is no longer practical, another method to monitor their population may be employed. Following sample processing, any live Zebra Mussel found will be dispatched. Notations of presence will also be made within TSUs for Zebra Mussel found on mussels or substrate. Attachment data can be used to track the severity of this invasive species threat to native mussels in the LOR and determine if there are differences in colonization rates between native species.

Zebra Mussel presence may also be monitored at 2 locations in the LOR using experimental samplers to monitor colonization rates (Figure 5). Samplers will be deployed by April of each year and placed in the mid-water column from a dock or other structure in pool habitat or another area with slow-moving current without debris to disrupt, displace, or destroy the sampler. Samplers will be collected each September and brought back to the laboratory for processing. Zebra Mussel will be counted on both center sampling plates (Figure 5). If a large number are attached to the plates, the number attached will be estimated by counting several 25x25 mm randomly selected square sections and calculating the average number per cm^2. These data will be helpful in understanding colonization rates and possibly correlate recruitment events with environmental variables. In addition to deploying samplers at 2 sites in the LOR, it would also be informative to install another sampler in Lake of the Ozarks to track the Zebra Mussel population upstream of Bagnell Dam to compare densities between the lake and the LOR.

Data Analysis and Reporting

After each monitoring effort, data will be entered into a Microsoft Excel spreadsheet and analyzed. The following parameters are calculated/described for each quantitative site: total number of species, average density (mussels/m^2), relative abundance of species (% of total), size frequency of mussels (length), and recruitment (abundance of mussels estimated to be $\leq$ 5 years old). Mean mussel densities and species richness from any survey data will be compared between study years for sites by conducting a one-way ANOVA and Tukey's test for pair-wise comparisons of the means (Conover and Iman 1981). We will continue to consult biometricians to develop methods to analyze data on species richness and species presence. These variables can be influenced by collector efficiency and environmental factors in the field.

Results will be summarized annually and discussed in a more detailed report approximately every 5 years. Reporting is identified as Project 08-6 of the LORPEP and provided within annual reports as prepared by the USFWS for Ameren Missouri.

This protocol will also be used to evaluate additional restoration actions, including additional releases of cultured, priority mussel species, to occur within established monitoring locations.

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Table 1. Federal and state status of freshwater mussel species found during previous studies in the lower Osage River*, Missouri.

Scientific name	Common name	Federal Status[1]	State Status[1]
Actinonaias ligamentina	Mucket		
Amblema plicata	Threeridge		
Arcidens confragosus	Rock Pocketbook		
Cyclonaias pustulosa	Pimpleback		
Cyclonaias tuberculata	Purple Wartyback		
Ellipsaria lineolata	Butterfly		
Elliptio crassidens	Elephantear		E
Eurynia dilatata	Spike		
Fusconaia flava	Wabash Pigtoe		
Lampsilis abrupta	Pink Mucket	E	E
Lampsilis brittsi	Northern Brokenray		
Lampsilis cardium	Plain Pocketbook		
Lampsilis siliquoidea	Fat Mucket		
Lampsilis teres	Yellow Sandshell		
Lasmigona complanata	White Heelsplitter		
Lasmigona costata	Fluted Shell		
Leptodea fragilis	Fragile Papershell		
Leptodea leptodon	Scaleshell	E	E
Ligumia recta	Black Sandshell		
Margaritifera monodonta	Spectaclecase	E	
Megalonaias nervosa	Washboard		
Obliquaria reflexa	Threehorn Wartyback		
Obovaria olivaria	Hickorynut		
Pleurobema sintoxia	Round Pigtoe		
Potamilus alatus	Pink Heelsplitter		
Potamilus ohiensis	Pink Papershell		
Pyganodon grandis	Giant Floater		
Quadrula nobilis	Gulf Mapleleaf		
Quadrula quadrula	Mapleleaf		
Reginaia ebenus	Ebony Shell		E
Strophitus undulatus	Creeper		
Theliderma metanevra	Monkeyface		
Tritogonia verrucosa	Pistolgrip		
Toxolasma parvum	Lilliput		
Truncilla donaciformis	Fawnsfoot		
Truncilla truncata	Deertoe		
Uniomerus tetralasmus	Pondhorn		
Utterbackia imbecillis	Paper Pondshell		
Venustaconcha ellipsiformis	Ellipse		

[1] E = Federal or state endangered

***References:**
Mussel records: Utterback 1916 and 1917, Grace and Buchanan 1981, Ecological Specialists, Inc. 2003
Status and Ranks: from MDC 2021

Table 2. Mussel monitoring sites in the lower Osage River by river kilometer. Previous site names are those used in preceding documents. Year of first sample is when the site was randomly picked and sampled during the first round of monitoring (i.e., initial 5 years) to establish the sequence of future sampling. Sites in bold are sampled triennially after the year of first sample. The remaining sites are sampled once every 5 years beginning with the year of first sample.

	Monitoring sites (river kilometers)	**Previous site names** (river miles)	**Year of first sample**	**Year of subsequent sample***
Group 1 (lower)	20.1	13 or 13.5	2016	2021
	28.1	17.5	2018	2023
	37.6	23.5	2017	2022
	43.4	26.5 or 27.5	2016	2019
	52.7	33 or 33.5	2014	2019
	65.5	41 or 41.5	2015	2020
Group 2 (middle)	71.3	45 or 45.5	2018	2023
	80.7	51	2016	2021
	84.5	53 or 53.5	2015	2020
	88.7	56	2014	2019
	91	57 or 57.5	2015	2018
	95.4	60.5	2017	2022
Group 3 (upper)	102.4	65	2018	2023
	107.4	68	2014	2019
	108.7	69 or 69.5	2017	2022
	112.9	71 or 71.5	2017	2020
	117	74	2015	2020
	123.3	77.5 or 78	2016	2021

*Years of subsequent sample provided for example only. Future sampling schedule may be affected by weather and other events.

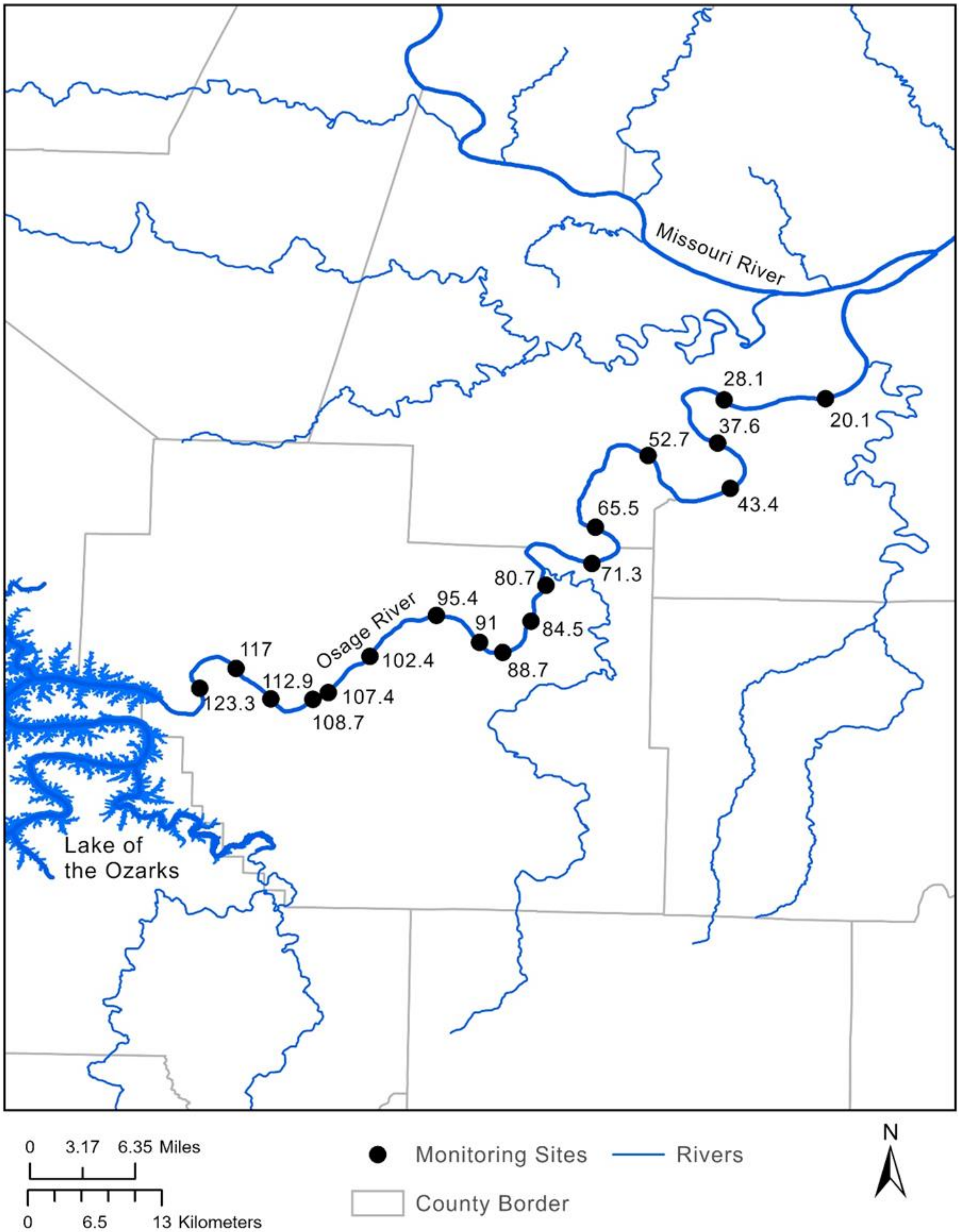

Figure 1. Freshwater mussel monitoring sites in the lower Osage River by river kilometer.

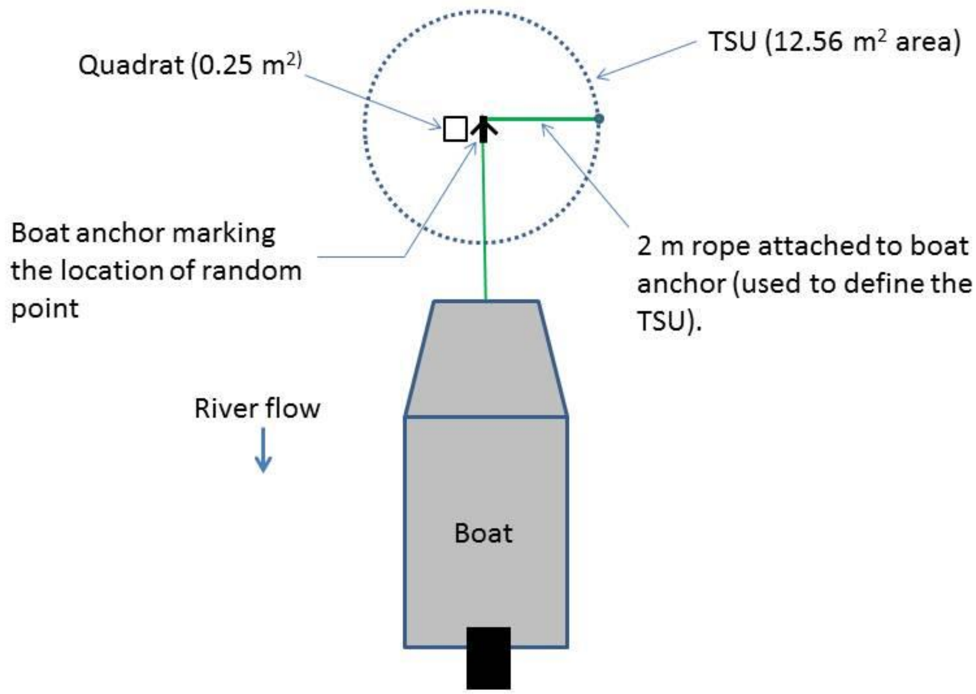

Figure 2. Diagram depicting the spatial relationship between the quadrat and timed search unit (TSU) used to sample freshwater mussels in the lower Osage River. Each of the 50 random points located within a mussel bed are marked with the boat anchor. The diver first collects substrate from within the quadrat and then conducts a random 5-minute search within the TSU using a 2 m rope to confine the search within the TSU.

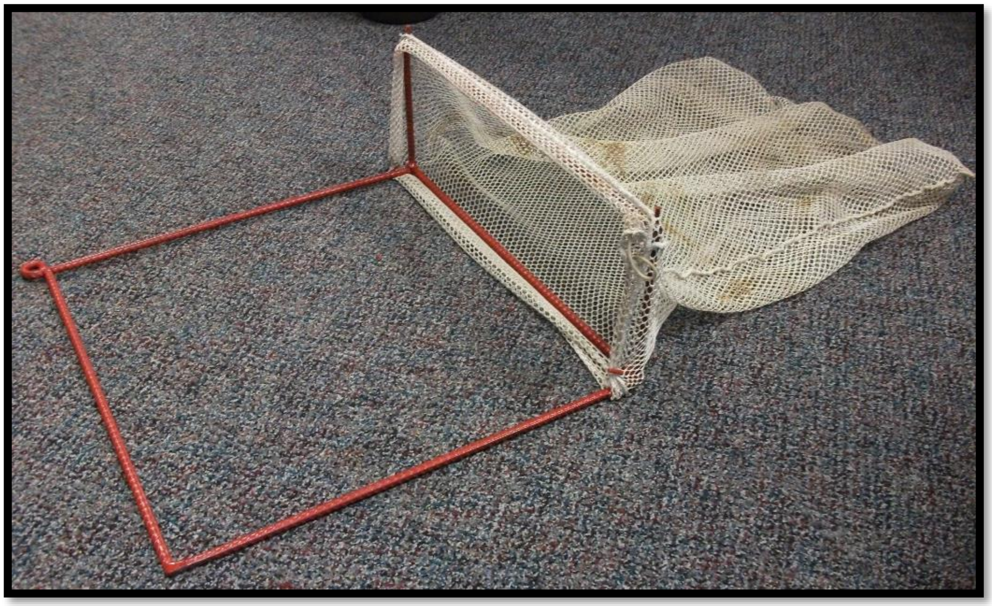

Figure 3. A 0.25 m^2 quadrat with attached mesh bag.

Figure 4. Quantitative sample being emptied on a sorting table mounted to a boat for processing.

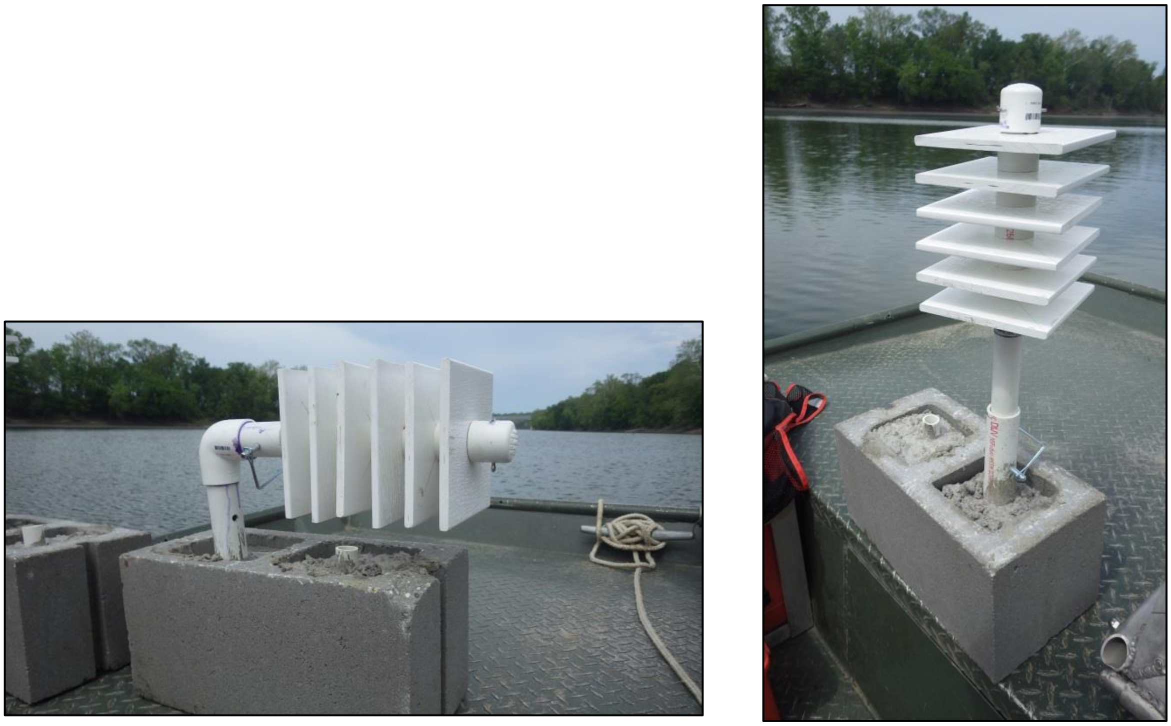

Figure 5. Experimental Zebra Mussel samplers for trial study in the lower Osage River.

Appendix A

Site Dimensions and Coordinates of Long-Term Mussel Monitoring Site Polygons in the Lower Osage River

Site dimensions and GPS coordinates of long-term mussel monitoring sites in the lower Osage River. The longitudes/latitudes listed for each site form a single polygon where random samples can be generated for comparative samples.

Site (River Km)	Length (m)	Width (m)	Area (m^2)	Longitude	Latitude
20.1	1,730	65	78,127	-92.039833	38.467336
				-92.040262	38.467215
				-92.040562	38.467267
				-92.041101	38.467222
				-92.041753	38.466966
				-92.042613	38.466513
				-92.043156	38.466169
				-92.045649	38.465778
				-92.046758	38.4657
				-92.048424	38.465403
				-92.048421	38.465639
				-92.049369	38.465721
				-92.051193	38.46596
				-92.052571	38.46581
				-92.055009	38.465892
				-92.055019	38.466327
				-92.05388	38.466306
				-92.053566	38.466141
				-92.053204	38.465977
				-92.051127	38.466221
				-92.0486	38.465964
				-92.047521	38.466067
				-92.045773	38.466265
				-92.043138	38.466853
				-92.040462	38.467665
				-92.035602	38.468894
				-92.035812	38.468597
				-92.035958	38.468349
				-92.037132	38.468073
				-92.03826	38.467783
				-92.03896	38.467515
				-92.039276	38.467518
				-92.039833	38.467336
28.1	1,165	102	111,727	-92.13709419	38.46795404
				-92.13604158	38.46829956
				-92.13478225	38.46714212
				-92.13323154	38.46530541
				-92.13171269	38.46393994
				-92.12952211	38.46239283
				-92.12793425	38.46127132
				-92.12720202	38.46075414
				-92.12764146	38.46036952
				-92.12897822	38.46091682
				-92.12954262	38.46140626

28.1 (con’t)				-92.13005722	38.46185251
				-92.13117086	38.46228732
				-92.1331008	38.46371471
				-92.134616	38.4653233
				-92.13709419	38.46795404
37.6	1,342	52	56,956	-92.13273839	38.42582137
				-92.13313696	38.42544281
				-92.1345818	38.42628804
				-92.13841059	38.42803305
				-92.14181721	38.42960793
				-92.14661799	38.43121671
				-92.1463727	38.43145337
				-92.14314184	38.43038064
				-92.14087788	38.4295772
				-92.13834459	38.4284856
				-92.13593148	38.42749022
				-92.13273839	38.42582137
43.4	170	86	12,390	-92.12671441	38.38766568
				-92.12780904	38.38686849
				-92.12857621	38.38755163
				-92.127712	38.38803577
				-92.12680707	38.38841584
				-92.12671441	38.38766568
52.7	169	150	23,410	-92.19786091	38.41663827
				-92.1990053	38.41563358
				-92.20039523	38.41674627
				-92.19934905	38.41755519
				-92.19786091	38.41663827
65.5	467	83	28,776	-92.24791474	38.35409568
				-92.24744871	38.354626
				-92.24544899	38.35414383
				-92.24434633	38.35336877
				-92.24319373	38.35218806
				-92.24505878	38.3529417
				-92.24543032	38.35333309
				-92.24627161	38.35359133
				-92.24791474	38.35409568
71.3	794	49	26,721	-92.24679072	38.32255513
				-92.24800359	38.32234831
				-92.24909292	38.32237914
				-92.25003191	38.32231361
				-92.25127503	38.32210695
				-92.25221254	38.32218446
				-92.2529377	38.32230831
				-92.25411637	38.32248273
				-92.25357036	38.32259848
				-92.25242098	38.32251962
				-92.24999894	38.32257569
				-92.24918102	38.32264199
				-92.24736356	38.32277341

71.3 con’t				-92.2460594	38.32302733
				-92.2451787	38.3233078
				-92.24512114	38.3230213
				-92.2458505	38.32273985
				-92.24679072	38.32255513
80.7	492	26	11,430	-92.28898152	38.30194259
				-92.2893664	38.30157294
				-92.28947407	38.30170235
				-92.28864747	38.30268453
				-92.28840279	38.30357008
				-92.28812277	38.30435526
				-92.28789875	38.30498341
				-92.28754582	38.30579677
				-92.28732978	38.30560946
				-92.28742209	38.30545265
				-92.28770156	38.30472469
				-92.28803689	38.303854
				-92.28816971	38.30326821
				-92.28844944	38.30251164
				-92.28898152	38.30194259
84.5	240	53	8,041	-92.30114116	38.27126584
				-92.30128442	38.27079276
				-92.30163071	38.27084951
				-92.30146633	38.27111284
				-92.30128673	38.27175887
				-92.30090552	38.27293231
				-92.30053593	38.27289365
				-92.30058331	38.27277545
				-92.30053786	38.27269316
				-92.30060879	38.27252953
				-92.30071371	38.27243901
				-92.30074927	38.27234808
				-92.30072746	38.27221124
				-92.30073998	38.27211106
				-92.30060265	38.27196442
				-92.30089455	38.27166539
				-92.30114116	38.27126584
88.7	226	28	4,571	-92.32482439	38.24496798
				-92.3248836	38.24471928
				-92.32566329	38.24476192
				-92.32650796	38.24470954
				-92.32742775	38.24464328
				-92.32742604	38.24482926
				-92.32716593	38.24483731
				-92.32688949	38.24484622
				-92.32658544	38.24486593
				-92.32651288	38.24486552
				-92.32635828	38.24490755
				-92.32614066	38.24489915
				-92.32562535	38.24493443

88.7 con't				-92.32482439	38.24496798
91	338	58	16,671	-92.34753956	38.25497569
				-92.3471042	38.25522373
				-92.34599064	38.25381721
				-92.34523661	38.25257206
				-92.34552718	38.25236876
				-92.34668464	38.25370722
				-92.34753956	38.25497569
95.4	845	75	38,950	-92.38569329	38.27725417
				-92.3854415	38.27716096
				-92.38485907	38.27711196
				-92.38404351	38.27706174
				-92.38307343	38.27690349
				-92.38216087	38.27683743
				-92.38163724	38.27672747
				-92.3802004	38.27662799
				-92.378744	38.27654372
				-92.37876682	38.27614565
				-92.37966008	38.27619632
				-92.38505657	38.27673012
				-92.38703958	38.27657203
				-92.38835947	38.27671676
				-92.38839692	38.27688542
				-92.38794919	38.27699029
				-92.38759949	38.27698846
				-92.3872691	38.27700205
				-92.38709297	38.27715428
				-92.38670403	38.2771982
				-92.38662645	38.27718247
				-92.38631522	38.27722679
				-92.38615916	38.27730255
				-92.38588717	38.27730113
				-92.38569329	38.27725417
102.4	697	39	23,313	-92.44348583	38.23841636
				-92.44357864	38.2382011
				-92.44387463	38.23823845
				-92.44364413	38.23857889
				-92.443387	38.2394225
				-92.44278664	38.24044424
				-92.44014023	38.24375705
				-92.43959434	38.24361064
				-92.44000721	38.24327108
				-92.44035211	38.24287728
				-92.44062877	38.24246517
				-92.44092767	38.24212506
				-92.44118085	38.24180272
				-92.44147947	38.24149856
				-92.44189259	38.24112305
				-92.44226177	38.24053162
				-92.44265387	38.23992233

102.4 con't				-92.44297664	38.23943853
				-92.4432298	38.23911618
				-92.44323227	38.23879264
				-92.44348583	38.23841636
107.4	600	72	31,579	-92.47862544	38.20914326
				-92.47986373	38.20817712
				-92.48022732	38.20827284
				-92.480421	38.20823555
				-92.48068667	38.20827487
				-92.48083037	38.20846628
				-92.4788877	38.20965949
				-92.47587257	38.2120497
				-92.47551087	38.21191455
				-92.47682491	38.21073767
				-92.47748219	38.21011107
				-92.47862544	38.20914326
108.7	118	30	2,811	-92.49108179	38.20409516
				-92.49206086	38.20343224
				-92.49234306	38.20358092
				-92.49195645	38.20377001
				-92.49148579	38.20409585
				-92.49125031	38.20427963
				-92.49108179	38.20409516
112.9	543	46	17,963	-92.52672398	38.20283515
				-92.52687358	38.20247539
				-92.52759981	38.20307065
				-92.52775825	38.20332392
				-92.5278315	38.20342138
				-92.52799057	38.20357748
				-92.52827182	38.20387982
				-92.52845527	38.20407488
				-92.5284912	38.20423049
				-92.52978978	38.20526563
				-92.52936994	38.20544856
				-92.52923687	38.20507881
				-92.5288816	38.20479561
				-92.52866102	38.20462954
				-92.52851426	38.20447349
				-92.52835457	38.20441455
				-92.52821969	38.20432656
				-92.52809693	38.20426777
				-92.52672398	38.20283515
117	336	54	14,704	-92.55956356	38.23223996
				-92.55917824	38.23198303
				-92.55878469	38.23159833
				-92.55834122	38.23116483
				-92.55803406	38.230751
				-92.55748993	38.23001788
				-92.55736572	38.22969107
				-92.55750127	38.22959222

117 con't				-92.55872698 -92.55979011 -92.55956356	38.23072522 38.23197113 38.23223996
123.3	246	48	7,715	-92.58942849 -92.58961014 -92.59081349 -92.59057401 -92.59049419 -92.58983503 -92.58942849	38.21264282 38.21258918 38.21443764 38.21454402 38.21481113 38.21346269 38.21264282