Quantitative Survey of Freshwater Mussels (Unionoidea) and Assessment of Sediment Contamination in the Big River, Missouri

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Abstract

The relationship between freshwater mussel density and heavy metal concentrations in river sediments was investigated in the Big River downstream of areas with past mineral mining. Quantitative mussel surveys were conducted and river sediments were analyzed for grain size and concentrations of lead (Pb), cadmium (Cd), and zinc (Zn) at 18 sites selected between river kilometer (RK) 2.5 and 127. Tissue samples were also collected of Asian clams (*Corbicula fluminea*) to determine body burdens of heavy metals and verify exposure of bivalves. Mussel density negatively corresponded to elevated concentrations of sediment Pb downstream of mining operations. A significant decrease in mussel density was observed downstream of mining sites between RK 113.5 and 67.5, where Pb concentrations were greater than the Probable Effects Concentration (PEC) of 128 mg/kg. As Pb concentrations decreased to near the PEC at RK 47.5 and further downstream, mussel density increased at most sites. While mussel densities recovered at RK 47, this did not correspond with a recovery of mussel richness until RK 16.5 where the mussel fauna is comparable to reference streams. *Corbicula fluminea* were found to be exposed to heavy metals, and tissue Pb concentrations were correlated with mussel density and sediment Pb concentrations. Lead concentrations in *C. fluminea* tissue correlated with Pb in sediment and mussel density more strongly than similar comparisons between Zn and Cd concentrations in tissues. Mussel population metrics were not correlated with sediment grain size and other substrate metrics. Comparisons of mussel species diversity using available data from other similar rivers in Missouri indicated a 70 to 75 percent decline of mussels in Pb contaminated areas of the Big River.

INTRODUCTION

Several state and federal agencies, in association with the U.S. Fish and Wildlife Service (USFWS) and the Missouri Department of Natural Resources (MDNR), have

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been studying the potential toxic effects to organisms from releases of heavy metals to the Big River as part of the Southeast Missouri Lead Mining District Natural Resources Damage Assessment (NRDA) process (Mosby et al. 2008, Allert et al. 2010, Besser et al. 2009, McKee et al. 2010, Roberts et al. 2010). This study and a companion report by Albers et al. (2016) (available at https://www.fws.gov/Midwest/es/ec/nrda/SEMONRDA/index.html.) provide additional methods and results on the effects of heavy metal contaminated sediment on freshwater mussels as part of the NRDA process. The work plan for this study was submitted for public review and posted on the USFWS web site listed above.

The Big River is within the Meramec River Basin, located in east-central Missouri (Figure 1). The clear, gravel-bottomed streams of this watershed drain the northeastern edge of the Ozark Highlands and support one of the most significant mussel fauna in the Midwest, including over 50 species of freshwater mussels (Buchanan 1979, Roberts and Bruenderman 2000, McMurray et al. 2012). While other streams in the basin contain relatively healthy mussel communities (Roberts and Brunderman 2000, Hinck et al. 2012), the Big River and its 37 native mussel species have been found to have reduced population metrics in over 100 km that contains heavy metal contamination (Table 1). The Big River watershed drains an area with a long history of lead and zinc mining called "the Old Lead Belt". Historically, this area provided the highest production of lead in the United States (U.S. Geological Survey 1998). While most mining activity has ceased, its legacy remains, including approximately 227 million metric tons of fine-grained dolomitic tailings that were produced and are now divided among 6 large piles adjacent to the Big River and its tributaries. An estimated 32% of mine tailings has been released to the Big River and its tributaries (Pavlowsky et al. 2010). In addition to Pb mining within the Old Lead Belt, the Big River watershed hosted the Washington County Barite District, which was one of the largest barium (Ba) mining areas in the country. While small dams were constructed to hold back mining wastes, most were improperly constructed or poorly maintained (Meneau 1997). As a result, continual releases of mine wastes have contaminated the sediments with lead (Pb), cadmium (Cd), and zinc (Zn) in more than 144 km (90 mi) of the Big River and its tributaries (Meneau 1997, MDNR 2007, Pavlowsky et al. 2010).

Heavy metal contaminated sediments have been shown to negatively affect mussel populations in the Big River downstream of mining areas (Besser et al. 2009, Roberts et al. 2010). In a 2008 assessment of heavy metal contamination and freshwater mussel populations in the Big River (Roberts et al. 2010), streambed sediment was collected at 39 locations in the lower 209 km (130 mi) of the river and tributaries, and mussel population and assemblage data (including number of species, number of individuals per species, number of live mussels, and recently dead shells) were collected at 19 of those sites. Sediment samples were considered contaminated with heavy metals at levels greater than the consensus-based Probable Effects Concentration (PEC) (MacDonald et al. 2000) for Pb at 128 mg/kg for more than 150 km (93 mi) downstream of mining areas. Sediment concentrations of Zn and Cd exceeded their respective consensus-based PECs of 458 mg/kg and 4.99 mg/kg for shorter distances (approximately 40 km (25 mi)). Sites with affected mussel communities were found

over a 158 km (98 mi) reach of the river, from river kilometer (RK) 181.8 - 23.2 [river mile (RM) 113 - 14.4]. Documented effects included reduced species richness and abundance compared to reference sites and reduced mussel densities corresponding with heavy metal concentrations above PECs. Stream reaches nearest to the Pb mining inputs from RK181.8 – 141 (RM113 – 87.7) demonstrated the greatest impacts to the mussel assemblage (Roberts et al. 2010), and toxicity of sediments to mussels was documented in laboratory tests (Besser et al. 2009). Mussel communities in the downstream 16 km (10 mi) of the Big River were similar to reference sites in terms of mussel abundance and species richness, and sediments in this reach did not consistently contain heavy metals at concentrations exceeding the PECs (Roberts et al. 2010).

The results of the 2008 sediment and mussel study (Roberts et al. 2010) revealed the need for additional information in areas not previously surveyed for mussels. Specifically, more sites were needed on the lower Big River (from RK16 to 141) to better characterize the extent and concentration of sediment contamination and mussel population response where more moderate concentrations of metals are found compared to heavily impacted reaches in St. Francois County (Besser et al. 2009). The current study also incorporated a more rigorous assessment to differentiate the effects of Pb and other heavy metal contamination on mussels from the effects of habitat variability (e.g., sediment characteristics, location in the watershed) in the Big River. The study by Albers et al. (2016) evaluated the same Big River mussel data using alternative statistical analyses to differentiate heavy metal contamination effects on mussels from sediment characteristics in the Big River. In summary, this study examined stream reaches downstream from the most severe documented impacts to the mussel community, where physical mussel habitat is of high quality, but metal concentrations are elevated relative to reference sites.

The objectives of this study were to:

1) Identify Big River study sites for quantitative mussel assessment by delineating areas that meet habitat needs for mussels located between previously surveyed mussel survey sites in the Big River.
2) Characterize the spatial (i.e. linear) extent of sediment contamination.
3) Relate mussel assemblage and population metrics at study sites and reference locations to heavy metal concentrations in sediment and biota and variability in substrate composition.
4) Compare mussel species richness from contaminated reaches of the Big River with expected reference or baseline conditions from stream systems without Pb contamination.

METHODS

Field work for this study took place in two phases. Phase I was a reconnaissance-level survey to identify sites in the lower 125 km of the Big River with characteristics typically suitable for the establishment of dense, multi-species assemblages of mussels

(generally termed mussel beds). Known mussel beds sampled in 2008 by Roberts et al. (2010) were not visited during this phase, which was intended to identify previously undocumented assemblages. Sites identified in Phase I were the subject of further site characterization in Phase II, including habitat assessment and quantitative mussel surveys.

Phase I: Mussel Habitat Reconnaissance (Objective 1)

The majority of the mussel species in the Meramec River Basin require permanent, flowing water above stable, gravel-dominated substrates intermixed with finer grained particles (e.g., sand). Within stream reaches providing these minimum habitat needs (as defined below), mussel beds can form over time (Roberts and Bruenderman 2000). During phase I, the Big River was traversed by boat from RK125 (RM77.7) to RK17 (RM10.6) to identify and delineate potential mussel habitat for sampling in Phase II. For this study, the following criteria determined if a site met minimum needs for establishment of a mussel bed: 1) a stable river channel (defined below); 2) permanent, flowing water (i.e., riffles, runs, glides), 3) stable, generally compact substrate that provided fine-grained materials ranging in size from 2-8 mm in diameter (i.e., sand and fine gravel), and 4) presence of a minimum of five live individual unionid mussels detected within one person-hour of searching. This design approach was an attempt to best characterize the quality of the site selection criteria and ensure the Phase II assessment included only the highest quality sites throughout the study area.

To obtain this information, crews traveled the length of the river and visually assessed reaches with a stable river channel [without horizontal channel migration exceeding ½ of the channel width according to interpretation of historic maps and optical imagery (Pavlowsky and Owen 2013)]. Within these reaches, stream segments with permanent, flowing water were identified from the boat as a riffle, run, and/or glide, and each of these areas were qualitatively assessed for stream armoring, indicating stable substrate conditions. If substrate was a compact, consolidated mixture of small particles (i.e., sand) and larger sized sediment (e.g., gravel or cobble), covered with a layer of diatoms or algae, it was deemed stable enough for mussel bed occurrence. In reaches meeting these minimum criteria, channel margins often contained macrophytic vegetation (e.g., *Justicia americana*), which supported our designation of the reach as horizontally stable. Further, the presence of living unionid mussels in reaches deemed suitable for mussel bed occurrence supported the use of these criteria for mussel community establishment. Reaches above RK 125 to RK 180 contain visible quantities of chat or tailings (Pavlowsky et al. 2010) and substrates with heavy metal concentrations toxic to mussels in laboratory studies (Besser et al. 2009). These sites were nearly devoid of live native mussels (Roberts et al. 2010); therefore, crews could not use mussel presence to validate these criteria. As a result of the findings of these earlier studies, this stretch of the river was excluded for consideration for this study.

In reaches deemed suitable for mussel bed establishment, timed searches were conducted to determine the presence of mussels. If the habitat was occupied by at least five living unionid mussels detected in one person hour of search time, all similar

habitat in that immediate area was delineated with a GPS to define the sampling boundary for additional sampling in Phase II (Table 2). All mussels found during the timed searches were identified and enumerated. Delineated sites were characterized such that only the portion of the channel with suitable, occupied mussel habitat was included for Phase II sampling. This strategy was intended to minimize variance in population estimates (Strayer and Smith 2003). Searches were conducted during base flow conditions to avoid stagnant or dry riverbed because mussels typically do not persist in portions of the channel exposed during dry periods.

Phase II: Sediment and Mussel Sampling (Objectives 2 and 3)

Phase II consisted of *C.fluminea* tissue sampling at 11 sites, sediment sampling for metal and grain size analysis, and intensive mussel surveys at the 14 sites delineated in Phase I and four previously delineated sites (including one reference site on the Meramec River, one reference site on the upper Big River upstream of mining operations, and two known sites located in the lower Big River downstream of the reconnaissance reach). The Meramec River reference site was previously surveyed and chosen based on similarity to mussel fauna in the Big River. The Big River reference site was found during the 2008 mussel study and was selected based on its position upstream of the first major mining inputs into the Big River. The two lower Big River sites were previously surveyed and included in the study because mussel abundance and species richness were comparable to references sites in 2008 (Roberts et al. 2010). A summary of specific information collected at each study site and of external data used in this analysis is shown in Table 3.

Sediment Sampling for Metals Analysis

Sediment sampling for metal analysis was conducted at each location where quantitative mussel data were obtained (Table 3). Two samples for metals analysis were collected at all sites. One set of samples was collected in shallow, slower water zones adjacent to the mussel bed (i.e. gravel bars or other depositional areas), designated as the "gravel bar sediment". A split sample was collected for confirmatory laboratory analyses of metals from three sites for gravel bar analyses. Finally, a second sample was collected from within the mussel bed itself, designated as the "mussel bed sediment". All samples were labeled with a unique sample identifier, site name, date, and name of collector. All sediment samples were recorded in a log book with a chain of custody form that was maintained and accompanied the samples to the laboratory. At the laboratory, the samples and chain of custody were signed over to the sample custodian.

An approximately 0.25 kg split sample of all gravel bar samples was collected by alternating scoops into a plastic bag concurrently with the bucket samples used for physical habitat characterization. The 0.25 kg bulk (un-sieved) sample was analyzed via x-ray fluorescence (XRF) at the USFWS facility in Columbia, Missouri. Six of 36 samples (consisting of 0.25 mm and 2 mm grain size fractions) were subjected to confirmatory quality assurance/quality control (QA/QC) analyses using Inductively

Coupled Plasma (ICP/MS) or Atomic Adsorption following EPA method 3050b "Acid Digestions of Sediment, Sludges, and Soils" at the U.S. Geological Survey (USGS) Environmental Research Center in Columbia, Missouri (CERC). Metals analyses included total Pb, Zn, Cd, and Ba. The split samples were collected for the purpose of evaluating the correlation between XRF and laboratory analyses. Additionally, sieved samples from CERC were returned to USFWS for XRF analysis of the 0.25 mm and 2 mm fractions.

Composite samples were collected within the mussel bed for metals characterization that consisted of a minimum of five aliquots (or subsamples) taken from points estimated to be evenly distributed throughout the delineated mussel habitat. These samples were collected by driving a 7.6 cm diameter PVC scoop (attached to a 1.2 m pole) into the substrate to a depth of 5 to10 cm, angling the opening upstream, and slowly raising the sampler to the surface to capture the sample. The aliquots were placed in an unused, 19 L plastic bucket, left to settle for 30 minutes, decanted, and placed in a sealed plastic bag. These samples were taken to the USFWS facility and analyzed for metals using a portable XRF meter

Samples for XRF analysis were thoroughly mixed within a bag by shaking and/or hand manipulation. Samples were dried at room temperature for at least seven days or until they contained less than 20% moisture. A portion of each sediment sample was sieved to obtain the <2mm and <250 micron fractions. The <2mm, <250 micron, and the bulk sample were analyzed by XRF. Each sample was analyzed for one minute with the XRF by placing the instrument directly against the bag, with the sediment in full contact with the XRF window. Three separate readings were collected for each sample. These results were recorded in a log book and stored electronically.

A suite of calibration verification check samples was used to assess the accuracy and precision of the XRF instrument. Check samples were analyzed at the beginning of each working day, during active sample analyses, and at the end of each working day. The criterion for a measured value for each target analyte was within ±20 percent (%D) of the true value for the calibration verification check to be acceptable. If a measured value fell outside this range, then the check sample was reanalyzed. If the value continued to fall outside the acceptable range, the instrument was recalibrated, and the batch of samples analyzed before the unacceptable calibration verification check was reanalyzed (USEPA 1998).

Quantitative Mussel Sampling

Freshwater mussels are considered good indicators of stream ecosystem health and are frequently used to assess toxicological stressors affecting bethic communities (Van Hassel and Farris 2007). Average mussel density (mussels/m^2) is a recommended metric for hypothesis testing and assessing the influence of anthropogenic impacts (Strayer and Smith 2003, Van Hassel and Farris 2007). Therefore, systematic sampling was conducted in this study to provide mussel density estimates (individuals/m^2) (Table

3). This involved searching for mussels within 150 0.25-m^2 quadrats spaced evenly within delineated mussel habitat with three random starts (Smith et al. 2001, Strayer and Smith 2003). To determine the systematic pattern of the 150 quadrats, first the distance between the quadrats was calculated by the following formula:

$$d = \sqrt{(L*W)/(n/k)}$$

where *d* is the distance between units, *L* and *W* are the length and width of the study site, *n* is the total number of quadrats, and k is the number of random starts. Second, the location of the three random starts was determined by using a random number table to pick the x and y grid coordinates of each random start within the delineated area such that each random start represented a separate systematic pattern of 50 quadrats within the delineated area. In the field, the three random start locations were located and sampling progressed by flipping the 0.25m^2 quad the appropriate number of times to measure the set distance (*d*) between each quadrat sampled.

The 150 quadrats at each site were searched using a double sampling design (Smith et al. 2001). This sampling technique uses exact mussel counts from excavated quadrats to calibrate a larger number of visual and tactile searches within quadrats. At each random sampling location, the quadrat was placed on the stream bottom, and all visible mussels were collected while removing any loose cobble and flat rocks lying on the surface. The remaining gravel substrate was searched by gently fanning/mixing the substrate to remove algae growth until no mussels were visible. For a subset of 50 quadrats (representing one random start pattern), a second, intensive mussel sampling effort was performed (within the same quadrat and location) to measure sampling efficiency of the visual quadrat searches (Smith et al. 2001). This involved removing the substrate to a depth of 10 cm (or to bedrock) and hand sorting the sample above the surface through a 6.35 mm sieve to find any mussels remaining below the surface. All living mussels collected within each quadrat were identified and measured while keeping the visual and excavated samples separate. Sampling efficiency is defined as $N_o/(N_o+N_e)$, where N_o is the number of mussels observed at the surface and N_e is the number of mussels found via excavation. After processing, the substrate and mussels were replaced into the quadrat location.

Dead shells (not represented by live individuals in the quantitative samples) were noted for species and classified as fresh-dead, dead, or subfossil. Fresh-dead shells represent individuals for which the soft anatomy is not fully decomposed, indicating recent mortality. Dead shells have some luster to the nacre (innermost layer of the shell) and a relatively intact periostracum (outermost layer of the shell). Subfossil shells have a 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 immersed in the water, 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.

Mean mussel densities from quantitative survey data were statistically compared among study sites. Analyses included a one-way ANOVA with rank-transformed data and Tukey's test for pair-wise comparisons of the means (Table 4) (Conover and Iman 1981).

Corbicula Tissue Sampling

Corbicula fluminea (the Asian Clam) were used as bivalve surrogates at 11 mussel sampling sites to determine body burdens of heavy metals (Schmitt et al. 2007) and verify exposure of the bivalve fauna to heavy metals (Table 3). *Corbicula fluminea* are bilvalved mollusks, occupy the same habitat in the Big River, and are good indicators of concentrations in sediment (Angelo et al. 2007). *Corbicula fluminea* were collected at sites that represent a range of sediment concentrations in mussel habitats. These animals were collected from random quadrats during quantitative mussel sampling as native mussels were processed from the substrate. Specimens within the 20 to 25 mm maximum shell diameter were collected and held in a small plastic bucket with fresh river water until all 50 quadrats were processed, following the USGS's National Water-Quality Assessment Program protocol (Crawford and Luoma 1993). After quadrat sampling, 30 *C. fluminea* specimens were selected by hand and held in a covered bucket of river water for 24 hours to allow mussels to expel stomach contents. The *C.fluminea* were then transferred to labeled high density polyethylene jars (one composite sample per jar) and frozen (-20°C) until thawed for analysis. Laboratory analysis of *C. fluminea* tissue samples was conducted at CERC using ICP-MS using methods according to Brumbaugh et al. (2005).

Substrate Physical Characterization

Pebble counts were conducted within the mussel sampling sites to characterize the substrate composition based on Wolman (1954). Pebble counts were conducted concurrently with the quantitative mussel sampling at the 100 visual 0.25 m^2 quadrats. After the quadrat was placed on the substrate, the diver (without looking) placed a finger on the substrate at the upper right corner of the quadrat. The first substrate touched was collected and measured along its intermediate axis. Sand or silt was only recorded and not measured. Substrate was divided into sand (<2mm), fine gravel (2-8mm), medium gravel (9-16mm), coarse gravel (17-64mm), cobble (65-256mm), and boulder (>256mm).

Sediment was collected for particle size fraction analysis to differentiate the <2mm size fraction and provide additional data for substrate composition. Each sample consisted of five aliquots taken at points distributed along the gravel bar in shallow water near or adjacent to the mussel habitat as described above for metals analyses. Approximately 10 kg of sample were placed in a 7.5 L plastic bucket. Bucket samples were sieved and

analyzed for grain size fraction at CERC. Grain size characterization was not completed for Big 67.5 and 68 because the remoteness of these sites did not allow sediment samples to be collected in time for analysis at CERC. The sediment samples were wet-sieved using Big River collection site water to determine metals content and the percentage of sediments that fell within the following fractions: <63 µm, 63-250 µm, 250 µm-2mm, and >2 mm. The composition of sediment sizes was analyzed by CERC by calculating the mass of each substrate size class after sieving.

To further illustrate substrate composition and conditions within the mussel habitat, underwater photos were taken of the substrate surface. This was conducted concurrently with quantitative mussel sampling at 25 of the 150 random quadrats (i.e., every other quadrat of the 50 samples where substrate was removed and sorted). Two photos were taken at each of the 25 quadrats with a Pentax® WG-3 underwater camera. A L-shaped bracket was inserted into the camera's tripod socket, and the end was placed on the stream bottom when photos were taken to maintain the camera 7.5 cm away from the substrate. The first photo was taken at the center of the quadrat before the substrate was disturbed. The second photo was taken after loose cobble and flat rocks were removed to expose the underlying substrate and finer materials where mussels are typically buried.

Comparison of Big River Data to Other Reference Streams (Objective 4)

To complete our fourth objective, we obtained data from outside of the lead-impacted area in the Big River drainage. The University of Missouri (MU) and Missouri Department of Conservation (MDC) have collected mussel and habitat data at additional locations on the Meramec, Bourbeuse and Gasconade Rivers that enable a more robust evaluation of reference conditions (MDC Unpubl. Mussel Database; Rosenberger, Lueckenhoff, and Schrum, unpubl. Data, University of Missouri; Lueckenhoff 2015). Independent of the USFWS mussel data collection, quantitative mussel sampling at 12 sites in the Bourbeuse and Meramec rivers took place in the summer of 2014 by the MU Cooperative Research Unit as part of an MDC-funded study to develop and validate standardized methods for sampling mussels. These data were used to provide basic information on assemblage richness and structure and habitat features of sites in the absence of Pb mining impacts.

For sites sampled by MU, relative densities of mussels were used to delineate the extent of the mussel bed in any area. Following delineation, a total of 30 locations within the mussel bed were selected for quadrat sampling via a set of random x y coordinates that corresponded to distance from the bank and distance from the start of the mussel bed. Quadrats (0.25-m^2) were placed at the center of these randomly selected locations and visually and tactilely searched for mussels at the substrate surface. Following data collection at the quadrat surface, quadrats were excavated to a depth of 10 cm and captured mussels were identified, recorded, and placed back in their original location. Ten quadrats were selected at random for inclusion of excavation data to correspond with FWS sampling methods. Substrate was also assessed at the same 30 random points and classified using the Wentworth particle size classification

(Wentworth 1922). Because different substrate classification methods were used by the USFWS and MU, classification methods were merged to differentiate sand, gravel-pebble, cobble, and boulder substrates (Table 5). Two sites from the 2008 USFWS mussel survey were located within the Big River reach of the present study (RK 49 and 32). These sites were sampled using similar methodologies as in the present study and were therefore included in the mussel assemblage analysis (Roberts et al. 2010). Although eight sites were surveyed in 2008, two were reference sites that were re-surveyed in the current study and other sites did not contain mussels.

Species richness data from MDC's Mussel Database (MDC Unpubl. Mussel Database) were used from sites in the Big, Bourbeuse, Meramec, and Gasconade rivers to compare species richness trends across a longitudinal gradient from headwaters to the lowest reaches sampled. The Gasconade River was added as an additional un-impounded reference stream with similar drainage area as the Meramec. Two time periods were evaluated for which robust mussel data existed: 1978-1993 and 1994-2013. If individual sites were sampled more than once within a time period, the highest richness estimate was retained as representing the most comprehensive survey of that site.

For both the USFWS 2013 (18 sites from Phases I and II) and MU 2014 mussel sampling locations described above, additional environmental variables of interest (e.g. drainage area, Euclidian distance between mussel sites) were gathered and calculated. Drainage area originated from the National Hydrography Dataset Plus for each stream segment where sampling took place.

Within a region, aquatic species richness has an overall positive relationship with drainage area, which serves as a useful surrogate for habitat size and diversity (e.g., Matthews and Robison 1998). It therefore can be considered as a baseline predictor of species richness for comparisons among systems. To validate the use of unaffected reaches in the Meramec River Drainage as appropriate references for this study, the Meramec River was compared to the nearby Gasconade River to confirm if the overall relationship between mussel species richness and drainage area manifests in both systems similarly. Potential impacts (unrelated to heavy metal contamination) that are common to these watersheds are bank and channel degradation, other contaminants, sedimentation, and indirect effects from mill dams (Roberts and Bruenderman 2000, Bruenderman et al. 2001, Blanc 2001, Blanc et al. 1998, Meneau 1997, Blanc 1999).

Analysis of covariance (ANCOVA) was used to assess if the relationship between drainage area and species richness (defined as the number of mussel species present) varied by river system. Specifically, the ANCOVA evaluates the differences in slopes and intercepts of regression lines describing this relationship. We expected richness to vary similarly by watershed size among rivers (Watters 1992); therefore, the Meramec and Gasconade Rivers were compared to confirm this overall pattern and the Bourbeuse and Big Rivers were compared to determine if this relationship was consistent for the Big River. Relationships between drainage area and species richness for historic (1978-1993) and current (1994-2014) time periods were evaluated for the

Bourbeuse and Big Rivers. Once the relationship between species richness and drainage area was confirmed with ANCOVA, we performed LOESS regression to examine continuous differences between the species richness-drainage area relationship among systems along the lengths for which we have data.
Mussel assemblage structure, as indicated by the relative abundance of species in sites, is a useful indicator of impact, potentially more responsive than simple measures of mussel density or richness (Dunn et al. 2006). Mussel assemblage data at all USFWS and MU sites were converted to relative abundance to compare assemblage similarity using Euclidean distance, standardizing for differences among sites in mussel densities. The matrix of similarity coefficients was clustered using the un-weighted pair-group with arithmetic averaging method to produce a dendrogram depicting clusters of sites with similar mussel assemblages in terms of relative abundance.   A bootstrap, or random resampling, approach to dendrogram evaluation was used to assess the reliability of the results through the approximately unbiased (AU) test (Suzuki and Shimodaira 2006). Ranging from 0 to 1, a high AU *p*-value indicates consistency between the resampled data sets (representing random resampling) and the original data set. AU values were based on 10,000 bootstrapped data sets. Calculation of the similarity index, cluster analysis and AU index were conducted using the R library 'pvclust' (Suzuki and Shimodaira 2015). To examine potential factors driving assemblage structure and similarity among sites, after distinct assemblages were identified, we averaged environmental characteristics of sites within the assemblage clusters and compared environmental conditions among clusters using box plots.

RESULTS AND DISCUSSION

Phase I: Mussel Habitat Reconnaissance and Site Selection (Objective 1)

In all, 40 sites were identified as potential mussel habitat (Appendix A) over the 110 km section of the Big River evaluated during the reconnaissance (Figure 1). Of the 40 sites evaluated with timed searches, 14 were found to have both suitable habitat and living mussels present and were delineated for further sampling in Phase II (Table 2). Choosing only the best sites with living mussels avoided the possibility of including sites that may not provide suitable habitat because of an unknown factor. This is considered a conservative approach because heavy metal contamination may still be a factor at sites where mussel presence is lacking.

Phase II: Sediment and Mussel Sampling

Sediment and quantitative mussel sampling was performed at the 14 sites delineated in Phase I, two reference sites (Mer75.6 and Big194), and two previously delineated sites on the Big River downstream of House Springs (Big2.5 and Big16.5) for a total of 18 sites on the Big River and one site on the Meramec River. *C. fluminea* were collected at 11 sites (Table 3).

Analysis of Heavy Metal Contamination in Sediment (Objective 2)

USGS ICP-MS data were used on a split sample to provide additional QA/QC verification of the XRF data. Six samples from Big2.5, Big16.5, and Big113.5 were analyzed for Pb, Zn, and Cd from the < 2 and < 250mm grain sizes (Table 3). Differences between the two methods were within an acceptable range of precision (±20%) with no clear bias, demonstrating XRF was either consistently higher or lower than the ICP-MS data (Appendix B). XRF and laboratory results demonstrated strong correlation ($R^2 > 0.99$) as shown by the figure in Appendix B. Therefore, no re-analyses of samples via XRF or adjustments to the XRF data were necessary.

In general, XRF and ICP/MS results demonstrated elevated bulk concentrations of Pb above the PEC as determined by MacDonald et al. (2000) along the entire study reach, with the exception of reference samples and the lower Big River sites including sites Big 16.5 and 2.5. However, the < 0.25 mm fraction was contaminated with Pb for the entire reach below the mining areas (from Big113.5 to Big2.5). Zinc exceeded the PEC only in the < 0.25 mm fraction from Big113 to 91, with the exception of Big106.5. The highest bulk Pb was at Big86 at 619 ppm, despite the highest Pb in the < 0.25 mm fraction found at Big113 (Tables 6a and 6b). Sediment metal results were generally consistent with those found by earlier studies by Besser (2009), Pavlowsky et al. (2010), and Roberts et al. (2010). However, many of the locations sampled in this study were not the same locations as earlier researchers, but were nearby reaches. .

Sediment concentrations for Pb, Zn, and Cd in the < 0.25 mm fraction by river kilometer frequently exceeded PECs (Appendix C). Meramec River sediments collected in 2014 did not exceed the PECs for any metal. Fine fraction (0.25 mm and smaller) were consistently higher in metal concentrations than bulk and < 2 mm samples. However, the pattern of increasing metal concentrations with decreasing grain-size was not consistent in a comparison between bulk and grain sizes < 2mm. Nine out of 18 bulk Pb samples and 10 out of 18 bulk Zn samples were greater than their respective metal concentrations in the < 2 mm fraction in mussel bed sediments.

In the bulk and < 2 mm fraction, data trend towards higher metal concentrations in the gravel bar samples as compared to the mussel bed (Appendix C). Within the < 0.25 mm fraction, this trend is more obvious and consistent, with 13 of 17 sites on the Big River containing higher concentrations of Pb in the gravel bar than in the associated mussel bed. The differences between the bar and mussel bed sediment samples could be attributed to differing hydrologic regimes between the two sampling areas.

XRF analysis of barium (Ba) was also recorded and reported in Appendix C. Barium concentrations ranged from 269 mg/kg in the Meramec to 1455 mg/kg at Big67.5 in the <2 mm fraction. Mean Ba concentrations were 466 mg/kg. The Ba concentrations are reflective of sediment inputs from barite mining within the Big River watershed in Washington and Jefferson Counties.   No PECs exist for Ba due to a paucity of data indicating toxicity. Further discussion of Big River Ba results and other metals in association with mussel populations are presented in Albers et al. (2016). Pavlowsky et

al. (2010) and Roberts et al. (2009) discuss relative inputs from Pb versus Ba in the Big River and found a large influx of Ba below the first major tributary of the Washington County Barite Mining District (approximately below RK 115), but no similar increase of Pb concentrations. All of the sampling done on the Big River for this study was below Big115, with the exception of the Big River reference site (Big194).

Mussel Abundance and Species Composition (Objective 3)

In all, 1,045 mussels were found in the quadrat surveys, representing 32 species (Table 7). The total number of living mussels observed ranged from 0 at Big113.5 to 379 individuals at Big2.5 (Table 7). Average mussel densities differed significantly among sites on the Big River (one way ANOVA with rank-transformed data [$p < 0.0001$] and Tukey's test for pair-wise comparisons of means) (Table 8, Figure 2). Ten sites downstream of mining areas from Big67.5 to Big113.5 grouped together with the lowest average densities (0 and 1.2 mussels/m^2). In contrast, mean mussel densities were significantly higher at sites located further downstream of this reach and at reference sites. The site with the highest mussel density was the most downstream Big River site (Big2.5) with an average density > 13 mussels/m^2. The reference site on the Meramec River (Mer75.6) was similar to two lower sites Big16.5 and Big47, with densities between 5.71 and 6.20 mussels/m^2. Finally, density at the upper reference site (Big 194) was similar to Big 30.7 and Big41 (between 2.9 and 3.8 mussels/m^2). The upper reference site grouped with two lower sites, despite its location in the head waters of the Big River. It is typical for mussel abundance to decrease with distance upstream (Roberts and Brunderman 2000). Nonetheless, density at this reference site is higher than sites downstream of mining areas down to Big41 (Table 8). Site Big20.5 is an outlier among the lower Big River sites (sites downstream of Big67.5), with an average mussel density of 0.02. This site is located in an atypical, predominately sandy reach of the Big River, which may have affected mussel density and distribution. Other Missouri streams with primarily sand substrate tend to naturally have low mussel densities (Andy Roberts pers. obs. and Roberts et al. 1997). In terms of overall patterns of mussel density in the Big River, we observed a significant decrease in mussel density downstream of mining sites until Big47, downstream of which all sites had high densities, with the exception of Big20.5 (Figure 2). In terms of overall density alone, these lower Big River sites (below Big47) were comparable to reference sites in the Meramec River and upper Big River.

Mussel density negatively corresponded to elevated levels of sediment Pb concentrations downstream of mining operations (Figures 3 and 4). Lead concentration was well above the PEC for sites downstream of mining areas between Big113.5 and Big67.5, and concentrations decreased to near the PEC at locations where mussel density began to increase. While mussel densities recovered at Big47, this did not correspond with a concomitant recovery of mussel richness and diversity. Sites between Big47 and Big30.7 were composed of predominantly one species (*Elliptio dilatata*) (Figure 5 and 6). This species comprised 57%, 88%, and 74% of the total number of species at sites Big30.7, Big41, and Big47, respectively. In contrast, the most dominant species at sites Mer75.6, Big2.5, and Big16.5 comprised 18% (*Amblema*

plicata), 32% (*Actinonaias ligamentina*), and 40% (*A. plicata*) of the total number of species respectively at those sites (Table 7). This, accompanied by an overall decrease in species richness, indicated lower mussel diversity (a product of evenness and richness) downstream of the Pb mining district when compared to reference sites (when taking into account expected downstream increases in mussel diversity) and the two most downstream Big River sites.

The negative correlations with mussel population metrics and sediment metals found in this study are consistent with other studies. Besser et al. (2009) showed general agreement between juvenile mussels exposed to Big River sediment in the laboratory and the observed impacts to mussel communities in the Big River by Roberts et al. (2010). However, mussels exposed in the laboratory were not as sensitive to heavy metal concentrations in sediment as observed in the field. This could be explained by the relatively short-term (28 days), sediment-only exposure of laboratory mussels compared to wild mussels that live for decades in the Big River while exposed to contaminated water, food, and sediment throughout their entire life cycle. Allert et al. (2013) evaluated crayfish densities in the Big River and survival in caged crayfish at locations near, distant and reference locations relative to the Pb mining area. Wild crayfish demonstrated a similar negative correlation between density and sediment metal concentrations as was observed for mussels in this study. Caged crayfish demonstrated reduced survival at sites with elevated metals near the mining areas.

Albers et al. (2016) evaluated the same mussel data set evaluated in this report for the Big River and conducted a more detailed evaluation of species assemblages in relationship to sediment characteristics and constructed metals concentration-response models. They found a number of species and mussel densities were negatively correlated with Pb and Ba and the sum PEQ for Pb, Zn, and Cd. The PEQ is the concentration of each metal divided by its respective PEC as determined by MacDonald et al. (2000). The best-fit (r^2=0.68) concentration-response model based on density estimated a 20 percent effect concentrations for Pb in the <2mm size fraction 136 mg/kg. This value is very close (within 10%) to the MacDonald et al. (2000) PEC. As noted previously, Ba concentrations are elevated below Big113.5, decline similarly to Pb in lower reaches of the Big River, but are not known to be particularly toxic in environmentally relevant concentrations.

Analysis of Heavy Metal Exposure to *Corbicula fluminea* (Objective 3)

A total of 330 *C. fluminea* were collected (33 composite samples) in the Big River for metal tissue analysis. Angelo et al. (2007) showed correlations between *C. fluminea* metal concentrations and metal concentrations in unionid fauna in the Tri-State Mining District. Therefore, metal concentrations in *C. fluminea* tissue should be representative of unionid exposure. In general, the Big River results demonstrated molluscan fauna exposure to heavy metals and metal bioavailability. The three analyzed metals (Zn, Cd, and Pb) were all detected in the *C. fluminea* tissue samples. Zinc concentrations ranged between 191 and 364 µ/g dry weight (dw), generally at higher levels than Cd or Pb, including the reference site (Figure 7). Zinc concentrations found in tissues at the

reference site were consistent with those analyzed for other comparable Ozark streams in non-mining areas (Schmitt et al. 2007). Zinc is an essential element for organisms and can be metabolized by animals. Tissue concentrations are typically regulated in narrow ranges, and are therefore, not a reliably consistent indicator of exposure. Further, Zn was well below the PEC in sediment samples along the sampled reaches of the Big River (Figure 7). Cadmium and Pb concentrations ranged from 0.61 to 23.17 and 1.09 and 133 µg/g dw, respectively (Figure 7). Cadmium and Pb concentrations in tissue samples were low at the upper Big River reference site, high downstream of mining sites, and decreased with distance downstream from mining sites (Figure 7). Tissue concentrations of Cd and Pb were lowest at the downstream-most Big River site at Big2.5, concurrent with the highest abundance and number of species out of all sites downstream of mining sites within the Big River. Figures 8a and b compare *C. fluminea* tissue Pb concentrations with mussel density. The results indicate a correlation of mussel density increasing with decreasing *C. fluminea* concentrations (R^2 = 0.44, including the reference site; R^2 =0.63 without the reference included). Mussel density correlated with the <2 mm sediment Pb concentrations in the mussel beds (R^2 = 0.53; Figure 9). *Corbicula fluminea* tissue Cd concentrations were not as strongly correlated with Cd sediment concentrations ($R^{2=}$ 0.19).

Angelo et al. (2007) evaluated metal concentrations in wild *C. fluminea* in the Tri-State Mining District in Missouri and Kansas. Arithmetic mean Pb (74 µ/g dw) and Cd (14.2 µ/g dw) concentrations in Big River *C. fluminea* (which included a reference site) exceeded the highest concentration found by Angelo et al. (2007) (24 µ/g dw Pb and 9.4 µ/g dw Cd). Big River *C. fluminea* concentrations of Zn were of a similar range as those found by Angelo, but did not approach some of the highest concentrations found in the Spring River and its tributaries (1400 µ/g dw). Notably, similar to the present Big River study, Angelo et al. (2007) found depressed mussel density and reduced species richness compared to reference streams of unionid mussels at sites co-located with *C. fluminea* with elevated tissue concentrations. Again these tissue comparisons between the two studies must be treated with caution because Angelo's data is for non-depurated *C. fluminea*, whereas the Big River animals in this study were depurated.

Czarneski (1987) and Schmitt and Finger (1982) introduced plain pocketbook mussels (*Lampsilis ventricosa*) in caged exposure studies. In both studies adult mussels were collected in the Bourbeuse River and placed in cages in the Big River above and below the mining areas and tissues were analyzed for metals two to twelve weeks after placement. Both studies showed an increase in Pb and Cd in soft tissues over time. Czarneski (1987) placed mussels immediately above and below the mining inputs in St. Francois County. The highest concentration of Pb and Cd occurred 12 weeks after placement and were 74.2 and 11.3 µ/g, respectively. Schmitt and Finger (1982) placed mussels throughout the river for eight weeks, including reaches near the current study at Washington State Park (RK~100) and Browns Ford (RK~80). Mean Pb and Cd concentrations at Washington State Park were 85 and 14.1 µ/g, respectively. Mean Pb and Cd concentrations at Browns Ford were 44 and 5.0 µ/g, respectively.

The concentrations observed for plain pocketbook are similar, but marginally lower than were measured for *corbicula* in this study, which could be due to the short term exposure of the caged mussels or interspecies differences.

Heavy-metal tissue concentrations from molluscan fauna in the literature were evaluated to determine whether *C. fluminea* in the Big River could be expected to exhibit a toxic response from metal exposure. Rainbow and Luoma (*in* Beyer and Meador 2011) compiled soft tissue metal concentration data from several authors in mollusks and other invertebrates and classified concentrations as "Typical" and "High" concentrations. "High" concentrations were defined as those that occur in atypically elevated bioavailability of metals for that localized habitat. The "High" concentration designation from Rainbow and Luoma (2011) can be interpreted to indicate absorbed concentrations that are elevated beyond the ability of an organism to regulate those metals. "Typical" dry weight concentrations for the marine mussel, *Mytilus edulis*, are given for the following metals:

Pb = 0.2-25 µ/g
Cd = 0.4-4.7 µ/g
Zn = 32-150 µ/g.

"High" dry weight concentrations for *Mytilus edulis* tissues are given below:

Pb = 58-105 µ/g
Cd = 21-65 µ/g
Zn = 198-579 µ/g.

Rainbow and Luoma (2011) discuss the very wide range of metals that occur as body burden between differing species and provide metal body burden in the tellinid clam, *Scrobicularia plana,* to illustrate this point. "Typical" dry weight concentrations of *Scrobicularia plana* are provided below:

Pb = 5-109 µ/g
Cd = 0.2-9.1 µ/g
Zn = 256-1514 µ/g.

"High" dry weight concentrations for *Scrobicularia plana* tissues are given below:

Pb = 225-3000 µ/g
Cd = 31.5-42.7 µ/g
Zn = 2060-4920 µ/g.

All but the most downstream *Corbicula fluminea* sample collected from Big River sites below the mining district had Pb tissue concentrations (52.37-133.0 µg/g Pb) that generally fit within the "High" concentration range for *Mytilus edulis* tissues found by Rainbow and Luoma (2011). However, all but one Big River *C. fluminea* tissue sample (23.17 µg/g Cd from Big113) were less than the "High" concentration range for Cd for

Mytilus edulis tissues. Tissue samples from the reference site in the Big River and the most downstream Big River site (Big2.5) had concentrations of 1.09 and 14.63 µg/g Pb and 0.61 and 3.54 µg/g Cd, respectively, that fit within the "Typical" concentration range of both species discussed in Rainbow and Luoma (2011).   Zn concentrations of Big River *C. fluminea* fit within the High range of *M. edulis* tissues and the "Typical" range for *S. plana.*

Liu et al. (2013) compared Zn body burdens and toxicity in laboratory exposures in the oyster (*Crassostrea hongkongensis*) collected from four different sites with varying degrees of contamination. They noted large differences in Zn sensitivity, and that oysters collected from the highly contaminated site had higher tolerance to Zn exposure. Oysters collected from a site with multi-metal contamination were more sensitive to Zn laboratory exposure than other sites. They concluded based on the variability of response that total body Zn concentration was not useful as a toxicity indicator. The species described by Rainbow and Luoma (2011) and Liu et al. (2013) are marine organisms and dramatic differences in ambient water chemistry could have an effect on ion regulation and presumably metal uptake between marine and freshwater organisms. As a result, care should be utilized in comparing Big River *C. fluminea* data to these marine reference species. Additionally, tolerance differences between wild organisms exposed to different concentrations over time make it difficult to make conclusions about toxic body burdens of metals between sites.

Sediment Textural Analyses of Habitat (Objective 3)

To isolate the effects of Pb contamination from mining-related alteration of substrate conditions, sampled habitats were examined to determine if they overlaid with mine tailings or fine sediment. Overall, all sites (including reference sites) contained a variety of substrate size classes for mussels as demonstrated by pebble counts (Table 9, Figure 10) grain size analysis (Figure 11), and substrate photos (Appendix D). This is characteristic of suitable mussel habitat in gravel bottom streams, as the presence of sand and fine gravel are important for mussels (Roberts and Bruenderman 2000, McMurray et al. 2012). Mussels are not typically found in high numbers where substrate is mostly composed of coarse materials or in depositional areas where fine sediments dominate. Sand and fine gravel-sized particles made up a relatively small percentage of the substrate at the surface for the majority of study sites, which substantiate that these habitats were not smothered with large volumes of fine gravel and sand. The diversity of sediment size classes and the presence of a coarse substrate layer overlaying a more mixed size class layer suggest the fluxes of sediment into and out of the sampling sites are in balance, providing the channel stability that is necessary for mussel establishment and longevity (Strayer 2008).

The grain-size analysis distinguishes sand from larger substrate and separates substrate smaller than sand (≤ 2mm dia.) into separate categories to characterize the smaller particle size classes (substrate fraction) typically associated with stable mussel habitat (Figure 11). The substrate size classes in this analysis include gravel (>2mm), medium to coarse sand (2 mm - 0.25 mm), fine sand (250 - 63 micron), and silt to clay

(<63 micron). Samples for particle size analysis were taken at all sites with the exception of Big67.5 and Big68 (see Methods Section). However, the grain sizes at these two sites would be expected to be similar to the sites immediately upstream and downstream based on pebble counts (Table 9). The overall grain size distribution among sites was variable with no clear longitudinal trends and was within the suitability ranges for mussels. Figure 12 shows the grain size distribution of the sites sampled by Roberts et al. (2010) in 2008. The same variability between sites was observed in 2008 as in 2014 when comparing figures 11 and 12. Only one of the sites (Big194, the upper reference site) was sampled in both 2009 and 2014. The sampling of that site shows similar results between 2008 and 2014. Similarly, Albers et al. (2016) did not find significant correlations between sand-size particles and mussel abundance. Abundance of two species were positively correlated with coarse substrates.   However, total mussel densities were more closely correlated with metals concentrations than with substrate variables.

Substrate photos also illustrate the suitability of habitat for mussels and document that substrate was not overlain with fines and/or mine tailings (Figure D1, Appendix D). Because of technical difficulties, photographs were not taken at three sites (Big20.5, Big113, and Big113.5), and 25 photos could not be taken at every site. The surface layer at most photo locations was composed of cobble and coarse gravel (covered with algae and diatoms) and was not covered with or impacted by finer substrate (Figure D2, Appendix D). This superficial layer was easily removed to reveal a substrate mixture of gravel, embedded cobble, and firm sand (Figure D2, Appendix D). Typically, living freshwater mussels were burrowed in this sandy, gravel mixture (Figure D3, Appendix D). Other areas within sample sites (including the Meramec River reference site) lacked the coarse armor layer (e.g. cobble), but provided firm substrate with a sand component that supported mussel burrowing. The substrate encountered at the Big River sampling sites was similar to substrate commonly found in Ozark streams possessing diverse mussel communities. A complete set of photographs taken at sampling sites are provided in Supplement A (https://www.fws.gov/Midwest/es/ec/nrda/SEMONRDA/index.html).

Patterns in Big River Mussel Species Richness Compared to Similar Missouri Streams (Objective 4)

Data sources and analyses used in comparing mussel and habitat characteristics between the Big River and other streams are shown in Table 4. As expected, we found a positive relationship between drainage area and richness in the Meramec and Gasconade River systems (F = 28.44, p < 0.0001, with overlapping overall species richness), supporting the use of the Meramec River and its tributaries unaffected by mining as an appropriate reference for comparison with the Big River (Figure 13). We observed no effect of river (no differences between the two streams) on the species-watershed area relationships for the Meramec and Gasconade rivers (F = 2.185, p = 0.141; Figure 13).

Species richness was positively related to drainage area in the Big and Bourbeuse rivers (F = 6.76, p < 0.01). However, in contrast to results from the Meramec and

Gasconade rivers, the effect of river was significant (large differences between the two streams) (F = 55.74, p < 0.0001; Figure 14). Linear regressions showed reduced species richness in the Big River by 5-7 species for any given watershed area in comparison with the Bourbeuse River. When the portions of the Big River that did not exceed the PEC were excluded, 70-75% lower richness was observed in affected portions of the Big River when compared to the mussel community associated with similar drainage areas in the Bourbeuse River.

LOESS fitted curves, which act as a moving indicator of the relationship between drainage area and species richness, show that sites furthest upstream in the Big and Bourbeuse rivers have a similar species richness-drainage area relationship for less than 400 km^2 of drainage area. Between 400 and 2000 km^2 of drainage area, species richness continues to increase in the Bourbeuse River with watershed size, levelling off at 1000 km^2, while species richness in the Big River remains low (Figure 15). At around 2350 km^2, the species richness-drainage area relationship in the Big River is again comparable to the relationship observed for the Bourbeuse River. The drainage area between 400-2000 km^2 corresponds with lead-affected reaches in the Big River. Changes in mussel species richness over time were not formally tested due to violations of statistical assumptions; however, plots indicate that species richness in the lower reaches of the Bourbeuse River decreased over the past 35 years (Appendix V). Over the same time period, species richness throughout most of the Big River (other than those sites noted above) remained low over the time period (Appendix V), consistent with Hinck et al. (2012).

The Effects of Habitat Features and Lead Concentrations on Mussel Assemblage Structure (Objective 4)

Based on the relative abundance of species, this analysis produced three robust clusters of sites, grouped based on assemblage similarity, at the AU alpha level of 0.9 (Figure 16). The first cluster contained four sites on the Big River from Big30.7 to Big49. A second cluster contained 10 sites on the Big River from Big32 to Big113. A third cluster represented sites on the Meramec and Bourbeuse rivers, the three most downstream sites on the Big River (Big2.5 to Big20.5), and the most upstream site (reference) on the Big River (Big194). Assemblages clustered according to proximity within the watershed, with the exception of the most upstream reference site in the Big River; instead of clustering with the adjacent sites downstream, it was most similar to the three most downstream sites on the Big River and reference sites on the Meramec and Bourbeuse Rivers. Assemblages of the first cluster were dominated by *E. dilatata* with an average species richness of 10 (Table 10). Assemblages of the second cluster had an average of five species present and were dominated by *L. cardium* and *L. brittsi*. The third assemblage had an average richness of 14 species and higher proportions of *Actinonaias ligamentina*, *Quadrula pustulosa*, and *Amblema plicata*. Among all sites within a cluster, a total of 14, 9 and 33 species were found in clusters 1, 2, and 3, respectively.

Based on examination of box plots (Figure 17) of environmental characteristics of sites within each the three clusters, some differences among clusters are apparent (Figure 17). Sites in the first cluster had intermediate Pb concentrations exceeding PEC and high percent cobble substrate. Cluster 2 contained sites with the highest Pb concentrations. Sites in cluster 3 had the lowest Pb concentrations, less cobble, and more sand. Overall, habitat features overlapped among the clusters. Lead contamination and percent cobble were the most distinct among clusters (Figure 17). Albers et al. (2016) evaluated the Big River 2014 data set and found that the presence of two mussel species was positively related to coarse substrates (*Pleurobema sintoxia* to fine gravel and *L. brittsi* to boulders). However, collectively their findings indicated that concentrations of Pb were negatively associated with the occurrence and density of individual mussel taxa, even when accounting for substrate variation (Albers et al. 2016).

CONCLUSIONS

Our overall analysis indicated that elevated Pb levels in sediment downstream of St. Francois County mine tailings along a significant portion of the Big River had a significant effect on mussels. Other metals did not appear to have as much impact longitudinally [for further discussions see Albers et al. (2016). Heavy metal concentrations in *C. fluminea* were negatively correlated with mussel density and positively correlated with sediment Pb concentrations. Lead concentration corresponded with multiple mussel assemblage characteristics in the Big River, namely, lower density of animals at the most contaminated sites, lower species richness, and an altered assemblage structure as measured by relative abundance. Densities were significantly lower in areas with sediment concentrations greater than the PEC. When compared to locations with a similar drainage area in the Bourbeuse River, the Big River had 70 to 75 percent fewer species in lead-contaminated areas in data collected between 1979 and the present.

Mussel assemblages in the Bourbeuse and Meramec rivers (without the impact of Pb mining) varied by substrate and drainage area, while these factors had reduced explanatory power in the Big River. In contrast, Pb sediment concentration in the Big River significantly corresponded with mussel community characteristics, perhaps overriding physical factors that would otherwise shape longitudinal patterns in mussel assemblage richness and structure (e.g., habitat size and diversity). Pb may interact with other habitat factors, particularly at sites dominated by *E. dilatata*.

Finally, the most upstream site on the Big River was clustered with assemblages in the Bourbeuse and Meramec rivers and the Big River downstream from areas with Pb contamination. Although this site is considerably further upstream than other sites included in the analysis, it is upstream of Pb mining and does not contain Pb contamination. This supports the designation of this site as a 'reference' and serves as additional evidence of the overriding impact of Pb on mussel assemblages in the Big River.

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Table 1: Federal and state status of freshwater mussel species found during previous studies in the Big River.[1]

Scientific name	Common name	Federal Status[2]	State Status[2]
Actinonaias ligamentina	Mucket		
Alasmidonta marginata	Elktoe		
Alasmidonta viridis	Slippershell Mussel		
Amblema plicata	Threeridge		
Cumberlandia monodonta	Spectaclecase	T	
Cyclonaias tuberculata	Purple Wartyback		
Ellipsaria lineolata	Butterfly		
Elliptio crassidens	Elephantear		E
Elliptio dilatata	Spike		
Fusconaia flava	Wabash Pigtoe		
Lampsilis abrupta	Pink Mucket	E	E
Lampsilis cardium	Plain Pocketbook		
Lampsilis siliquoidea	Fatmucket		
Lampsilis brittsi	Northern Brokenray		
Lampsilis teres	Yellow Sandshell		
Lasmigona complanata	White Heelsplitter		
Lasmigona costata	Fluted Shell		
Leptodea fragilis	Fragile Papershell		
Leptodea leptodon	Scaleshell	E	
Ligumia recta	Black Sandshell		
Megalonaias nervosa	Washboard		
Obliquaria reflexa	Threehorned Wartyback		
Plethobasus cyphyus	Sheepnose	E	
Pleurobema sintoxia	Round Pigtoe		
Potamilus alatus	Pink Heelsplitter		
Potamilus ohiensis	Pink Papershell		
Pyganodon grandis	Giant Floater		
Quadrula metanevra	Monkeyface		
Quadrula pustulosa	Pimpleback		
Quadrula quadrula	Mapleleaf		
Quadrula verrucosa	Pistolgrip		
Strophitus undulatus	Creeper		
Toxolasma parvum	Lilliput		
Truncilla donaciformis	Fawnsfoot		
Truncilla truncata	Deertoe		
Utterbackia imbecilis	Paper Pondshell		
Venustaconcha ellipsiformis	Ellipse		

[1] Buchanan 1979, Buchanan 1980, Oesch 1995, Roberts and Bruenderman 2000, Roberts et al. 2010, Williams et al. in review, McMurray et al. 2012.
[2] E = Federal or state endangered, T = Federally threatened

Table 2: Sediment and mussel Phase II survey sites in the Big and Meramec rivers sampled in 2013 and 2014.

River Name	River Kilometer	Site Name	County	Site Description
Meramec	75.6	Mer75.6	Jefferson	1.5 mi E of Pacific at Pacific Palisades Conservation Area
Big	2.5	Big2.5	Jefferson	3 mi. S of Eureka, along Route W
Big	16.5	Big16.5	Jefferson	1.5 mi. NW of House Springs at Rockford Beach
Big	20.5	Big20.5	Jefferson	2.7 mi. W of House Springs
Big	30.7	Big30.7	Jefferson	1 mi. W of Cedar Hill, downstream of Route 30
Big	41.0	Big41	Jefferson	2.7 mi NE of Morse Mill
Big	47.0	Big47	Jefferson	0.9 mi NE of Morse Mill
Big	67.5	Big67.5	Jefferson	3.3 mi SE of Grubville
Big	68.0	Big68	Jefferson	2.8 mi SE of Grubville
Big	86.0	Big86	Jefferson	1.4 mi NE of Fletcher
Big	91.0	Big91	Jefferson	2 mi SE of Fletcher
Big	105.7	Big105.7	Jefferson	3.7 mi NW of Blackwell
Big	106.5	Big106.5	Jefferson	3.5 mi NW of Blackwell
Big	107.5	Big107.5	Jefferson	3 mi NW of Blackwell
Big	108.0	Big108	Jefferson	2.8 mi NW of Blackwell
Big	113.0	Big113	Jefferson	1.3 mi NE of Blackwell
Big	113.5	Big113.5	Jefferson	1 mi NE of Blackwell
Big	194.0	Big194	Washington	2.3 mi SW of Irondale

Site Name	Metals Analysis of Sediment Sample: gravel bar adjacent to mussel habitat	Metals Analysis of Sediment Sample: In-mussel bed	Quantitative Mussel Sampling: Number of Visual/Sieved Quadrats	Quantitative Mussel Sampling: Number of Visual Quadrats Searched	Pebble Count	Grain Size Analysis	*Corbicula* Tissue Sample	Substrate Photos
Mer75.6	X	X	50	100	100	X	-	X
Big2.5	X*	X*	50	100	100	X	X	X
Big16.5	X*	X*	50	100	100	X	-	X
Big20.5	X	X	107	188	100	X	-	-
Big30.7	X	X	50	101	100	X	X	X
Big41	X	X	50	100	100	X	X	X
Big47	X	X	50	90	100	X	X	X
Big67.5	X	X	50	100	100	-	-	X
Big68	X	X	50	100	100	-	-	X
Big86	X	X	53	98	98	X	X	X
Big91	X	X	50	100	100	X	-	X
Big105.7	X	X	50	100	100	X	X	X
Big106.5	X	X	52	102	100	X	X	X
Big107.5	X	X	50	108	100	X	X	X
Big108	X	X	50	100	100	X	X	X
Big113	X	X	50	100	100	X	X	-
Big113.5	X*	X*	50	100	100	X	X	-

| Big194 | X | X | 50 | 100 | 100 | X | X | X |

Table 3: Summary of specific data collected at each study site in the Big River. * = quality control sample analyzed separately by the U.S. Geological Survey via Atomic Adsorption for metals confirmation.

Table 4: Study design identifying data sources and analyses used in evaluating mussel characteristics.

Mussel characteristic	Data source	Analysis
Species richness	MDC	Analysis of covariance
Density	USFWS 2013	Analysis of variance, Tukey's
Assemblage structure	USFWS 2008 & 2013 MU 2014	Cluster analysis
Assemblage and habitat relationships	USFWS 2008 & 2013 MU 2014	Canonical correspondence analysis

Table 5: Wolman and Wentworth substrate classifications used in data collection and simplified classification scheme used in this report.

Size range	Wolman	Wentworth	Current Report
<0.059	*Sand*	*Silt and Clay*	*Sand*
<2		Sand	
2-8	Fine gravel	Gravel	Gravel-Pebble
9-16	Medium gravel		
17-64	Coarse gravel	Pebble	
64-256	Cobble	Cobble	Cobble
>256	Boulder	Boulder	Boulder

Table 6: Concentrations of Pb and Zn in <250 µm, <2 µm, and bulk sediments collected from gravel bars (a) and within mussel beds (b) in the Big River as determined by XRF. LOD = limit of detection.

a.

Site Name	<250µm (ppm) Pb	Zn	<2 mm (ppm) Pb	Zn	Bulk (ppm) Pb	Zn
Mer75.6	< LOD	18	2	18	5	20.72
Big2.5	322	136	108	55	110	50.1
Big16.5	62	25	48	29	39	22
Big20.5	1962	611	154	67	110	47
Big30.7	988	326	162	70	310	137
Big41	1477	534	189	89	158	109
Big47	1309	298	322	84	278	70
Big67.5	864	211	452	111	365	125
Big68	629	190	391	137	312	130
Big86	1148	292	274	107	619	262
Big91	2179	714	186	81	357	132
Big105.7	2011	658	471	197	346	136
Big106.5	927	313	174	73	368	204
Big107.5	2177	607	334	134	NA	NA
Big108	2427	686	344	120	NA	NA
Big113	3081	988	391	154	306	136
Big113.5	892	406	495	230	488	248
Big194	41	49	11	4	15	< LOD

b.

Site Name	<250µm (ppm) Pb	Zn	<2 mm (ppm) Pb	Zn	Bulk (ppm) Pb	Zn
Mer75.6	< LOD	12	< LOD	1	< LOD	< LOD
Big2.5	208	81	110	52	180	90
Big16.5	258	84	94	47	67	30
Big20.5	599	228	92	51	123	66
Big30.7	252	104	175	78	156	67
Big41	545	180	234	92	268	110
Big47	297	106	169	60	187	76
Big67.5	546	147	245	214	197	108
Big68	300	88	214	71	211	72
Big86	858	291	427	166	478	358
Big91	533	179	248	84	230	91
Big105.7	1596	435	708	228	500	183
Big106.5	987	381	358	155	391	165
Big107.5	851	276	294	113	538	195
Big108	1049	304	544	193	488	191
Big113	905	355	348	137	429	149
Big113.5	1481	679	298	111	323	347
Big194	55	47	21	27	12	< LOD

Table 7: Species and numbers of mussels found during quantitative sampling at each site in the Big River in 2013 and 2014. D = dead shell, SF = Subfossil shell.

Scientific name	Mer75.6	Big2.5	Big16.5	Big20.5	Big30.7	Big41	Big47	Big67.5	Big68	Big86
Actinonaias ligamentina	19	121	5	-	SF	SF	SF	-	-	-
Alasmidonta marginata	2	4	2	-	4	1	10	1	4	2
Alasmidonta viridis	-	-	-	-	-	-	-	SF	-	-
Amblema plicata	31	6	71	1	1	-	1	SF	-	-
Cumberlandia monodonta	-	1	-	-	-	-	-	-	-	-
Cyclonaias tuberculata	-	3	-	-	-	SF	SF	-	-	-
Ellipsaria lineolata	1	9	2	-	-	-	-	-	-	-
Elliptio crassidens	-	-	-	-	SF	-	-	-	-	-
Elliptio dilatata	-	118	12	-	44	103	127	-	SF	-
Fusconaia flava	1	1	1	1	6	2	8	7	3	2
Lampsilis brittsi	-	-	-	-	1	1	4	8	6	2
Lampsilis cardium	10	4	12	1	9	8	8	7	6	3
Lampsilis teres	-		-	-	-	-	-	-	-	-
Lasmigona costata	-	4	1	-	1	-	-	-	-	-
Leptodea fragilis	5	3	6	1	1	-	1	-	-	-
Leptodea leptodon	6	1	-	-	1	-	-	-	-	-
Ligumia recta	2	7	1	-	SF	-	-	-	-	-
Megalonaias nervosa		2	-	-	-	-	-	-	-	-
Obliquaria reflexa	20	24	9	2	-	-	-	-	-	-
Plethobasus cyphyus	5	-	-	-	-	-	-	-	-	-
Pleurobema sintoxia	14	33	1	-	SF	SF	SF	-	1	-
Potamilus alatus	2	4	5	D	1	-	2	6	1	-
Pyganodon grandis	-	-	-	-	D	-	-	-	-	-
Quadrula metanevra	26	-	-	-	-	-	-	-	-	-
Quadrula pustulosa	24	11	23	5	1	1	6	-	-	-
Quadrula quadrula	1		-	-	-	-	-	-	-	-
Quadrula verrucosa	-	15	5	-	SF	SF	1	SF	SF	SF
Strophitus undulatus	1	2	-	-	2	1	3	D	4	SF
Toxolasma parvum	-	-	-	-	-	-	-	D	-	-
Truncilla donaciformis	3		1	-	-	-	-	-	-	-
Truncilla truncata	12	3	20	-	1	-	-	-	-	-
Venustaconcha ellipsiformis		3	-	-	-	-	-	-	-	-
Total number of living individuals	185	379	177	11	77	117	171	29	25	9
Total number of live species	19	22	17	6	13	6	11	5	7	4

Table 7 con't: Species and numbers of mussels found at each site in the Big River in 2013 and 2014. D = dead shell, SF = Subfossil shell.

Scientific name	Big91	Big105.7	Big106.5	Big107.5	Big108	Big113	Big113.5	Big194
Actinonaias ligamentina	-	SF	-	-	-	-	-	-
Alasmidonta marginata	-	3	-	1	1	-	-	-
Alasmidonta viridis	1	1	-	-	-	-	-	-
Amblema plicata	-	SF	-	SF	-	-	-	-
Cumberlandia monodonta	-	-	-	-	-	-	-	-
Cyclonaias tuberculata	-	-	-	-	-	-	-	-
Ellipsaria lineolata	-	-	-	-	-	-	-	-
Elliptio crassidens	-	-	-	-	-	-	-	-
Elliptio dilatata	SF	-	-	SF	-	-	-	-
Fusconaia flava	4	1	-	SF	1	4	-	-
Lampsilis brittsi	3	5	4	-	2	9	-	36
Lampsilis cardium	4	3	6	5	14	10	D	20
Lampsilis teres	-	-	-	-	-	-	-	-
Lasmigona costata	-	-	-	-	-	-	-	-
Leptodea fragilis	1	-	-	-	-	1	-	-
Leptodea leptodon	-	-	-	-	-	-	-	-
Ligumia recta	-	-	-	-	-	-	-	-
Megalonaias nervosa	-	-	-	-	-	-	-	-
Obliquaria reflexa	-	-	-	-	-	-	-	-
Plethobasus cyphyus	-	-	-	-	-	-	-	-
Pleurobema sintoxia	SF	-	-	-	-	-	-	-
Potamilus alatus	3	-	-	-	3	-	-	-
Pyganodon grandis	-	-	-	-	-	-	-	-
Quadrula metanevra	SF	-	-	-	-	-	-	-
Quadrula pustulosa	SF	-	-	-	-	-	-	-
Quadrula quadrula	-	-	-	-	-	-	-	-
Quadrula verrucosa	SF	-	-	-	-	-	-	-
Strophitus undulatus	1	-	-	-	-	-	-	11
Toxolasma parvum	-	-	-	-	D	D	-	-
Truncilla donaciformis	-	-	-	-	-	-	-	-
Truncilla truncata	-	-	-	-	-	-	-	-
Venustaconcha ellipsiformis	-	-	-	-	-	-	-	61
Total number of living individuals	17	13	10	6	21	24	0	128
Total number of live species	7	5	2	2	5	4	0	4

Table 8: Statistical results for overall mean mussel density (mussels/m^2) found at quantitative survey sites in the Big River and reference sites. Means with same letter are not significantly different (One way ANOVA with rank-transformed data [p<0.0001] and mean comparisons with Tukey's test). "n" = number of samples.

Site Name	Stream	Mean mussel density	n	Standard error	Minimum	Maximum	Tukey's test
Mer75.6	Meramec	6.2	150	0.12	0	6	b
Big2.5	Big	11.3	150	0.19	0	11	a
Big16.5	Big	6.11	150	0.61	0	7	b
Big20.5	Big	0.20	295	0.08	0	2	d
Big30.7	Big	2.92	151	0.51	0	5	c
Big41	Big	3.55	150	0.48	0	6	c
Big47	Big	5.71	140	0.62	0	6	b
Big67.5	Big	0.77	150	0.14	0	2	d
Big68	Big	1.2	150	0.29	0	2	d
Big86	Big	0.38	150	0.12	0	1	d
Big91	Big	0.51	150	0.12	0	2	d
Big105.7	Big	0.5	150	0.15	0	1	d
Big106.5	Big	0.21	154	0.14	0	2	d
Big107.5	Big	0.15	158	0.09	0	1	d
Big108	Big	0.46	150	0.15	0	2	d
Big113	Big	0.68	150	0.08	0	2	d
Big113.5	Big	0	150	0	0	0	d
Big194	Big	3.8	150	0.42	0	4	c

Table 9:   Results of Wolman pebble counts within mussel sampling sites on the Big and Meramec Rivers. Sample size N=100 at each site.

Site	Substrate						
	No. of Samples	Sand (<2mm)	Fine Gravel (2-8mm)	Medium Gravel (9-16mm)	Coarse Gravel (17-64mm)	Cobble (65-256mm)	Boulder (>256mm)
Mer75.6	100	32	14	18	34	2	0
Big2.5	100	9	12	8	27	37	6
Big16.5	100	9	22	24	39	6	0
Big20.5	100	42	14	19	24	1	0
Big30.7	100	7	5	3	41	31	13
Big41	100	0	2	14	33	30	21
Big47	100	6	7	11	37	34	5
Big67.5	100	6	0	6	42	36	10
Big68	100	6	9	8	44	26	7
Big86	98	3	4	10	65	16	2
Big91	100	5	0	4	24	37	30
Big105.7	100	2	3	9	55	24	7
Big106.5	100	14	4	10	48	23	1
Big107.5	100	8	6	10	65	11	0
Big108	100	10	1	5	45	37	2
Big113	100	11	4	8	24	44	9
Big113.5	100	86	4	5	5	0	0
Big194	100	13	3	1	20	45	18

Table 10: Average proportional abundance and species richness for sites in each cluster (% numerical abundance).

| Common name	Scientific name	Cluster 1	Cluster 2	Cluster 3
Mapleleaf	*Quadrula quadrula*	0.00	0.00	0.94
Monkeyface	*Quadrula metanevra*	0.00	0.00	1.80
Sheepnose	*Plethobasus cyphyus*	0.00	0.00	0.51
Washboard	*Megalonaias nervosa*	0.00	0.00	0.54
Fatmucket	*Lampsilis siliquoidea*	0.00	0.00	0.36
Purple wartyback	*Cyclonaias tuberculata*	0.00	0.00	0.04
Spectaclecase	*Cumberland monodonta*	0.00	0.00	1.18
Slippershell	*Alasmidonta viridis*	0.00	1.51	0.00
Fawnsfoot	*Truncilla donaciformis*	0.00	0.00	2.54
Ellipse	*Venustaconcha ellipsiformis*	0.00	0.00	9.15
Pistolgrip	*Quadrula verrucosa*	0.19	0.00	3.42
Black sandshell	*Ligumia recta*	0.00	0.00	0.72
Scaleshell	*Leptodea leptodon*	0.43	0.00	1.29
Fluted shell	*Lasmigona costata*	0.43	0.00	0.09
Butterfly	*Ellipsaria lineolata*	0.00	0.00	2.33
Mucket	*Actinonaias ligamentina*	0.00	0.00	11.76
Threehorn wartyback	*Obliquaria reflexa*	0.00	0.00	5.68
Round pigtoe	*Pleurobema sintoxia*	0.00	0.44	8.86
Deertoe	*Truncilla truncata*	0.43	0.00	3.39
Spike	*Elliptio dilatata*	73.15	0.00	5.25
Threeridge	*Amblema plicata*	0.63	0.00	10.62
Pimpleback	*Quadrula pustulosa*	3.62	0.00	10.97
Fragile papershell	*Leptodea fragilis*	0.63	1.12	2.03
Creeper	*Strophitus undulatus*	1.74	2.43	1.00
Pink heelsplitter	*Potamilus alatus*	0.82	6.29	2.85
Elktoe	*Alasmidonta marginata*	3.97	9.58	0.95
Northern brokenray	*Lampsilis brittsi*	1.50	24.10	1.32
Wabash pigtoe	*Fusconaia flava*	4.73	12.33	3.98
Plain pocketbook	*Lampsilis cardium*	7.73	42.19	4.03
Pink Mucket	*Lampsilis abrupta*	0.00	0.00	0.16
Snuffbox	*Epioblasma triquetra*	0.00	0.00	1.13
White Heelsplitter	*Lasmigona complanata*	0.00	0.00	0.24
Yellow Sandshell	*Lampsilis teres*	0.00	0.00	0.56
Lilliput	*Toxolasma parvum*	0.00	0.00	0.32
Average species richness		10	5	14
Number of species in cluster		14	9	33
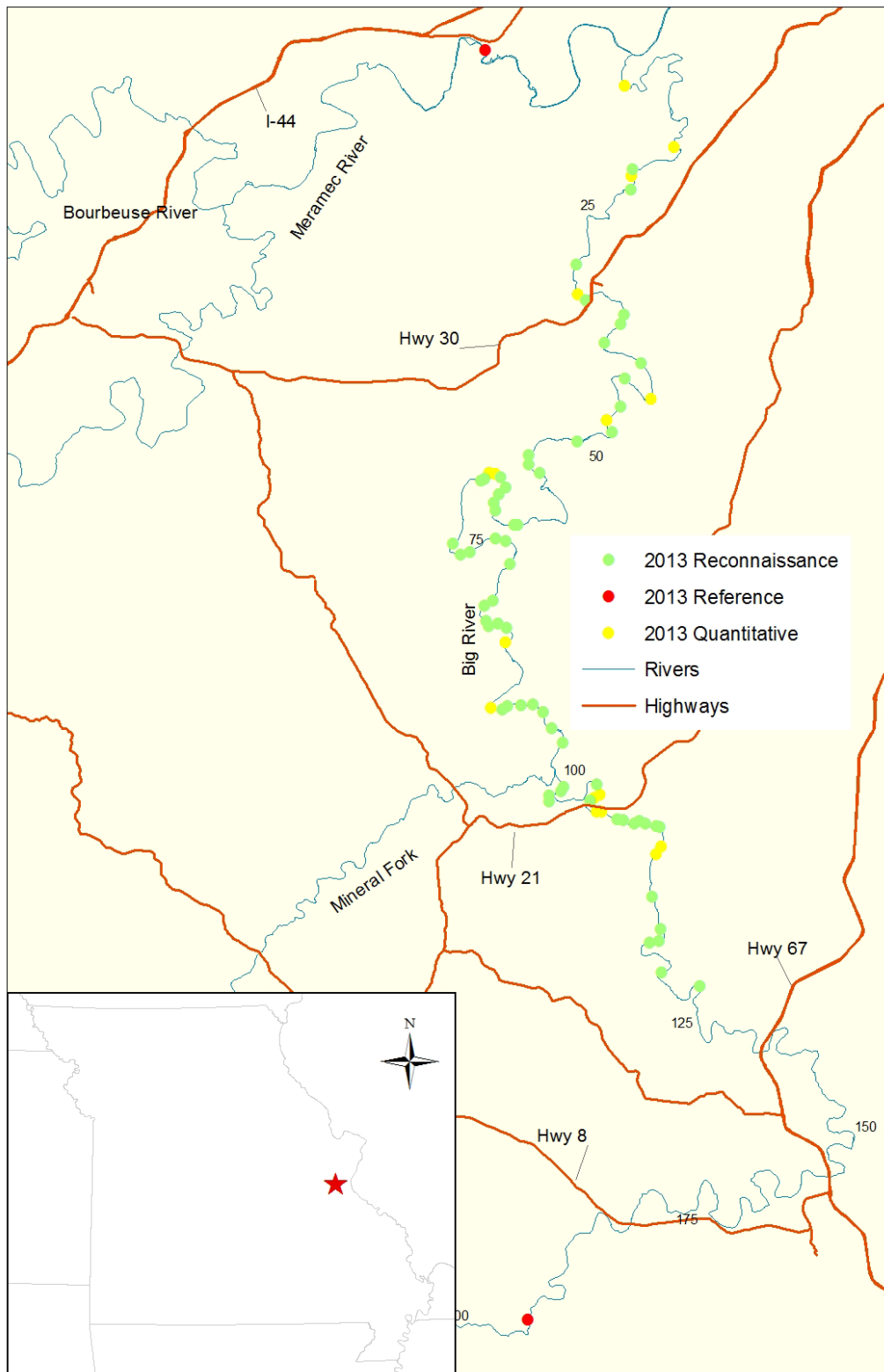
Figure 1: Map of study area showing reconnaissance, quantitative, and reference quantitative mussel sampling sites on the Big and Meramec rivers. River kilometers are shown for the Big River.
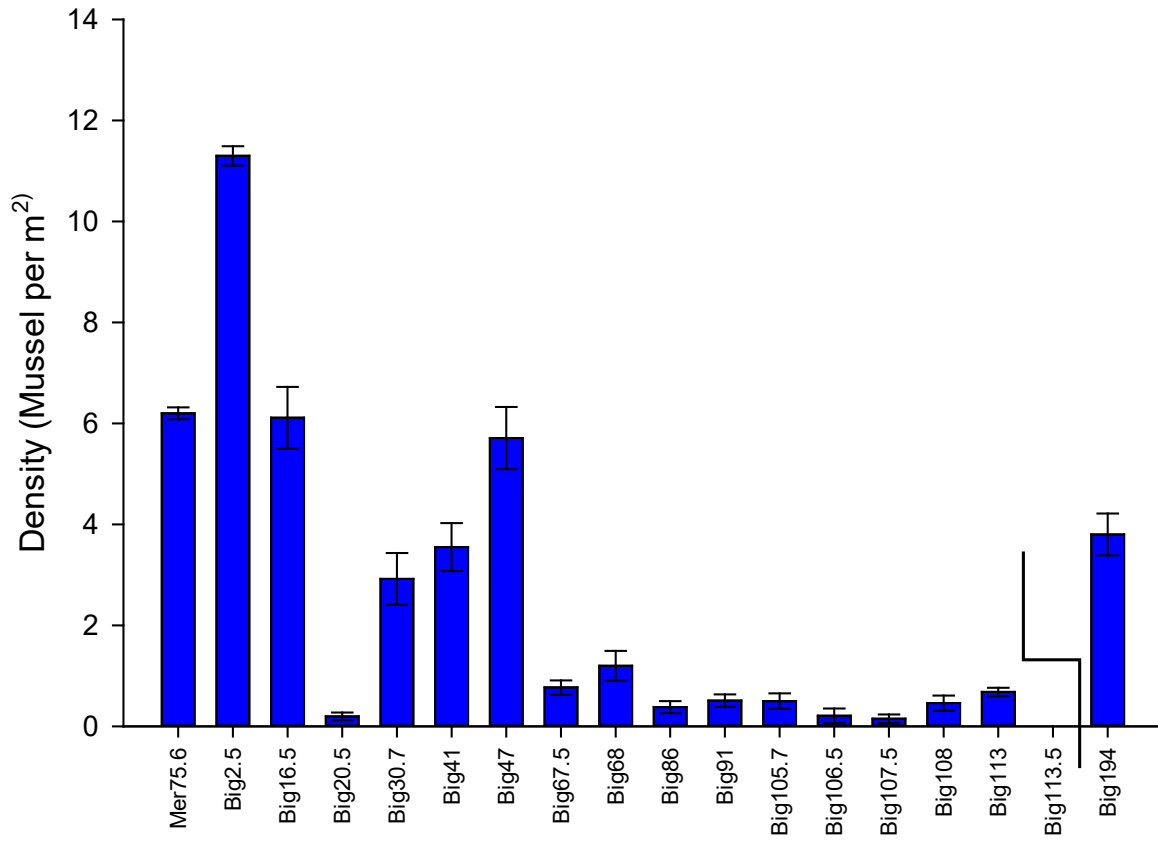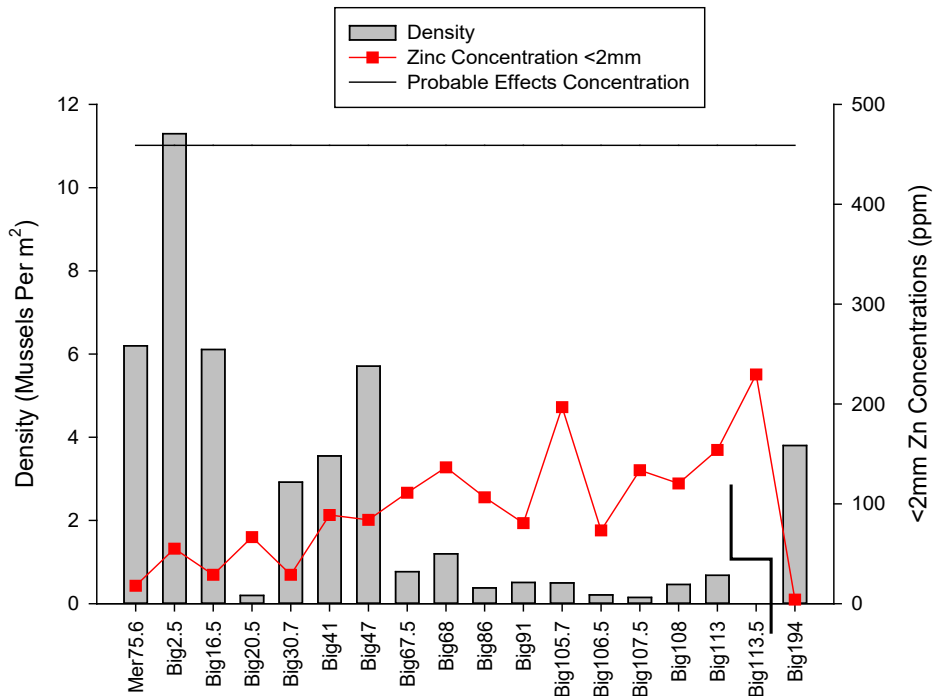
Figure 2: Mean mussel density estimated by quantitative sampling in the Meramec (Mer) and Big Rivers with standard error bars. ⌐ = large break in stream kilometers.
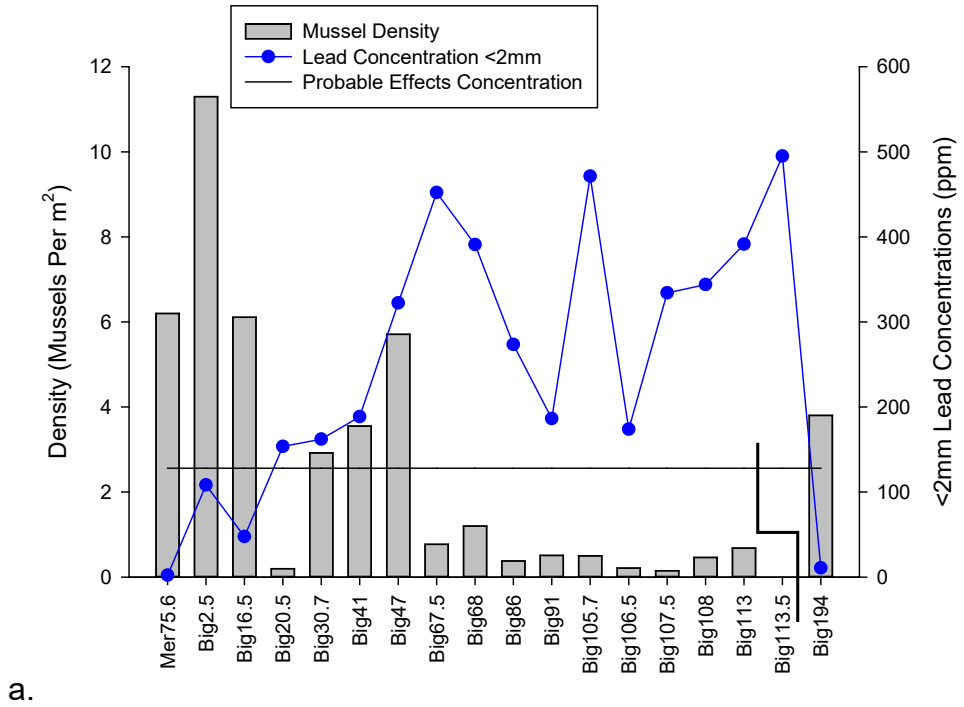
a.

b.

Figure 3 a and b: Mean mussel density (mussels/m^2) and concentration of Pb (a.) and Zn (b.) in <2 mm <u>gravel bar sediments</u> in the Big River. ⌐ = large break in the stream kilometers.
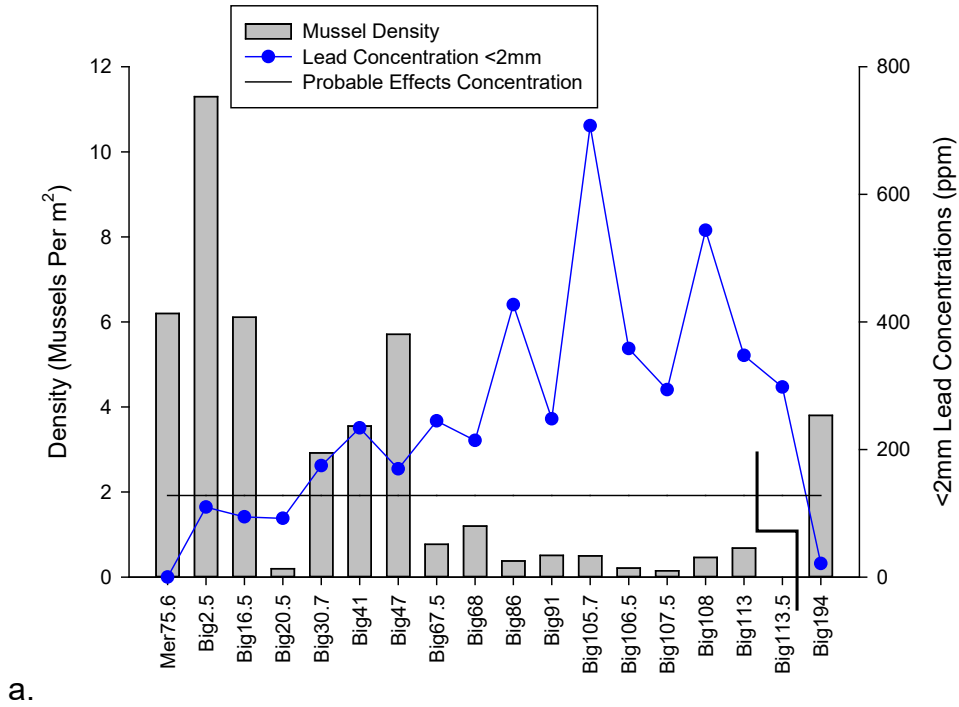
a.
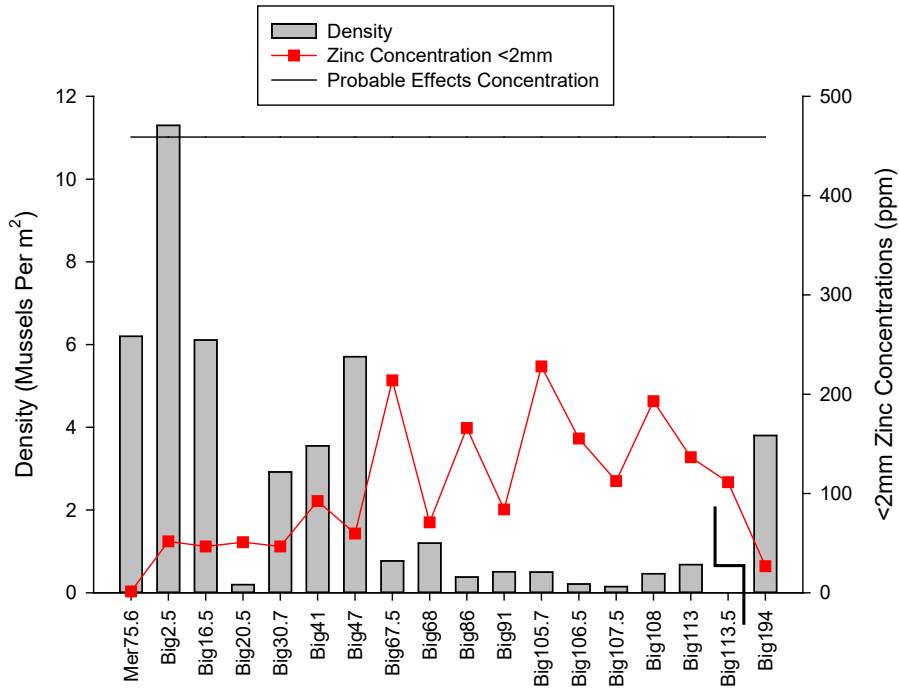
b.

Figure 4 a. and b.: Mean mussel density (mussels/m^2) and concentration of Pb (a.) and Zn (b.) in <2 mm <u>mussel bed sediments</u> in the Big River. ⌐ = large break in the stream kilometers.
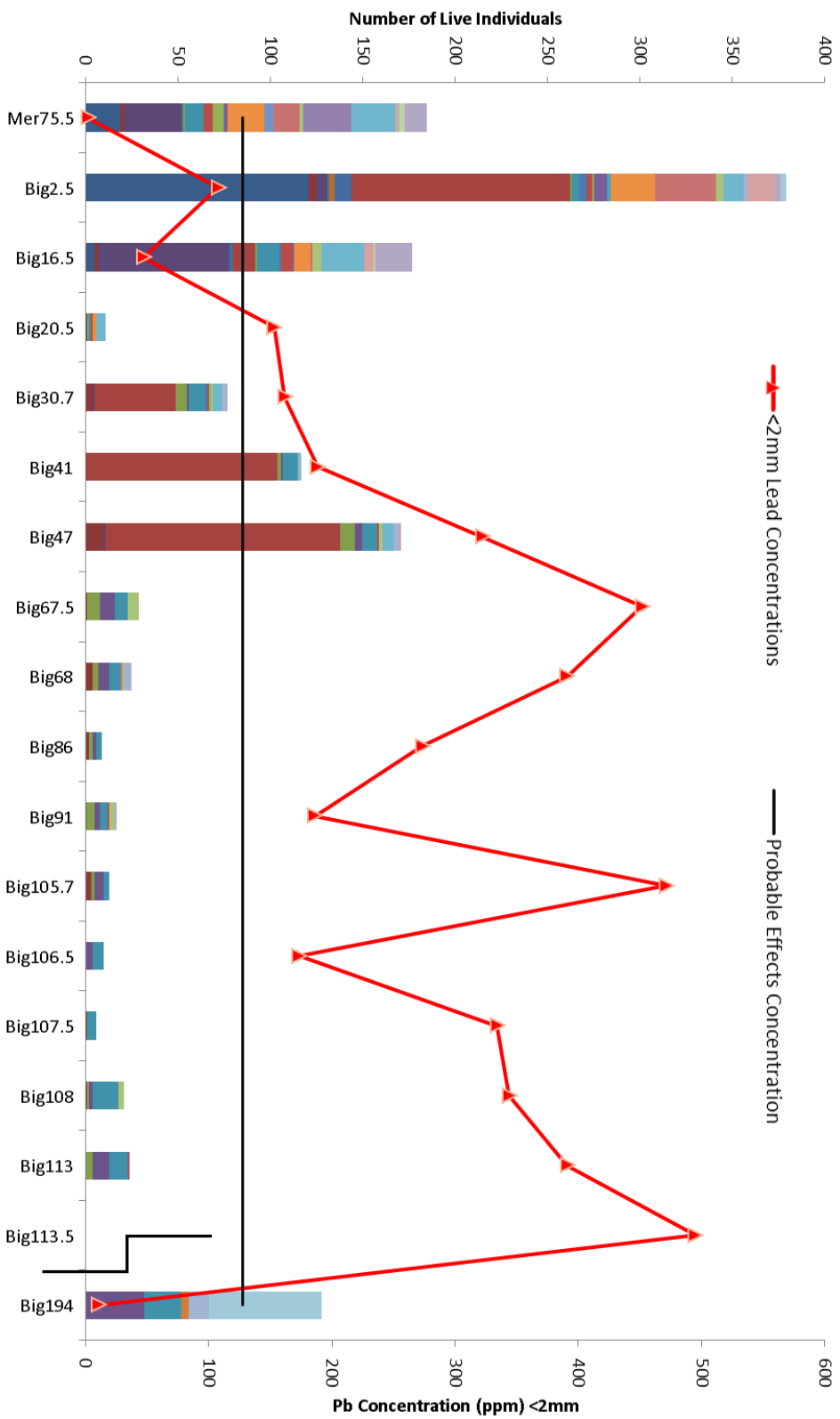
Figure 5: Number of individuals collected in the Big and Meramec rivers during quantitative surveys and concentration of Pb in <2mm mussel bed sediments. Each bar color represents a living species found at the site (note: colors do not correspond to species in Figure 6). ⌐= large break in the stream kilometers.
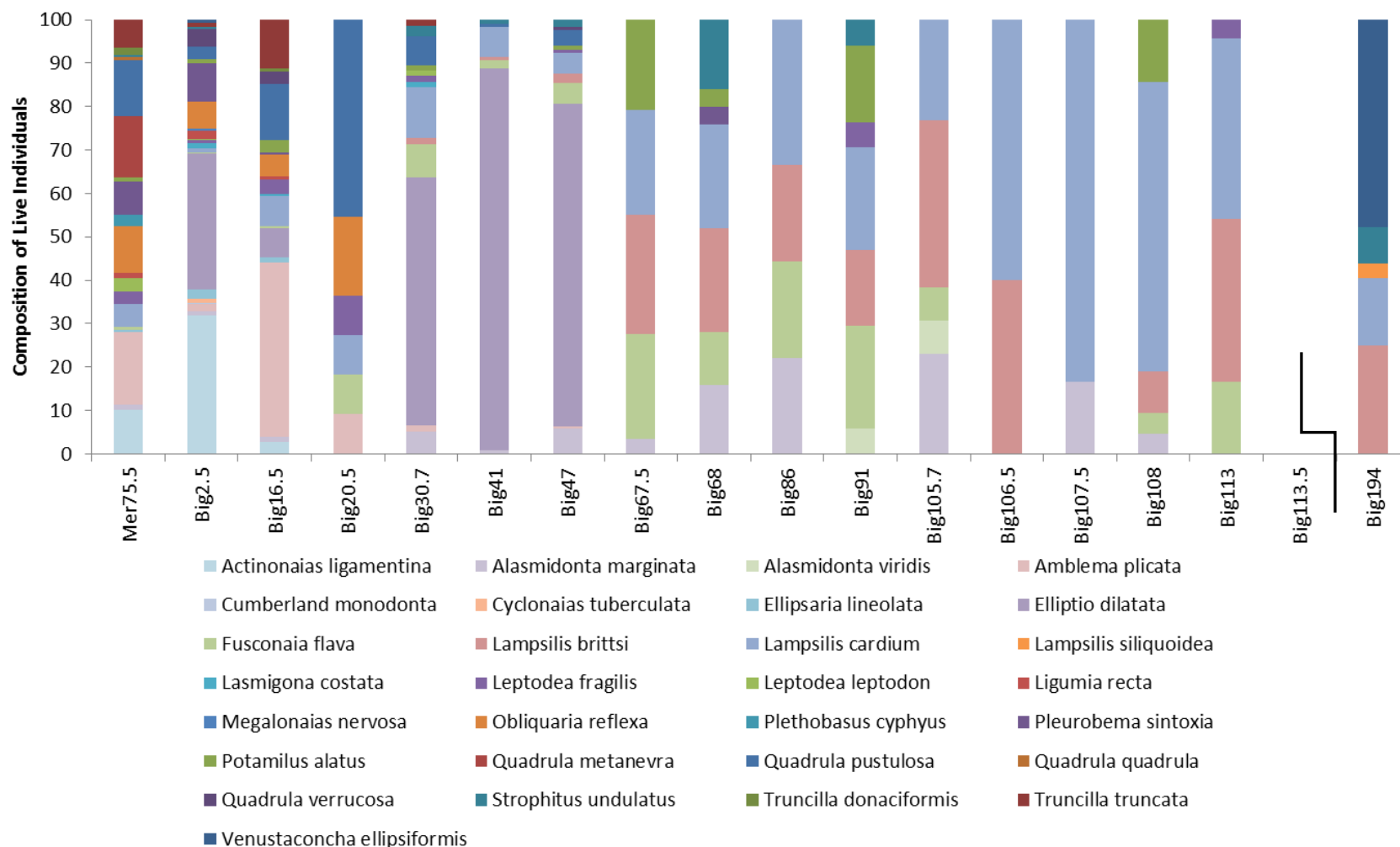
Figure 6. Species composition of mussels collected during quantitative surveys in the Big River by river kilometer. Species are arranged on the bars in alphabetical order from bottom to top. ⌐ = large break in the stream kilometers.
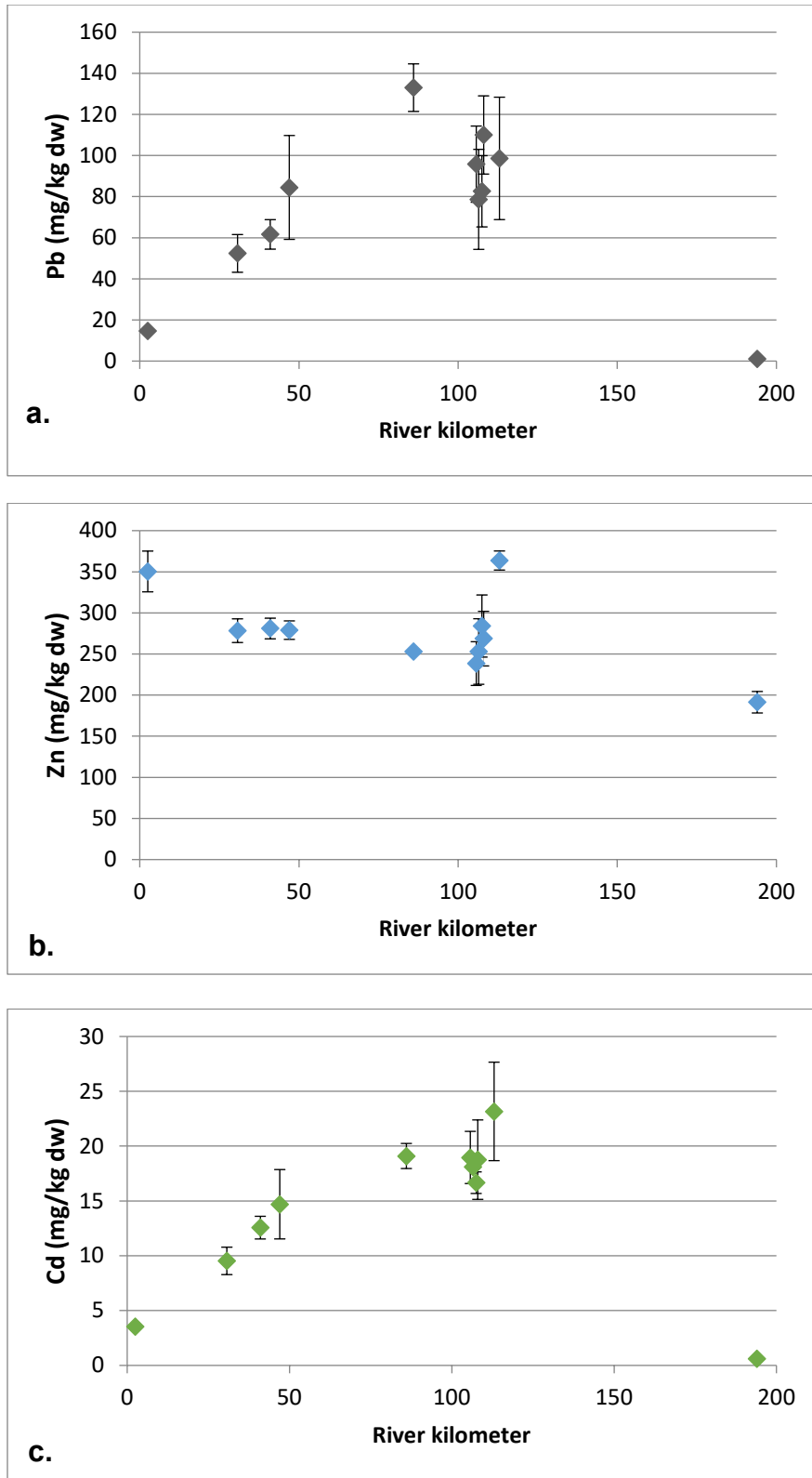
**Figure 7 a.b.c**: Site mean concentrations (µg/g dry weight) of Pb, Zn, and Cd in *Corbicula fluminea* tissue samples collected in the Big River (Error bars ± 1 SD).

Mean *Corbicula fluminea* Pb Tissue vs Mussel Density

y = -5.9327x + 91.867
R² = 0.4357

Mussel Density

Corbicula Tissue Pb in mg/kg

a.

Mean *Corbicula fluminea* Pb Tissue vs Mussel Density without reference site

y = -5.6306x + 97.798
R² = 0.6264

Mussel Density

Corbicula Tissue Pb in mg/kg

b.
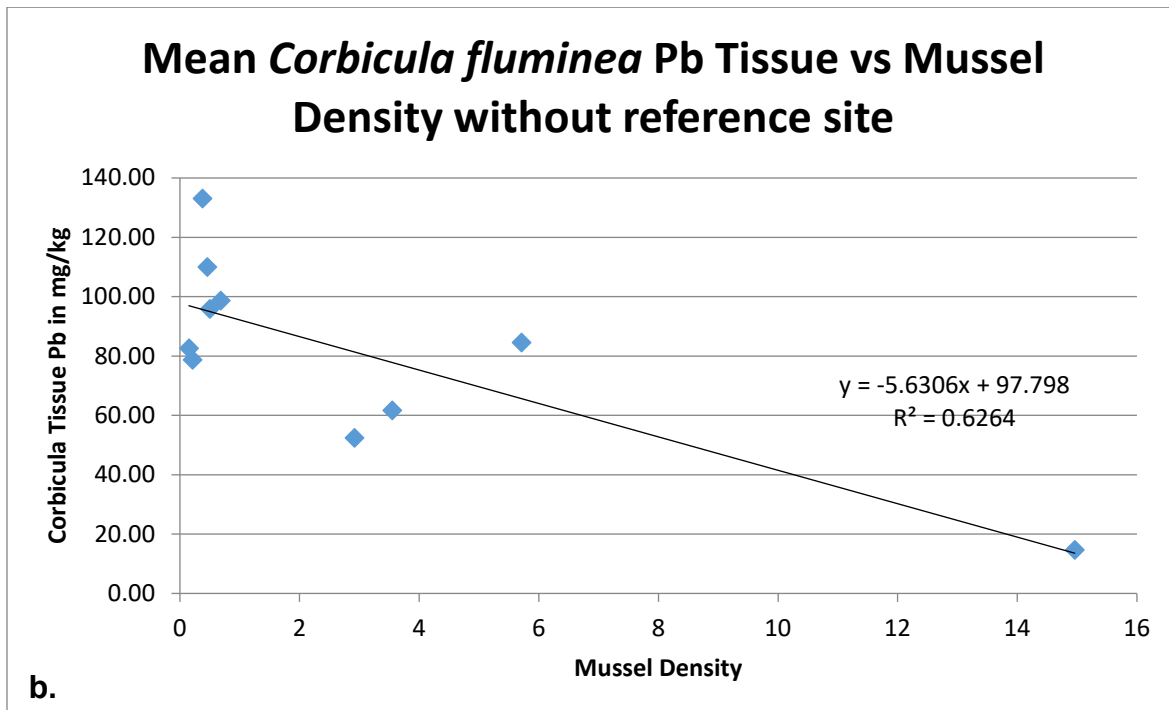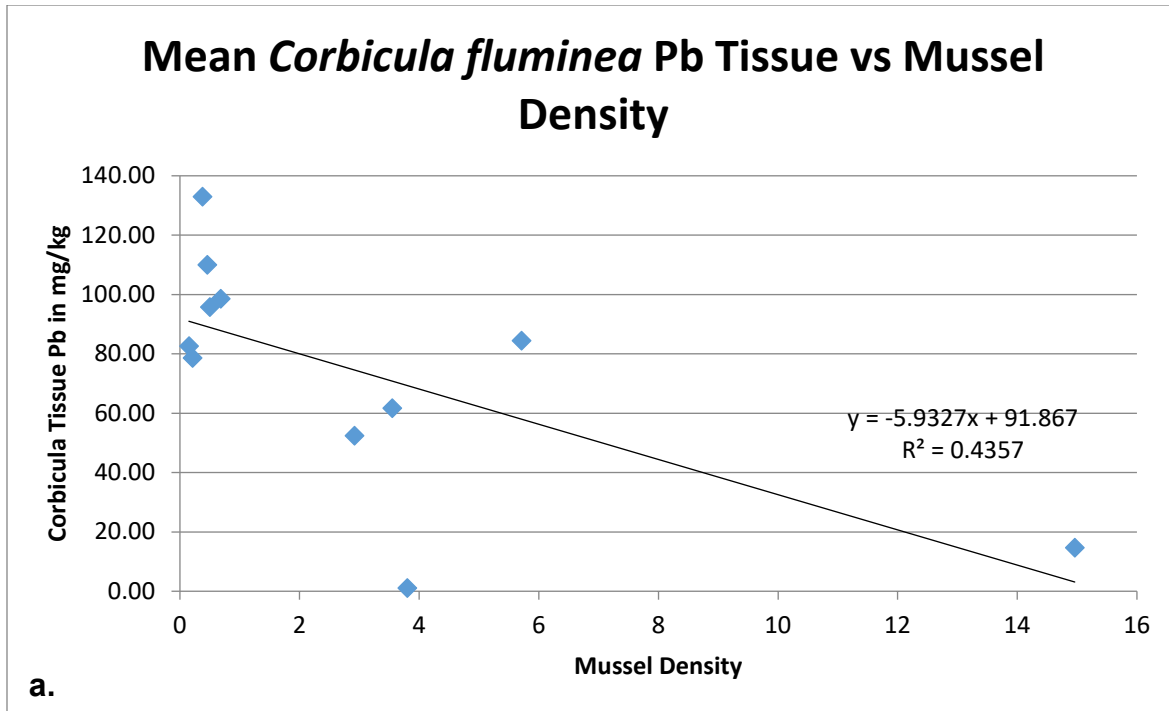
Figure 8. Mussel density vs. *Corbicula fluminea* tissue concentrations (ug/g dry weight) with (a.) and without (b.) reference sites, respectively.
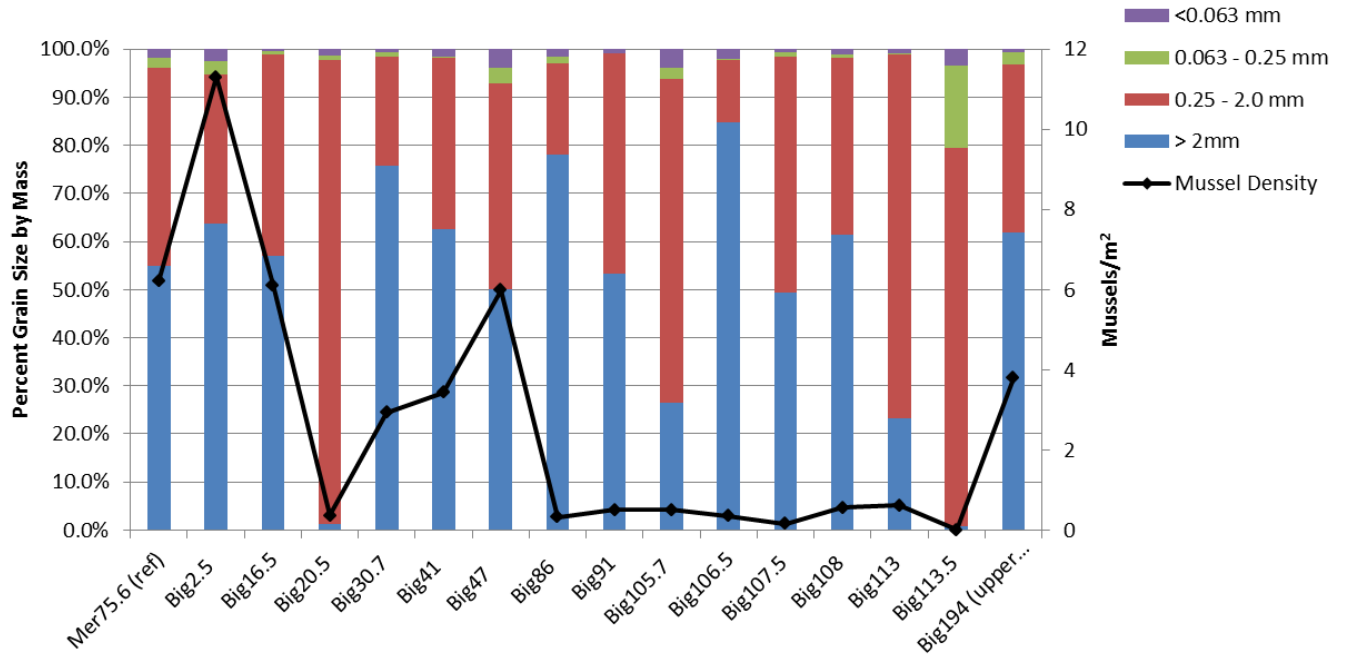
Mean *Corbicula fluminea* Tissue vs < 2mm Sediment Pb

y = 0.1492x + 26.815
R² = 0.5318
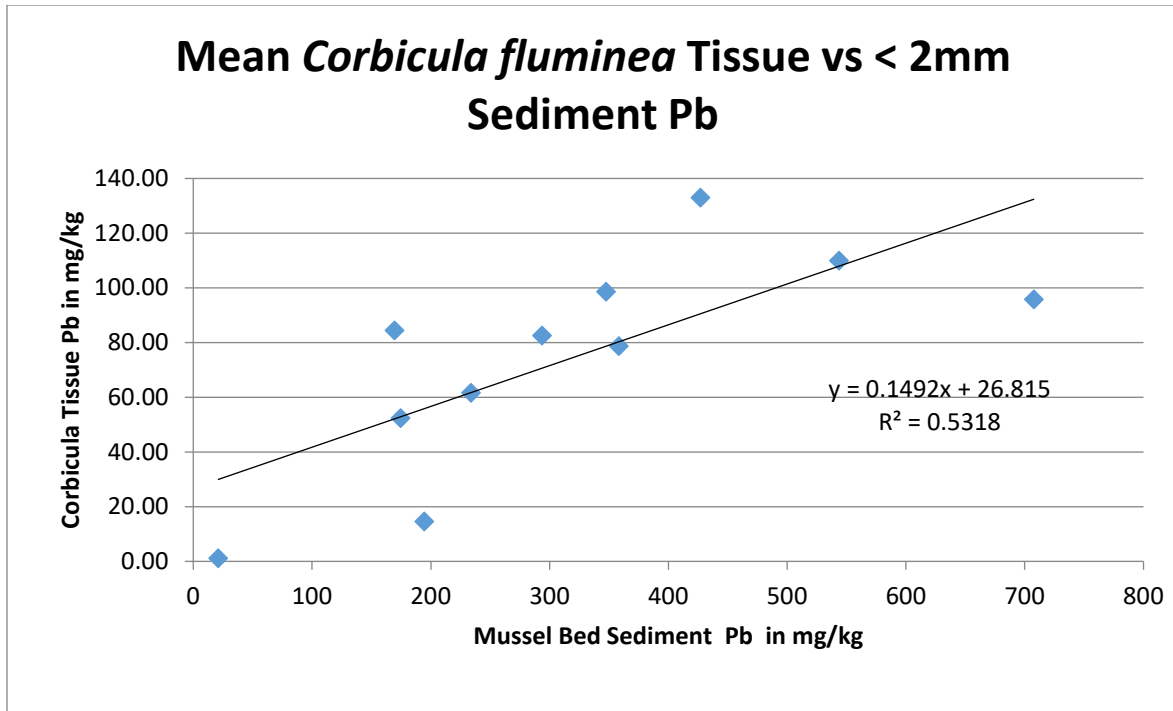
Figure 9. Mean *Corbicula fluminea* tissue Pb vs. <2mm mussel bed sediment Pb concentration.
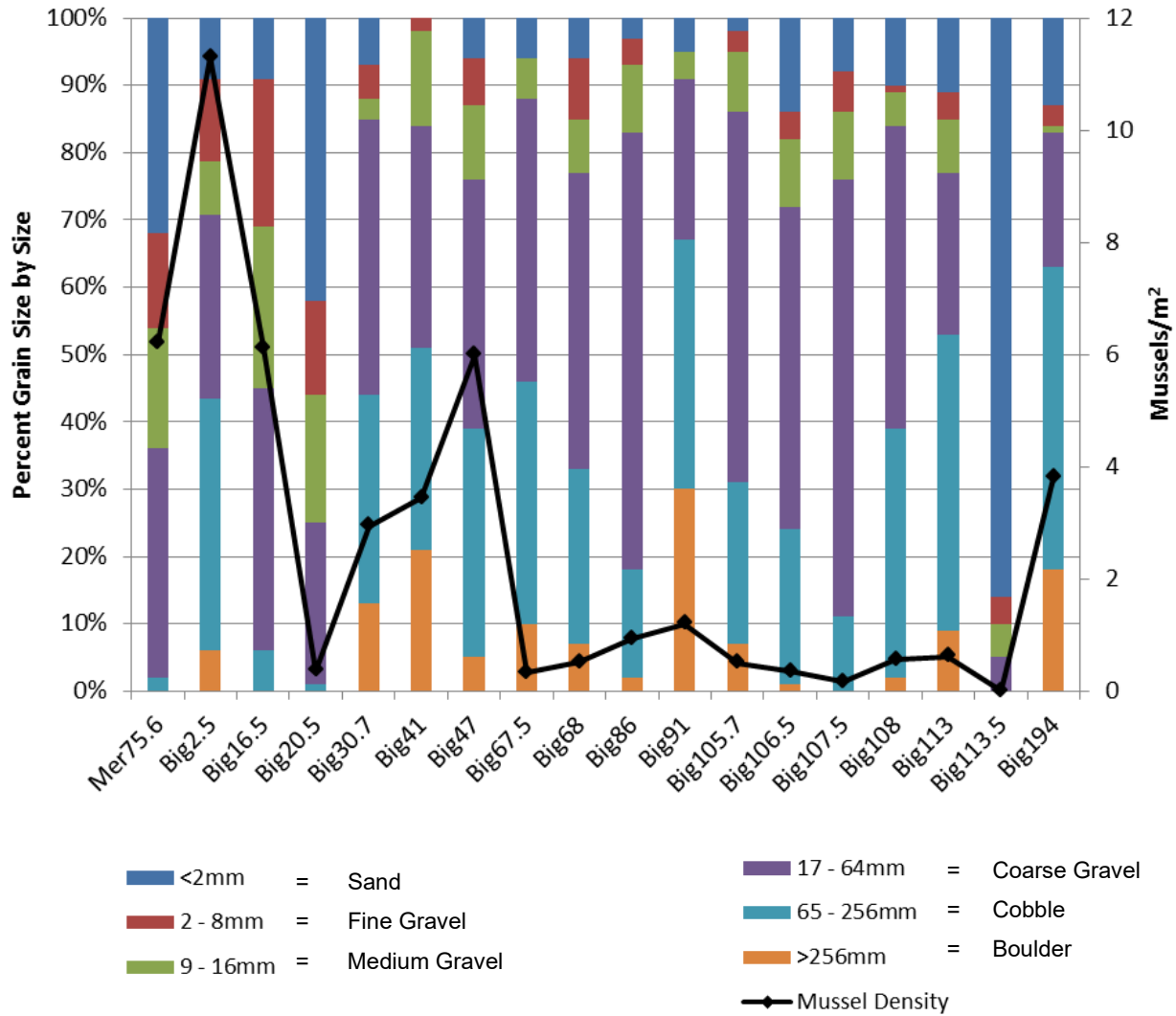
Figure 10: Substrate composition from Wolman pebble counts and mussel density collected in the Big River and Meramec Rivers. The Wolman pebble count method does not subdivide size classes < 2mm.
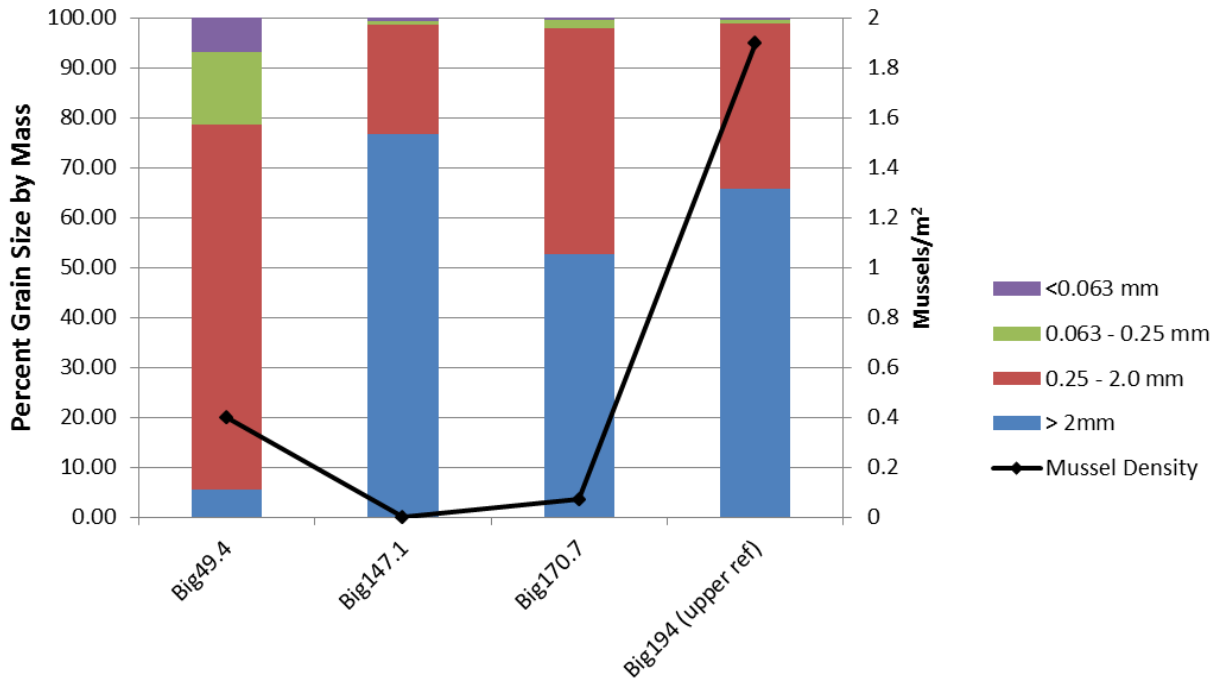
Figure 11: Grain size distribution of sediments by percent mass and mussel density collected in the Big River and Meramec Rivers. The USGS grain size fraction analysis does not subdivide size classes > 2mm.
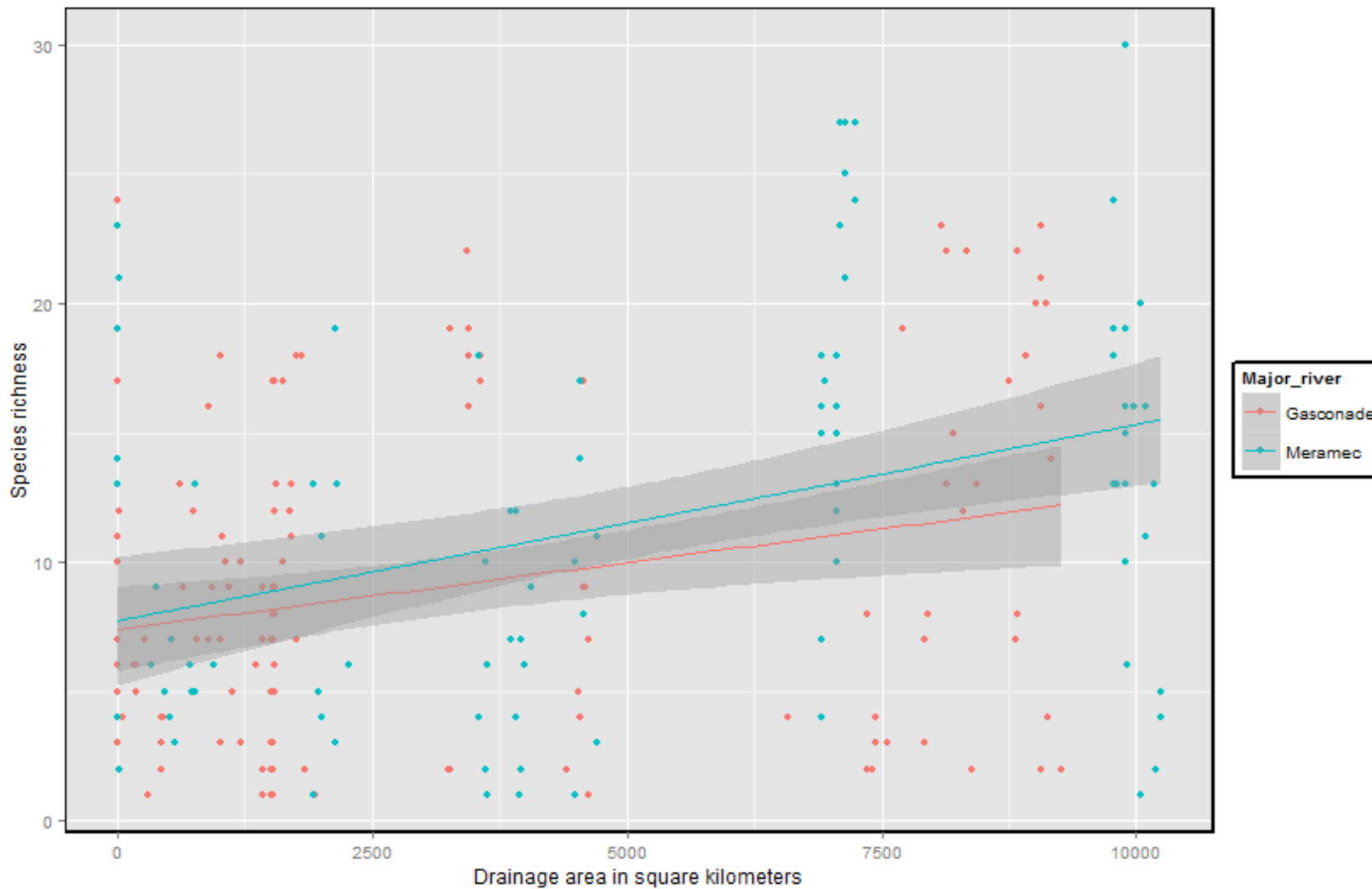
Figure 12: Grain size distribution by percent mass and mussel density for Big River sites where both these data were collected in 2008 (Roberts et al. 2010). The USGS grain size fraction analysis does not separate size classes > 2mm.

Figure 13: Linear regressions and 95% confidence interval of species richness by drainage area for the Meramec and Gasconade rivers indicated anticipated similarity among the two rivers in this relationship.
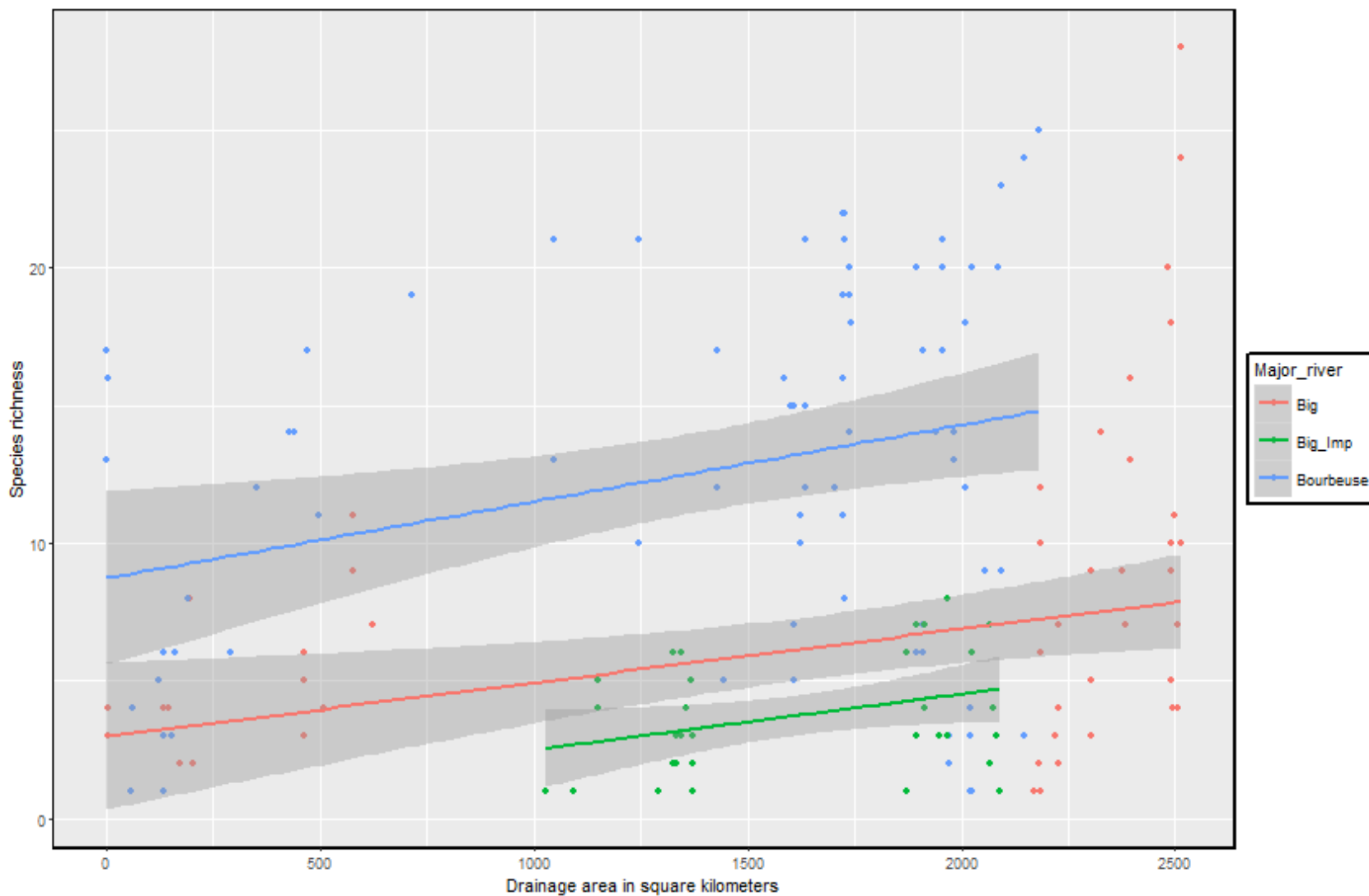
Figure 14. Linear regressions and 95% confidence interval of species richness by drainage area in the Big and Bourbeuse rivers indicated species richness in the Big River was significantly lower than in the Bourbeuse River for a given watershed size. Those sites identified as greater than the PEC are also shown separately in green.
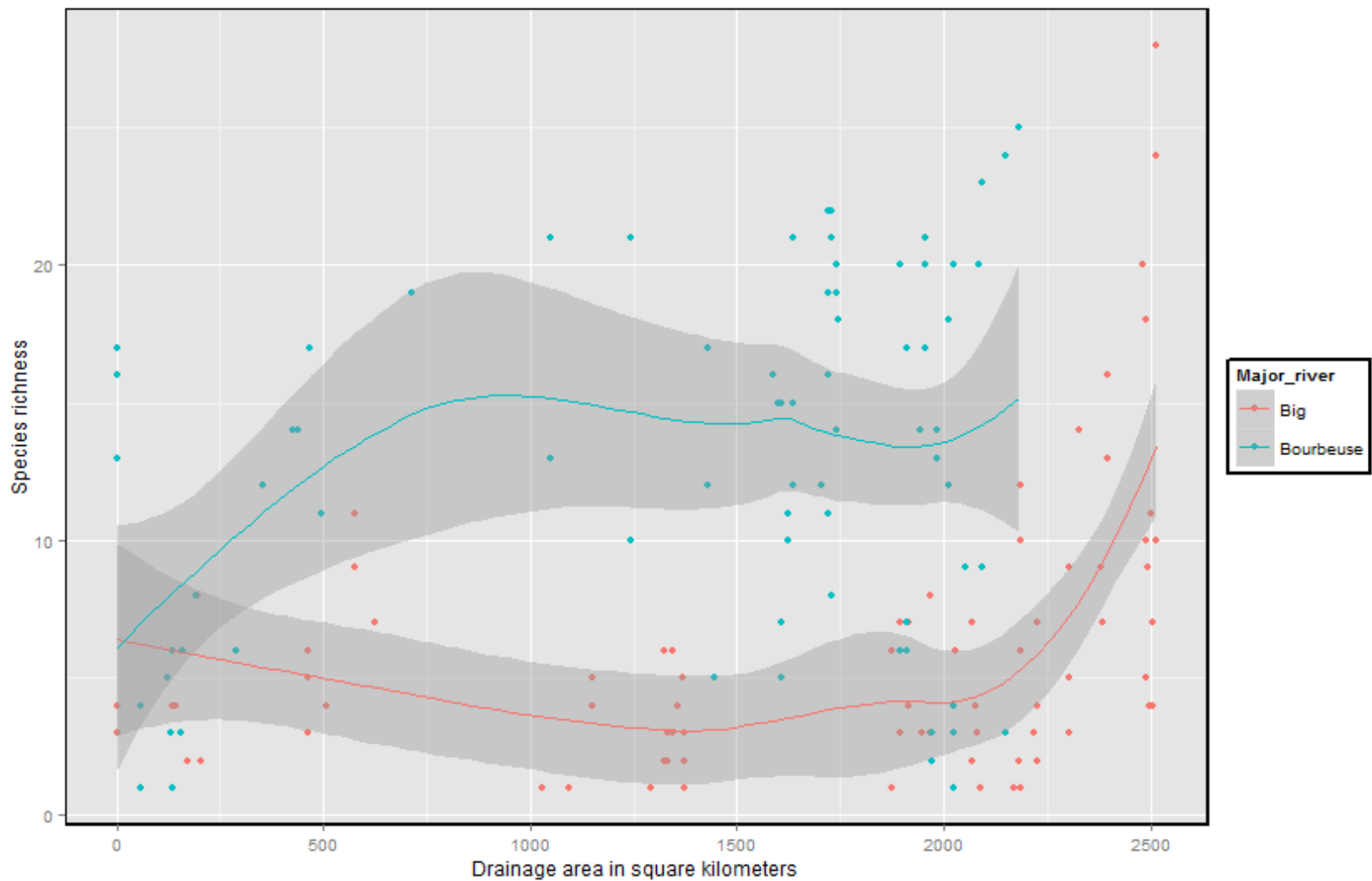
Figure 15. A LOESS smoothing function and 95% confidence interval shows a difference in both the magnitude of species richness by watershed area between rivers and a difference in the shape of the relationship.
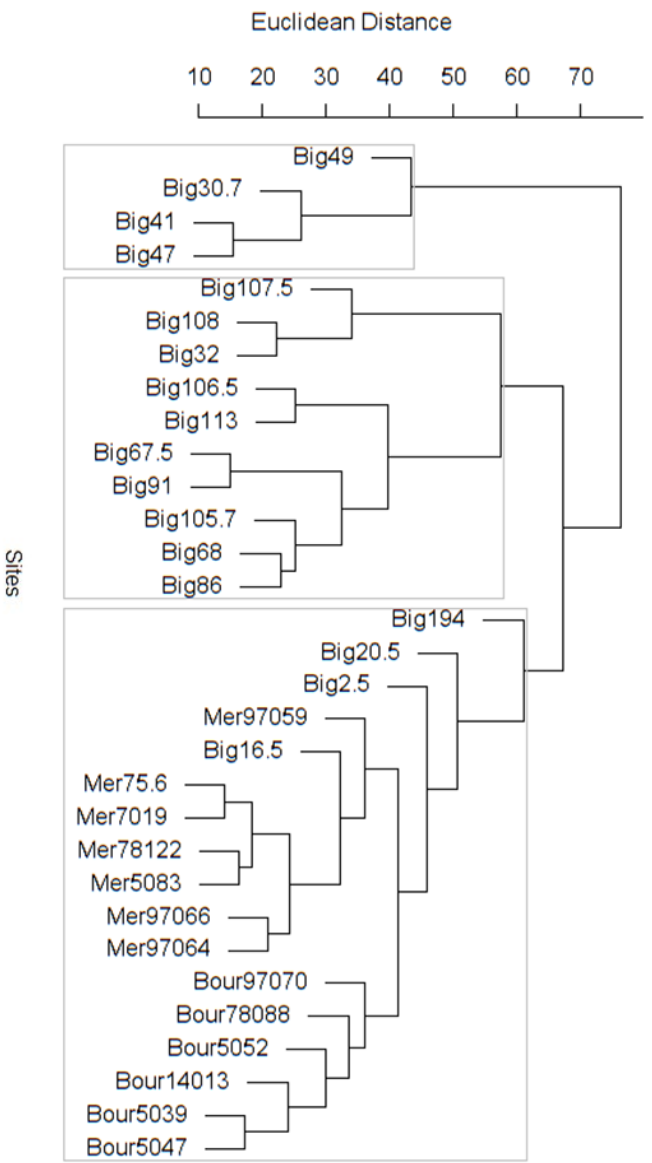
Figure 16: Dendrograms of mussel assemblage similarity in the Big, Meramec, and Bourbeuse rivers. Boxed clusters represent different types of mussel assemblages identified using the approximately unbiased (AU) test. Sites are named based on their location along the river corridor. The map shows sites in cluster 1 as light yellow, cluster 2 as green, and cluster 3 as orange.
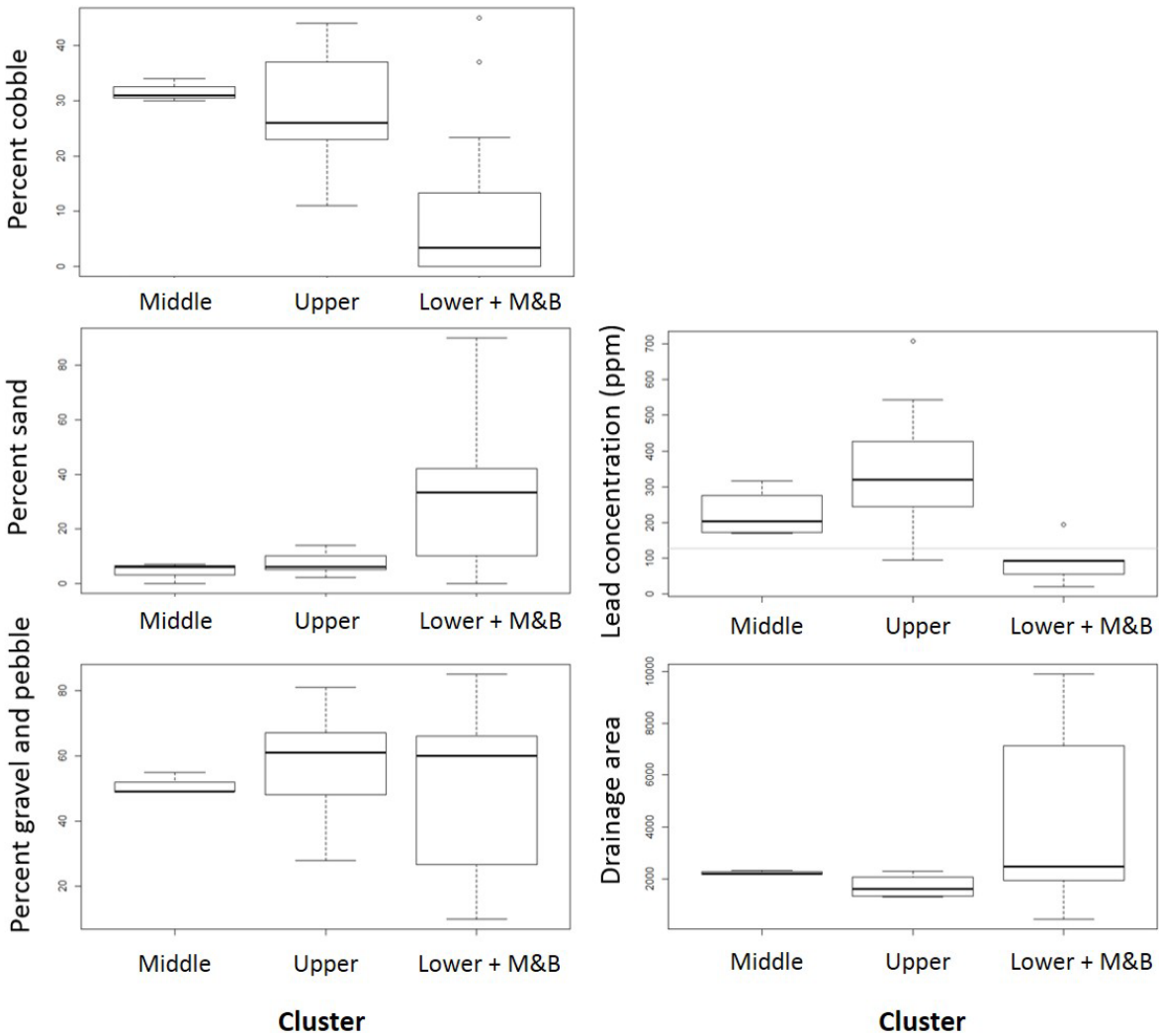
Figure 17: Box plots of environmental variables grouped by the assemblage cluster. Cluster 1 (middle) represents sites in the Big River with intermediate assemblage richness. Cluster 2 (upper) represents sites with the lowest richness of mussels. Cluster 3 (lower + M&B) represents reference sites in the Meramec, Bourbeuse, and Big rivers and the lower-most Big River sites. Habitat features overlap among clusters; lead contamination and % cobble are most distinct among clusters.

APPENDIX A

Scientific name	Big20.5	Big30.7	Big36.7	Big39.2	Big41	Big43.5	Big46	Big47	Big48	Big50.5	Big54.5	Big66.5	Big67.5
Actinonaias ligamentina	-	SF	SF	-	-	SF	-	D	-	-	-	-	-
Alasmidonta marginata	-	1	-	-	-	-	1	SF	-	-	1	SF	1
Alasmidonta viridis	-	-	-	-	-	-	-	-	-	-	-	-	-
Amblema plicata	2	SF	SF	SF	SF	SF	SF	SF	FD	-	-	SF	SF
Cumberlandia monodonta	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyclonaia tuberculata	-	SF	-	-	-	-	-	-	-	SF	-	-	-
Ellipsaria lineolata	-	-	-	-	-	-	-	-	-	-	-	-	-
Elliptio dilatata	SF	27	SF	4	26	1	16	6	SF	-	-	1	SF
Fusconaia ebena	-	-	SF	-	-	-	-	-	-	-	-	-	-
Fusconaia flava	D	2	-	SF	2	SF	1	SF	SF	-	-	SF	3
Lampsilis brittsi	-	-	-	-	-	2	-	-	D	-	-	SF	2
Lampsilis cardium	5	13	1	7	9	1	1	4	2	4	FD	SF	8
Lampsilis siliquoidea	-	-	-	-	-	-	-	-	-	-	-	-	3
Lampsilis teres	-	-	-	-	-	-	-	-	-	-	-	-	-
Lasmigona costata	-	2	-	-	-	-	-	-	-	-	-	-	-
Leptodea fragilis	-	-	-	-	-	-	-	-	-	-	-	-	-
Ligumia recta	-	-	-	-	-	SF	-	-	-	-	-	-	-
Megalonaias nervosa	-	-	-	-	-	-	SF	-	SF	SF	-	-	-
Obliquaria reflexa	FD	-	-	-	-	-	-	-	-	-	-	-	-
Plethobasus cyphyus	-	-	-	-	-	-	-	-	-	-	-	-	-
Pleurobema sintoxia	-	1	SF	-	SF	SF	1	D	SF	SF	-	SF	-
Potamilus alatus	3	1	1	1	-	-	-	-	1	-	-	1	7
Quadrula metanevra	-	SF	-	-	-	-	-	-	-	-	-	-	-
Quadrula pustulosa	FD	4	SF	2	-	-	3	-	-	-	-	-	-
Quadrula quadrula	-	-	-	-	-	-	-	-	-	-	-	-	-
Quadrula verrucosa	SF	SF	SF	SF	SF	-	SF	SF	SF	-	-	SF	SF
Strophitus undulatus	-	SF	-	-	1	-	SF	-	SF	-	-	-	3
Toxolasma parvum	-	-	-	-	-	-	-	-	-	-	-	-	-
Truncilla donaciformis	-	-	-	-	-	-	-	-	-	-	-	-	-
Truncilla truncata	D	-	-	SF	-	-	SF	D	SF	-	-	D	-
Uniomerus tetralasmus	-	-	-	-	-	-	-	-	-	-	-	-	-
Venustaconcha ellipsiformis	-	-	-	-	-	-	-	-	-	-	-	-	-
Live Individuals	10	51	2	14	38	4	23	11	3	4	1	2	27
Live Species	3	8	2	4	4	3	6	3	2	1	1	2	7
Total Species	9	14	9	8	7	8	11	11	11	4	2	10	10

Met Site Selection Criteria	X		X			X		X					X

Number of each species found during reconnaissance field surveys on the Big River (Phase I). FD = Fresh dead shell, D = dead shell, SF = Subfossil shell.

Scientific name	Big68	Big70	Big73.5	Big74.5	Big77	Big77.5	Big81	Big81.5	Big82.5	Big84	Big86	Big91	Big93	Big94.5
Actinonaias ligamentina	-	-	-	-	-	-	-	-	-	-	-	-		-
Alasmidonta marginata	2	-	-	-	-	-	-	-	-	-	-	-		-
Alasmidonta viridis	-	-	-	-	-	-	-	-	-	-	-	SF		-
Amblema plicata	SF	SF	SF	-	-	-	-	-	-	-	SF	SF		SF
Cumberlandia monodonta	-	-	-	-	-	-	-	-	-	-	-	-		-
Cyclonaia tuberculata	-	-	-	-	-	-	-	-	-	-	-	-		-
Ellipsaria lineolata	-	-	-	-	-	-	-	-	-	-	-	-		-
Elliptio dilatata	SF	SF	SF	-	SF	D	-	-	-	-	SF	SF		-
Fusconaia ebena	-	-	-	-	-	-	-	-	-	-	-	-		-
Fusconaia flava	SF	SF	D	D	SF	SF	-	SF	SF	1	SF	3		1
Lampsilis brittsi	2		-	D	SF	-	-	-	-	1	1	SF		2
Lampsilis cardium	1	1	D	D	SF	D	-	-	SF	5	7	2		2
Lampsilis siliquoidea	-	-	-	-	-	-	-	-	-	-	-	SF		-
Lampsilis teres	-	-	-	-	-	-	-	-	-	-	-	-		-
Lasmigona costata	-	-	-	D	-	-	-	-	-	-	-	-		-
Leptodea fragilis	-	-	-	-	-	-	-	-	-	-	-	-		-
Ligumia recta	-	-	SF	-	-	-	-	-	-	-	-	-		-
Megalonaias nervosa	-	-	-	-	-	-	-	-	-	-	-	-		-
Obliquaria reflexa	-	-	-	-	-	-	-	-	-	-	-	-		-
Plethobasus cyphyus	-	SF	-	-	-	-	-	-	-	-	-	-		-
Pleurobema sintoxia	-	SF	SF	SF	-	-	-	-	-	-	-	SF		SF
Potamilus alatus	3	1	-	-	D	-	-	-	-	-	-	-		-
Quadrula metanevra	-	SF	-	-	-	-	-	-	-	-	-	SF		-
Quadrula pustulosa	-	-	-	-	-	-	-	-	-	-	-	SF		-
Quadrula quadrula	-	-	-	-	-	-	-	-	-	-	-	-		-
Quadrula verrucosa	SF	-	-	-	-	-	-	-	-	SF	SF	SF		-
Strophitus undulatus	-	1	-	-	-	-	-	-	-	-	-	-		-
Toxolasma parvum	-	-	-	-	-	-	-	-	-	-	-	-		-
Truncilla donaciformis	-	-	-	-	-	-	-	-	-	-	-	-		-
Truncilla truncata	-	-	-	-	-	-	-	-	-	SF	SF	-		-
Uniomerus tetralasmus	-	-	-	-	-	-	-	-	-	1	-	-		-
Venustaconcha ellipsiformis	-	-	-	-	-	-	-	-	-	-	-	-		-
Live Individuals	8	3	0	0	0	0	0	0	0	8	8	5	0	5

Live Species	4	6	0	0	0	0	0	0	0	4	2	2	0	3
Total Species	8	9	6	5	5	3	0	1	2	6	7	11	0	5
Met Site Selection Criteria	X										X	X		

Scientific name	Big100	Big101.5	Big105.7	Big106.5	Big107.5	Big108	Big109.3	Big109.5	Big111	Big112	Big113	Big113.5	Big127.5
Actinonaias ligamentina	SF	-	-	-	SF	-	-	-	-	-	-	-	-
Alasmidonta marginata	-	-	-	SF	SF	1	-	-	-	-	-	-	-
Alasmidonta viridis	-	-	D	1	-	-	-	-	-	D	D	-	-
Amblema plicata	SF	SF	D	-	-	-	SF	-	-	SF	-	-	SF
Cumberlandia monodonta	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyclonaia tuberculata	-	-	-	-	-	-	-	-	-	-	-	-	-
Ellipsaria lineolata	-	-	-	-	-	-	-	-	-	-	-	-	-
Elliptio dilatata	SF	SF	-	-	SF	-	-	-	-	-	-	-	-
Fusconaia ebena	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusconaia flava	-	1	D	1	D	2	SF	SF	2	SF	1	D	D
Lampsilis brittsi	SF	SF	2	1	D	2	-	-	FD	-	5	D	1
Lampsilis cardium	-	2	3	9	3	7	SF	-	1	SF	8	4	-
Lampsilis siliquoidea	-	-	-	-	-	-	-	-	-	-	-	-	-
Lampsilis teres	-	-	-	-	-	-	-	-	-	-	-	-	-
Lasmigona costata	-	-	-	-	SF	-	-	-	-	-	-	-	SF
Leptodea fragilis	-	-	-	-	-	-	-	-	-	-	-	-	-
Ligumia recta	-	-	-	-	SF	-	-	-	-	-	-	-	-
Megalonaias nervosa	-	-	-	-	-	-	-	-	-	-	-	-	-
Obliquaria reflexa	-	-	-	-	-	-	-	-	-	-	-	-	-
Plethobasus cyphyus	-	-	-	-	SF	-	-	-	-	-	-	-	-
Pleurobema sintoxia	-	-	-	-	D	-	SF	-	-	-	-	-	-
Potamilus alatus	-	2	-	-	1	3	SF	-	-	-	1	1	SF
Quadrula metanevra	-	SF	-	-	SF	-	-	-	-	-	-	-	-
Quadrula pustulosa	-	SF	-	-	SF	-	-	-	-	-	-	-	-
Quadrula quadrula	-	-	-	-	-	-	-	-	-	-	-	-	-
Quadrula verrucosa	SF	-	-	-	SF	-	-	-	-	-	-	-	-
Strophitus undulatus	-	SF	D	1	-	-	-	-	-	SF	-	-	-
Toxolasma parvum	-	-	-	-	-	D	-	-	-	-	-	-	-
Truncilla donaciformis	-	-	-	-	-	-	-	-	-	-	-	-	-
Truncilla truncata	-	SF	D	-	-	-	-	-	-	-	-	-	-
Uniomerus tetralasmus	-	-	-	-	-	-	-	-	-	-	-	-	-
Venustaconcha ellipsiformis	-	-	-	-	-	-	-	-	-	-	-	D	-
Live Individuals	0	5	5	13	4	15	0	0	3	0	14	5	1
Live Species	0	3	2	5	2	5	0	0	2	0	4	2	1
Total Species	5	10	7	6	14	6	5	1	3	5	5	5	5

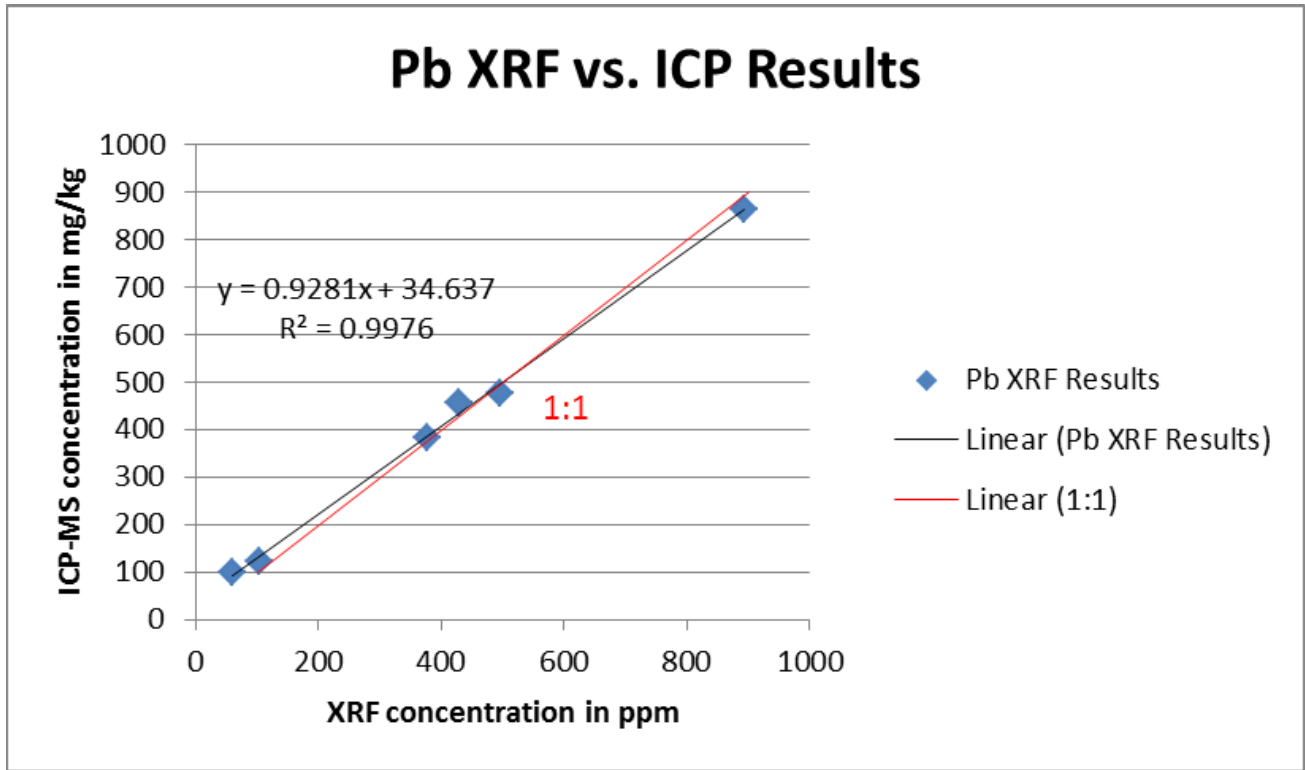|									
Met Site Selection Criteria		X	X	X	X			X	X

APPENDIX B

Results of quality control tests: XRF Pb vs. ICP-MS Pb in <2 and 0.25 mm sediment

APPENDIX C

Concentrations of heavy metals by river km in gravel bars and mussel bed sediments along the Big River according to multiple methods (Figures C1 – C3) and in relation to probable effects on biota (Figure C4). Table C1 shows concentrations of Cd and Ba in gravel bars and mussel bed sediments.
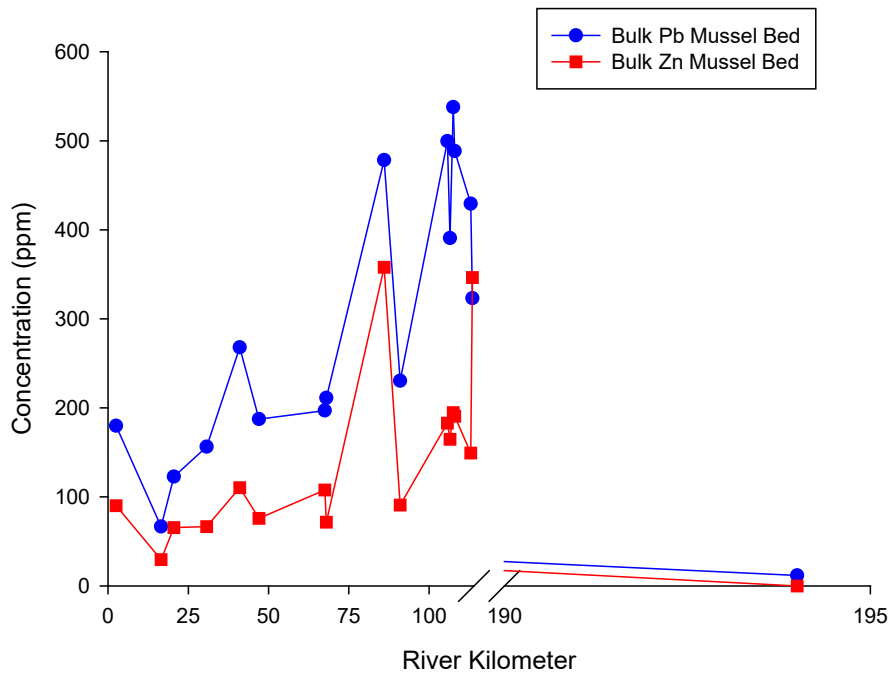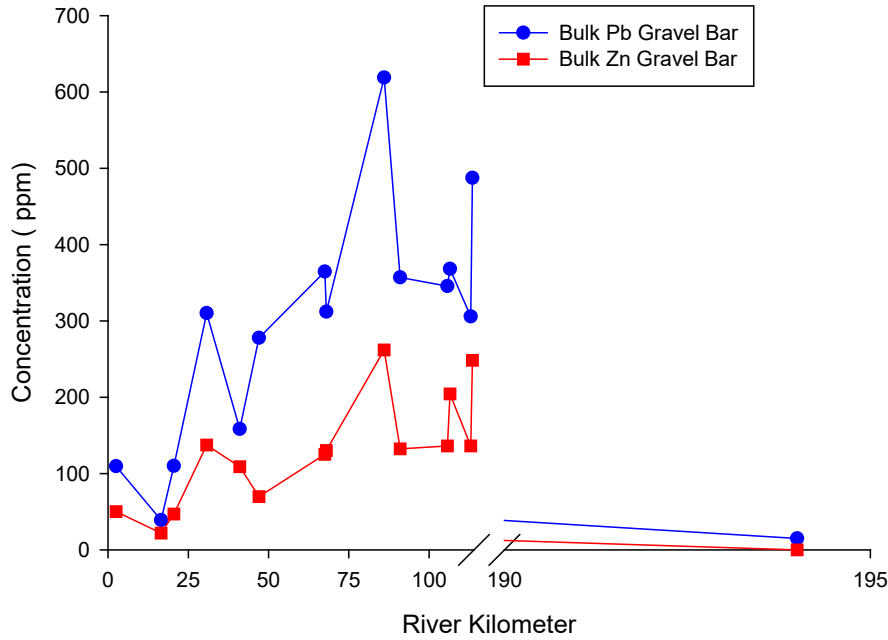
Figure C1: Concentrations of Pb and Zn, as determined by XRF in bulk sediments collected from gravel bars and within mussel beds in the Big River by river kilometer.
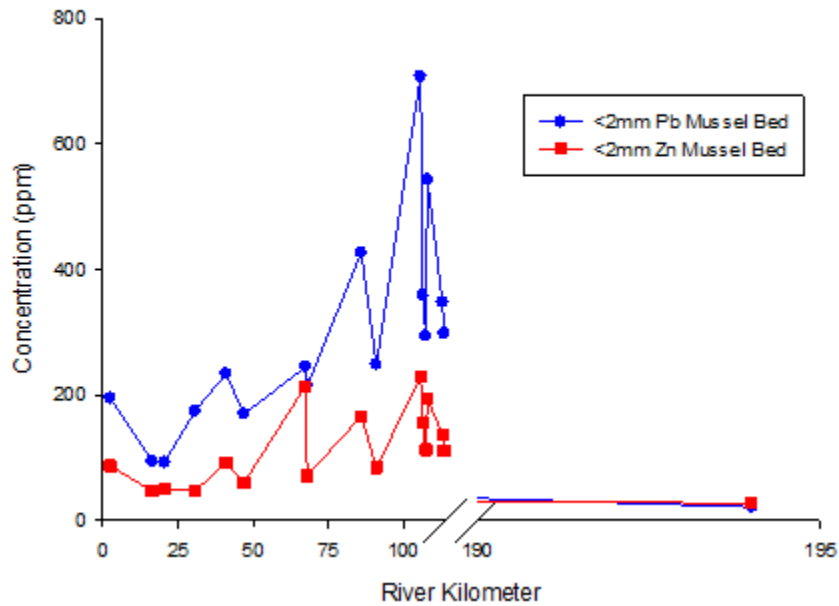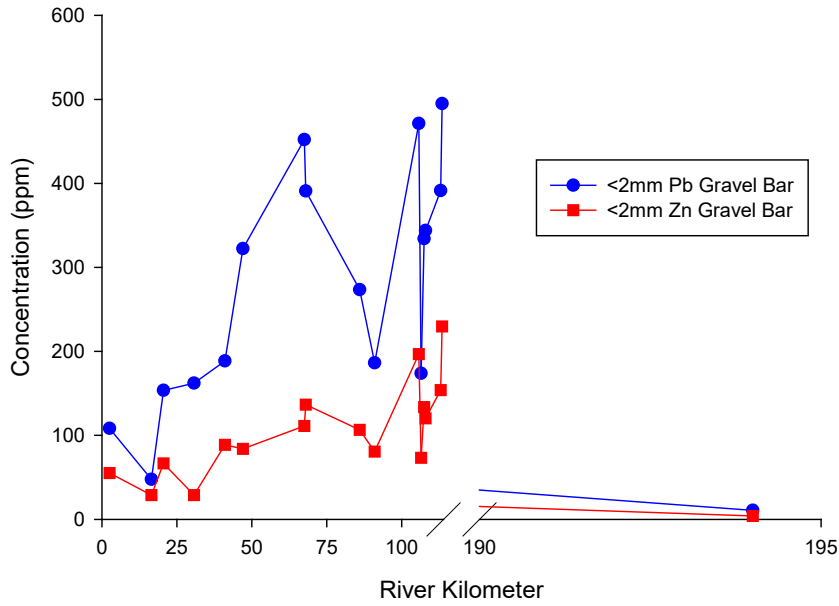
Figure C2: Concentrations of Pb and Zn, as determined by XRF in < 2 mm for sediments collected from gravel bars and within mussel beds in the Big River by river kilometer.
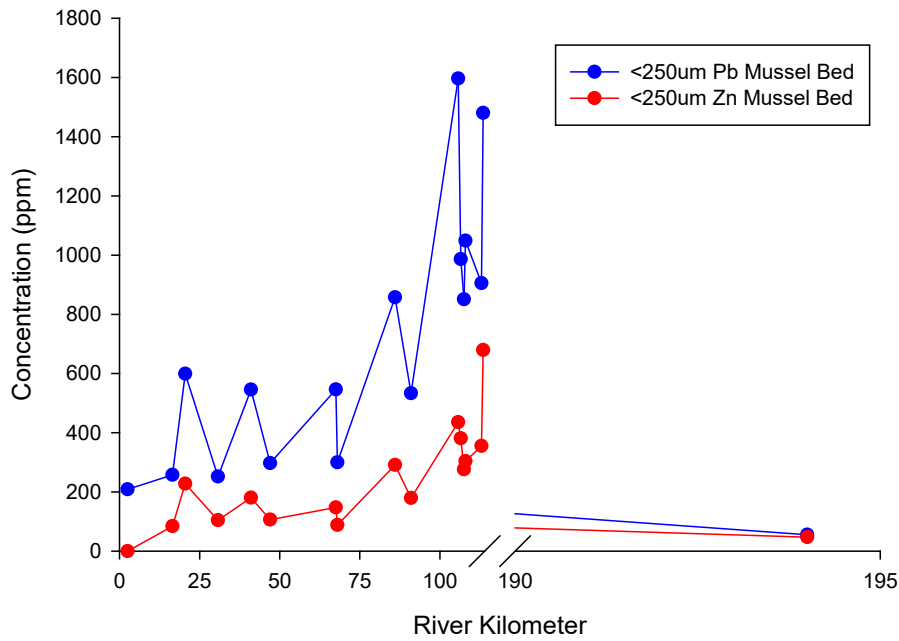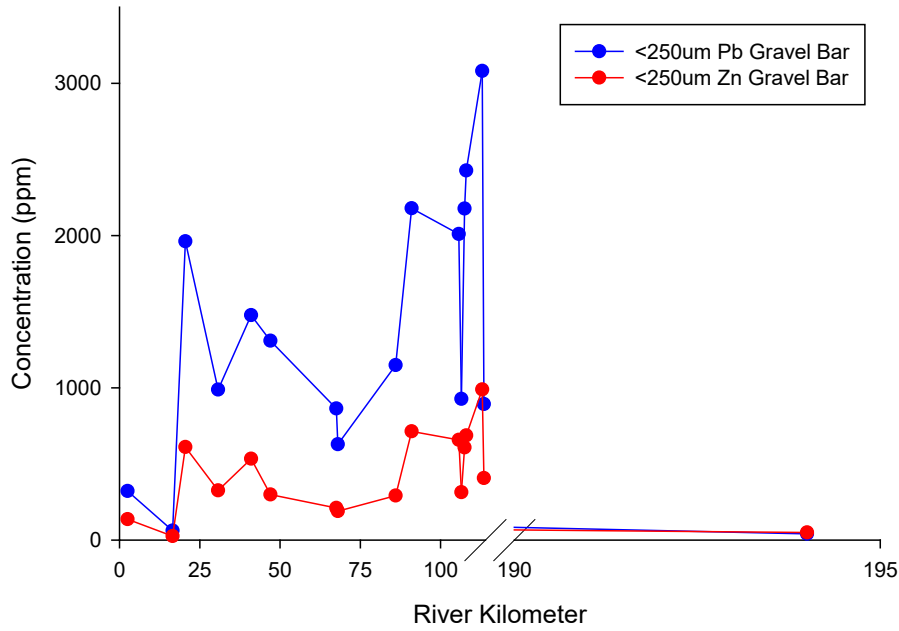
Figure C3: Concentrations of Pb and Zn, as determined by XRF in < 250 μm for sediments collected from gravel bars and within mussel beds in the Big River by river kilometer.
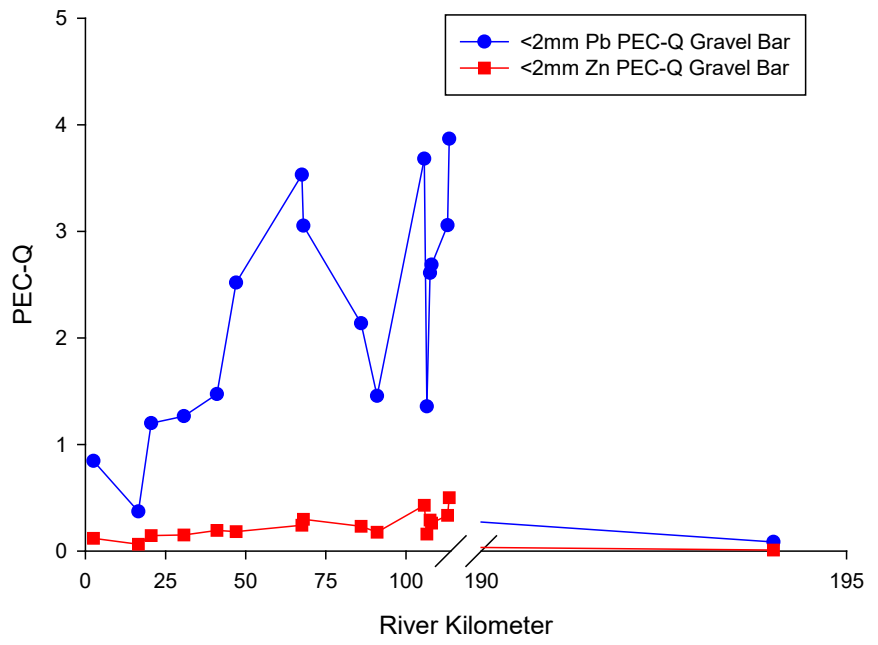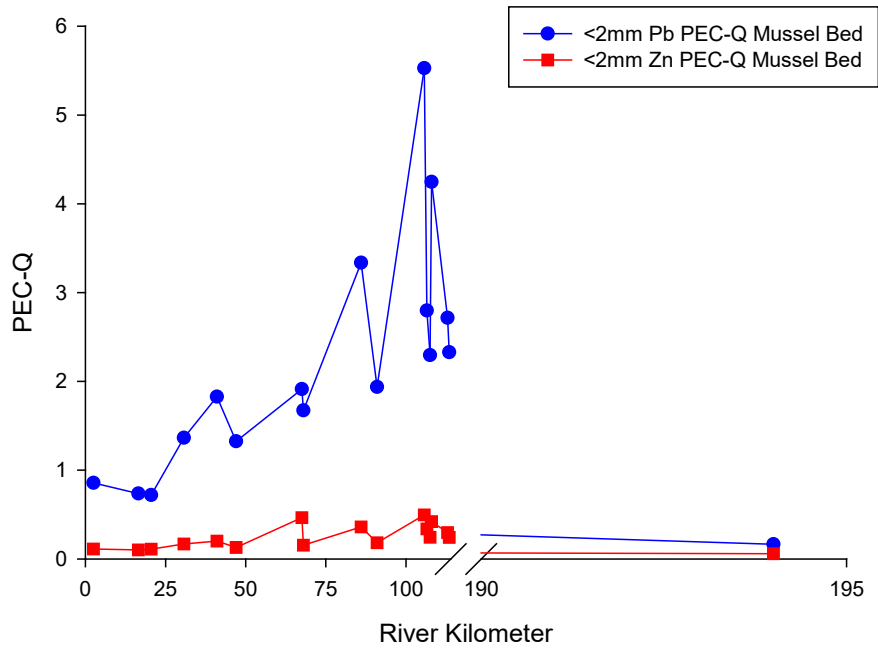
Figure C4: Probable Effects Quotients of Pb and Zn in <2 mm in sediments collected from gravel bars and mussel beds in the Big River by river kilometer.

a.

Site Name	<250µm (ppm) Cd	<250µm (ppm) Ba	<2 mm (ppm) Cd	<2 mm (ppm) Ba	Bulk (ppm) Cd	Bulk (ppm) Ba
Mer75.6	< LOD	265	< LOD	234	< LOD	199
Big2.5	< LOD	366	< LOD	319	< LOD	311
Big16.5	< LOD	282	< LOD	270	< LOD	273
Big20.5	6.9	794	0.2	285	< LOD	302
Big30.7	11.3	519	0.7	287	< LOD	480
Big41	10.3	671	0.5	291	< LOD	268
Big47	< LOD	676	< LOD	367	< LOD	272
Big67.5	< LOD	1036	< LOD	745	< LOD	577
Big68	< LOD	934	6.7	217	< LOD	372
Big86	< LOD	677	< LOD	327	< LOD	390
Big91	< LOD	625	< LOD	268	< LOD	375
Big105.7	6.4	696	0.5	366	< LOD	358
Big106.5	< LOD	528	< LOD	281	< LOD	376
Big107.5	7.9	621	0.3	307	NA	NA
Big108	< LOD	312	< LOD	312	NA	NA
Big113	15.4	731	0.2	324	< LOD	307
Big113.5	4.4	1000	1	691	< LOD	638
Big194	< LOD	347	< LOD	278	< LOD	286

b.

| Site Name	<250um (ppm) Cd	<250um (ppm) Ba	<2 (ppm) Cd	<2 (ppm) Ba	Bulk (ppm) Cd	Bulk (ppm) Ba
Mer75.6	< LOD	265	< LOD	244	< LOD	239
Big2.5	9.9	324	1.6	319	< LOD	279
Big16.5	< LOD	282	< LOD	371	9.5	360
Big20.5	< LOD	472	< LOD	276	< LOD	258
Big30.7	< LOD	380	< LOD	337	< LOD	336
Big41	< LOD	605	10.5	422	< LOD	316
Big47	< LOD	414	< LOD	317	< LOD	370
Big67.5	< LOD	1131	< LOD	1455	< LOD	502
Big68	< LOD	1444	< LOD	550	< LOD	573
Big86	< LOD	728	< LOD	499	9.9	449
Big91	< LOD	1374	< LOD	370	< LOD	304
Big105.7	< LOD	624	< LOD	531	< LOD	475
Big106.5	< LOD	4026	9.7	798	< LOD	1004
Big107.5	< LOD	1189	< LOD	366	10.8	451
Big108	< LOD	797	< LOD	369	< LOD	363
Big113	< LOD	2463	< LOD	461	< LOD	476
Big113.5	9.9	1541	< LOD	452	< LOD	470
Big194	< LOD	68	< LOD	307	< LOD	323

Table C1. Concentrations of Cd and Ba in gravel bars (a) and mussel bed (b) sediments as measured by XRF meter. LOD is limit of instrument detection.
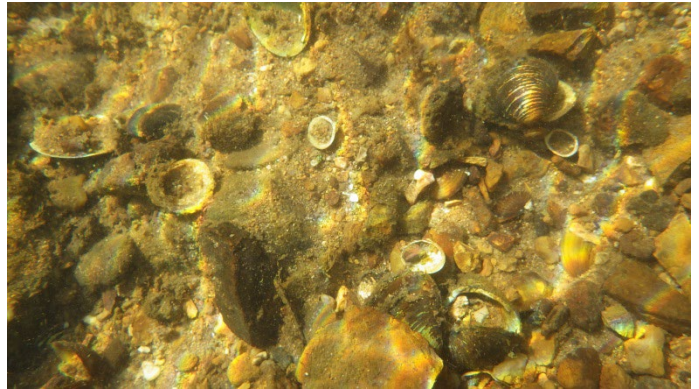
APPENDIX D

Illustrative photographs of mussel bed habitat from sites in the Big River (Figures D1 – D3).
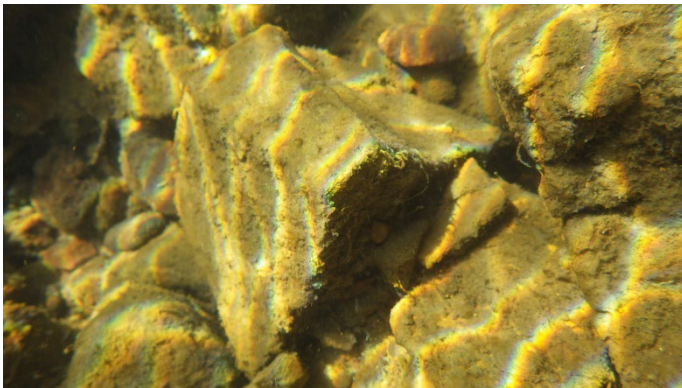
Big194 (Reference)   Photo 1

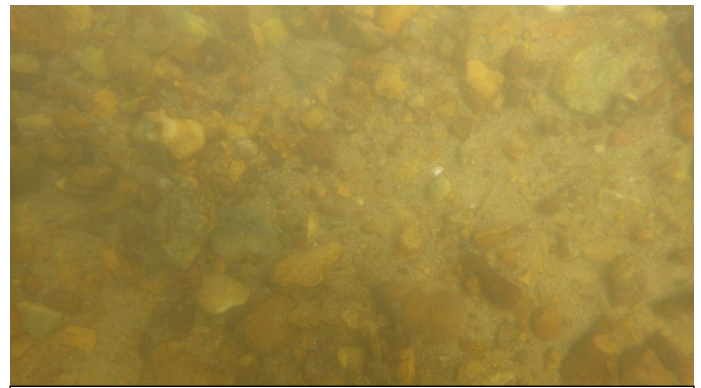Big194 (Reference)   Photo 2

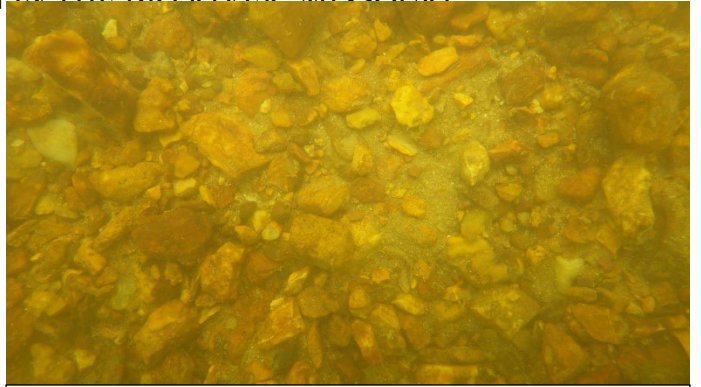Big107.5   Photo 1

Big107.5   Photo 2

Big106.5   Photo 1

Big106.5   Photo 2

Figure D1: Sample of substrate photos taken at the center of quadrats before searching for mussels at sampling sites on the Big River at river km 194, 107.5, and 106.5. First photo was taken before substrate was disturbed. Second photo was taken after a loose, superficial layer was removed, exposing finer substrate.
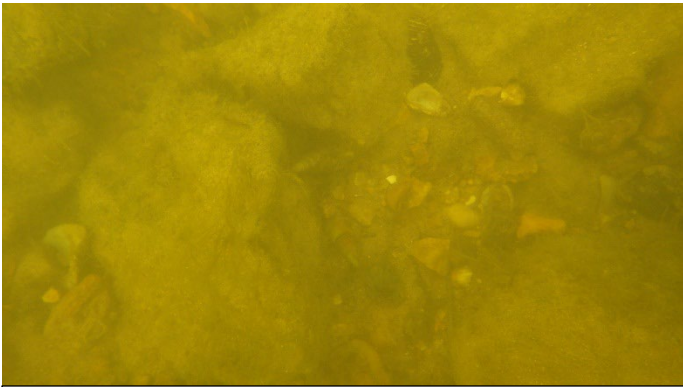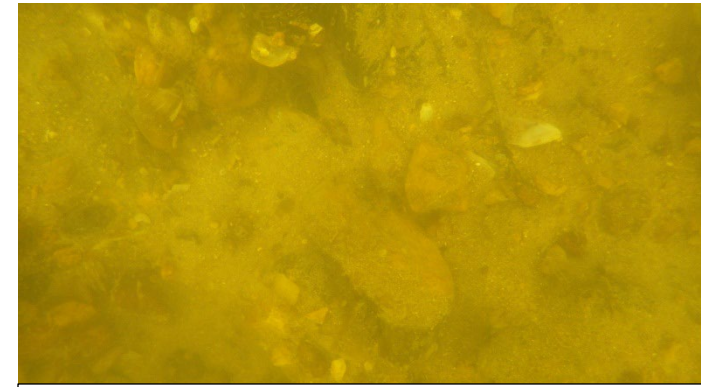
Big91   Photo 2

Big86   Photo 2
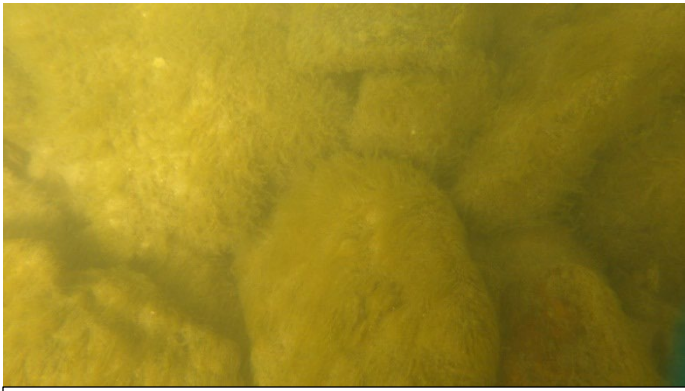
Big67.5   Photo 1

Big67.5   Photo 2

Figure D1 (con't): Sample of substrate photos taken at the center of quadrats before searching for mussels at sampling sites on the Big River at river km 91, 86, and 67.5. First photo was taken before substrate was disturbed. Second photo was taken after loose, superficial layer was removed, exposing finer substrate.

Big68 — Photo 1

Big68 — Photo 2

Big47 — Photo 1

Big47 — Photo 2

Figure D1 (con't): Sample of substrate photos taken at the center of quadrats before searching for mussels at sampling sites on the Big River at river km 68, 47, and 41. First photo was taken before substrate was disturbed. Second photo was taken after loose, superficial layer was removed, exposing finer substrate.

| Big30.7 | Photo 1 |

| Big30.7 | Photo 2 |

| Big16.5 | Photo 1 |

| Big16.5 | Photo 2 |

Mer75.6   Photo 2
Meramec River reference Site

Figure D1 (con't): Sample of substrate photos taken at the center of quadrats before searching for mussels at sampling sites on the Big and Meramec rivers at river km 30.7, 16.5, and 75.6 (Meramec). First photo was taken before substrate was disturbed. Second photo was taken after loose, superficial layer was removed, exposing finer substrate.

Figure D2: Substrate photos taken at 1/4m^2 quadrat locations at river km 47 in the Big River. Top photo was taken before superficial layer of loose cobble and gravel removed. Bottom photo was taken after top layer of substrate removed showing unionoid mussel (*Elliptio dilatata*) living in gravel sand mixture.

Figure D3: Photo of substrate in the mussel bed at the Meramec River reference site located at river km 75.6 (top), and a federally endangered scaleshell mussel (*Leptodea leptodon*) found living in the sand/gravel substrate below the surface (bottom).

APPENDIX V

Species richness by drainage area for the (a) Bourbeuse and (b) Big rivers at two time periods: 1977-1993 (post lead-contamination) and 1994-2014. The overall decline in species richness in the Bourbeuse River, likely due to ongoing habitat alterations in that system, is not matched by the Big River, which shows a more consistent depression in species richness. All data collected post-lead contamination.