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An assessment of the relation between metal contaminated sediment and freshwater mussel populations in the Big River, Missouri

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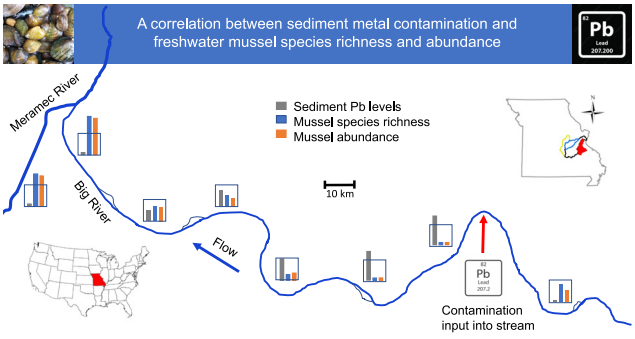
HIGHLIGHTS

- ~170 km of the Big River in Southeast Missouri, USA, is contaminated with Pb.
- ~140 km of river suffers toxic effects to mussels from Pb contaminated sediments.
- Pb in river sediment negatively correlated with mussel species richness and abundance.
- Pb toxicity to mussels from a field study was lower in concentration than a lab study.
- Pb is likely responsible for depressed mussel populations rather than habitat factors.

GRAPHICAL ABSTRACT



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ABSTRACT

The Big River in southeast Missouri drains the largest historical lead mining area in the United States. Ongoing releases of metal contaminated sediments into this river are well documented and are suspected of suppressing freshwater mussel populations. We characterized the spatial extent of metal contaminated sediments and evaluated its relationship with mussel populations in the Big River. Mussels and sediments were collected at 34 sites with potential metal effects and 3 reference sites. Analysis of sediment samples showed that lead (Pb) and zinc (Zn) concentrations were 1.5 to 65 times greater than background concentrations in the reach extending 168 km downstream from Pb mining releases. Mussel abundance decreased acutely downstream from these releases where sediment Pb concentrations were highest and increased gradually as Pb sediment concentrations attenuated downstream. We compared current species richness with historical survey data from three reference rivers with similar physical habitat characteristics and human effects, but without Pb-contaminated sediment. Big River species richness was on average about one-half that expected based on reference stream populations and was 70–75 % lower in reaches with high median Pb concentrations. Sediment Zn and cadmium, and particularly Pb, had significant negative correlations with species richness and abundance. The association of sediment Pb concentrations with mussel community metrics in otherwise high-quality habitat indicates that Pb toxicity is likely responsible for depressed mussel populations observed within the Big River. We used concentration-response regressions of mussel density verses sediment Pb to determine that the Big River mussel community is adversely affected when sediment Pb concentrations are above 166 ppm, the concentration associated with

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50 % decreases in mussel density. Based on this assessment of metals concentrations sediment and mussel fauna, our findings indicate that sediment in approximately 140 km of the Big River with suitable habitat has a toxic effect to mussels.

1. Introduction

Freshwater mussels (Bivalvia: Unionida) are important components of river ecosystems that are declining in many areas of the United States. Declines are attributed to several factors such as impoundment, sedimentation, channelization, water pollution, and invasive species (Haag, 2012). An increasing number of studies show that mussels are sensitive to environmental contaminants, including metals (e.g., Havlik and Marking, 1987; Naimo, 1995; Markich, 2017; Wang et al., 2010). Thus, mussels have been the subject of increased scientific focus in the field of ecotoxicology and have been found to be reliable indicators of the overall ecological integrity of aquatic systems (Farris and Hassel, 2007; Grabarkiewicz and Davis, 2008; Besser et al., 2015; Sohail et al., 2017; Timpano et al., 2022). Juvenile mussels have been developed as a standard test organism for water (Ingersoll et al., 2007; MacDonald et al., 2000; Wang et al., 2010). Wang et al. (2010) conducted acute and chronic toxicity tests with juvenile *Lampsilis siliquoidea* and *L. rafinesqueana* with lead (Pb), zinc (Zn), and cadmium (Cd) and concluded that freshwater mussels were sensitive to toxicity of metals.

The Big River is located within the Meramec River Basin in southeast Missouri, USA (Fig. A1), which supports one of the most diverse mussel faunas in the Midwest, including over 50 species basin-wide. However, the Big River drains the Southeast Missouri Lead Mining District (SEMOLMD), which is the largest historical Pb mining area in the United States (Long et al., 1998). Various metals from the mining process have been released into the Big River from historical mining areas mainly through direct discharge or erosion of mine and mill waste called chat and tailings, which have become incorporated into stream sediments over several decades (Pavlowsky et al., 2017; Noerpel et al., 2020). Metal contamination has long been suspected to adversely affect mussel populations and other aquatic biota downstream from Pb mining, milling, and smelting facilities. Elevated levels of bioavailable metals have been documented in the water, sediments, and tissues of various aquatic biota of affected streams in SEMOLMD (Gale et al., 1973; Zachritz, 1978; Schmitt and Finger, 1982; Duchrow, 1983; Czarnezki, 1985; Niethammer et al., 1985; Gale and Wixson, 1986; Czarnezki, 1987; Schmitt et al., 1987; Menau, 1997; Gale et al., 2002; Missouri Department of Natural Resources (MDNR), 2003; Besser et al., 2007; Besser et al., 2015). Big River sediments have been used in toxicity testing in laboratory studies to determine chronic effects on survival, growth, and biomass of the juvenile freshwater mussel *L. siliquoidea* (Besser et al., 2015). In this study, they found that mussel toxicity endpoints were strongly associated with sediment metal concentrations and that the sensitivity of mussels to metals can be similar or greater than standard test organisms.

Researchers have noted the lack of mussel diversity and abundance in the Big River from extensive survey work in the Meramec River Basin and suggested the potential cause to be metals contaminated sediment and toxic releases of metals from the contaminated sediment (Oesch, 1995; Buchanan, 1979; Roberts and Bruenderman, 2000; Hinck et al., 2012). To investigate the causes of the reduced diversity and abundance we formulated the primary objectives of this study to: (1) characterize the spatial (i.e., longitudinal) extent of sediment metal contamination, (2) evaluate the relationships between Pb concentrations in sediments and mussel density and species richness, and (3) account for other possible threats to mussels in the Big River. We hypothesize that elevated Pb in sediment is the limiting factor for mussel density and species richness in areas of otherwise suitable habitat in the Big River in the SEMOLMD. The analysis in this report was conducted as part of the Natural Resource Damage Assessment and Restoration process for the Big River historical mining area in the SEMOLMD.

2. Materials and methods

The primary data presented here are from surveys of mussel communities, sediment characteristics, and physical habitat of the Big River collected during two Phases. Phase I was conducted in 2008 to provide a broad characterization of stream sediment contamination and health of the mussel populations throughout the length of the Big River (Roberts et al., 2009). Phase II (conducted in 2013/2014) was planned according to Phase I results to focus sampling on the river where sediment Pb levels attenuate to moderate concentrations within the gradient of Pb concentrations observed, and the most downstream reaches where mussel populations are more comparable to reference sites (Roberts et al., 2016). Therefore, during Phase II we sampled mostly previously undocumented mussel assemblages between the Phase I study sites. Phase I was conducted concurrently with a study of Big River sediment toxicity conducted by the U.S. Geological Survey (Besser et al., 2015) and in-channel sediment deposits by Pavlowsky et al. (2017). In fact, our 2008 mussel population data was incorporated into Besser et al. (2015) to compare lab toxicity with our field data. For this paper, we combined our the mussel survey sediment data with these two other data sources to better represent sediment toxicity in the Big River as described in Section 2.4.2 below. Another data source is watershed-scale surveys of mussel communities in four watersheds of Southeast Missouri[1] (unpubl. Missouri Mussel Database, MDC). Our 2008 sediment data and 2014 sediment and mussel data has not appeared in a peer reviewed journal previously. Sediment data from Besser et al. (2015) Pavlowsky et al. (2017) have been published, but have not been evaluated comprehensively with the current data set. This paper represents a substantial and compressive synthesis and analysis of the 2008 sediment datasets mentioned above and incorporation of all new data from the 2013/2014 work.

2.1. Phase I data collection

Sediment and mussels were sampled in the Big River from July through October of 2008. Sites were selected based on previous reports of mussel abundance (Oesch, 1995; Buchanan, 1979; Roberts and Bruenderman, 2000) and the presence of suitable mussel habitat defined as riffle/run complexes with stable substrates containing a mixture of gravel and sand. A summary of site selection criteria is provided in the online supplementary material (Appendix A). Additional sites were surveyed as necessary to gain a more complete geographic coverage of the river and representation of present conditions. Nineteen sites were sampled on the Big River, including 18 downstream from known mining releases and 1 reference site upstream from known mining operations (Fig. A1). Two additional reference sites were included, one in the Meramec River and one in the Bourbeuse River, to better represent the lower Big River sites with higher stream order (Fig. A1). The additional reference sites were added to represent longitudinal differences in the mussel community (i.e., representation of middle and lower Big River sites) because mussel diversity and abundance naturally increase in a downstream direction (Bearden et al., 2019; Watters, 1992). The Bourbeuse and Meramec Rivers are a part of the drainage area of the Big River, are of similar size, physical habitat, and have similar mussel communities (Buchanan, 1979; Menau, 1997; Blanc et al., 1998; Blanc, 1999; Rosenberger and Lindner, 2022).

[1] The Missouri Mussel Database is managed by the MDC and includes all available mussel survey data in Missouri, including data from both large-scale and small-scale surveys between 1979 and 2008 (data available upon request to and subject to the approval of the MDC, 3500 East Gans Road, Columbia, MO, 65201).

2.1.1. Sediment collection

Sediments were collected from shallow, slower water zones adjacent to the mussel bed (i.e., gravel bars or other depositional areas). Each sample was a composite of no less than five subsamples collected within an approximately 100 m^2 area, from water <15 cm deep. Subsamples were collected with a PVC scoop, deposited into a high-density polyethylene (HDPE) 2 L mixing vessel, homogenized, and then spooned into a Ziploc® brand 3.8-L freezer bag. Samples were labeled and placed in a cooler for further analysis (given the persistence of metals in sediment and soils, typical hold times are not applicable). Approximately 0.5–1.0 kg (wet weight) of sediment was collected at each location. Additional sediment was collected for quality control (QC) at a rate of every tenth sample, or one QC sample per day, whichever was greater. Each sediment sample was accompanied by "site water" collected at the same site and sealed in a clean, 19-L plastic bucket for wet-sieving in the laboratory (see Instrumentation below).

2.1.2. Mussel surveys

Timed searches were used in Phase I to evaluate species richness (i.e., number of species), abundance, and spatial distribution of mussel assemblages. Timed searches are commonly used to determine species presence, including the detection of rare species (Strayer and Smith, 2003). Abundance from timed searches were expressed as catch per unit effort (CPUE), defined here as the number of live individual mussels per person hour of search time. While snorkeling, searchers disturbed and fanned gravel substrates by hand and moved cobbles and large flat rocks to increase collections of juveniles, smaller species, and individuals buried in the substrate. All suitable habitats were searched at each site until at least 1.5 person-hours of search time failed to increase species richness. All mussels were classified as live (including fresh-dead with tissue still attached to the shell), dead, or subfossil as described by Buchanan (1979). All sites were surveyed during base-flow conditions by at least two biologists with expertise in mussel sampling and the regional fauna.

Quantitative mussel sampling was conducted at eight of the survey sites to provide estimates of overall mussel densities (i.e., individuals/m^2). These sites included six of the Big River sites downstream from mining operations and two reference sites (upper Big River and lower Bourbeuse River). To obtain the most accurate density estimate, each site was delineated as described in Appendix A, such that only the portion of the channel with suitable, occupied mussel habitat was sampled. A simple random sampling design was used and conducted by randomly placing 0.25 m^2 quadrats on substrate within the delineated area of each site (Strayer and Smith, 2003). All visible mussels were collected by surveyors. Following this initial search, cobble and flat rocks were removed by hand and gravel substrates were searched by mixing and fanning by hand until no mussels remained. Mussels were identified, enumerated, and returned to the substrate within the quadrat location. Mean mussel densities from quantitative surveys were statistically compared among study sites with a one-way analysis of variance (ANOVA) with rank-transformed data and Tukey's test for pair-wise comparisons of the means (Conover and Iman, 1981).

Physical habitat was evaluated at each site using the protocol described by Barbour et al. (1999). This assessment procedure generates a numerical score representing the overall physical habitat quality by rating various parameters on a scale of 0 to 20 with greater scores indicating better habitat quality. Habitat parameters included in the analysis generally reflect accepted conditions that are important to riverine aquatic life. Therefore, these scores and environmental chemistry data from sediment samples provided a general basis for distinguishing between contaminant-limited and physical habitat-limited mussel populations. Evaluated habitat parameters included epifaunal substrate/available cover, embeddedness, velocity/depth regime, sediment deposition, channel flow status, channel alteration, frequency of riffles, bank stability, bank vegetation, and riparian zone width. Ratings for each parameter were determined by averaging the values independently assigned by three surveyors familiar with the regional stream conditions. The final physical habitat score is the sum of average ratings for each habitat parameters (maximum = 200).

2.2. Phase II data collection

Sediment and mussel sampling in Phase II took place between August 2013 and October 2014 to sample undocumented mussel assemblages between Phase I sites in middle and lower reaches (i.e., lower 125 km) of the Big River. Sediment Pb levels in this reach attenuate to more moderate Pb concentrations relative to the higher concentrations observed in Phase I near Pb mining releases. In addition, more mussel community data were needed to better define population responses. Site selection involved a reconnaissance effort to identify sites with suitable habitat using the criteria described in Appendix A. During this effort, the lower 125 km of the Big River was traversed by boat during base-flow conditions to identify sites occupied with mussels and with characteristics typically suitable for the establishment of dense, multi-species assemblages of mussels (generally termed mussel beds). A total of 14 sites that met the selection criteria were found during the reconnaissance effort. All 14 sites were delineated to establish site boundaries for additional sampling. In addition, four Phase I sites were chosen for Phase II sampling including the Meramec River reference site, upstream Big River reference site, and the two downstream-most Big River sites (Fig. A1). The Meramec River reference site was chosen based on similarity to mussel fauna in the Big River. The upper Big River reference site was selected based on its position upstream from the first major mining-related input of metals. Two lower Big River sites were included because mussel abundance and species richness were comparable to reference sites surveyed in 2008 (Roberts et al., 2009). In all, 18 sites were selected for further site characterization in Phase II, which included sediment sampling, quantitative mussel surveys, habitat assessment, and tissue metals analysis of *Corbicula fluminea* as described below.

2.2.1. Sediment collection

Phase II sediment collection was conducted as described above for Phase I. However, in Phase II, a second set of composite samples for metals analysis was collected per site. One set of samples was collected from the streambed adjacent to occupied mussel habitat and designated as the "gravel bar sediment" (as done in Phase I). A portion of this sample was used for confirmatory laboratory analyses of metals in gravel bars from three sites. A second composite sample was collected from within the mussel bed itself, designated as the "mussel bed sediment." This was done to confirm that bar samples were representative of sediments where mussels were living nearby. An approximately 0.25-kg split sample of all gravel bar samples was collected by alternating scoops directly into a plastic bag instead of a mixing bowl. The 0.25-mm and 2 mm fractions from sieved samples were dried and analyzed by X-ray fluorescence (XRF). Composite samples from the mussel bed consisted of a minimum of five subsamples taken from evenly distributed points 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 to 10 cm, angling the opening upstream, and slowly raising the sampler to the surface to capture the sample. The subsamples were placed in a clean, 19-L plastic bucket, allowed to settle for 30 min, then decanted and placed in a sealed Ziploc® brand 3.8-L freezer bag.

2.2.2. Mussel surveys

Because of the remoteness of Phase II sites, we used an intensive quantitative sampling method in place of separate timed and simple quantitative sampling techniques as conducted in Phase I. Systematic quantitative sampling was conducted to estimate mussel density (individuals/m^2) and species richness (Strayer and Smith, 2003). This sampling design is comparable to Phase I methods in estimating density. The main difference is that systematic sampling evenly distributes the random points across the site compared to simple random sampling, which tends to cluster some points (Strayer and Smith, 2003). Another advantage to this method is that the evenly spaced points require less time to locate, allowing us to increase the sample size at each site. This sampling effort 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). We found that this sample size was also sufficient to estimate species richness and comparable to Big River sites previously sampled via timed searches (Roberts et al., 2009; Roberts et al., 2016). To determine the systematic pattern of the 150 quadrats, first the distance between the quadrats was calculated by the following formula:

$$d = \sqrt{\frac{L \cdot W}{n/k}}$$

where d is the distance between units, L and W are the length and width, respectively, of the delineated 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 using a random number table to select the x and y coordinates of each random start, which represented a separate systematic pattern of 50 quadrats. After the three random start locations were determined, sampling progressed by flipping the 0.25-m^2 quadrat 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. This method has the potential to underestimate species richness in quadrats that are calibrated. However, the large sample size we used ensured species detection, and the high efficiency of our surveyors resulted in similar numbers between visual and excavated 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 algal 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 shallower if bedrock was encountered) and hand sorting the sample above the surface through a 6.4-mm sieve to find any individuals remaining not detected by visual methods. All living mussels collected within each quadrat were identified and recorded separately for visual and excavated samples. Dead shells of species not detected live at each site were classified as either dead or subfossil (Buchanan, 1979). Sampling efficiency was 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.

2.2.3. Sediment habitat sampling

Pebble counts and size fraction analysis were conducted in Phase II to evaluate variability in substrate composition, and specifically evaluate the negative physical habitat impacts from mining wastes. Pebble counts were conducted concurrently with the quantitative mussel sampling in the 100 visual 0.25-m^2 quadrats to characterize the substrate composition based on Wolman (1954). 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 (<2 mm), fine gravel (2–8 mm), medium gravel (9–16 mm), coarse gravel (17–64 mm), cobble (65–256 mm), and boulder (>256 mm). Sediment was also collected for particle-size fraction analysis to differentiate the <2 mm size fraction and provide additional data for substrate composition using the same methods as Phase I. Grain-size characterization was not completed for BR67.5[2] and BR68 because the remoteness of these sites did not allow sufficient volume of sediment samples to be collected in time for analysis.

[2] Site nomenclature: stream abbreviation (BR = Big River, MR = Meramec, and Bou = Bourbeuse) followed by river km.

2.2.4. Corbicula fluminea sampling

Corbicula fluminea were used as biomonitors to determine tissue concentrations of metals and verify exposure of the bivalve fauna to metals. *Corbicula fluminea* are bivalves that occupy the same habitat as native mussels in the Big River and are relatively tolerant of Pb and other metals (Labrot et al., 1999). Angelo et al. (2007) found *C. fluminea* to be good indicators of metals concentrations in sediment and mussels in the Tri-State Mining District of Southwest Missouri. Therefore, metals concentrations in *C. fluminea* tissue are expected to be representative of unionid exposure, and as an invasive species, they are a more desirable target for lethal collection as opposed to native mollusks. *Corbicula fluminea* were collected at sites that represented a range of sediment Pb concentrations in delineated mussel habitats (sites BR2.5, BR30.7, BR41, BR47, BR86, BR105.7, BR106.5, BR107.5, BR108, BR113, BR113.5, and BR194). Live individuals were collected from random quadrats during quantitative mussel sampling as native mussels were processed from the substrate. Specimens within a range of 20 to 25-mm maximum shell diameter were collected and held in a small plastic bucket with fresh site water until all 50 quadrats were processed following Crawford and Luoma (1993). Individuals in this size range were the largest size classes available in the Big River and presumed to be adults, which can live up to seven years (Aguirre and Poss, 1999). After quadrat sampling, 30 specimens were blindly selected and held in a covered bucket of river water for 24 h to allow individuals to expel stomach contents. A total of 330 individual *C. fluminea* were collected from the Big River to constitute 33 composite samples for analysis of metals in tissue. The samples were transferred to labeled HDPE jars (one composite sample per jar) and frozen at −20 °C. Samples were thawed prior to analysis by inductively coupled plasma-mass spectroscopy (ICP-MS) using a Perkin-Elmer/Sciex ELAN DRC-e ICP-MS and methods as described in Brumbaugh et al. (2005).

2.3. Sediment metal analytical methods

In the laboratory, sediments were wet sieved using site water to <2 mm to achieve a common grain size for comparison across sites for Phase I and Phase II. Metals tend to concentrate in the finer fraction of the sediment, which tends to increase their bioavailability (Strom et al., 2011, Sadeghi et al., 2012, Zhang et al., 2014). An evaluation of metals concentrations within various grain-size fractions in Big River sediments can be found in Roberts et al. (2009) and Roberts et al. (2016). Sieved samples were homogenized and air-dried to <20 % moisture prior to analysis by XRF using a 2007 Thermo Niton Xl3t 600 XRF (Thermo Scientific, Billerica, Massachusetts) (Margui' and Grieken, 2013). Samples were fully mixed between each of three separate readings, with the mean used as the best estimate of the metal concentrations. A suite of calibration verification samples was used to check the accuracy of the XRF and to assess the stability and consistency of the analysis for the analytes of interest. Quality control (QC) analyses for the XRF consisted of using three certified reference materials (CRMs) from the National Institute of Standards and Technology (NIST; Gaithersburg, Maryland) to verify instrument performance. The CRMs were soil or sediment matrices that had Pb concentrations ranging from 27 mg of Pb per kilogram (mg/kg) of CRM to 5532 mg/kg.

Confirmatory QC samples were analyzed at U.S. Geological Survey (USGS) Columbia Environmental Research Center for total recoverable Pb, Zn, and Cd by ICP-MS (as described for *C. fluminea*) (Brumbaugh et al., 2007). Sediment samples were digested by microwave-assisted heating at 180 °C with 5 mL of concentrated nitric acid and 0.5 mL of hydrochloric acid. The correlation coefficient of combined Phase I and Phase II data was $r^2 = 0.88$ with an average relative percent difference (RPD) of 1 %. However, two samples from the low end of Pb concentrations observed in this study (1.3 and 87 ppm) had very high RPD (−285 and −124 %, respectively) compared to ICP-MS values.[3] Samples were wet-sieved using

[3] Samples analyzed using XRF (Pavlowsky et al., 2017; Roberts et al., 2009, 2016) are reported in ppm. Samples analyzed using ICP-MS (*Corbicula fluminea* tissue samples, Besser et al., 2015 and QC samples within Roberts et al., 2009, 2016) are reported from respective laboratories as μg/kg for C. fluminea and mg/kg for sediment. To simplify the discussion and display of data, we have used ppm as an equivalent representation of all the sediment metals data.

site water to determine the percentage of sediments (and associated metals concentrations) in the particle size fractions <63 μm, 62–250 μm, 250 μm-2 mm, and >2 mm. Additional information on quality assurance/quality control and XRF and laboratory analyses of this study can be found in Roberts et al. (2009) and Roberts et al. (2016).

2.4. Data analysis

2.4.1. Sediment data

Because sediment transport, deposition, and aggradation in streams is a dynamic process, we included sediment data from separate but concurrent Big River studies and added geographically and temporally proximate data to better represent mussel exposure to Pb from sediments at the survey sites. We calculated median Pb values from all individual 2008 samples reported from Besser et al. (2015) and Pavlowsky et al. (2017) in addition to our samples (Tables A1 and A2). In order to ensure data comparability, archived sediment samples collected during the 2008 study (Roberts et al., 2009) were wet-sieved in 2020 using site water to obtain the <2 mm fraction (and bulk sediment for comparison) for analysis using XRF. *Corbicula fluminea* tissue concentration data were evaluated by simple untransformed correlations (coefficient of determination, r^2) with sediment and mussel density metrics collected from the same sites.

2.4.2. Mussel sampling data

One-way ANOVA was performed to compare densities of freshwater mussels among sampling sites during each sampling period. Density data were rank-transformed before ANOVA and mean comparisons. A p-value of <0.05 for the overall ANOVA indicated significant overall differences in density among sites (i.e., statistically significant difference between at least two means). Tukey's test was used to compare mean density between all pairs of site means, with critical p-value of 0.05 indicating significant difference between site pairs. Results of the Tukey's test were used to determine which sites had mean mussel density significantly different than reference sites. The longitudinal pattern in mussel species richness was evaluated by comparing the number of live mussel species collected during Phase I and Phase II sampling to the total number of mussel species documented (live or dead shells) from 50 sites in the Big River (historical regression). This analysis was based on all available data from the Missouri Department of Conservation (MDC) between 1979 and 2008 (unpubl. Missouri Mussel Database, MDC). This determination was complicated by the natural increase in mussel species expected to occur in streams with distance downstream from the headwaters (Watters, 1992; Bearden et al., 2019), and the absence of mussel survey data prior to mining and associated releases of metals in the basin. The first factor was addressed by performing a linear regression of all past species richness data versus river km (i.e. historical regression), which approximated the natural decrease in species with distance upstream. To estimate the natural decrease of mussel species richness in the Big River with distance from its confluence with the Meramec River, only sites in the database where searches were conducted as part of an official timed search within suitable habitat (e.g. no data from cursory searches or incidental collections were used) were plotted versus river km, with the X-axis log transformed to produce a linear relation. This regression was assumed to be a conservative estimate of the expected mussel species richness in the Big River, and sites that fell below 50 % of the regression-predicted species richness were considered impacted.

Rank correlation analysis was used to evaluate associations of timed mussel survey data (species richness and CPUE) with sediment metals concentrations and habitat scores using SAS/STAT (version 9.2) (SAS; Cary, North Carolina) with statistical significance based on a type I error rate of <5 % ($p \leq 0.05$). Rank correlation analyses (PROC CORR) examined relationships of taxa richness and CPUE with Pb, Zn, and Cd sediment concentrations and with habitat variables, including the total habitat scores and individual scores for the 13 individual habitat metrics. To evaluate the effects of sediment Pb on mussel density, we used a 'threshold sigmoidal' regression model (TRAP; Erickson, 2015). This analysis is similar to a logistic regression, but it defines finite thresholds for 0 % and 100 % effects. This concentration-response model allows estimation of Pb concentrations associated with defined percent reduction in mussel community parameters, provided the exposure concentration (in this case sediment Pb concentrations) explains a large portion of the variation in the biological response.

A primary objective of this study was to assess factors other than metal toxicity potentially affecting mussel populations in the Big River. To accomplish this, we compared Big River mussel species richness to regional reference streams without mining-related Pb releases and with otherwise very similar land uses and human effects. We obtained all available mussel survey data from the Big River and comparable streams outside of the Pb-affected area including the Bourbeuse, Meramec, and Gasconade Rivers (unpubl. Missouri Mussel Database, MDC). The Gasconade River was included in this analysis because it is also a free-flowing Ozark stream adjacent to the Meramec River Basin with similar land-use, topography, and mussel communities (Menau, 1997; Blanc et al., 1998; Blanc, 1999; Blanc, 2001; Bruenderman et al., 2001). Species richness data from the Big, Bourbeuse, Meramec, and Gasconade Rivers were compared across a longitudinal gradient from headwaters to downstream reaches where sampling sites were located. The time period evaluated, for which robust mussel data existed, included data from 1978 to 2013. A total of 444 data points were included in this analysis for the Meramec (62), Bourbeuse (53), Big (57), and Gasconade (101) Rivers (Fig. A2). Drainage area was determined from the National Hydrography Dataset Plus (https://www.epa.gov/waterdata/nhdplus-national-hydrography-dataset-plus) for each stream segment where sampling took place. An analysis of covariance (ANCOVA) was used to assess if the relation between drainage area and species richness varied by river system. Specifically, the ANCOVA evaluated differences in slopes and intercepts of regression lines describing this relationship. The Meramec and Gasconade rivers were compared to confirm patterns in species richness verses drainage area, and the Bourbeuse and Big Rivers were compared to determine if this relation was consistent for the Big River.

3. Results and discussion

3.1. Sediment analysis

Sediment analysis results demonstrated that Big River sediment is contaminated with Pb and Zn throughout its length downstream from areas where mine and mill waste has been released from historical mining operations. Median Pb and Zn concentrations in the <2 mm size fraction were <20 ppm at the upper Big River reference site and < 10 ppm at the Bourbeuse and Meramec River reference sites (Table A3, Fig. 1). However, metals concentrations increased abruptly at the first site downstream from mining releases in the Big River. Median Pb in sediment exceeded background concentrations (represented by reference sites) by 2–65 times from BR170.5 to BR2.5, which is near the Meramec River confluence. Median Zn exceeded background concentrations by 1.5–65 times in this same reach (Fig. 1). Median Pb concentrations peaked at over 1200 ppm at BR147, and then decreased gradually over the downstream course of the river (Table A3, Fig. 1). Sediment Pb concentrations in the Big River remained greater than the consensus-based probable effects concentration (PEC = 128 ppm) established by MacDonald et al. (2000) for over 125 km downstream from BR170.5, peaking at nearly 10 times the PEC at BR147. The PEC is defined as the concentration of a metal in sediment above which adverse effects to multiple benthic faunae are expected to occur (MacDonald et al., 2000). Median Zn concentrations initially followed a similar pattern with a peak over 900 ppm at BR156 (Fig. 1). However, Zn concentrations decreased to less than the PEC (458 ppm) at BR136.5 and remained less than the PEC throughout the remaining length of the river. Cadmium, which is often correlated with Zn concentrations in the SEMOLMD, was also detected in sediment samples. Concentrations of Cd were typically less than the quantification level of the XRF, except for the most upstream mining affected sites. Besser et al. (2015) quantified Pb, Zn, and Cd in Big River sediments (<2 mm) at 21 sites and found that

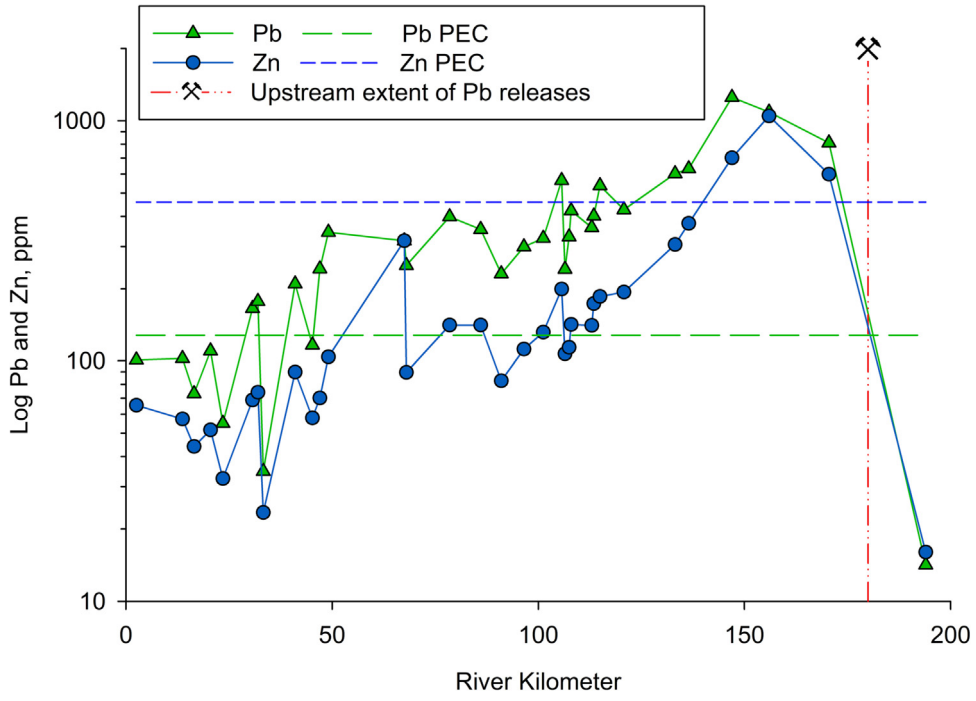

Fig. 1. Median lead (Pb) and zinc (Zn) sediment concentrations in the Big River from all sediment studies (Phases I and II of current study, Besser et al., 2015, and Pavlowsky et al., 2017). PEC = probable effects concentration. Not shown: Pb and Zn concentrations at the Bourbeuse and Meramec River reference sites <10 ppm.

Pb exceeded the PEC at 20 of these sites, whereas Cd and Zn exceeded PECs in only 6 and 4 sites, respectively. None of these sediments exceeded PECs for Zn or Cd without Pb also exceeding the PEC. Based on this information, Pb was the primary focus of this toxicity investigation. However, this does not negate the possibility that additive, synergistic, or antagonistic toxicity of metals may influence mussel populations in portions of the middle to upper river. No systematic differences in metals concentrations were found between sediment samples from gravel bars and within mussel beds in 2013 (Table A4, Fig. A3). Therefore, we used median Pb concentrations in gravel bar sediment samples for investigating associations between mussel metrics, habitat metrics, and sediment metals.

3.2. Big river mussel community

No known pre-mining data are known for the Big River mussel community to allow direct comparisons with post-mining data. Utterback (1916, 1917) published the first species list for the Meramec River Basin, but he did not specify which species were found in the Big River. The first known surveys of the Meramec River Basin were not conducted until the 1960s and 1970s (Missouri Water Pollution Board, 1964; Rychman, Edgerley, Tomlinson and Associates, Inc., 1973; Buchanan, 1979; Oesch, 1995). Of these efforts, Buchanan (1979) published the first detailed account of mussels in the basin. According to the most recent taxonomy (Williams et al., 2017), Buchanan reported 39, 38, and 34 species in the Meramec, Bourbeuse, and Big Rivers, respectively. Mussel surveys conducted in the Meramec River Basin since 1979 included a resurvey of the basin in 1997 (Roberts and Bruenderman, 2000), studies of survey methods in 2015 by Lueckenhoff (2015) and Schrum (2017), and various other site-and objective-specific work. With all data sources combined, 38 mussel species have been reported from the Big River mainstem.

Our study is the most thorough mussel survey of the Big River since Buchanan (1979) and included an extensive quantitative data-set. In all, we collected 31 live species and found evidence of 36 of the 38 species previously reported in the Big River by all other surveys combined (unpubl. Missouri Mussel Database, MDC). Regressions of historical species richness for the Big River by river km indicated patterns whereby downstream sites had greater mussel species richness than upstream sites (r^2 = 0.4566, Fig. 2). While this distributional pattern is expected, a scatterplot of only recent data from the current study indicated a much reduced richness across the longitudinal gradient relative to the historical regression (Fig. 2). Community metrics revealed suppressed mussel populations downstream from inputs of metals from mining sites even when compared to the upper reference site, which is expected to have the lowest species richness and density because of its position in the headwaters. Results of quantitative sampling show reductions in both species richness and mussel abundance (CPUE and density) at most of the metal contaminated sites compared to reference sites (Table A5, Fig. 3). Fifteen of 19 contaminated sites sampled in Phase I had fewer than 50 % of the taxa predicted by the historical species richness regression by river km. All 15 of these low-richness sites were located closest to historical mining sites and moderate to high relative Pb sediment concentrations (353–1251 ppm Pb). Sites surveyed in Phase II were generally located farther downstream from historical mining operations and relatively low to moderate Pb sediment concentrations (61–495 ppm Pb), yet 10 of 17 sites surveyed also fell below the 50 % historical regression line. Only the upstream reference site and three sites near the confluence of the Meramec River had species richness that fell at or above the historical regression line (Fig. 2). Our results were consistent with Buchanan (1979), who found less than half as many species per site in the Big River than in the Meramec or Bourbeuse Rivers.

Our multi-stream species richness comparison revealed positive correlations between drainage area and species richness in both the Meramec and Gasconade River systems (r^2 = 0.115) with overlapping overall species richness between the two river basins. This supports the use of the Meramec and Bourbeuse Rivers as reference streams unaffected by mining for comparison with the Big River (Fig. 4). We observed no differences in the species-watershed area relations between the Meramec and Gasconade Rivers (r^2 = 0.124; Fig. 4). Species richness was also positively correlated to drainage area in both the Big and Bourbeuse Rivers (r^2 = 0.031), but in contrast to results from the Meramec and Gasconade Rivers, there were significant differences between these two streams (r^2 = 0.287; Fig. 4). Linear regressions showed reduced species richness in the Big River by an average of seven species in comparison to sites with comparable drainage areas in the Bourbeuse River. In the portions of the Big River where sediment exceeded the Pb PEC, species richness was 70–75 % lower

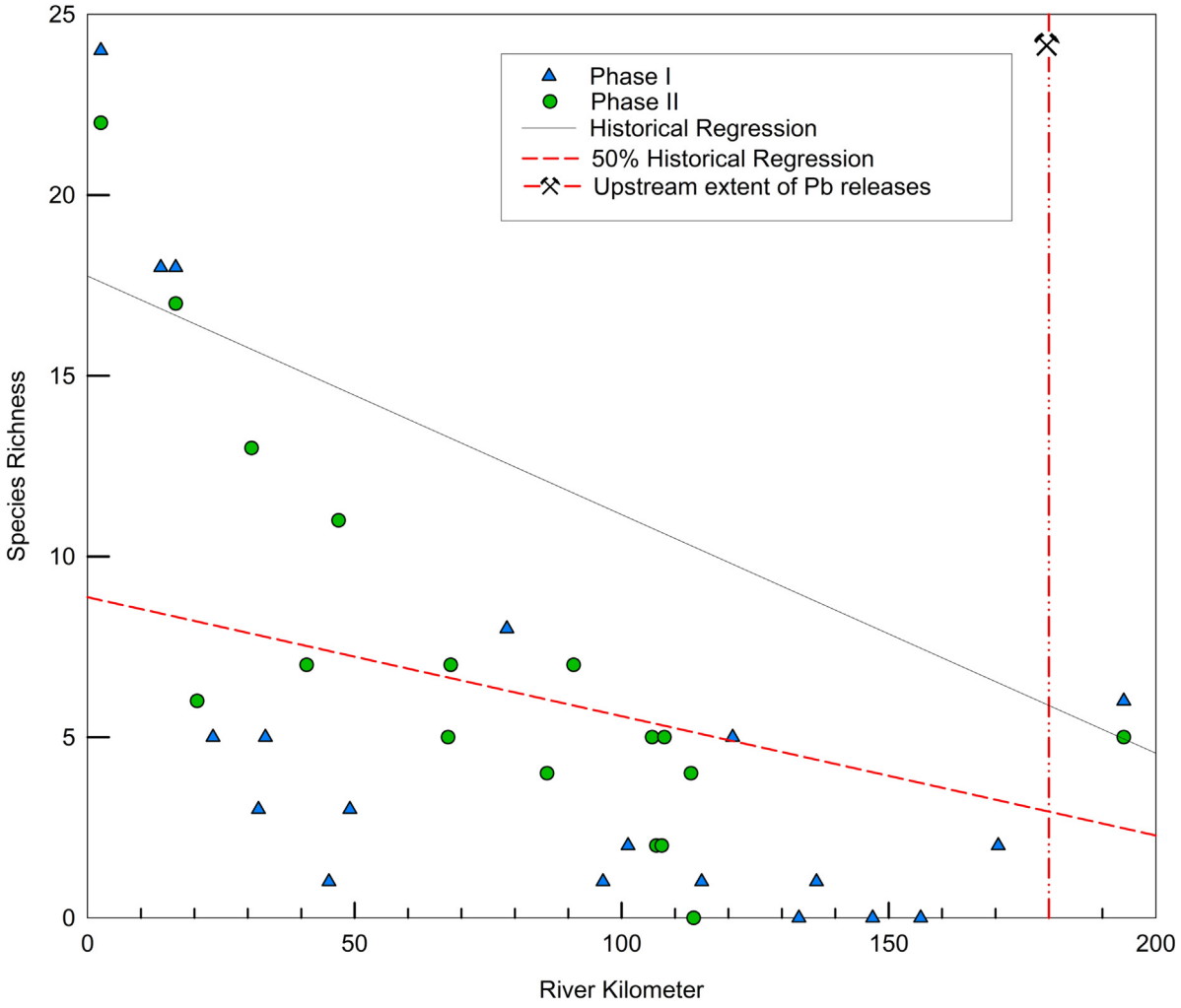

Fig. 2. Comparison of Big River live mussel taxa collected from Phases I and II with a regression of all available historical species-richness data (1979–1997). Solid line indicates predicted decreases in taxa richness with distance from mouth of Big River based on historic regression; dashed line indicates 50 % reduction in taxa relative to the regression.

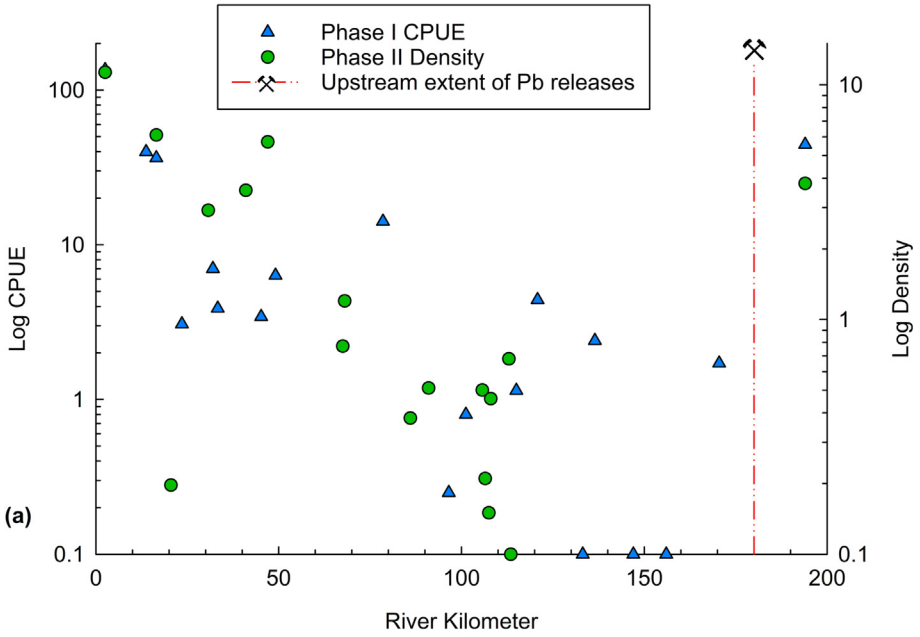

Fig. 3. Big River Catch per unit effort (CPUE; mussels/person-hour) and density (mussels/m^2) of freshwater mussels by river kilometer **(a)** and species richness of freshwater mussels by river kilometer **(b)**.

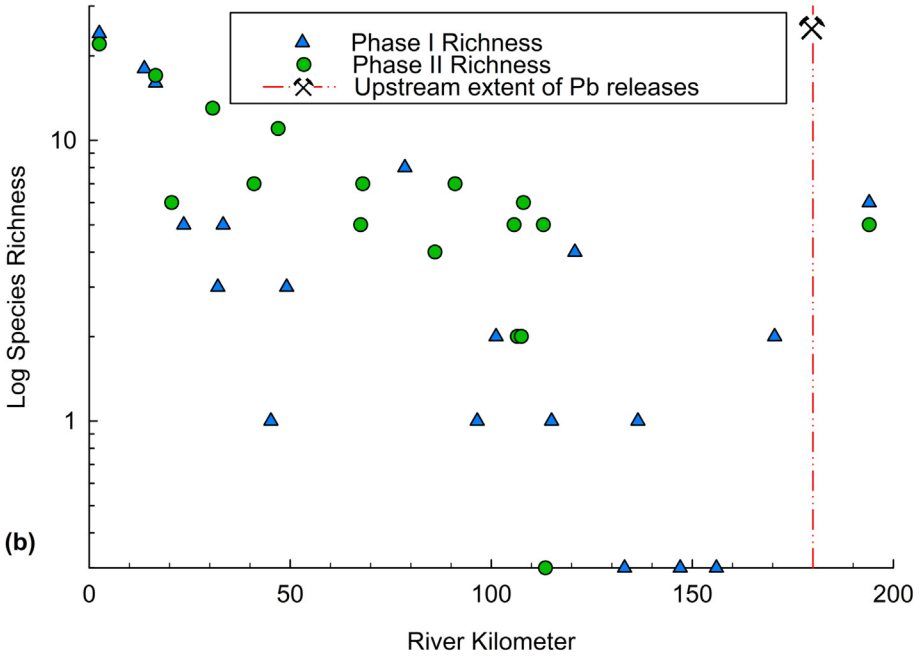

Fig. 3 (*continued*).

(i.e., 9–11 species) compared to similar drainage areas in the Bourbeuse River (Roberts et al., 2016).

3.3. Habitat analysis

Overall physical habitat scores from evaluations conducted in Phase I varied among the mussel survey sites, ranging from 165.7 (82.9 % of the theoretical maximum score) at BR194 to 103.7 (51.9 % of the theoretical maximum) at BR115 (Table A3, Fig. A4). Habitat scores at the Bourbeuse and Meramec Rivers reference sites were within this same range at 158.3 (79.2 % of theoretical maximum) and 137.7 (68.9 % of theoretical maximum), respectively. The average score among all sites, including reference sites, was 138.1 (69.0 % of the theoretical maximum). In general, these habitat scores do not show consistent upstream to downstream patterns

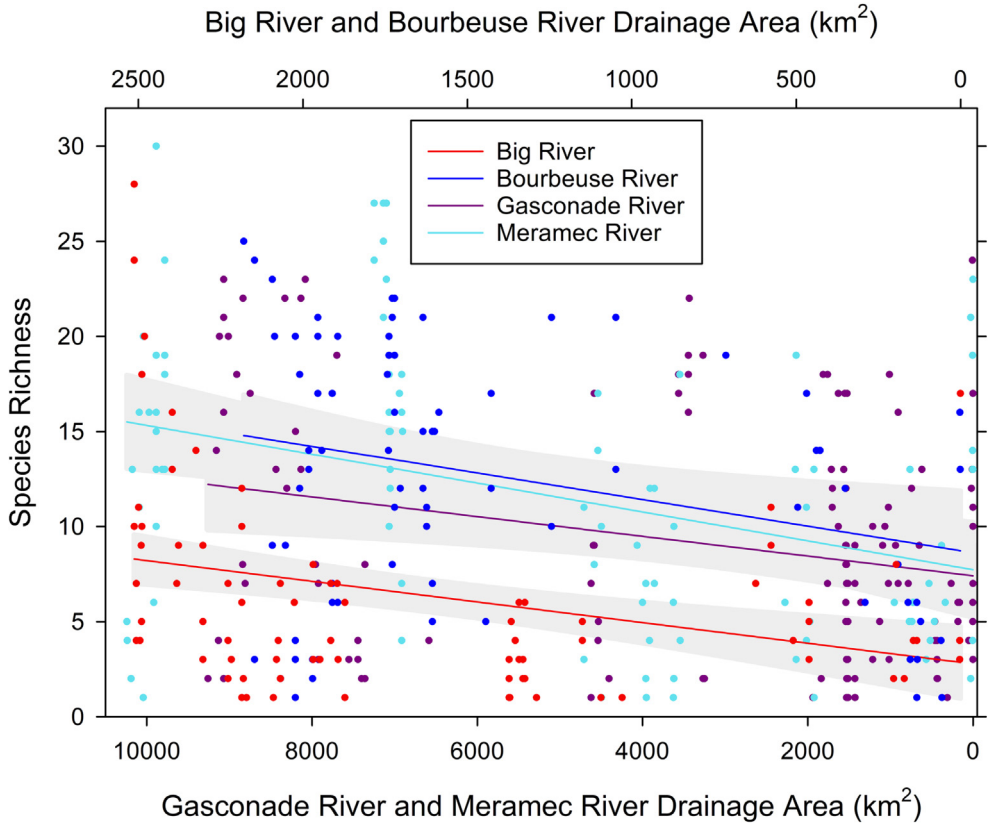

Fig. 4. Comparison of mussel species richness in the Big, Bourbeuse, Gasconade, and Meramec Rivers by linear regressions of species richness verses drainage area based on all available historical data.

in the Big River but indicate the presence of intact, suitable physical habitat within the range of reference conditions, throughout the length of the Big River. Given the suspected effects to mussel communities within the Big River due to Pb concentrations, and our selection of suitable habitat throughout a range of sediment Pb concentrations demonstrated to be toxic to mussels, as suspected, we did not find consistent associations of mussel community metrics when compared to the full suite of habitat parameters (CPUE or richness) (Table 1).

Three of 13 individual Phase I habitat parameters (i.e., embeddedness, sediment deposition, and channel flow status) had significant, positive correlations with both mussel species richness and CPUE ($p < 0.05$, Table 1). Note that the degree of these three habitat parameters observed in the river is inversely related to the score. These associations with the habitat scores indicated that mussel species richness and CPUE were greater at sites with lesser deposition of fine sediments, lesser embeddedness of coarse substrates, and lesser degree to which the channel is aggraded with sediment. While excess fine sediment in the river channel (e.g., sediment deposition and channel flow status) can have negative physical effects on mussels (i.e., can physically smother mussels), this was only observed in pools and other depositional areas within survey reaches and was not encountered within areas of suitable mussel habitat (well-established riffles and runs). Embeddedness (the degree to which gravel substrate is infused with smaller particles like sand) was observed at nearly all the survey sites. Gravel and sand mixtures are supportive of diverse mussel beds and naturally form in the Meramec River Basin (Buchanan, 1979; Roberts and Bruenderman, 2000). Sand and other smaller particles, unless contaminated, create a favorable burrowing substrate for mussels and creates stability (Strayer, 2008). However, embeddedness is not a favorable habitat quality for other macroinvertebrates as the surface areas and interstitial spaces needed for living is decreased, and subsequently embeddedness contributes to a negative habitat score in the Barbour et al. (1999) habitat assessment. The correlation analysis for these individual habitat parameters does not provide information on the relative importance of metal contamination and habitat parameters in determining mussel community status. The strength of significant positive correlations of mussel variables (richness and CPUE) with the three habitat variables (r^2 from 0.467 to 0.830) was similar to correlations of mussel variables with sediment metals (r^2 from −0.603 to −0.824). This result is because the same sites that had the highest metal concentrations also had the highest degrees of these habitat variables.

Table 1
Rank correlation coefficients (r) for associations between mussel community metrics, scores for habitat characteristics, and sediment metal concentrations at mussel survey sites in the Big River. Values in bold text indicate significant correlations ($p < 0.05$). [CPUE = catch per unit effort.]

Variable	Phase I		Phase II	
	Number of live mussel species	Live mussel CPUE	Number of live mussel species	Live mussel density
Total habitat score	0.286	0.417	–	–
Epifaunal substrate/cover	0.178	0.334	–	–
Embeddedness	**0.467**	**0.557**	–	–
Velocity/depth regime	−0.185	−0.208	–	–
Sediment deposition	**0.572**	**0.628**	–	–
Channel flow status	**0.714**	**0.830**	–	–
Channel alteration	0.058	−0.006	–	–
Frequency of riffles	−0.273	−0.141	–	–
Left bank stability	−0.049	−0.073	–	–
Right bank stability	−0.211	−0.076	–	–
Left bank vegetation	0.081	0.101	–	–
Right bank vegetation	0.081	0.161	–	–
Left bank riparian zone width	0.094	0.034	–	–
Right bank riparian zone width	0.099	0.098	–	–
Median pebble size	–	–	0.057	0.322
Grain size	–	–	−0.264	−0.332
Lead (<2 mm) sediments	**−0.686**	**−0.654**	**−0.602**	**−0.559**
Zinc (<2 mm) sediments	**−0.824**	**−0.766**	**−0.626**	**−0.539**
Cadmium (<2 mm) sediments	**−0.689**	**−0.603**	–	–

To better isolate the effects of Pb contamination from mining-related physical alteration of substrate conditions (i.e., sedimentation), pebble counts and grain-size analysis were conducted during Phase II to determine if sites with sediment Pb concentrations above the PEC were overlaid with mine tailings or fine sediment (pebble counts) and to characterize sediment particle size classes in the upper layer of the substrate where mussels live (grain-size analysis). Overall, pebble counts indicated all sites (including reference sites) contained a variety of substrate size classes within sampled suitable mussel habitat (Tables A3 and A6), and substrate size showed no correlation with species richness or mussel density (Table 1). This result is consistent with a 2016 reevaluation of the data from the current study, which showed that physical sediment parameters were not associated with mussel population metrics (Janice Albers, USGS, written communication, 2016). Sand and fine gravel-sized particles made up a relatively small percentage of the substrate at the surface for most study sites, which further supports that these habitats were not covered with large volumes of fine gravel and sand (Table A6, Fig. A5). The diversity of sediment size classes and the presence of a coarse substrate layer overlaying a more mixed-size class layer indicates that 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 results were similar to pebble counts with no clear longitudinal patterns. Additionally, there was no correlation between pebble size and mussel metrics (Table 1). Smaller grain sizes (i.e., sand) were present at all sites (Table A3, Fig. A6). The diversity of particle size among survey sites in the Big River does not support the hypothesis that physical effects to mussel habitat (as opposed to toxicological effects) had a strong negative influence on mussel habitat suitability. Similarly, Albers (Janice Albers, USGS, written communication, 2016) did not find significant correlations between ratios of sand-size particles and mussel abundance. Abundance of two species was positively correlated with coarse substrates, but total mussel densities were more closely correlated with metals concentrations than with substrate variables. Further, Roberts et al. (2022) also found that both robust and depauperate mussel beds in the Big River exhibited increased stability (associated with decreased shear stress and velocities) and exhibited frequent flushing flows that transported fine sediments out of the mussel habitats.

The physical effects of Pb mine tailings and other sedimentation on aquatic habitat does not explain the depressed mussel diversity and abundance observed at our Big River sampling sites, particularly in the middle and lower reaches. However, other factors reported to negatively affect mussels, such as channel destabilization, impoundments, and non-Pb related water quality degradation also warrant consideration (Roberts and Bruenderman, 2000). These issues commonly occur in the Meramec, Bourbeuse, and Gasconade River Basins, which were used as reference streams in this study (Fig. A2). Land use within these basins is similar including row crops, pasture, and urban areas (Menau, 1997; Blanc, 1999; Blanc, 2001; Blanc et al., 1998; Roberts and Bruenderman, 2000; Bruenderman et al., 2001). The mainstem of the Meramec, Bourbeuse, and Gasconade Rivers are free of large dams, which are known to affect mussels by altering flow, habitat, and fish host populations that mussels depend upon for their life cycle (Haag, 2012). Both the Bourbeuse and Big Rivers have intact mill dams. The Big River has five historical mill dams located between river km 7.9 and 29. Three of these mill dams (river km 7.9, 18.8, and 29) have been breached for several decades and are in varying degrees of disrepair while two dams at river km 9.4 and 13.8 remain intact (Menau, 1997). The Bourbeuse River has two intact mill dams, located at river km 18.8 and 92.5 (Blanc, 1999). Considering the Meramec, Gasconade, Bourbeuse, and Big Rivers have similar non-Pb effects, the Big River fauna is conspicuously lower in species richness, particularly in its middle reaches (Fig. 4), indicating an effect unique to the Big River. In fact, Rosenberger and Lindner (2022) compared models of suitable habitat developed by Key et al. (2021) and water quality factors in the Big and Bourbeuse rivers and determined that, although the two streams had very similar physical habitat characteristics important for diverse mussel populations, the Big River contained more and longer

contiguous reaches of suitable habitat and less agricultural and wastewater inputs than the Bourbeuse River.

3.4. Metals, Corbicula fluminea, and mussel community metrics

Corbicula fluminea were observed at nearly all sites sampled in our study and were abundant at some. Analyses of *C. fluminea* tissues demonstrated that metals in Big River sediment are bioavailable to bivalves, indicating that the appropriate chemical, physical, and biological interactions exist that allow mussels to uptake metals present in sediments in the Big River. The analyzed metals (Zn, Cd, and Pb) were all detected in *C. fluminea* tissue samples. Tissue Pb concentrations were significantly correlated with the sediment Pb concentrations at mussel sampling sites ($r^2 = 0.69$; Fig. A7). This relation was not significant for Cd ($r^2 = 0.19$) or Zn ($r^2 = 0.03$). Tissue Pb concentrations ranged from 0.97 ppm at the upstream reference (BR194) site to 144 ppm at BR86. Cadmium showed a similar pattern with concentrations of 0.44 ppm at the upstream reference site to 28.7 ppm at BR113. Depending on the concentration, organisms can regulate Zn, and tissue concentrations did not follow longitudinal patterns for sediment concentrations, ranging from 175 ppm Zn at the upstream reference site to 383 ppm at BR2.5, which was the most downstream mussel site and among the lowest sediment Zn concentration (52 ppm). Czarnezki (1987) and Schmitt and Finger (1982) demonstrated the uptake of metals by native bivalves more directly by introducing *L. cardium* collected from the Bourbeuse River in caged exposure studies in the Big River. In both studies, caged adult mussels were placed upstream and downstream from mining areas and tissues were analyzed for metals 2–12 weeks after placement. Czarnezki (1987) found the highest concentrations of Pb and Cd (74.2 and 11.3 ppm, respectively) in caged animals directly downstream from mining areas. Both studies showed an increase in Pb and Cd in soft tissues over time. Schmitt and Finger (1982) had sites that directly corresponded with our study and found mean Pb and Cd concentrations at approximately river km 100 were 85 and 14.1 ppm, respectively, and mean Pb and Cd concentrations at ~river km 80 were 44 and 5.0 ppm, respectively. Our *C. fluminea* tissue results were similar, but marginally higher than those found in past studies. This could reflect a lifetime of exposure experienced by the *C. fluminea* as opposed to the relatively short exposure of the caged mussels. Resident populations of native mussels in the Big River could be expected to have a higher body burden of metals because they have a lifespan measured in decades (Roberts and Bruenderman, 2000; Haag and Rypel, 2011; Sansom et al., 2016), whereas *C. fluminea* has an average life span of 4–7 years (Pacific Northwest National Laboratory, 2003). Also, the differences could be related to selection of different sized and types of food particles of the two bivalve groups, and differences in feeding, metabolism, and growth rates (i.e. not solely caused by exposure time).

The presence of bioavailable and toxic levels of metals in Big River sediments are likely the cause of the low mussel species richness and abundances. We observed significant relations between metals concentrations in sediment, *C. fluminea* tissues, and mussel community variables in the Big River. Results of Phase I timed surveys at sites in the Big River showed reductions in mussel species richness and CPUE compared to reference conditions, which corresponded to elevated sediment Pb concentrations in much of the Big River downstream from mining-related Pb releases (Table A5, Fig. 5). Characteristics of mussel communities in the Big River were significantly correlated with metal concentrations in sediments. Rank correlation coefficients for Phase I species richness/CPUE and Phase II species richness/density of live mussels indicated significant negative associations with Pb, Zn, and Cd in sediments ($p < 0.05$, Table 1). These correlations indicated significant trends for lower species richness, CPUE, and density at sites with greater sediment metal concentrations. In addition, mussel density was negatively correlated with *C. fluminea* tissue Pb concentrations downstream from known mining-related Pb releases ($r^2 = 0.63$). Our

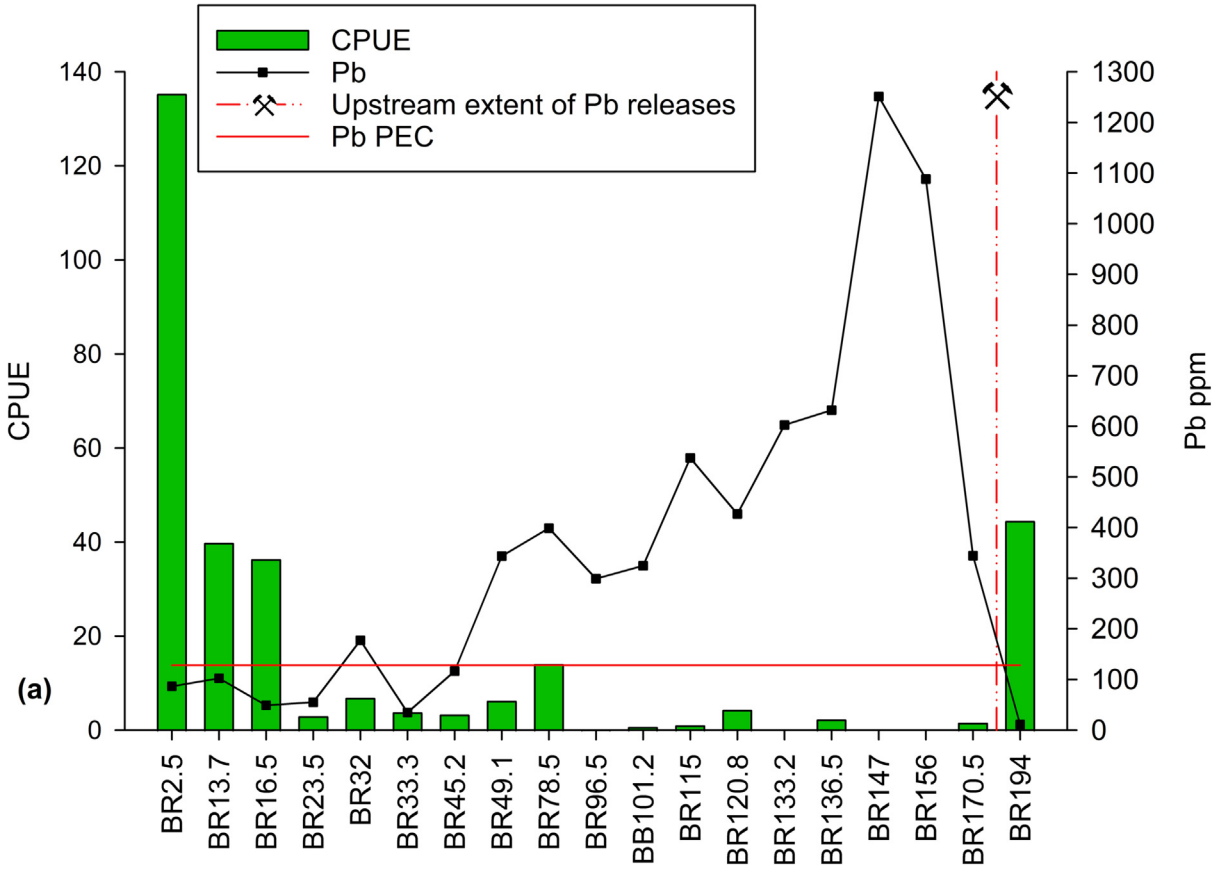

Fig. 5. Big River catch per unit effort (CPUE; mussels/person hour) **(a)** and species richness **(b)** versus lead (Pb) sediment concentration at Phase I survey sites. Sites labeled by river kilometer. BR = Big River. PEC = probable effects concentration.

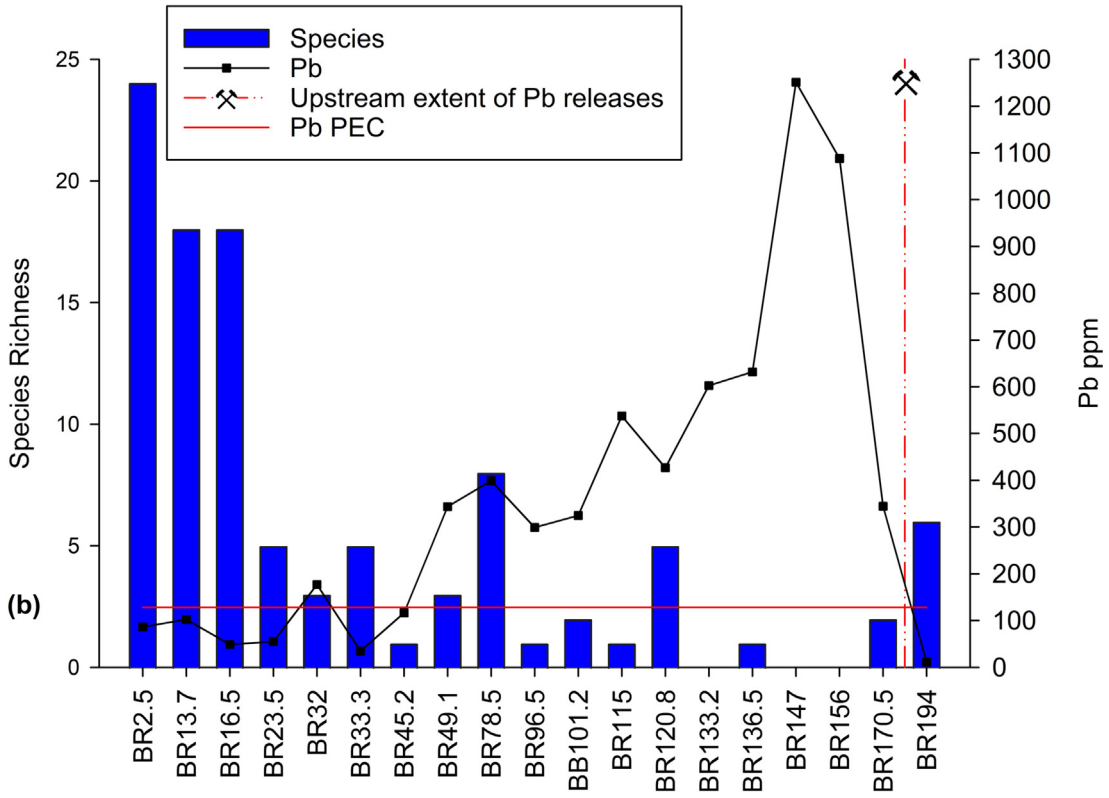

Fig. 5 (*continued*).

correlation results (Table 1) as well as those reported by Besser et al. (2015) show significant negative correlations of lead, zinc, and cadmium in Big River sediments with both richness and abundance of freshwater mussel assemblages and toxic effects on juvenile mussels in laboratory tests. The relative contributions of these three metals to adverse effects on Big River mussels are difficult to determine because they are strongly intercorrelated, and indeed all three metals may contribute to effects observed in the field and in the laboratory. However, Besser et al. (2015) noted that toxic effects on mussels were greater in Big River sediments, which were dominated by lead, compared to sediments dominated by zinc. These results led us to further explore associations of sediment lead with abundance and richness of mussels in the Big River.

The mode of toxicity of Pb in freshwater mussels has not been thoroughly investigated. However, in general Pb replaces calcium when absorbed and therefore, can affect many metabolic pathways including growth, filtration efficiency, enzyme activity, and behavior (Pattee and Pain, 2003). The most common sites of metal uptake in mussels is the gill, the mantle, and the kidney (Naimo, 1995). This study does not characterize toxic contributions from exposure to water-borne Pb or other metals in the Big River. Dissolved Pb, Zn, and Cd concentrations exceeding EPA's aquatic life criteria have been found in the Big River, but tend to be associated with high flow events (Missouri Department of Natural Resources (MDNR), 2010). In addition, contaminated bed sediment is the source of elevated metals in sediment pore water (Besser et al., 2015) and is likely a major contributor to elevated metals in the water column during high flow (Missouri Department of Natural Resources (MDNR), 2010). Ultimately, mussels are exposed to Pb all three contaminated media: sediment, pore water, and the water column. However, sediment metal contamination is the most persistent and probable controlling factor of the contaminant concentrations of the other two media.

Average mussel densities were significantly reduced at survey sites downstream from releases of Pb compared to reference sites. Rank-transformed mussel density data from Phase I sites produced a significant one-way ANOVA ($p < 0.0001$) and significant Tukey's mean comparisons ($p < 0.05$, Table A7). Mean mussel densities at all sites downstream from mining were significantly less than densities at reference sites (Tukey's test; $p < 0.05$). For Phase II sites, one-way ANOVA with ranked density data also showed significant differences in mean density among sites ($p < 0.0001$). Tukey's tests with Phase-II data demonstrated longitudinal trends in density in Big River sites (Table A8). Mussel densities in the reach downstream from historical mining operations (river km 113.5 to river km 67.5; 0.0 to 1.2 mussels/m^2) were significantly less than densities at both reference sites (3.8 to 6.2 mussels/m^2). Mussel densities generally increased in the reach farther downstream from mining-related inputs of metals (river km 47 to km 2.5) and only one site in this lower reach (river km 20.5; 0.2 mussels/m^2) had densities significantly less than the reference sites. The site with the highest mussel density was the most downstream Big River site (BR2.5), which had a mean density > 11 mussels/m^2. The reference site on the Meramec River (MR75.6) was similar to lower Big River sites (BR16.5 and BR47), with densities between 5.71 and 6.11 mussels/m^2 (Table A5). Finally, density at the upper reference site (BR194) was similar to densities at BR30.7 and BR41 (2.92–3.55 mussels/m^2). The upper reference site grouped with two of the lower sites despite its location in the head-waters of the Big River, where lower densities and lower richness are expected (Watters, 1992; Bearden et al., 2019). Nonetheless, density at this reference site is greater than sites with Pb concentrations greater than the PEC located downstream from releases Pb as far as site BR67.5. Site BR20.5 appears to be an outlier among the downstream Big River sites, with a mean density of 0.20 mussels/m^2. This site is in an atypical reach of the Big River with a substrate dominated by shifting sand, which may have affected mussel density and distribution. Some Missouri streams with primarily unstable sand substrates tend to naturally have low mussel densities (Andrew Roberts, U.S. Fish and Wildlife Service [USFWS], pers. obs. 1996 and Roberts et al., 1997). In terms of overall patterns of mussel density in the Big River, we observed a significant decrease in density downstream from historical mining areas with sediment Pb concentrations from 242 to 1251 ppm until BR47, downstream from which all sites except BR20.5 had higher densities (Fig. 6). In terms of overall mussel density, these lower Big River sites (downstream from BR47) were comparable to reference sites in the Meramec River and upper Big River. Sediment Pb

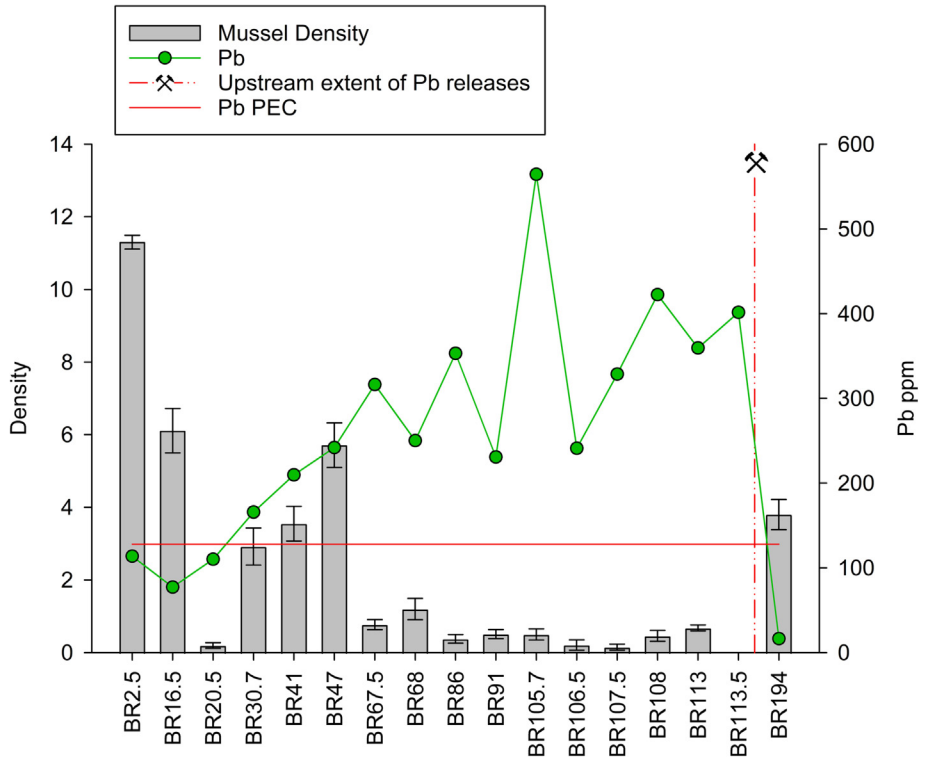

Fig. 6. Big River Phase II mussel density (mussels/m^2) versus lead (Pb) sediment concentration. Sites labeled by river kilometer. BR = Big River. PEC = probable effects concentration.

concentrations were well above the PEC for sites downstream from mining areas between BR113.5 and BR67.5, and Pb concentrations decreased to near the PEC at locations where mussel densities began to increase starting at RK47 (Fig. 6).

Although overall mussel densities at BR47 did not differ from reference densities, this did not correspond with increased mussel richness. Sites between BR47 and BR30.7 were composed of predominantly one species (*Eurynia dilatata*) (Figs. A8 and A9). This species comprised 57 %, 88 %, and 74 % of the total number of species at sites BR30.7, BR41, and BR47, respectively. At BR30.7, the next most abundant species was *L. cardium*, which made up 12 % of CPUE. At sites BR41 and BR47, the next most abundant species next to *E. dilatata* made up <7 % of total species observed. In contrast, dominant species at the reference site MR75.6 on the Meramec River and lower Big River sites BR2.5 and BR16.5 comprised lower percentages including 18 % (*Amblema plicata*), 32 % (*Actinonaias ligamentina*), and 40 % (*A. plicata*), respectively, of the total number of species at those sites. These and several other common species (e.g., *Cyclonaias pustulosa, Obliquaria reflexa, Pleurobema sintoxia*), were either very rare or absent at sites BR30.7-BR47, but large numbers of subfossil shells were observed indicating they once occurred at these sites. This dominance of *E. dilatata*, together with an overall decrease in species richness at sites downstream from the Pb mining releases, indicated lower mussel diversity (a product of evenness and richness), compared to reference sites and the two most downstream Big River sites.

The consistent longitudinal pattern of suppressed mussel community metrics in the Big River followed by their abrupt increase at BR47 indicate that increased sediment Pb concentrations associated with metals released associated with historical mining activities are a primary control on mussel communities (Fig. 7). The Pb model created in Fig. 7 explains 40 % of the variation in mussel density ($r^2 = 0.403$) and estimates a half maximal effective concentration (EC50) for mussel density of 166 ppm (the concentration of toxicant that induces a 50 % effect or a response halfway between the baseline and maximum). The model has a high uncertainty for steepness, indicating that extrapolation from the EC50 to lesser effect concentrations such as the EC20 would be less reliable. However, relatively small differences were estimated between the EC20 and the EC50 (161 versus 166 ppm, respectively). The site-specific EC50 for effects of Pb on mussel density in the Big River has narrow 95 % confidence limits (153–181 ppm) and is consistent with the PEC threshold of 128 ppm for Pb in sediment (MacDonald et al., 2000). The EC20 and EC50 are only slightly greater than the PEC, indicating that conditions in the Big River (e.g., low dissolved organic carbon, high pH, high hardness) produce small reductions in Pb bioavailability in sediment. Sediment Pb concentrations that are reliably below the EC50 values do not occur until approximately river km 30.

Notably, the analysis of correlations between field mussel population metrics and Pb concentrations in sediment is a more sensitive indicator of

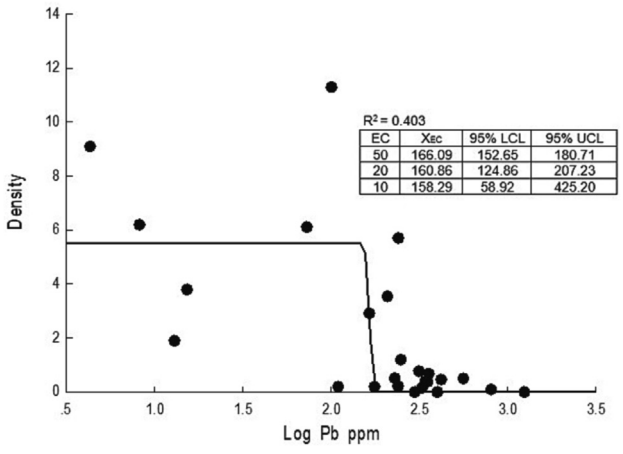

Fig. 7. Concentration-response model for effects of sediment lead on mussel density in the Big River. Line is nonlinear regression model ('threshold sigmoid' model; TRAP software, version 1.30) based on median lead concentrations in <2 mm sediment from Phases I and II quantitative sampling sites in Big River and reference sites in Bourbeuse River and Meramec River. EC = percent effect, X_{EC} = concentration associated with 'EC' percent effect, LCL/UCL = lower and upper confidence limits.

Pb toxicity than laboratory toxicity tests with juvenile mussels conducted using Big River sediment (Besser et al., 2015). Besser et al. (2015) compared toxicity testing done with juvenile *L. siliquoidea* with Big River sediments for 28 days with field mussel population metrics described in this paper. They found overall good agreement between laboratory and field metrics and noted that five of the co-located sediment samples were associated with reduced biomass of juvenile mussels in their laboratory study and reduced species richness from field studies (Roberts et al., 2009). However, four co-located sites had lower sediment toxicity to juvenile mussels in the laboratory that showed depressed mussel population metrics in Roberts et al. (2009; i.e., the current study). The lowest metal concentrations that indicated toxicity to juvenile mussels in Besser et al. (2015) was 250 ppm Pb, 810 ppm Zn, and 11 ppm Cd, with a sum probable effects quotient (PEQ) of 5.9 (PEQ = total recoverable metal concentration/PEC, MacDonald et al., 2000). As stated above we determined an EC50 of 166 ppm Pb. Zinc and estimated Cd concentrations associated with this Pb concentration (found in a sediment sample at BR13.7 in Roberts et al., 2009) are 74 ppm Zn and 2.0 ppm Cd for a sum PEQ of 1.8. Further, Besser et al. (2015) laboratory toxicity studies with juvenile mussels demonstrated toxicity only where Zn and Cd were also above their respective PEC, which is not consistent with depleted population metrics observed in this study. The evidence from Besser et al. (2015) indicates that elevated Cd and Zn concentrations have an additive toxic effect to juvenile mussels. This is further supported by Salerno et al. (2020) who found additive toxicity to juvenile *L. fasciola* mussels and *Villosa iris* (= *Cambarunio iris*) glochidia using a variety of common co-occurring inorganic contaminants including copper, ammonia sulfate, and potassium chloride. The evidence from laboratory toxicity and field results could also indicate that concentrations of Cd and Zn in Big River sediments are acutely toxic, but Pb effects are more chronic in nature. Wang et al. (2010) reported that freshwater mussels (*L. siliquoidea* and *L. rafinesqueana*) are highly sensitive to Pb Cd, and Zn.

Our study indicates that for sediment in the Big River where Pb is the predominant contaminant, field analyzed population metrics are a more sensitive indicator of toxicity to the native mussel fauna than are laboratory toxicity tests. In field studies conducted by Angelo et al. (2007) in Pb, Zn, and Cd contaminated streams in the Tristate Mining District, depressed mussel populations were correlated with metal contamination at lower concentrations than were found to be toxic in the laboratory. Because many mussel species live for several decades, toxicity tests may not capture either the effects of long-term exposure of metals to mussels throughout their life span or periods of acute exposure associated with past releases of metals at legacy mining sites. In addition, toxicity testing with juvenile mussels also does not account for toxic effects to glochidia or other stages in the mussel reproductive processes.

4. Conclusions

In our study, we found that Big River sediments are contaminated with bioavailable Pb for its entire length downstream from areas where metals have been released from historical Pb mining, a distance of 170 km. Sediment Pb and Zn concentrations were high near mining sources and attenuated with distance downstream. This downward trend in sediment Pb levels had a significant negative correlation with mussel abundance and species richness. We accounted for other possible known effects to mussels that could also be contributing to this relation in several ways. First, we assessed the overall aquatic habitat conditions, which were similar between reference and affected sites and showed no correlation with the reduced mussel metrics. Pebble counts and substrate composition analysis both showed that sedimentation, including mine tailings, is also not responsible for the reduced community metrics observed in the Big River. Lastly, mussel community metrics were found to be reduced in the Big River compared to a reference site upstream from metal releases and three other reference streams. This is an important comparison because reference streams all have similar land use and effects to habitat, but do not have sediments contaminated with Pb. Albers (Janice Albers, USGS, written communication, 2016), Rosenberger and Lindner (2022), and Roberts et al. (2022) have all evaluated habitat factors in the Big River and concluded that habitat is not the limiting factor in mussel communities. The association of sediment Pb concentrations with mussel density and richness indicates that Pb toxicity is likely responsible for changes in mussel community metrics. Big River mussel communities are adversely affected when sediment Pb concentrations are above 166 ppm, the concentration associated with 50 % decreases in mussel density, which occurs at approximately river km 30. Thus, based on sediment metal and mussel fauna sampling, our findings indicate that mussels in approximately 140 km of the Big River with suitable mussel habitat suffer toxic effects from Pb contaminated sediments.

Importantly, sediment Pb concentrations found to be limiting to mussels in our field surveys are similar to PEC toxicity thresholds found in the literature (128 ppm) and were more sensitive than toxic concentrations determined using laboratory tests conducted on mussels with co-located sediment. Paired laboratory toxicity and field surveys would be beneficial to determine whether field results may be a better indicator of long-term mussel toxicity than short-term laboratory testing. Additionally, future studies on juvenile mussel recruitment at sites downstream from mining-related Pb releases compared to reference sites could help elucidate potential effects of Pb on mussel reproduction and juvenile survival. Our study provides important information about sediment Pb contamination and mussel populations that could inform efforts to remove metals from stream sediments and potentially restore mussel populations via artificial propagation. Actions that include removal of contaminated sediment, restoration of mussel habitat, and mussel reintroduction in the Big River are being considered by multiple government agencies.

Disclaimer

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the USFWS, but do represent the views of the USGS. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

CRediT authorship contribution statement

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Data availability statement

Data collected for this study are available at https://www.fws.gov/media/assessment-freshwater-mussel-bivalvia-margaritiferidae-and-unionidae-populations-and-heavy and https://www.fws.gov/media/quantitative-survey-freshwater-mussels-unionoidea-and-assessment-sediment-contamination-big.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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