In cooperation with the U.S. Fish and Wildlife Service

Mussel Community Associations with Sediment Metal Concentrations and Substrate Characteristics in the Big River, Missouri, U.S.A.

Janice L. Albers, John M. Besser, Christopher J. Schmitt, Mark L. Wildhaber

Administrative Report

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Executive Summary

The Big River, which drains the Old Lead Belt (OLB) subdistrict of the southeastern Missouri Lead Mining District, has been heavily contaminated by mine tailings containing high concentrations of lead (Pb), zinc (Zn) and other metals from historical mining. Separation of ecological effects of metals from effects caused by the physical presence of the sandy tailings is important in the context of natural resource damage assessment. Accordingly, the objective of this study was to determine whether there were associations between characteristics of mussel communities of the Big River and sediment metal concentrations after accounting for substrate variation. Our analyses addressed the following specific questions:

(1) Are the distribution and abundance of mussels in the Big River influenced by substrate characteristics, sediment metal concentrations, or both? We found the presence of 10 species, the density of all species combined, and the density of 4 biological species groups were negatively related to concentrations of Pb and barium (Ba) and to an index of toxicity hazards known as PEQ for the

mixture of cadmium (Cd), Pb, and Zn (summed probable effect quotients [PEQs]). The presence of *Ligumia recta*, a species of conservation concern, was negatively related with Ba concentrations and PEQ. The presence of seven additional species was also negatively related with Ba concentrations, two of which also were negatively related to PEQ. Although sand is a necessary component of suitable mussel habitat, none of the 23 species or 17 biological species groups evaluated were negatively or positively related with the abundance of sand-sized particles, the size range associated with OLB mine tailings. The presence of two species was positively related with coarse substrates. Several other mussel variables decreased with increasing Pb concentrations and increased with fine gravel percentages.

(2) Do metal concentrations in sediment influence mussel abundance, after accounting for variations associated with the substrate variables? We found a negative relation between the density of *Lampsilis cardium* with Pb in sediment after accounting for substrate variation. In addition, the density of two species groups (those using Percidae as hosts and those that display a lure) significantly decreased as metal concentrations increased after accounting for substrate variation.

(3) Can concentration-response models based on sediment metal concentrations reliably estimate thresholds for adverse effects on mussel communities in the Big River? Total mussel densities in sediments were negatively associated with concentrations of Pb and other metals in Big River sediments. Logistic regression models based on Pb concentrations in the less than (<) 2 millimeter (mm) sediment fraction explained more variation in mussel density among sites than models based on Pb in the <250 micrometer (μm) fraction or models based on PEQ. Mussel density also had (weaker) negative associations with Ba in Big River sediments, but Ba concentrations in sediment and pore water were substantially less than estimated toxicity thresholds, and sites with reduced mussel density and elevated Ba concentrations also had Pb concentrations greater than probable effect concentrations (PEC) for Pb of 128 micrograms per gram (μg/g). Concentration-response models using several modeling options

estimated 20 percent effect concentrations (EC20s) for effects of Pb (in <2 mm sediments) on mussel density that ranged from 116 to 173 µg/g. The model with the combination of best overall fit (r^2=0.68) and narrowest confidence limits estimated an EC20 of 136 µg/g, similar to the Pb PEC.

Collectively, these findings indicate that concentrations of Pb were significantly associated with the occurrence and density of individual mussel taxa, even after accounting for substrate variation.

Introduction

The Big River, located in the Meramec River watershed of southeastern Missouri, drains the Old Lead Belt (OLB) subdistrict of the southeastern Missouri Lead Mining District, an area of historically productive lead (Pb) - zinc (Zn) mines (fig. 1; Kleeschulte, 2008). The Big River has been heavily contaminated with elevated concentrations of Pb, Zn, and other metals from the erosion of OLB mine tailings into the stream channel and leaching of metals from mine tailings (Schmitt and Finger, 1982; Besser and Rabeni, 1987). Further downstream, the Big River drains the former Washington County Barite District. Freshwater mussels and other aquatic organisms inhabiting the Big River are sensitive to metals released from historical mining and related activities in the OLB (Schmitt and others, 1984, 1993, 2007a, 2007b; Allert and others, 2013; Wang and others, 2010; Besser and others, 2015). Previous studies indicated concordance between sediment toxicity (reduced growth and biomass in laboratory tests) and injury to Big River mussel communities (reduced species richness; Roberts and others, 2010; Besser and others, 2009, 2015). The small number of sites quantitatively sampled during these studies, however, limited the analysis of associations between physical riverine habitat and mussel community characteristics. Follow-up studies completed in 2013/2014 provided additional data for examination of relations between mussel distribution and abundance, substrate characteristics, and sediment metal concentrations.

Data collected from 2008 and 2013/2014 indicated an inverse relation between sediment Pb concentrations and mussel density along the length of the Big River (figs. 2 and 3; table 1; Roberts and others, 2016). The Big River, in areas without contamination from the OLB, supported a diverse and productive mussel community; however, densities declined to near zero in the reach downstream from the OLB. Sediment Pb concentrations in the reach between 170 and 50 kilometer (km, measured upstream from the mouth) peak at more than 1,000 µg/g (dry weight), decreasing gradually with distance downstream. In the reach between km 50 and km 20, Pb concentrations decrease to 100–200 µg/g, and mussel densities fluctuated widely: near 0 mussels per meter squared (mussel/m^2) at km 28.5 and km 32, but 3–6 mussel/m^2 at other sites in this reach. Downstream from km 20, sediment Pb concentrations decrease to less than 100 µg/g and mussel density remains more than 6/m^2, similar to the range observed elsewhere.

Attributing effects on Big River mussel communities exclusively to the release of toxic metals from the OLB is complicated by the alteration of stream substrates resulting from erosion of metal-contaminated tailings into the stream channel, which is most evident in St. Francois County between km 137 and 171 (Pavlowsky and others, 2010). Tailings from the OLB typically consist of 91–99 percent sand-sized particles (0.05–2.0 mm particle diameter; Jennett and others, 1981; Schmitt and Finger, 1982). Since at least the late 1970s, tailings from the OLB have become distributed throughout the Big River downstream of inputs (Pavlowsky and others, 2010). Recent studies have concluded that in general, mussel distributions are related more to stream geomorphology and hydrology than to substrate size at a local scale (Gangloff and Feminella 2007, Morales and others, 2006; Allen and Vaughn 2010). Nevertheless, substrate size selectivity can occur in some freshwater mussel species (for example

Huehner 1987; Brim-Box and others, 2002), and the substrate particle size distribution is determined by sediment inputs, stream hydrology and geomorphology; therefore, alteration of stream substrates by tailings may have affected the distribution and abundance of mussels in the Big River downstream of the OLB.

Substrates at most Big River sites sampled in 2013/2014 were dominated by medium to coarse gravel, with cobbles more dominant in the upper reach and fine gravel more abundant in the lower reach (table 2). Sand-sized particles made up less than 10 percent of the substrate at most sites, but upstream sites had slightly higher percent sand, and sand represented the largest percentage at three sites: the Meramec River reference site (32 percent sand), and Big River sites at location 20.5 km (42 percent) and 113.5 (86 percent) upstream from the mouth. The latter was the nearest site to the OLB sampled in 2013/2014, and this high sand content may reflect the influence of tailings particles.

The objectives of this study were to compare the associations of mussel community characteristics with sediment metal concentrations and substrate characteristics. Specifically, we attempted to answer the following questions: (1) Are the distribution and abundance of mussels in the Big River influenced by substrate characteristics, sediment metal concentrations, or both? (2) Do metal concentrations in sediment influence mussel abundance after accounting for variations associated with the substrate variables? (3) Can sediment metal concentrations explain observed reductions in density of mussel communities in the Big River?

Methods

Sites

The primary data for this study were obtained from 17 sites along the Big River located at 2.5, 16.5, 20.5, 30.7, 41.0, 47.0, 67.5, 68.0, 86.0, 91.0, 105.7, 106.5, 107.5, 108.0, 113.0, 113.5, and 194.0 km upstream from the mouth (figs. 1, 2, and 3). Each site was sampled once between the fall of 2013 and fall 2014. All Big River study sites were initially investigated in a reconnaissance survey in 2013 and quantitatively sampled in 2013 and 2014. Sites were specifically chosen because mussels were found to be present during reconnaissance surveys (Roberts and others, 2016), because the habitat was judged to be favorable for mussels by the scientists conducting the survey, or both. Factors considered during site selection included the presence of suitable riffles and runs, substrate composition and stability, presence of a minimum number of five live mussels during a reconnaissance survey, and permanence of the channel. Sites dominated by sand or bedrock or where the channel has relocated within the floodplain were avoided where possible. Site selection was concentrated in areas where the influence of tailings as a textural component was unlikely. Although this method of site selection increased efficiency by minimizing the number of zero mussel values, it intentionally biased the results toward sites where mussels were known or likely to be found. It also precluded collection of habitat characteristics from sites where mussels were not likely to be found.

Additional data on mussel density and sediment metal concentrations for seven sites sampled from July to October, 2008 were obtained from Roberts and others (2010; table 1). Several sites without known metal contamination were considered to be reference sites: the upstream Big River site located 94 km upstream from the mouth (sampled in 2008 and 2013/2014), a reference site in the Meramec River (km 75.6), about 20 km upstream from the confluence with the Big River (sampled in 2014), and a reference site on the Bourbeuse River (km 0.4, sampled in 2008). The latter is a Meramec River tributary that drains no mined lands (Hinck and others, 2012).

Even though the Meramec River reference site has a similar mussel fauna to the lower Big River (Roberts and Bruendeman 2000), it is unknown whether the Meramec River reference site represents other sites in the Meramec River or what condition it represents in the Big River. Because there was only one Meramec River reference site in the 2013/2014 data collection, differences between the Meramec and Big Rivers and reference and contaminated sites were not evaluated. All analyses of the 2013/2014 only data were performed with and without the Meramec River reference site (number of sites was 16 and 15, respectively). We only discuss results of the analyses with the Meramec River site, but all results are reported in the tables and appendixes. The Bourbeuse River reference site was only used in analyses that included 2008 data.

Mussel Response Variables

In both the 2008 and 2013/2014 sampling, individual mussels were excavated from at least 50 0.25-m^2 quadrats at each site (ranged 50–107 quadrats at a site), where all live species of mussels on the substrate surface were counted and then the substrate was excavated to a depth of 10 centimeters (cm) and all live individual mussels were identified to species and counted (tables 1, 3, and 4). In the 2013/2014 sampling, an additional 40 to 81 - 0.25-m^2 quadrats were sampled at each site, during which only individual mussels at the substrate surface were identified and counted. Quadrats were located either completely randomly within the delineated mussel habitat site (2008) or systematically placed within the delineated mussel habitat within a site using three random starts (Smith and others, 2001; Strayer and Smith, 2003). Using surface and subsurface species counts from the quadrats, overall site-level mussel species density (numbers of animals/m^2) were predicted using statistical models developed by Smith and others, (2001; more details of this procedure are available in Nedeau and others, 2003 and Strayer and Smith, 2003).

In the study, individual mussel species densities and presence, biological guild densities, and an overall summed mussel density were evaluated. We included only species that occurred at more than one site for individual species tests and eliminated seven species that occurred at only one site from consideration in species assemblage evaluations (table 3). We evaluated the density of biologically relevant groups based on brood type, infestation type, tribe, and fish host (tables 3 and 4). Brood types included bradytictic (winter brooding) and tachytictic (summer brooding) species. Infestation type, which describes the mode of reproduction or how glochidia are released, included use of lures to attract fish host, release of conglutinates on the bottom of the stream, broadcast of glochidia into the stream, and storage of glochidia in the mantle. Mussel species were grouped into four tribes based loosely on shell thickness: Amblemini, Anodontini, Lampsilini, Pleurobemini, and Quadrulini. Lastly, we grouped mussels by fish host family to investigate the interactions between mussels and the ichthyofauna: Catostomidae, Centrarchidae, Cyprinidae, Fundulidae, Ictaluridae, Percidae, and Sciaenidae. We also evaluated responses using common community metrics computed from the measured species densities. Community metrics computed for each site included Brillouin’s Index, Fisher’s Alpha, Simpson's Index, Inverse Simpson's Index, Pielou's Evenness, Shannon-Wiener Index (base 10 and natural log), number of species (species richness), Variety, Equitability, Rarefaction, and Redundancy (table 5).

Continuous Predictor Variables

Mussels and other aquatic organisms are sensitive to metals contamination from mining (Schmitt and others, 1993; Allert and others, 2013; Wang and others, 2010; Besser and others, 2015). Accordingly, dry weight concentrations of barium (Ba), cadmium (Cd), Pb, and Zn were determined from each of two composite substrate samples at each site (fig. 3, table 1). One composite sample was collected within the mussel bed itself and the second was collected in shallow water outside of the main current on the nearest gravel bar to the mussel bed. The individual aliquots collected (minimum of five) to make up a composite sample were distributed throughout the gravel bar or mussel bed in a manner that would reflect the conditions within that habitat feature. From each composite sample, subsamples representing three substrate size fractions, bulk (containing all particle sizes present, including gravel-sized and larger particles) and two smaller sized categories collected by sieving: <2 mm (coarse sand and smaller particles); and <250 µm (only fine sand, silt and clay-sized particles; see table 6 for list of all categories tested). Sediments were air dried in the laboratory, wet sieved, and air dried again. Dry-weight metal concentrations (µg/g) were determined using a portable x-ray fluorescence meter (XRF; Thermo Niton XL3t XRF Analyzer). A subsample was quality checked by inductively coupled plasma mass spectrometry, as reported in previous studies (Besser and others, 2009; Roberts and others, 2010). Details of these procedures and the results of quality assurance associated with them are available elsewhere (Roberts and others, 2016; Besser and others, 2009). In this study, we used only sediment categories where metals were detected at more than one site, which precluded analysis of Cd in mussel bed sediment <250 µm and Cd in bulk gravel bar sediment. In addition, two sites (Big River, km 107.5 and Big River, km 108) were excluded in the analysis of the 2013/2014 data because there were no gravel bar bulk samples. Where a metal was not detected at a site by XRF, values were set to the detection limit of 1 µg/g. To determine whether predictor variables were related with one another, a Spearman rank-order correlation test was used on all predictor variables (SAS 9.4 TS1M0, Proc Corr; SAS Institute, Cary, North Carolina). Highly related variables contribute equivalent information, so one member of the pair was excluded from all analyses examining associations between biota and metals or habitat variables if the linear correlation coefficient was greater than or equal to (≥) 0.9.

We evaluated whether mussel density, occurrence, or community composition were related to the cumulative effects of multiple metals based on PEQs (Ingersoll and others, 2001; Besser and others, 2009, 2015). The PEQ for a single metal is defined as the measured concentration divided by its probable effect concentration or PEC (MacDonald and others, 2000). The PEC is an empirical estimate of the threshold concentration above which there is an increased frequency of chronic toxicity, based on a large sediment toxicity database (Cd PEC is 4.98 µg/g, Pb PEC is 128 µg/g, and Zn PEC is 459 µg/g). We summed PEQs for Cd, Pb, and Zn for each site, substrate type and size fraction and used this summed PEQ in all model testing. As an example, the PEQ for site Big River km 113.5 mussel bed sediment, <250 µm fraction was computed as (Cd: 1010/4.98 + Pb: 14811/128 + Zn: 679/459) = 15.03. Only sites at which Cd, Pb, and Zn were measured were used in this analysis. There is no consensus PEC for Ba, so it was not included in PEQ analysis

Substrate was characterized in six particle size categories: sand and smaller (<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) based on pebble counts completed at each of the 0.25 m^2 quadrats sampled in the mussel bed at each site (table 2; based on Wolman, 1954). After each quadrat was placed on the substrate, the diver placed a finger, without looking, on the substrate at the upper right corner of the quadrat and the first substrate particle touched was measured along its intermediate axis and recorded. If a fine-grained particle was touched it was tallied into the sand substrate category. The total number of particles in each size category at each site was used as predictor variables in the models.

Statistical Analyses with Selection Procedure

The first set of analyses sought to determine whether substrate characteristics, metal concentrations, or some combination of these groups of variables were related to mussel distribution.

We evaluated every combination of substrate and metal variables and identified combinations that were statistically significant using a nonparametric linear regression model with forward selection. During forward selection, variables were added one at a time to a model containing just an intercept, retaining only the variable with the highest statistical significance. The process was repeated with the remaining variables until none of the added variables were statistically significant. This procedure can result in either a regression model with a single predictor substrate or metal variable (in which case the variation explained is reported using a lower case ‘r’) or a regression model containing multiple predictor substrate or metal variables (where variation explained is reported using a capital ‘*R*’). Because the distributions of most continuous response variables were non-normal, we used distribution-free permutation Least Absolute Deviation Regression (LAD procedure in BLOSSOM/R, 10,000 permutations; Talbert and Cade, 2013; The R Foundation, Version 2.15.3 x64). Using this hypothesis testing procedure, we selected predictor variables with forward selection and inclusion criteria of *P*-value less than or equal to (≤) 0.01 and an increase in the adjusted coefficient of determination, R^2_{adj} ≥0.03. The R^2_{adj} is defined as

$$R^2_{adj} = 1 - \left(\frac{SAF}{SAR}\right) \times \frac{n-1}{n-p-1},$$

where *n* is the number of observations, *p* is the number of parameters in the model, *SAF* is sums of absolute deviation of full model, and *SAR* is the sum of absolute deviations of the reduced model (also denoted as r^2_{adj} if the model contains only one predictor variable). The full model contained an intercept and the predictor variables of interest and the reduced model included only the intercept. One at a time, we regressed all 27 predictor variables (6 substrate variables, plus 21 metal variables, see table 6 for list of predictor variables) with each of the 54 continuous response variables (see tables 3 and 4 for list of mussel species, guilds, and groups) to determine if one or more in combination were significantly related with mussel density or community.

Using the same process, we also used a logistic regression analysis to determine whether the presence of mussel species was related to substrate characteristics, metals, or both. The permutation procedure prr.test (Werft and Benner, 2010) was used to conduct the tests using 10,000 permutations for all tests and the quasi-binomial distribution family (to accommodate dispersed data). Predictor variables were selected using the forward selection procedure described previously with a selection criterion of *P*-value ≤0.01. We determined the goodness-of-fit for these models using R^2_{pseudo}, defined as

$$R^2_{pseudo} = \frac{deviance_{reduced} - deviance_{full}}{deviance_{reduced}},$$

where the full model included a predictor variable of interest and the reduced model included only an intercept (also denoted as r^2_{pseudo} when there is only one predictor variable in the model). Since R^2_{pseudo} is derived from the same likelihood values as the *P*-value, R^2_{pseudo} is redundant and was not used in the selection procedure. The 27 predictor substrate or metal variables, or both were regressed against the presence or absence of the 22 species that occurred at more than one site to determine if one or more variables were significantly related with mussel presence.

Analyses Accounting for Substrate Variation

We used nonparametric linear regression to determine whether the continuous mussel response variables (that is, mussel density or community composition metrics) were related to metal concentrations after accounting for variation associated with the substrate variables. All 6 substrate variables were included in each regression test, then each of the 21 predictor metal variables were added one at a time (see table 6 for list of predictor variables tested). The reduced model included an intercept and six substrate variables and the full model included an intercept, six substrate variables, and one metal variable of interest. We repeated this test for each of the 54 response variables (see tables 3 and 4 for list of response variables). We used a distribution-free permutation LAD procedure in BLOSSOM/R with 10,000 permutations (Talbert and Cade, 2013; The R Foundation, Version 2.15.3 x64) because, as previously noted in the Statistical Analyses with Selection Procedure section, the distributions of most response variables were non-normal. We used the quantile rank score statistic within the hypothesis test procedure, which reduced the sensitivity of the test to heterogeneous error distributions (Talbert and Cade, 2013; The R Foundation, Version 2.15.3 x64). We determined the goodness-of-fit for these models using R^2_{quasi}, which was calculated as

$$R^2_{quasi} = 1 - \left(\frac{SAF}{SAR}\right)$$

(Talbert and Cade, 2013; The R Foundation, Version 2.15.3 x64). The *P*-value from the rank score test was used to determine whether any metals were related with mussel density or community composition.

We completed a similar series of analyses using logistic regression to determine if mussel presence/absence data were related to sediment metal concentrations after controlling for substrate variation. All 6 substrate variables were included in each logistic test (reduced model) and then each of the 21 predictor metal variables were added one at a time (full model). We repeated these tests for each of the 22 species that occurred at more than one site to determine if metals explained more variation in mussel presence than substrate alone, where the reduced model included an intercept and 6 substrate variables and the full model included an intercept, 6 substrate variables, and 1 metal variable of interest. By comparing full and reduced models, we determined the relation between species presence and metals after the substrate variation was controlled. We used the permutation procedure prr.test (Werft and Benner, 2010) to determine significance using 10,000 permutations and the quasi-binomial distribution family (to accommodate dispersed data). We determined the goodness-of-fit for these models using R^2_{pseudo}. The P-value was based on the chi-squared distribution with a dispersion parameter of zero.

Concentration-Response Models

The third set of analyses examined relations between metal concentrations in mussel bed sediments and the abundance of all mussels (expressed as density, the number of live mussels per square meter [m^2]), without attempting to model associations with substrate characteristics. These analyses were completed with combined data from the 2008 and 2013/2014 sampling periods, which consisted of 25 sites of mussel density matched with concentrations of metals analyzed in multiple sediment size fractions. The merged dataset (table 1) included results from 18 sites sampled in 2013/2014 and seven sites sampled in 2008. Four of the 25 sites (two from each sampling period) were reference sites located in stream reaches known to be substantially free of metal pollution.

The objective of this analysis was to estimate thresholds for toxic effects of metals on mussel density in the Big River based on concentration-response models. Our approach was to compare models based on different metals and different size fractions of mussel bed sediment, analyzed separately and in combination, to find the strongest relations between metal concentrations and reductions in density of mussel communities. We analyzed concentration-response relations using the U.S. Environmental Protection Agency's Toxicity Relationship Analysis Program (TRAP; Erickson, 2010) to estimate threshold metal concentrations associated with reductions in mussel density. We used these models to estimate EC10 and EC20 values defined as concentrations associated with 10 percent and 20 percent reductions in mussel density, compared to reference sites or other sites with low metal concentrations. We assumed that 10 percent or 20 percent reductions in mussel density represent biologically meaningful changes in mussel populations. Sediment samples with concentrations of Cd, Pb, or Zn less than XRF detection limits were set to 1 μg/g for concentration-response analyses. We used nonlinear regression to fit two types of sigmoid (S-shaped) concentration-response curves: logistic (based on the lognormal distribution, with infinite tails), and threshold sigmoid, which assumes defined thresholds for 0 percent effect and 100 percent effect. All models were based on $\log_{10}$-transformed data for the concentration (X) axis. We compared models constructed with untransformed density (Y) data versus models in which the values of density were weighted using square roots to reduce the influence of observations with greater (and more variable) mussel density. Models were evaluated based on goodness of fit, as indicated by convergence of the regression model, the width of 95-percent confidence limits, the significance (P-value) of the regression model, and coefficient of determination (r^2). The coefficient of determination for TRAP models was estimated as

$$r^2 = 1 - \frac{sum\ of\ squares\ for\ error}{total\ sum\ of\ squares}.$$

Qualifiers

As previously noted, the results of these analyses completed with data from the 2013–14 surveys are relevant only to sites at which mussels were found or presumed to be found because of bias associated with site selection. No sites were sampled in 2013–14 from the reach immediately downstream from the OLB mining area where mussels are completely absent and where sediment metal concentrations are greatest (Besser and others, 2015); sampling in these years was focused at sites where mussel populations were known to be present based on reconnaissance surveys (Roberts and others, 2016). Within the reach that was sampled, mussel surveys and associated sediment characterization were only completed in habitats typically inhabited by freshwater mussels. As a result of these intentional biases in site selection, inferences about these data should not be extrapolated to locations without mussels that may contain high sediment metal concentrations or unsuitable substrate characteristics for freshwater mussels.

The Spearman rank correlation tests indicated that two Pb variables (<2 mm Pb mussel bed and <250 µm Pb mussel bed) were correlated. In addition, all Pb variables were highly correlated with Zn (<250 µm Zn mussel bed with <250 µm Pb mussel bed, <2 mm Zn mussel bed with <2 mm Pb mussel bed, <250 µm Zn gravel bar with <250 µm Pb gravel bar, and <2 mm Zn gravel bar with <2 mm Pb gravel bar). Therefore, all Zn variables and the <2 mm Pb mussel bed variables were removed from the LAD and prr.test analyses to reduce multicollinearity during multiple regression tests. Also, because PEQ is calculated using Cd, Pb, and Zn, values were expected to be related with these metal variables. Consequently, models containing PEQ were run separately from those that contained individual metals.

Cadmium was detected in most substrate fractions using XRF; however, Cd in the mussel bed <250 µm fraction was only detected at one site (Big River 133.5). Because of this limitation, no results from Cd in mussel bed, <250 µm fraction are reported, but they are included in appendix 1 and 2 for future reference.

The analyses reported here quantify relations between variables that neither prove nor disprove causation. In addition, a common practice when testing multiple hypotheses is to adjust the level of significance according to the number of tests to reduce the likelihood of a Type I error (that is, decreasing the rate of false positives). In the analyses reported here, adjusting the *P*-value of the hypothesis tests and increasing the rate of a Type II error (false negatives) would reduce the number of significant relations between predictor variables and mussel densities. A highly conservative correction could be used, such as the Bonferroni correction where an alpha of 0.05 is decreased to 0.0023 (0.05 divided by the number of tests, 22), but it would eliminate all statistically significant correlations. Use of the marginally significant Bonferroni-corrected alpha of 0.0045 (that is, 0.10/22) would result in only one significant test (a negative correlation between the Lampsilini mussel tribe and Pb in the gravel bar <250 µm fraction using data without the Meramec River reference site; see appendix 1).

We used a less conservative approach than the Bonferroni corrected P-value by discussing results of tests with an alpha of 0.01 or less, and also presenting all model results with P-values ≤ 0.1 in appendix 1 for future reference. In addition, relations between variables can be statistically significant but explain little of the variation in the response and may not be biologically meaningful. Therefore, we also restricted our discussion to models for which the goodness of fit was at least 10 percent, that is, the model reduced the R^2_{adj} or R^2_{quasi} in the LAD models and R^2_{pseudo} in logistic models by at least 10 percent. Note that none of these measures is the same as coefficient of determination (r^2 or R^2) used in least squares regression models; however, they all represent the amount of uncertainty reduced by the models.

Results and Discussion

Associations Using Selection Procedure

We describe only results for which the mussel response was related to the predictor variables at the 0.01 level (without Bonferroni adjustment) and that included the Meramec River reference site (table 7, appendix 2):

Concentrations of metals in several sediment fractions were significantly associated with the density or presence of individual mussel species, all mussel species, and mussel species groups (table 7). Among the OLB metals considered individually (Cd, Pb, and Zn), only concentrations of Pb were significantly associated with mussel response variables. Consistent with metal toxicity, most of the associations were negative. As the concentration of Pb in the mussel bed <250 µm fraction increased, the density of all species of mussels decreased (r^2_{adj} = 0.25), as did the density of *Potamilus alatus* (r^2_{adj} = 0.15), *Strophitus undulatus* (r^2_{adj} = 0.16), bradytictic mussels (r^2_{adj} = 0.17), mussel diversity as indicated by Brillouin's Index (r^2_{adj} = 0.18), and mussels with Centrarchidae (r^2_{adj} = 0.13) and Fundulidae (r^2_{adj} = 0.16) hosts. So also did the presence of *S. undulatus* (r^2_{pseudo} = 0.47). In addition, the density of mussel species that broadcast their glochidia was negatively related to the bulk Pb concentration in the mussel bed sediment (r^2_{adj} = 0.15).

Many of the mussel variables were negatively related with PEQ, which represents the potential cumulative toxicity of OLB metals (table 7). We determined negative relations between PEQ in the gravel bar <250 µm fraction and the presence of *Actinonaias ligamentina* (r^2_{pseudo} = 0.58), *Ellipsaria lineolata* (r^2_{pseudo} = 0.58), *Ligumia recta* (r^2_{pseudo} = 0.58), and *Pleurobema sintoxia* (r^2_{pseudo} = 0.49). The presence of *Obliquaria reflexa* also was negatively related to PEQ in the gravel bar bulk sediment

(r^2_{pseudo} = 0.50). Other significant negative relations explained less variation. The PEQ of the mussel bed <250 µm fraction was negatively related with the density of all species (r^2_{adj} = 0.22), *P. alatus* (r^2_{adj} = 0.15), *S. undulatus* (r^2_{adj} = 0.15), bradytictic mussels (r^2_{adj} = 0.16), mussel diversity as indicated by Brillouin's Index (r^2_{adj} = 0.18), mussels with a Centrachidae (r^2_{adj} = 0.11) or Fundulidae host (r^2_{adj} = 0.15), and the presence of *S. undulatus* (r^2_{pseudo} = 0.47). The PEQ of the mussel bed <2 mm fraction was negatively related with the presence of *Amblema plicata* (r^2_{pseudo} = 0.39), mussels that broadcast glochidia (r^2_{adj} = 0.15), and a Variety of mussel species (r^2_{adj} = 0.23).

Two mussel community characteristics had positive associations with substrate variables (table 7). As the fine gravel percentage increased so also did the presence of *P. sintoxia* (r^2_{pseudo} = 0.52) and mussel Variety (r^2_{pseudo} = 0.33), and as boulder percentage increased so did the presence of *Lampsilis brittsi* (r^2_{pseudo}= 0.42). Notably, there were no associations with the percentage of sand-sized particles, which dominate OLB tailings.

Four mussel groups were best explained by models that included a metal variable and a substrate variable (appendix 2). The density of *Quadrula pustulosa* (R^2_{adj} = 0.35), the densities of mussel species with an Ictaluridae host (R^2_{adj} = 0.39), species that store conglutinates (R^2_{adj} = 0.40), and species that belong to the tribe Quadrulini (R^2_{adj} = 0.32) were negatively related with Pb in the gravel bar bulk sediment and positively related with the percentage of fine gravel.

None of the 39 mussel densities tested were related to Ba concentrations; however, the presence of 8 of the 19 mussel species were negatively associated with Ba concentrations (table 7). The concentration of Ba in the gravel bar <250 µm fraction was negatively associated with the presence of *A. ligamentina* (r^2_{pseudo} = 0. 74), *E. lineolata* (r^2_{pseudo} = 0.74), *L. recta* (r^2_{pseudo} = 0.74), and *Truncilla truncata* (r^2_{pseudo} = 0.53), with the first three species having the same relation with PEQ. Additionally, as the concentration of Ba in the mussel bed <2 mm fraction increased, the presence of *A. plicata* (r^2_{pseudo} = 0.66), *Leptodea fragilis* (r^2_{pseudo} = 0.44), *O. reflexa* (r^2_{pseudo} = 0.67) and *Q. pustulosa* (r^2_{pseudo} = 0.47) decreased. These eight logistic models had some of the highest r^2 values in explaining single species presence with a sediment metal concentration. *Truncilla truncata*, *O. reflexa*, *Q. pustulosa* and *E. lineolata* were found at sites downstream from km 47, which is expected because these species typically inhabit mid-river to mouth-river segments. In addition to *E. lineolata* presence also being related to PEQ, these results could be from Ba concentrations, PEQ, or could include selection of larger-scale riverine habitat by these species (Buchanan, 1980; Bruenderman and others, 2002). *Actinonaias ligamentina*, *Ligumia recta*, and *A. plicata* were only found at sites downstream from km 47, where Ba concentrations in the gravel bar <250 µm fraction were low (~ <360 µg/g) or in mussel bed <22 mm fraction were lower (~ <337 µg/g). *Actinonaias ligamentina* and *L. recta* are usually found in all river segments (headwater, mid and mouth river; Buchanan, 1980) and their absence also is related to PEQ, consequently these relations might indicate a sensitivity of these species to either PEQ or Ba. Another species that is usually found in all river segments is *L. fragilis*, which we found as far upstream as km 133 but only when Ba concentrations were less than ~461 µg/g in the mussel bed <22 mm sediment fraction; this result could indicate a response by this species with Ba concentrations but more investigations would be necessary because there is little evidence of Ba toxicity in the literature (see Concentration-Response Model section for further discussion).

In contrast, we found positive relations between Equitability and Ba concentration in the gravel bar <250 µm fraction (r^2_{adj} = 0.16) and between Equitability and PEQ in the <250 µm mussel bed sediment (r^2_{adj} = 0.17; table7). Sites with higher equitability also had higher Ba concentrations in the gravel bar and higher PEQ in the mussel bed, but these were the same sites that had low species richness. Equitability tends to be high at sites with low species richness. Here, Equitability is probably decreasing as species richness increased at sites with low Ba and PEQ.

Metal Associations after Accounting for Substrate Variation

The objective of these analyses was to determine whether metals significantly contributed to variation in the mussel variables after accounting for substrate variation. With all substrate variables accounted for, several mussel response variables had significant negative associations with metal concentration variables at the 0.01 level (table 8, appendix 1). After accounting for variation associated with the substrate variables, Pb concentrations in the <250 µm fraction of gravel bar sediments were negatively associated with densities of *Lampsilis cardium* (R^2_{quasi} = 0.45), species with Percidae host (R^2_{quasi} = 0.11), and species that display lures (R^2_{quasi} = 0.12). Additionally, the density of species that display a lure was negatively related with PEQ in the <250 µm gravel bar sediments (R^2_{quasi} = 0.12).

Concentration-Response Models

We developed concentration response models based on concentrations of Ba, Cd, Pb, and Zn in mussel bed sediments to determine what metal or metal mixture best predicted the density of mussels in the Big River. Based on results of previous laboratory and field studies with mussels (Roberts and others, 2010, Besser and others, 2009, 2015) and the fact that concentrations of these metals frequently exceed PECs in Big River sediments, we expected to find relations with Cd, Pb, or Zn, or some combination. The previously described permutation analyses also found significant associations of mussel community endpoints with Pb and with sediment particle size distributions, both of which are also known or suspected to influence mussel communities. In contrast, significant associations between sediment Ba concentrations and the distribution of certain mussel species were unexpected because there is little evidence that Ba is toxic at environmentally realistic concentrations in sediment or water. As of 2016, there is currently no national water quality criterion for Ba in the United States for protection of aquatic life, and there are no consensus sediment quality guidelines, such as PECs, established for Ba in sediments. We were unable to find any data reporting toxic levels of Ba in freshwater sediments.

We evaluated hazards of Ba toxicity in Big River sediments based on published data on the toxicity of Ba in water. A search of the U.S. Environmental Protection Agency 'ECOTOX' Database (accessed at http://cfpub.epa.gov/ecotox/ on February 8, 2016) for studies of toxic effects of Ba on survival, growth, or reproduction of freshwater organisms retrieved 33 entries with results of acute toxicity tests (<7 days duration) and 7 entries that reported results of chronic toxicity tests (greater than [>] 7 days; appendix 3). The four lowest acute effect concentrations for waterborne Ba ranged from 46,000 milligrams per liter (mg/L) to 88,800 micrograms per liter (µg/L), with all other acute effect concentrations being greater than 100,000 µg/L. The lowest chronic effect concentration was an EC50 of 8.9 mg/L for reduced reproduction of *Daphnia magna* in a 21-day study (Biesenger and Christiansen, 1972), with five other chronic tests reporting LC50s for fish and crayfish ranging from 39 to 61 mg/L. Besser and others, (2009) reported Ba concentrations ranging from 0.14 to 0.84 mg/L in pore waters from 21 Big River sites. These limited data indicated that Ba concentrations in Big River pore waters were at least 50 fold less than acute toxicity values and at least 10 fold less than chronic toxicity values, suggesting low hazard of Ba toxicity to benthic organisms.

We only found one published report that attempted to establish toxicity thresholds for Ba in sediment. The Dutch Ministry of Environment (Lizjen and others, 2001) estimated a "serious risk concentration” (SRC) for Ba in freshwater sediment using an equilibrium partitioning approach. This approach assumes that Ba toxicity in sediments is related to toxic concentrations of Ba in pore water. The SRC for sediment of 7,200 micrograms per kilogram was derived by dividing the SRC for water by a distribution coefficient (Kd = Ba concentration in sediment/Ba concentration in water) of 1,000 liters per kilogram (L/kg). A similar threshold (7,120 µg/g) can be estimated from the lowest chronic toxicity value reported above (8,900 µg/L) and a typical distribution coefficient for Ba in Big River sediments (about 800 L/kg). The highest Ba concentration measured in Big River mussel bed sediments during 2008–2013/2014 (4,026 and 1,455 µg/g in <250 µm and <2 mm sediments, respectively; table 1) was less than the sediment SRC by about a factor of two to five, suggesting low hazards of Ba toxicity in Big River sediments.

We estimated thresholds for adverse effects of sediment metals on mussel density (total number of live mussels/m^2), a metric that reflects the number of mussel species occurring at a site and the abundance of each species. Longitudinal trends in mussel density and sediment metal concentrations in the Big River are summarized in figures 2 and 3. Mussel density was 2 to 4/m^2 at the headwater reference site, decreased to 1/m^2 or less in a 100-km reach downstream of the OLB, and gradually recovered further downstream to reach 6/m^2 at sites near the mouth of the Big River. Lead concentrations in <2 mm and <250 µm sediment size fractions followed very different trends, with low Pb in the headwaters, peak Pb concentrations downstream from the OLB, and gradual decreases in the downstream reach. Sediment Ba concentrations show a broad peak in the middle reach of the Big River (km 150 to km 50). Elevated concentrations of Ba (>400 µg/g) only occurred in sediments with Pb concentrations greater than the probable effect concentration for Pb of 128 µg/g. Thirteen of the 15 Big River sites with severely reduced mussel densities (<1.0/m^2) had sediment Pb concentrations greater than the PEC (table 1).

Concentration-response models based on sediment Pb concentrations had the strongest relations with mussel density. Logistic regression models based on Pb in the <2 mm mussel bed sediment fraction explained a greater proportion of the variation in mussel density (r^2=0.44; fig. 4*A*) than models based on Pb in the <250 µm mussel bed sediment fraction (r^2 = 0.30; fig. 4*B*) or models based on Ba in either size fraction (r^2 = 0.26 for the <250 µm fraction; fig. 4*C*). Models based on summed PEQs, which were intended to reflect the combined contributions of Cd, Pb, and Zn to sediment toxicity, did not explain more variation in mussel density than models based on Pb alone (r^2 = 0.44 for sum-PEQ in <2 mm sediments; fig. 4*D*). The similarity of concentration-response models for summed PEQs to those for Pb reflected the dominant contribution of Pb to summed PEQs for Big River samples (for example, median PEQ for Pb was 8.3 times greater than that for Zn in <2 mm mussel bed sediments). The weaker explanatory power of the Ba models reflected the wide variation in mussel density at low Ba concentrations. Sediments with the lowest Ba concentrations included several sites with mussel density at or near zero as well as sites with very high density (fig. 4*C*). In contrast, sediments with the lowest Pb concentrations (or lowest sum-PEQs) had moderate to high mussel densities (>2/m^2), and sites sediment Pb concentrations at or above the Pb PEC—or sum-PEQ values near 1.0—had mussel densities less than 1/m^2. These Pb concentration results indicated that concentration response models based on Pb in sediment were most likely to reliably predict decreases in mussel density in the Big River.

We examined the influence of different model characteristics on the goodness of fit of concentration-response models and the reliability of EC10 and EC20 values. Table 9 summarizes effects concentrations based on several different model options: (1) different size fractions in mussel sediments (<2 mm or <250 µm); (2) different model type (logistic or threshold sigmoid); (3) with or without weighting of observations (using the square root of density) to reflect increased variation with increased density; and (4) with or without two potentially influential observations (Big River sites at 20.5 and 30 km; indicated by pink symbols in fig. 4). Neither threshold models nor weighted models consistently increased the explanatory power of logistic concentration-response models; however, models that excluded two data points produced better fit than models based on the full dataset. For example, logistic models based on Pb in the <2 mm mussel bed sediment size fractions had r^2 = 0.67 for the reduced dataset (n = 23) and r^2 = 0.44 for the full dataset (n = 25). For the full and reduced datasets, three of the four model options based on Pb in the <2mm sediment fraction (highlighted in table 9) had better fit than the fourth option (threshold model with weighted data). The average effects concentrations for these two groups of models were similar (mean EC20s of 154 and 151 µg/g for full and reduced datasets, respectively), but both the ranges among effect concentrations within each set of models and confidence limits for individual effect concentrations were narrower for the reduced dataset. The weighted logistic model with the reduced dataset had the best combination of overall model fit (r^2 = 0.68) and narrow confidence limits. This model produced an EC20 of 136 µg/g and the same model options applied to the full dataset estimated an EC20 of 116 µg/g. Both of these Pb EC20 values were close to the Pb PEC for sediment of 128 µg/g. The decision regarding which of these best-fit models is most appropriate should probably be based on the judgment of whether effects on mussel density at two Big River sites, km 20.5 and km 30, were strongly influenced by factors other than metals (for example, unfavorable habitat type, which would favor using the reduced model) or whether the occurrence of severe effects at these sites with low Pb concentrations primarily reflects uncertainty in the concentration values determined by XRF (which would favor using the full model).

Summary and Conclusions

The objective of this study was to determine whether there were associations between characteristics of mussel communities of the Big River (species presence/absence, density, and community metrics) and metal concentrations in sediments that are independent of associations of mussel communities with variables that define substrate composition.

We found that the overall density of mussels in the Big River was influenced by sediment dry weight metal concentrations and none of the species distributions and abundances were correlated with fine substrate characteristics indicative of Old Lead Belt mine tailings. We used nonparametric regression analyses to evaluate the relations between the mussels and all of the environmental variables collected and determined negative associations existed between mussel variables and metal concentrations in sediment, which were consistent with the hypothesis of metal toxicity, but we determined no significant associations between mussel variables and the substrate size fraction less than (<) 2 millimeters (mm) commonly associated with mine tailings. Mussel Variety and the presence of two species, however, were positively related with coarse substrates: *Pleurobema sintoxia* was positively related with fine gravel, and *Lampsilis brittsi* was positively related with boulders. Metals appear to affect community structure, as evident by several mussel variables (density of *Quadrula pustulosa* and the densities of mussel species that have an Ictaluridae host, species that store conglutinates, and species that belong to the tribe Quadrulini) having negative relations with lead (Pb) in the gravel bar bulk sediment and positive relations with the percentage of fine gravel. The presence of seven species, the density of all species combined, density of two individual species, and the density of six biological groups were negatively related with concentrations of Pb or PEQ, or both. The presence of *Ligumia recta*, a species of conservation concern, was negatively related with Ba concentrations in addition to the presence of seven other species; only Ba results from two species require more inquiry. Nevertheless, our analysis of Big River barium (Ba) concentrations relative to extant information on Ba toxicity indicates that the relations between mussel variables and Ba do not reflect Ba toxicity. Overall, these results indicate that mussel density and presence are associated with Pb or probable effect quotients, or both, in addition to coarse substrate and not sand-sized substrate.

We also determined that metal concentrations in sediment influenced mussel abundance even after accounting for variation associated with all the substrate variables. We used a nonparametric regression analysis model that accounted for the variation associated with the substrate variables during testing for metal effects. We determined negative relations between the density of *Lampsilis cardium* with Pb after accounting for substrate variation. In addition, the density of two groups (those using Percidae as hosts and those that display a lure) significantly decreased as metal concentrations increased after accounting for substrate variation. Collectively, these results indicate that concentrations of Pb and possibly other metals had significant negative associations with mussel species occurrence and density after accounting for substrate variation.

We were also able to estimate thresholds for injury to mussel communities in the Big River using the associations between sediment metal concentrations and overall mussel density. Merged data from quantitative mussel surveys completed from 2008 through 2013/2014 were analyzed by nonlinear regression to derive concentration-response models based on relations between total mussel density and sediment metal concentrations. The best concentration-response models based on Pb concentrations in <2 mm mussel bed sediments with total mussel density explained 45–68 percent of variation among sites and produced estimates of Pb EC20s—concentrations associated with 20 percent reduction in mussel density relative to low-metal sites—of 116 micrograms per gram (μg/g) and 136 μg/g (table 9), consistent with the published probable effect concentration for Pb toxicity in sediment (128 μg/g).

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Figure 1. Map of study area showing Big and Meramec River sites; reconnaissance, quantitative, and reference quantitative mussel sampling sites completed in 2013/2014 (used by permission from Roberts and others, 2016; OLB indicates the start of the Old Lead Belt inputs).

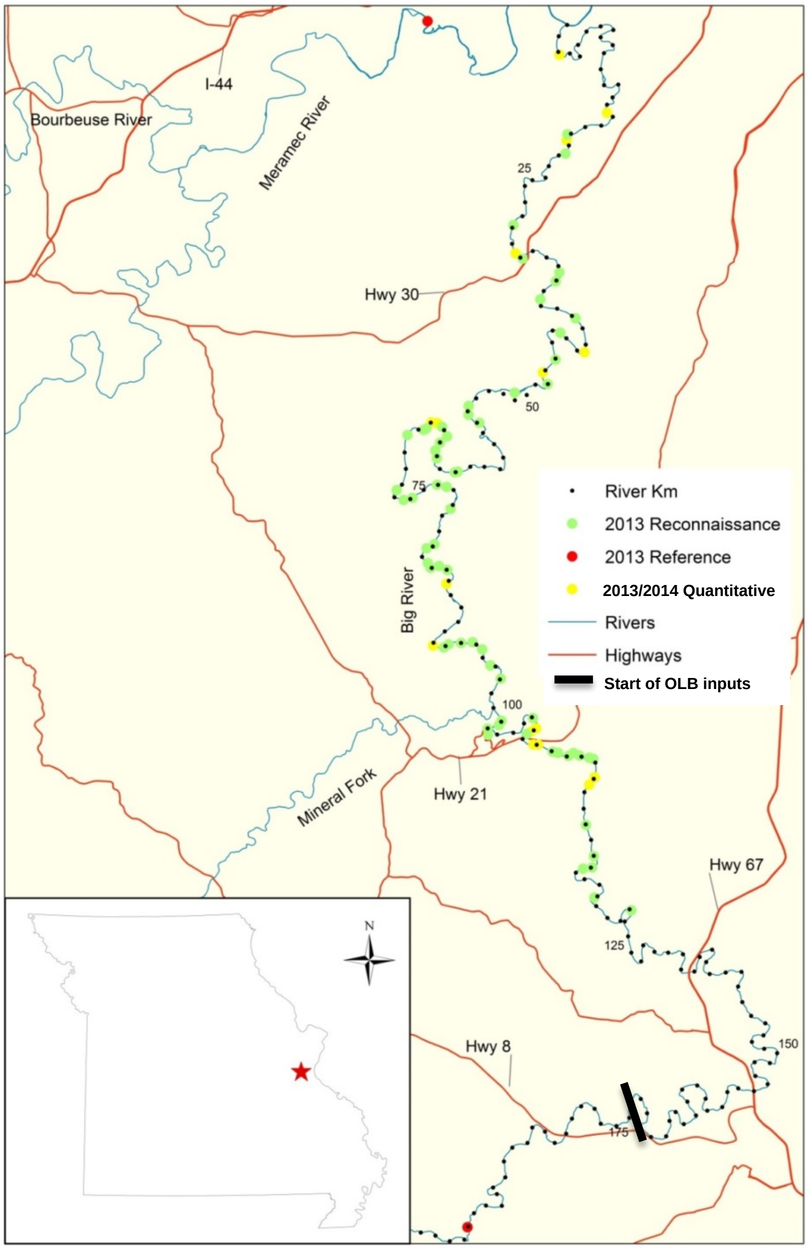

Figure 2. Longitudinal variation in mussel density in the Big and Meramec Rivers, Missouri, found in this study. Meramec and Bourbeuse River reference site values also included (m^2 = meter squared).

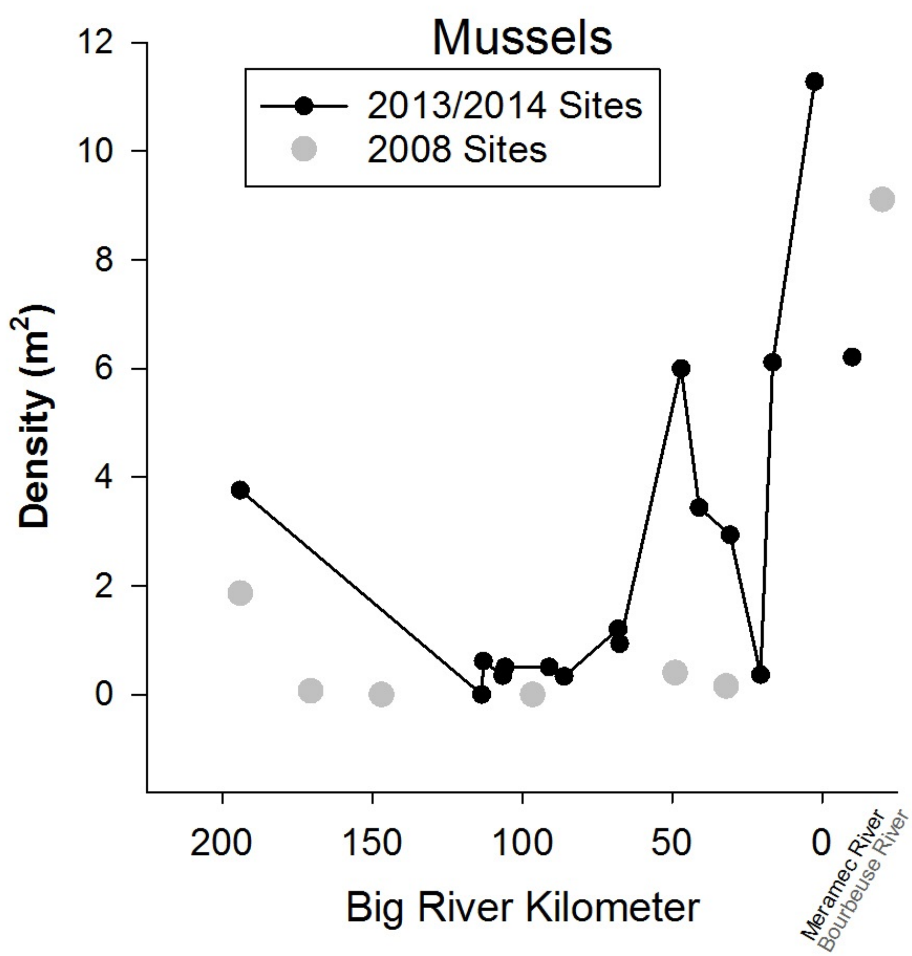

Figure 3. Longitudinal variation in dry weight lead and barium concentrations and PEQ in mussel bed and gravel bar sediments in the Big River, Missouri. Meramec and Bourbeuse River reference site values also included. The Probable Effects Quotient (PEQ) is the summed PEQ of the three metals of interest: lead, zinc, and cadmium; where the PEQ for a single metal is defined as the measured concentration divided by its probable effect concentration (MacDonald and others, 2000; micrograms per gram [µg/g]). Gray points (open circle less than [<] 2 millimeter [mm] sediment, solid circle <250 micrometer [µm] sediment) indicate data collected in 2008 and added in the concentration response analysis.

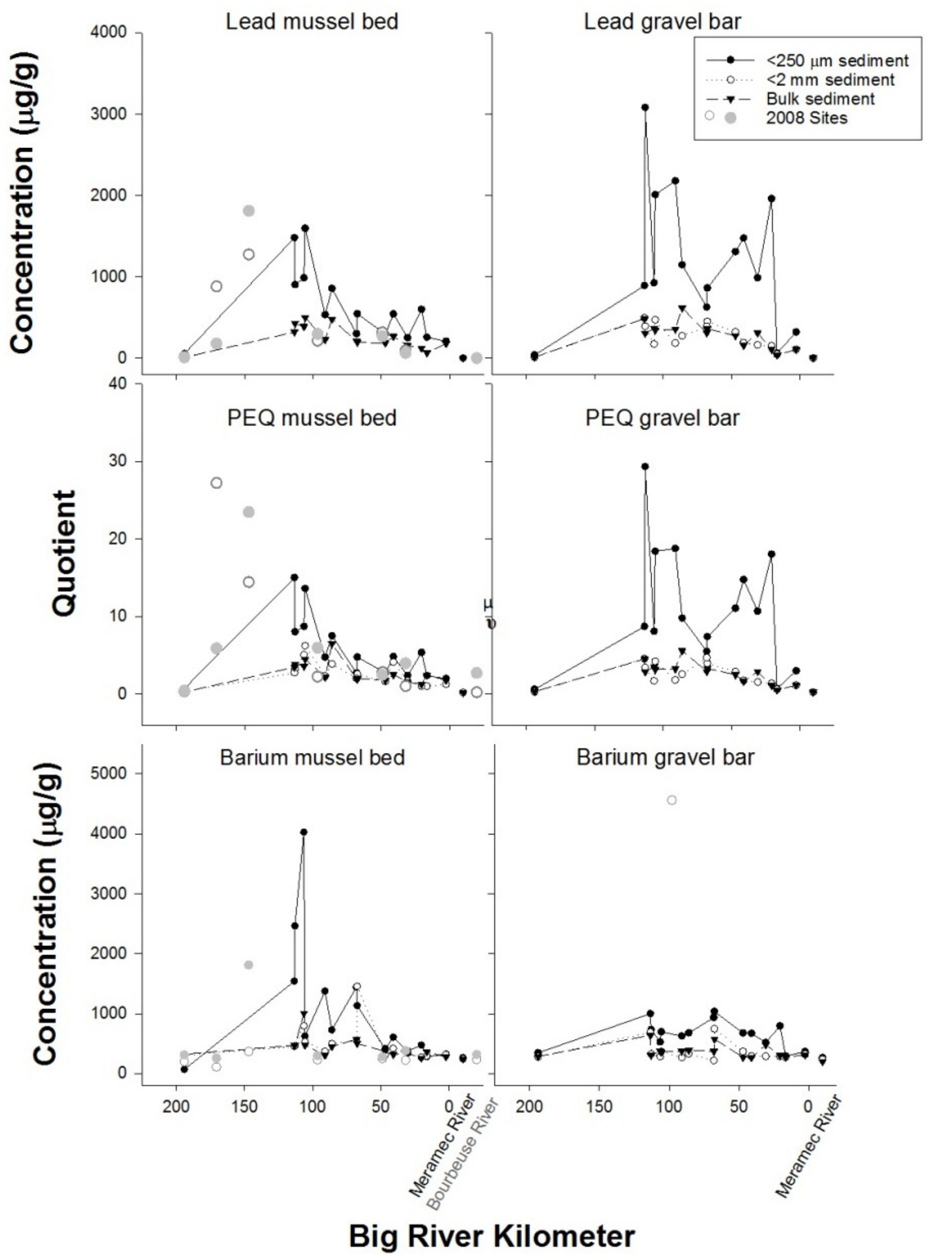

Figure 4. Concentration-response plots and logistic regression models for associations between dry weight sediment metal concentrations and mussel density in the Big River, 2008 and 2013/2014: *A*. Lead in less than (<) 2 millimeter (mm) sediment fraction; *B*. Lead in <250 micrometer (µm) sediment fraction; *C*. Barium in <250 µm sediment fraction; D. Sum of probable effect quotients (PEQ) for lead, zinc, and cadmium in <2 mm sediment fraction. Pink symbols represent sites excluded from some concentration-response models (see text); micrograms per gram (µg/g); *p* is the *P*-value. Three-parameter log-logistic regression models (blue lines) generated by SigmaPlot, version 12.55.

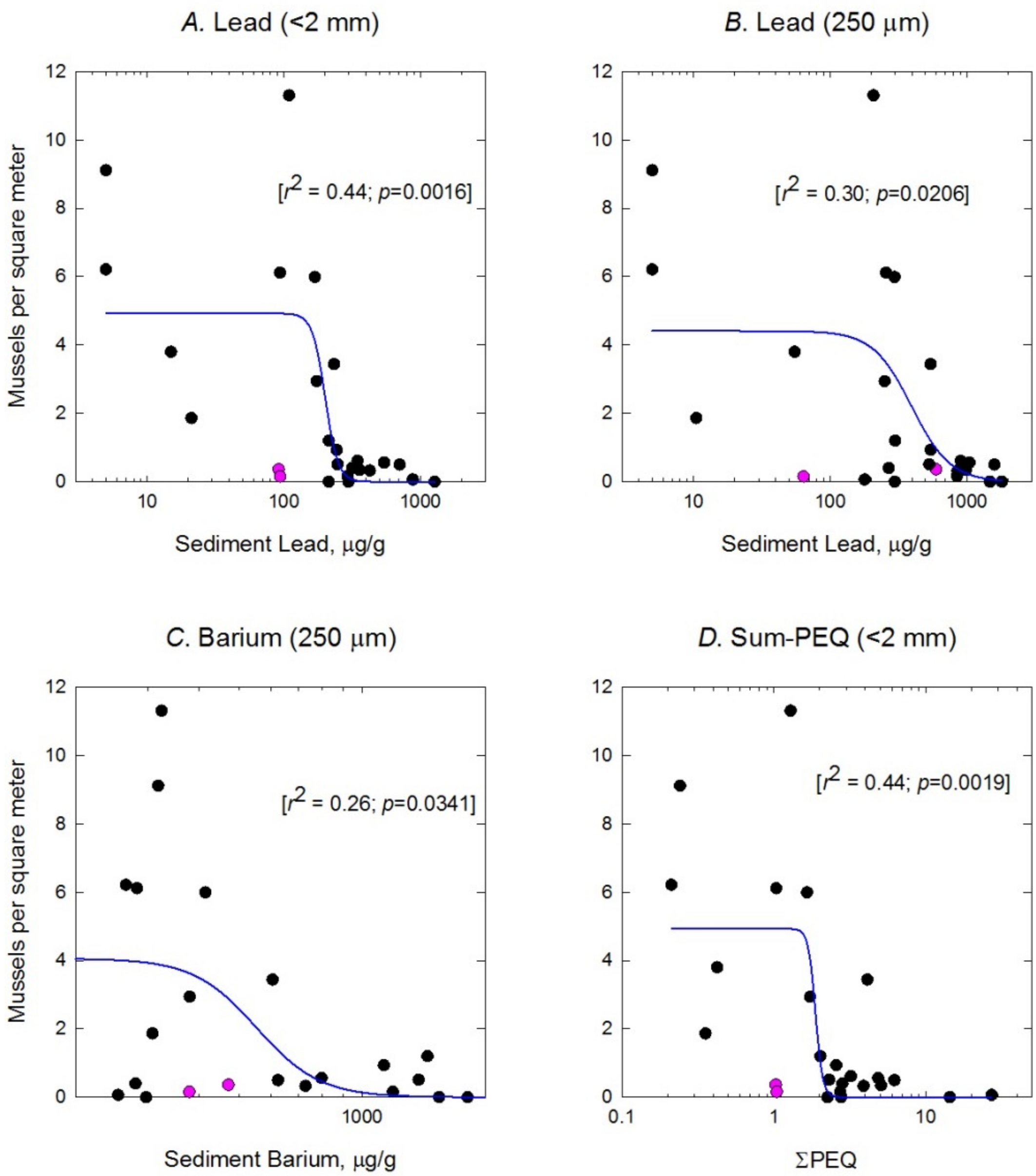

Table 1. Mean mussel density (per square meter[m²]) and metal concentrations (microgram per gram [µg/g] dry weight) in mussel bed sediment size fractions in Big River, Missouri and reference sites, 2008, 2013/2014 (millimeter [mm], cadmium [Cd], lead [Pb], zinc [Zn], summed probable effects quotient [∑PEQ]; Roberts and others, 2010; 2016).

			Metals (<2 mm; µg/g)				Metals (<250 µm; µg/g)				ΣPEQ (Pb, Zn, Cd)	
Year	Site (river name, kilometer from mouth)	Mussels per m^2	Pb	Zn	Cd	Ba	Pb	Zn	Cd	Ba	<2 mm	<0.25 mm
2014	Big2.5	11.30	110	52	2	319	208	81	1*	324	1.29	2.00
2014	Big16.5	6.11	94	47	1*	291	258	84	1*	282	1.04	2.40
2013	Big20.5	0.36	92	51	1*	276	599	228	1*	472	1.03	5.38
2013	Big30.7	2.94	175	78	1*	337	252	104	1*	380	1.73	2.39
2008	Big32	0.16	94	50	1*	222	64	28	17	379	1.05	3.98
2013	Big41	3.44	234	92	11	422	545	180	1*	605	4.14	4.85
2013	Big47	5.99	169	60	1*	317	297	106	1*	414	1.65	2.75
2008	Big49	0.40	317	67	1*	249	269	85	1*	280	2.83	2.49
2013	Big67.5	0.94	245	214	1*	1455	546	147	1*	1131	2.58	4.79
2013	Big68	1.20	214	71	1*	550	300	88	1*	1444	2.03	2.73
2013	Big86	0.33	427	166	1*	499	858	291	1*	728	3.90	7.53
2013	Big91	0.51	248	84	1*	370	533	179	1*	1374	2.32	4.76
2008	Big96.5	0	214	174	1*	231	297	107	17	297	2.26	5.97
2013	Big105.7	0.50	708	228	1*	531	1596	435	1*	624	6.23	13.62
2013	Big106.5	0.35	358	155	10	798	987	381	1*	4026	5.08	8.74
2013	Big107.5	0.16	294	113	1*	366	851	276	1*	1189	2.74	7.45
2013	Big108	0.56	544	193	1*	369	1049	304	1*	797	4.87	9.06
2013	Big113	0.61	348	137	1*	461	905	355	1*	2463	3.21	8.05
2013	Big113.5	0	298	111	1*	452	1481	679	10	1541	2.77	15.03
2008	Big146.9	0	1275	588	16	367	1810	1913	26	1810	14.45	23.46
2008	Big170.5	0.07	881	3213	66	108	179	126	21	254	27.20	5.89
2008	Big194	1.86	15	17	1*	195	11	83	1*	308	0.36	0.46
2013	Big194	3.80	21	27	1*	307	55	47	1*	68	0.42	0.73
2008	Bourbeuse0.64	9.11	1*	15	1*	235	1*	1*	14	318	0.24	2.72
2014	Meramec75.6	6.21	1*	1	1*	244	1*	12	1*	265	0.21	0.24

* Indicates default value for samples less than detection limits.

Table 2. Substrate particle size distribution in mussel beds estimated from pebble counts, 2013/2014. One substrate particle was selected at random from each quadrat sample and assigned to the appropriate size category (millimeter [mm]).

Site Name*	Sand (<2 mm)	Fine gravel (2-8mm)	Medium gravel (9-16mm)	Coarse gravel (17-64mm)	Cobble (65-256mm)	Boulder (>256 mm)	Number of samples
Mer75.6	32	14	18	34	2	0	100
Big2.5	9	12	8	27	37	6	99
Big16.5	9	22	24	39	6	0	100
Big20.5	42	14	19	24	1	0	100
Big30.7	7	5	3	41	31	13	100
Big41	0	2	14	33	30	21	100
Big47	6	7	11	37	34	5	100
Big67.5	6	0	6	42	36	10	100
Big68	6	9	8	44	26	7	100
Big86	3	4	10	65	16	2	100
Big91	5	0	4	24	37	30	100
Big105.7	2	3	9	55	24	7	100
Big106.5	14	4	10	48	23	1	100
Big107.5	8	6	10	65	11	0	100
Big108	10	1	5	45	37	2	100
Big113	11	4	8	24	44	9	100
Big113.5	86	4	5	5	0	0	100
Big194	13	3	1	20	45	18	100

*Distance upstream from the mouth of the river.

Table 3. Median and maximum species density and each species' assigned biological guilds, 2013/2014. Only species found at more than one site were included in the individual species analysis. All minimum densities were zero; bold species names indicate mussel species of conservation concern (meters squared [m^2]).

	Without Meramec reference site (*n*=15)			With Meramec reference site (*n*=16)			Biological guild			
Species	Median density (m^2)	Maximum (m^2)	Number of sites occupied	Median density (m^2)	Maximum (m^2)	Number of sites occupied	Fish host family	Tribe	Infestation type	Brood type
All species combined	0.935	11.277	14	1.069	11.277	15	NA	NA	NA	NA
Actinonaias ligamentina	0	3.308	2	0	3.308	3	Centrarchidae	Lampsilini	Releases conglutinates	Bradytictic
Alasmidonta marginata	0.027	0.337	9	0.040	0.337	10	Catostomidae	Anodontini	Broadcast of free glochidia	Bradytictic
Alasmidonta viridis	0	0.027	2	0	0.027	2	Cottidae, same as individual species	Anodontini	Broadcast of free glochidia	Bradytictic
Amblema plicata	0	2.000	5	0	2.000	6	Centrarchidae	Amblemini, same as individual species	Broadcast of free glochidia	Tachytictic
Cumberlandia monodonta	0	0.026	1	0	0.026	1	Unknown	Margaritiferidae, same as individual species	Broadcast of free glochidia	Both
Cyclonaias tuberculata	0	0.079	1	0	0.079	1	Ictaluridae	Quadrulini	Mantle storage of conglutinates	Tachytictic
Ellipsaria lineolata	0	0.238	2	0	0.238	3	Sciaenidae	Lampsilini	Only Sciaenidae, same as fish host Sciaenidae	Tachytictic
Elliptio dilatata	0	4.376	5	0	4.376	5	Centrarchidae	Pleurobemini	Releases conglutinates	Tachytictic
Fusconaia flava	0.053	0.301	12	0.040	0.301	13	Centrarchidae	Pleurobemini	Releases conglutinates	Tachytictic

Lampsilis brittsi	0.080	0.909	11	0.066	0.909	11	Centrarchidae	Lampsilini	Displays lure	Brady-tictic
Lampsilis cardium	0.213	0.533	14	0.214	0.533	15	Percidae	Lampsilini	Displays lure	Brady-tictic
Lampsilis siliquoidea	0	0.107	1	0	0.107	1	Centrarchidae	Lampsilini	Displays lure	Brady-tictic
Lasmigona costata	0	0.106	3	0	0.106	3	Centrarchidae	Anodontini	Broadcast of free glochidia	Brady-tictic
Leptodea fragilis	0	0.160	7	0.008	0.160	8	Sciaenidae	Lampsilini	Only Sciaenidae, same as fish host Sciaenidae	Brady-tictic
Leptodea leptodon	0	0.027	2	0	0.311	3	Sciaenidae	Lampsilini	Only Sciaenidae, same as fish host Sciaenidae	Brady-tictic
Ligumia recta	0	0.185	2	0	0.185	3	Centrarchidae	Lampsilini	Displays lure	Brady-tictic
Megalonaias nervosa	0	0.053	1	0	0.053	1	Ictaluridae	Quadrulini	Broadcast of free glochidia	Tachy-tictic
Obliquaria reflexa	0	0.726	3	0	0.817	4	Cyprinidae	Lampsilini	Releases conglutinates	Brady-tictic
Plethobasus cyphyus	0	0	0	0	0.133	1	Percidae	Pleurobemini	Releases conglutinates	Tachy-tictic
Pleurobema sintoxia	0	0.874	3	0	0.874	4	Cyprinidae	Pleurobemini	Releases conglutinates	Tachy-tictic
Potamilus alatus	0	0.160	7	0.013	0.160	8	Sciaenidae	Lampsilini	Only Sciaenidae, same as fish host Sciaenidae	Brady-tictic
Quadrula metanevra	0	0	0	0	0.735	1	Centrarchidae	Quadrulini	Releases conglutinates	Tachy-tictic
Quadrula pustulosa	0	0.877	6	0	0.877	7	Ictaluridae	Quadrulini	Mantle storage of conglutinates	Tachy-tictic
Quadrula quadrula	0	0	0	0	0.027	1	Ictaluridae	Quadrulini	Releases conglutinates	Tachy-tictic
Strophitus undulatus	0	0.293	7	0.013	0.293	8	Fundulidae	Anodontini	Broadcast of free glochidia	Brady-tictic

Tritogonia verrucosa	0	0.485	3	0	0.485	3	Ictaluridae	Quadrulini	Mantle storage of conglutinates	Tachy-tictic
Truncilla donaciformis	0	0.027	1	0	0.080	2	Sciaenidae	Lampsilini	Only Sciaenidae, same as fish host Sciaenidae	Brady-tictic
Truncilla truncata	0	0.974	3	0	0.974	4	Sciaenidae	Lampsilini	Only Sciaenidae, same as fish host Sciaenidae	Brady-tictic
Venustaconcha ellipsiformis	0	1.942	2	0	1.942	2	Percidae	Lampsilini	Displays lure	Brady-tictic

Table 4. Median, minimum and maximum density of mussel biological guilds, 2013/2014. One sampling site found no mussels so community metrics were only calculated for sites where individuals were collected (meters squared [m^2]).

		Without Meramec reference site (*n*=15)				With Meramec reference site (*n*=16)			
Group	Level	Median density (m^2)	Minimum (m^2)	Maximum (m^2)	Number of sites occupied	Median density (m^2)	Minimum (m^2)	Maximum (m^2)	Number of sites occupied
Fish host family									
	Centrarchidae	0.462	0	7.483	14	0.486	0	7.483	15
	Catostomidae	0.027	0	0.337	9	0.040	0	0.337	10
	Cottidae	0	0	0.027	2	0.000	0	0.027	2
	Cyprinidae	0	0	1.618	4	0.000	0	1.618	5
	Fundulidae	0	0	0.293	7	0.014	0	0.293	8
	Ictaluridae	0	0	1.120	6	0.000	0	1.120	7
	Percidae	0.239	0	6.200	14	0.240	0	6.200	15
	Sciaenidae	0.027	0	0.717	9	0.027	0	1.287	10
Tribe									
	Anodontini	0.053	0	0.553	11	0.067	0	0.553	12
	Amblemini	0	0	2.000	5	0	0	2.000	6
	Quadrulini	0	0	1.120	6	0	0	1.530	7
	Lampsilini	0.473	0	5.159	14	0.485	0	5.159	15
	Pleurobemini	0.107	0	4.635	12	0.189	0	4.635	13
Infestation type									
	Releases conglutinates	0.107	0	4.635	12	0.189	0	4.635	13
	Mantle storage of conglutinates	0	0	1.120	6	0	0	1.120	7
	Broadcast of free glochidia	0.107	0	2.083	12	0.134	0	2.083	13
Brood type									
	Displays lure	0.346	0	3.468	14	0.347	0	3.468	15
	Bradytictic	0.507	0	5.396	14	0.574	0	5.396	15
	Tachytictic	0.267	0	5.912	12	0.268	0	5.912	13
Diveristy Indexes									
	Brillouin's Index	1.685	0.673	2.779	15	1.793	0.673	3.339	16
	Equitability	1.025	0.288	1.360	15	0.981	0.288	1.360	16
	Pielou's Evenness	0.850	0.271	0.987	15	0.841	0.271	0.987	16
	Fisher's Alpha	2.867	0.752	5.403	15	2.975	0.752	5.403	16
	Inverse Simpson's Index	3.711	1.282	5.453	15	3.756	1.282	9.709	16
	Rarefaction	1.789	1.222	1.868	15	1.791	1.222	1.902	16
	Redundancy	0.150	0.013	0.729	15	0.159	0.013	0.729	16
	Shannon-Wiener Index *ln*	1.461	0.528	2.059	15	1.484	0.528	2.478	16
	Shannon-Wiener Index Log_{10}	2.108	0.762	2.970	15	2.140	0.762	3.575	16
	Simpson's Index	0.731	0.220	0.817	15	0.734	0.220	0.897	16
	Species richness	6.5	2.0	22.0	15	7.0	2.0	22.0	16
	Variety	1.712	0.434	3.537	15	1.864	0.434	3.537	16

Table 5. Formulations of the community metrics calculated in this study.

Community metric	Formula	Parameter definitions	
Species richness	$\sum_i p_i$		
Redundancy	$R = \frac{\ln \sum_i p_i}{\ln 2}$	Number of species	p_i
Equitability	$E_H = \frac{\sum p_i \ln p_i}{\frac{\ln p_i}{\ln 2}}$	Number of items in a group	N
Rarefaction	$f_n = K - \left(\frac{N}{n}\right)^{-1} \sum_{i=1}^{K} \left(\frac{N - N_i}{n}\right)$	Number of individuals	β
Simpson's Index	$\lambda = \sum_i p_i^2$	Total number of groups	K
Inverse Simpson's Index	$\frac{1}{\lambda} = \sum_i \frac{1}{p_i^2}$		
Variety	$V = \frac{\sum_i p_i - 1}{\ln \sum \beta}$		
Shannon-Wiener Index $\log_{10}$	$H' = -\sum p_i \, log_{10} p_i$		
Shannon-Wiener Index *ln*	$H' = -\sum p_i \ln p_i$		
Pielou's Evenness	$J' = \frac{\sum p_i \ln p_i}{H_{max}}$		
Fisher's Alpha	$\alpha = \frac{\beta(1 - x)}{x}$		
Brillouin's Index	$B' = \frac{1}{N^{\cdot}} \ln \frac{N!}{\pi N_i}$		

Table 6. Median, minimum and maximum sediment composition, Probable Effects Quotient (PEQs), and metal concentrations in the Big River 2013/2014 for different sized substrate fractions: bulk, <2 millimeter (mm), and <250 micrometer (µm). The PEQ is the summed PEQ of the three metals of interest (lead, zinc, and cadmium), where the PEQ for a single metal is defined as the measured concentration divided by its probable effect concentration (MacDonald and others, 2000).

Substrate predictor variable	Without Meramec reference site (n=15)			With Meramec reference site (n=16)		
	Median	Minimum	Maximum	Median	Minimum	Maximum
			Substrate (total count per site)			
Sand or smaller	7	0	86	8	0	86
Fine gravel	4	0	22	4	0	22
Medium gravel	8	1	24	9	1	24
Coarse gravel	37	5	65	36	5	65
Cobble	30	0	45	28	0	45
Boulder	7	0	30	7	0	30
Mussel bed			**Barium (concentration µg/g)**			
<250 µm	624	68	4026	614	68	4026
<2 mm	422	276	1455	396	244	1455
Bulk	370	258	1004	365	239	1004
Gravel bar						
<250 µm	676	292	1036	673	265	1036
<2 mm	291	217	745	289	217	745
Bulk	358	268	638	335	199	638
Mussel bed			Cadmium **(concentration µg/g)**			
<2 mm	1*	1*	11	1*	1*	11
Bulk	1*	1*	10	1*	1*	10
Gravel bar						
<250 µm	1*	1*	15	1*	1*	15
<2 mm	1*	1*	7	1*	1*	7
Mussel bed			**Lead (concentration µg/g)**			
<250 µm	545	55	1596	539	1*	1596
Bulk	211	12	500	204	1*	500
Gravel bar						
<250 µm	988	41	3081	957	1*	3081
<2 mm	189	11	495	187	2	495
Bulk	310	15	619	308	5	619
Mussel bed			Probable Effects Quotient			
<250 µm	4.788	0.735	15.033	4.772	0.235	15.033
<2 mm	2.320	0.424	6.226	2.174	0.211	6.226
Bulk	2.198	0.295	6.506	2.103	0.211	6.506
Gravel bar						
<250 µm	9.808	0.627	29.318	9.274	0.249	29.318
<2 mm	1.832	0.294	4.697	1.799	0.257	4.697
Bulk	2.923	0.320	5.607	2.904	0.284	5.607

* Limit of detection.

Table 7. Results from model selection permutation tests where substrate predictor variables or metal variables, or both could be included in the final model (alpha less than or equal to 0.01). Response variables in bold indicate differences between the analysis with and without the Meramec River reference site.

		Without Meramec reference site (*n*=15)		With Meramec reference site (*n*=16)	
Class	Predictor variable	Positive associations	Negative associations	Positive associations	Negative associations
Barium (Ba)	<250 µm Ba gravel bar			**Equitability**	**Presence of *Actinonaias ligamentina*, presence of *Ellipsaria lineolata*, presence of *Ligumia recta*, presence of *Truncilla truncata***
	<2 mm Ba mussel bed		Presence of *Amblema plicata*, presence of *Leptodea fragilis*, presence of *Quadrula pustulosa*		Presence of *Amblema plicata*, presence of *Leptodea fragilis*, **presence of *Obliquaria reflexa***, presence of *Quadrula pustulosa*
Lead (Pb)	<250 µm Pb mussel bed		Density of all species, density of *Strophitus undulatus*, presence of *Strophitus undulatus*, Brillouin's Index, brood guild bradytictic, fish host Centrarchidae, fish host Fundulidae		Density of all species, **density of *Potamilus alatus***, density of *Strophitus undulatus*, presence of *Strophitus undulatus*, Brillouin's Index, brood guild bradytictic, fish host Centrarchidae, fish host Fundulidae
	Bulk Pb mussel bed				**Infestation broadcast**
Substrate	Fine gravel	**Infestation broadcast**		**Presence of *Pleurobema sintoxia*, Variety**	
	Boulder			**Presence of *Lampsilis brittsi***	
Probable Effects Quotient	<250 µm gravel bar				**Presence of *Actinonaias ligamentina*, presence of *Ellipsaria lineolata*, presence of *Ligumia recta*, presence of *Pleurobema sintoxia***
	Bulk gravel bar				**Presence of *Obliquaria reflexa***

<250 µm mussel bed	Density of all species, density of *Strophitus undulatus*, presence of *Strophitus undulatus*, Brillouin's Index, brood guild bradytictic	Equitability	Density of all species, density of *Potamilus alatus*, density of *Strophitus undulatus*, presence of *Strophitus undulatus*, Brillouin's Index, brood guild bradytictic, fish host Centrarchidae, fish host Fundulidae
<2 mm mussel bed	Infestation broadcast		Presence of *Amblema plicata*, Variety, infestation broadcast

Table 8. Results from permutation tests after substrate variation was controlled and the variable of interest was tested (alpha less than or equal to 0.01). Response variables in bold indicate differences between the analysis with and without the Meramec River reference site.

		Without Meramec reference site (*n*=15)		With Meramec reference site (*n*=16)	
Class	Predictor variable	Positive associations	Negative associations	Positive associations	Negative associations
Lead (Pb)	<250 µm Pb gravel bar		Density of *Lampsilis cardium*, infestation displays lure, **tribe Lampsilini**		Density of *Lampsilis cardium*, **fish host Percidae**, infestation displays lure
	<2 mm Pb gravel bar	**Shannon-Wiener Index Log10, Shannon-Wiener Index *ln***			
Probable Effects Quotient	<250 µm gravel bar		Infestation displays lure		Infestation displays lure

Table 9. Lead concentration-response models and effect concentrations for reductions in mussel density (mussel per meter squared) from data collected in 2008, 2013/2014 in the Big River, Missouri, using nonlinear regression (EC10 and EC20 is 10 and 20 percent effect concentrations; CL = confidence limits; Gray highlights indicate preferred models [see text]).

Model characteristics					Lead effect concentrations (µg/g)			
N	Model	Weighting	*P*-value	r^2	EC10	95 percent CL	EC20	95 percent CL
				Sediments <2 mm				
25	Logistic	None	0.0015	0.44	158	(82-306)	173	(105-284)
25	Logistic	Square root	0.0015	0.45	92	(52-161)	116	(75-179)
25	Threshold	None	0.0015	0.44	159	(82-308)	172	(104-286)
25	Threshold	Square root	0.0082	0.35	170	(138-208)	182	(156-213)
23*	Logistic	None	<0.0001	0.67	147	(97-222)	161	(118-220)
23*	Logistic	Square root	<0.0001	0.68	118	(92-150)	136	(113-164)
23*	Threshold	None	<0.0001	0.68	140	(80-248)	155	(103-233)
23*	Threshold	Square root	0.0001	0.59	164	(143-188)	176	(159-196)
				Sediments <250 µm				
25	Logistic	None	0.0205	0.30	193	(36-1045)	252	(69-917)
25	Logistic	Square root	0.0128	0.33	128	(51-317)	185	(93-366)
25	Threshold	None	0.0210	0.29	216	(40-1149)	268	(76-953)
25	Threshold	Square root	0.0345	0.26	356	(121-823)	422	(258-689)

* Analyses excluded data from sites at km 21.5 and km 32.

Appendix 1. Summary of model results that included all six substrate variables and one metal variable of interest (alpha = 0.1). Results are sorted by P-value, where the P-value and R^2 are associated with the variable of interest. See table 3 for species abbreviations; PEQ, Probable Effects Quotient.

Reference site included	Model type	Response variable	R^2	*P*-value	Parameter coefficients: Intercept	Sand	Fine gravel	Medium gravel	Coarse gravel	Cobble	Boulder	Metal	Predictor variable
No	Logistic	Presence of all species	0.31	<0.0001	-295.85	2.6760	3.5086	3.5111	3.1495	3.3341	3.1242	-5.1654	<250 µm Cd mussel bed
No	Logistic	Presence of *Lampsilis cardium*	0.31	<0.0001	-295.85	2.6760	3.5086	3.5111	3.1495	3.3341	3.1242	-5.1654	<250 µm Cd mussel bed
Yes	Logistic	Presence of all species	0.31	<0.0001	-295.85	2.6760	3.5086	3.5111	3.1495	3.3341	3.1242	-10.4351	<250 µm Cd mussel bed
Yes	Logistic	Presence of *Lampsilis cardium*	0.31	<0.0001	-295.85	2.6760	3.5086	3.5111	3.1495	3.3341	3.1242	-10.4351	<250 µm Cd mussel bed
No	LAD	Density of *Lampsilis cardium*	0.54	0.0051	-40.10	0.4014	0.4053	0.4098	0.3993	0.4142	0.3959	-0.0001	<250 µm Pb gravel bar
No	LAD	Fish host Percidae	0.07	0.0052	35.08	-0.3461	-0.5784	-0.0880	-0.3781	-0.3360	-0.1997	-0.0008	<250 µm Pb gravel bar
No	LAD	Tribe Lampsilini	0.12	0.0055	351.14	-3.5110	-3.4321	-3.5346	-3.5129	-3.4846	-3.5102	-0.0003	<250 µm Pb gravel bar
Yes	LAD	Density of *Lampsilis cardium*	0.45	0.0064	-40.10	0.4014	0.4053	0.4098	0.3993	0.4142	0.3959	-0.0001	<250 µm Pb gravel bar
Yes	LAD	Fish host Percidae	0.11	0.0064	7.71	-0.0741	-0.2074	0.0787	-0.0935	-0.0625	0.0076	-0.0005	<250 µm Pb gravel bar
Yes	LAD	Infestation displays lure	0.12	0.0065	-38.42	0.3847	0.3995	0.3861	0.3823	0.4043	0.3753	-0.0001	<250 µm Pb gravel bar
No	LAD	Infestation displays lure	0.13	0.0066	-28.75	0.2879	0.2978	0.2909	0.2857	0.3062	0.2785	-9.392E-05	<250 µm Pb gravel bar
No	LAD	Shannon-Wiener Index ln	0.27	0.0076	32.99	-0.3236	-0.1931	-0.3900	-0.3194	-0.3393	-0.2703	0.0017	<2 mm Pb gravel bar

No	LAD	Infestation displays lure	0.12	0.0080	-29.27	0.2932	0.3002	0.2992	0.2904	0.3123	0.2825	-0.0110	<250 µm PEQ gravel bar
Yes	LAD	Density of *Lasmigona costata*	0.00	0.0084	10.60	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	2.084E-16	<2 mm PEQ mussel bed
No	LAD	Shannon-Wiener Index Log10	0.27	0.0089	47.60	-0.4668	-0.2785	-0.5626	-0.4608	-0.4896	-0.3900	0.00241	<2 mm Pb gravel bar
No	LAD	Inverse Simpsons Index	0.27	0.0092	25.96	-0.2388	0.1048	-0.4617	-0.2241	-0.2774	-0.1495	0.00454	<2 mm Pb gravel bar
Yes	LAD	Density of *Leptodea leptodon*	0.00	0.0095	2.65	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	2.882E-19	<250 µm Ba gravel bar
Yes	LAD	Infestation displays lure	0.12	0.0098	-30.47	0.3052	0.3127	0.3111	0.3023	0.3245	0.2944	-0.0113	<250 µm PEQ gravel bar
Yes	LAD	Density of *Lasmigona costata*	0.00	0.0112	10.60	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	6.18E-17	<250 µm PEQ mussel bed
Yes	LAD	Density of *Lasmigona costata*	0.00	0.0136	10.60	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	2.02E-18	<250 µm Pb mussel bed
No	LAD	Density of *Venustaconcha ellipsiformis*	0.00	0.0141	13.35	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	5.559E-16	Bulk PEQ mussel bed
No	LAD	Tribe Lampsilini	0.12	0.0156	367.87	-3.6783	-3.6149	-3.6923	-3.6813	-3.6526	-3.6804	-0.0245	<250 µm PEQ gravel bar
Yes	LAD	Fish host Percidae	0.04	0.0157	-15.73	0.1601	0.1520	0.1755	0.1559	0.1676	0.1598	-0.0006	<2 mm Pb gravel bar
No	LAD	Density of *Lampsilis cardium*	0.45	0.0158	-42.18	0.4224	0.4295	0.4277	0.4202	0.4347	0.4192	-0.0109	<250 µm PEQ gravel bar
No	LAD	Fish host Percidae	0.06	0.0166	-10.22	0.1037	0.0502	0.1718	0.0951	0.1139	0.1403	-0.0268	<250 µm PEQ gravel bar
Yes	LAD	Density of *Lampsilis cardium*	0.31	0.0175	-31.98	0.3217	0.3251	0.3256	0.3199	0.3298	0.3149	-0.0004	<2 mm Pb gravel bar

No	LAD	Inverse Simpsons Index	0.25	0.0179	78.53	-0.7721	-0.5070	-0.8928	-0.7565	-0.8055	-0.6465	0.4256	<2 mm PEQ gravel bar
No	LAD	Shannon-Wiener Index Log10	0.19	0.0184	77.83	-0.7705	-0.6702	-0.7897	-0.7580	-0.7837	-0.7059	0.0820	<2 mm PEQ gravel bar
Yes	LAD	Fish host Percidae	0.10	0.0192	-9.93	0.1018	0.0043	0.2249	0.0849	0.1135	0.1726	-0.0453	<250 µm PEQ gravel bar
Yes	LAD	Density of *Lampsilis cardium*	0.38	0.0200	-40.67	0.4073	0.4080	0.4191	0.4044	0.4210	0.4004	-0.0122	<250 µm PEQ gravel bar
Yes	LAD	Density of *Lampsilis cardium*	0.25	0.0200	-31.28	0.3148	0.3183	0.3182	0.3132	0.3223	0.3088	-0.0508	<2 mm PEQ gravel bar
No	LAD	Shannon-Wiener Index ln	0.19	0.0202	53.95	-0.5341	-0.4646	-0.5474	-0.5254	-0.5432	-0.4893	0.0568	<2 mm PEQ gravel bar
Yes	LAD	Density of *Venustaconcha ellipsiformis*	0.00	0.0202	13.35	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	4.714E-16	<2 mm PEQ mussel bed
No	Logistic	Presence of *Lampsilis brittsi*	0.18	0.0211	-4065.92	40.4260	39.0704	40.3886	41.0660	41.4583	40.3044	1.5042	<2 mm Cd gravel bar
Yes	LAD	Fish host Percidae	0.03	0.0223	-16.16	0.1638	0.1632	0.1714	0.1616	0.1709	0.1605	-0.0550	<2 mm PEQ gravel bar
No	LAD	Density of *Leptodea fragilis*	0.19	0.0233	2.85	-0.0293	-0.0223	-0.0292	-0.0295	-0.0285	-0.0280	0.0001	Bulk Pb gravel bar
Yes	LAD	Density of all species	0.28	0.0250	699.69	-6.9954	-6.9900	-6.7133	-7.0299	-6.9092	-6.9850	-0.0016	<250 µm Pb gravel bar
No	LAD	Density of *Ligumia recta*	0.00	0.0260	18.54	-0.1854	-0.1854	-0.1854	-0.1854	-0.1854	-0.1854	-1.415E-18	<250 µm PEQ mussel bed
No	LAD	Density of *Actinonaias ligamentina*	0.00	0.0262	330.83	-3.3083	-3.3083	-3.3083	-3.3083	-3.3083	-3.3083	-2.524E-17	<250 µm PEQ mussel bed
No	LAD	Density of *Ellipsaria lineolata*	0.00	0.0265	23.84	-0.2384	-0.2384	-0.2384	-0.2384	-0.2384	-0.2384	1.671E-17	<250 µm Pb mussel bed
No	LAD	Density of *Ellipsaria*	0.00	0.0267	23.84	-0.2384	-0.2384	-0.2384	-0.2384	-0.2384	-0.2384	-1.819E-18	<250 µm PEQ mussel bed

		lineolata											
No	LAD	Rarefy	0.16	0.0268	5.46	-0.0371	-0.0068	-0.0606	-0.0380	-0.0453	-0.0233	0.0007	<2 mm Pb gravel bar
No	LAD	Simpsons Index	0.16	0.0271	2.00	-0.0139	0.0229	-0.0395	-0.0140	-0.0199	0.0001	0.0006	<2 mm Pb gravel bar
No	LAD	Brillouin Index	0.11	0.0275	31.39	-0.3145	-0.1146	-0.4046	-0.3011	-0.3159	-0.2521	0.0015	<2 mm Pb gravel bar
Yes	LAD	Density of *Elliptio dilatata*	0.05	0.0277	351.58	-3.5279	-3.5225	-3.4769	-3.5217	-3.5117	-3.5193	0.0908	<250 µm Cd mussel bed
No	LAD	Density of *Actinonaias ligamentina*	0.00	0.0282	330.83	-3.3083	-3.3083	-3.3083	-3.3083	-3.3083	-3.3083	2.319E-16	<250 µm Pb mussel bed
No	Logistic	Presence of *Venustaconcha ellipsiformis*	0.43	0.0287	3640.50	-36.6398	-34.5105	-37.9663	-37.5277	-36.0692	-36.4188	-0.1224	<250 µm Ba gravel bar
Yes	Logistic	Presence of *Venustaconcha ellipsiformis*	0.43	0.0287	3640.50	-36.6398	-34.5105	-37.9663	-37.5277	-36.0692	-36.4188	-0.1224	<250 µm Ba gravel bar
Yes	LAD	Density of *Lasmigona costata*	0.00	0.0290	10.60	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	3.202E-18	Bulk Pb mussel bed
No	LAD	Density of *Ligumia recta*	0.00	0.0292	18.54	-0.1854	-0.1854	-0.1854	-0.1854	-0.1854	-0.1854	1.3E-17	<250 µm Pb mussel bed
Yes	LAD	Fish host Centrarchidae	0.18	0.0297	632.03	-6.3210	-6.4037	-6.1015	-6.3430	-6.2870	-6.3182	-0.0007	<250 µm Pb gravel bar
No	LAD	Density of *Leptodea fragilis*	0.19	0.0314	2.28	-0.0237	-0.0158	-0.0236	-0.0238	-0.0228	-0.0221	0.0132	Bulk PEQ gravel bar
Yes	LAD	Density of all species	0.23	0.0315	697.79	-6.9747	-7.0162	-6.6419	-7.0200	-6.8770	-6.9846	-0.1815	<250 µm PEQ gravel bar
Yes	LAD	Brood guild tachytictic	0.11	0.0322	582.10	-5.8238	-5.8764	-5.5792	-5.8352	-5.8430	-5.7510	-0.0021	Bulk Pb mussel bed
Yes	LAD	Density of *Elliptio dilatata*	0.00	0.0331	340.50	-3.4077	-3.3805	-3.4056	-3.4068	-3.4077	-3.4016	0.0002	<2 mm Ba gravel bar

Yes	LAD	Density of *Lampsilis brittsi*	0.05	0.0334	-23.08	0.2320	0.2339	0.2239	0.2321	0.2372	0.2253	-0.0092	<250 µm Cd mussel bed
Yes	LAD	Redundancy	0.07	0.0336	20.50	-0.2070	-0.2083	-0.1883	-0.2060	-0.2032	-0.1968	-0.0002	Bulk Pb gravel bar
Yes	LAD	Brood guild tachytictic	0.06	0.0338	541.45	-5.4148	-5.4783	-5.1464	-5.4424	-5.4221	-5.3664	-0.0006	<250 µm Pb mussel bed
Yes	LAD	Density of *Leptodea leptodon*	0.00	0.0341	2.65	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	-1.074E-18	<2 mm Pb gravel bar
Yes	LAD	Equitability	0.06	0.0344	-12.49	0.1450	0.1328	0.1074	0.1456	0.1264	0.1289	8.635E-05	<250 µm Ba gravel bar
Yes	LAD	Density of *Lampsilis brittsi*	0.09	0.0346	-25.53	0.2561	0.2592	0.2484	0.2571	0.2625	0.2495	-0.0191	Bulk PEQ mussel bed
Yes	LAD	Fish host Centrarchidae	0.10	0.0352	648.27	-6.4768	-6.4727	-6.3595	-6.4910	-6.4876	-6.4154	-0.0008	<250 µm Pb mussel bed
Yes	LAD	Density of all species	0.18	0.0354	660.79	-6.5547	-6.4246	-6.5459	-6.5863	-6.5638	-6.5941	-0.0039	<250 µm Pb mussel bed
Yes	LAD	Density of all species	0.18	0.0357	620.77	-6.1939	-6.0566	-6.0458	-6.2417	-6.1161	-6.2283	-0.0016	<250 µm Ba mussel bed
No	LAD	Density of *Ellipsaria lineolata*	0.00	0.0374	23.84	-0.2384	-0.2384	-0.2384	-0.2384	-0.2384	-0.2384	3.763E-16	Bulk PEQ mussel bed
Yes	LAD	Equitability	0.23	0.0376	-18.00	0.1981	0.2007	0.1698	0.1890	0.1822	0.1951	0.0011	Bulk Pb gravel bar
Yes	LAD	Redundancy	0.04	0.0393	19.36	-0.1958	-0.1919	-0.1770	-0.1958	-0.1928	-0.1809	-6.433E-05	<250 µm Ba gravel bar
Yes	LAD	Brood guild tachytictic	0.13	0.0393	518.72	-5.1932	-5.2401	-4.9365	-5.2146	-5.1825	-5.1684	-0.0004	<250 µm Pb gravel bar
Yes	LAD	Pielou's Evenness	0.04	0.0396	-18.36	0.1958	0.1919	0.1770	0.1958	0.1928	0.1809	6.433E-05	<250 µm Ba gravel bar
Yes	LAD	Redundancy	0.07	0.0398	20.29	-0.2049	-0.2062	-0.1863	-0.2039	-0.2010	-0.1951	-0.0259	Bulk PEQ gravel bar
No	LAD	Density of *Fusconaia flava*	0.32	0.0402	-6.80	0.0651	0.0780	0.0597	0.0670	0.0680	0.0688	0.0003	<250 µm Ba gravel bar

No	LAD	Density of *Ligumia recta*	0.00	0.0408	18.54	-0.1854	-0.1854	-0.1854	-0.1854	-0.1854	-0.1854	2.927E-16	Bulk PEQ mussel bed
Yes	LAD	Fish host Percidae	0.03	0.0414	-24.81	0.2525	0.2559	0.2523	0.2496	0.2609	0.2425	-0.0004	<250 µm Ba gravel bar
No	LAD	Density of *Actinonaias ligamentina*	0.00	0.0415	330.83	-3.3083	-3.3083	-3.3083	-3.3083	-3.3083	-3.3083	5.222E-15	Bulk PEQ mussel bed
Yes	LAD	Density of Tritogonia verrucosa	0.00	0.0415	48.48	-0.4848	-0.4848	-0.4848	-0.4848	-0.4848	-0.4848	-3.486E-17	Bulk Ba gravel bar
Yes	LAD	Equitability	0.22	0.0428	-17.99	0.1980	0.2007	0.1694	0.1889	0.1820	0.1953	0.1254	Bulk PEQ gravel bar
Yes	LAD	Pielou's Evenness	0.07	0.0435	-19.50	0.2070	0.2083	0.1883	0.2060	0.2032	0.1968	0.0002	Bulk Pb gravel bar
No	LAD	Density of *Pleurobema sintoxia*	0.04	0.0450	86.29	-0.8630	-0.8614	-0.8631	-0.8630	-0.8631	-0.8627	6.108E-07	<250 µm Ba mussel bed
Yes	LAD	Density of all species	0.06	0.0450	650.57	-6.5135	-6.1997	-6.5083	-6.5192	-6.4736	-6.4050	-0.1836	<2 mm PEQ mussel bed
Yes	LAD	Density of *Lampsilis brittsi*	0.15	0.0451	-31.25	0.3153	0.3183	0.2967	0.3175	0.3200	0.3085	-0.0484	Bulk PEQ gravel bar
Yes	LAD	Density of *Lampsilis cardium*	0.25	0.0452	-38.10	0.3854	0.3888	0.3852	0.3824	0.3937	0.3754	-0.0004	<250 µm Ba gravel bar
Yes	LAD	Pielou's Evenness	0.07	0.0452	-19.29	0.2049	0.2062	0.1863	0.2039	0.2010	0.1951	0.0259	Bulk PEQ gravel bar
Yes	LAD	Fish host Centrarchidae	0.13	0.0457	673.66	-6.7335	-6.7473	-6.6115	-6.7349	-6.7375	-6.6856	-0.0026	Bulk Pb mussel bed
Yes	LAD	Density of *Leptodea leptodon*	0.00	0.0458	2.65	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	-1.572E-17	<2 mm PEQ gravel bar
No	LAD	Density of *Truncilla truncata*	0.00	0.0463	7.95	-0.0795	-0.0795	-0.0795	-0.0795	-0.0795	-0.0795	1.174E-16	<250 µm PEQ mussel bed

Yes	LAD	Density of all species	0.24	0.0468	865.14	-8.6203	-8.7327	-8.3828	-8.6101	-8.5903	-8.6570	-0.0121	Bulk Pb mussel bed
Yes	LAD	Fish host Centrarchidae	0.14	0.0474	626.06	-6.2609	-6.3458	-6.0377	-6.2856	-6.2236	-6.2652	-0.0718	<250 µm PEQ gravel bar
No	LAD	Density of *Lasmigona costata*	0.00	0.0475	10.60	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	6.993E-16	<2 mm PEQ mussel bed
No	LAD	Density of *Lasmigona costata*	0.00	0.0478	10.60	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	1.566E-16	<250 µm PEQ mussel bed
Yes	LAD	Density of *Lampsilis brittsi*	0.16	0.0478	-32.36	0.3265	0.3303	0.3064	0.3290	0.3312	0.3197	-0.0004	Bulk Pb gravel bar
Yes	LAD	Density of *Elliptio dilatata*	0.01	0.0484	329.03	-3.3000	-3.2617	-3.2948	-3.2977	-3.2916	-3.2957	0.0012	Bulk Ba gravel bar
No	LAD	Density of *Leptodea fragilis*	0.06	0.0485	1.80	-0.0182	-0.0129	-0.0186	-0.0184	-0.0176	-0.0171	0.0007	Bulk Cd mussel bed
Yes	LAD	Density of all species	0.16	0.0486	685.98	-6.8032	-6.6759	-6.7938	-6.8427	-6.8292	-6.8098	-0.4004	<250 µm PEQ mussel bed
No	Logistic	Presence of *Obliquaria reflexa*	0.26	0.0489	4075.03	-41.0742	-39.3505	-39.5251	-41.3482	-41.3421	-40.8879	-4.6324	<250 µm Cd mussel bed
Yes	Logistic	Presence of *Obliquaria reflexa*	0.26	0.0489	4075.03	-41.0742	-39.3505	-39.5251	-41.3482	-41.3421	-40.8879	-9.3584	<250 µm Cd mussel bed
Yes	LAD	Shannon-Wiener Index ln	0.13	0.0500	48.97	-0.4844	-0.4039	-0.5052	-0.4772	-0.4936	-0.4386	0.0008	<2 mm Pb gravel bar
Yes	LAD	Shannon-Wiener Index Log10	0.13	0.0505	70.64	-0.6988	-0.5827	-0.7289	-0.6885	-0.7122	-0.6327	0.0011	<2 mm Pb gravel bar
Yes	LAD	Brood guild tachytictic	0.06	0.0506	541.27	-5.4122	-5.4743	-5.1465	-5.4411	-5.4208	-5.3635	-0.0653	<250 µm PEQ mussel bed
No	LAD	Density of *Pleurobema sintoxia*	0.06	0.0512	86.43	-0.8644	-0.8628	-0.8645	-0.8644	-0.8645	-0.8640	1.443E-05	Bulk Ba mussel bed
Yes	LAD	Inverse Simpsons Index	0.12	0.0516	95.58	-0.9206	-0.5439	-1.2634	-0.8748	-1.0280	-0.7718	0.0026	<2 mm Pb gravel bar

Yes	LAD	Equitability	0.06	0.0518	-18.15	0.2025	0.1915	0.1639	0.2008	0.1856	0.1810	0.0002	<2 mm Pb gravel bar
No	LAD	Density of *Lasmigona costata*	0.00	0.0524	10.60	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	-0.1060	1.665E-18	<250 µm Pb mussel bed
Yes	LAD	Pielou's Evenness	0.02	0.0524	-23.43	0.2472	0.2479	0.2246	0.2467	0.2446	0.2291	5.241E-05	<2 mm Pb gravel bar
Yes	LAD	Infestation broadcast	0.23	0.0525	-41.40	0.4053	0.5293	0.3958	0.4084	0.4113	0.4222	0.0006	<2 mm Ba gravel bar
Yes	LAD	Fish host Centrarchidae	0.09	0.0527	667.69	-6.6714	-6.6503	-6.5804	-6.6764	-6.6990	-6.5776	-0.0706	<250 µm PEQ mussel bed
No	LAD	Density of *Leptodea leptodon*	0.00	0.0528	2.65	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	4.429E-18	<250 µm PEQ gravel bar
No	LAD	Density of *Truncilla truncata*	0.00	0.0529	7.95	-0.0795	-0.0795	-0.0795	-0.0795	-0.0795	-0.0795	5.245E-16	<2 mm PEQ mussel bed
No	LAD	Density of *Venustaconcha ellipsiformis*	0.00	0.0529	13.35	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	2.042E-16	Bulk Cd mussel bed
No	LAD	Infestation broadcast	0.23	0.0543	-47.51	0.4655	0.5987	0.4554	0.4690	0.4713	0.4845	0.0007	<2 mm Ba gravel bar
No	LAD	Simpsons Index	0.11	0.0546	9.24	-0.0868	-0.0728	-0.0916	-0.0856	-0.0899	-0.0762	0.0256	<2 mm PEQ gravel bar
Yes	LAD	Density of *Lampsilis cardium*	0.22	0.0549	-35.43	0.3537	0.3749	0.3486	0.3533	0.3622	0.3506	0.0118	<2 mm Cd mussel bed
No	LAD	Brillouin Index	0.04	0.0551	54.31	-0.5454	-0.4162	-0.5687	-0.5287	-0.5369	-0.4930	0.0237	<2 mm PEQ gravel bar
No	LAD	Density of all species	0.26	0.0557	699.69	-6.9954	-6.9900	-6.7133	-7.0299	-6.9092	-6.9850	-0.0016	<250 µm Pb gravel bar
Yes	LAD	Brood guild tachytictic	0.10	0.0559	481.38	-4.8192	-4.8389	-4.5905	-4.8404	-4.7998	-4.8227	-0.0003	<250 µm Ba mussel bed
Yes	LAD	Tribe Anodontini	0.13	0.0560	27.84	-0.2759	-0.2553	-0.3014	-0.2740	-0.2753	-0.2740	-0.0003	Bulk Ba gravel bar

Yes	LAD	Brood guild tachytictic	0.06	0.0560	586.69	-5.8720	-5.9210	-5.6289	-5.8768	-5.9001	-5.7708	-0.1861	<2 mm PEQ mussel bed
Yes	LAD	Brood guild tachytictic	0.11	0.0560	513.59	-5.1417	-5.1855	-4.8860	-5.1650	-5.1290	-5.1209	-0.0466	<250 µm PEQ gravel bar
No	LAD	Density of *Truncilla truncata*	0.00	0.0566	7.95	-0.0795	-0.0795	-0.0795	-0.0795	-0.0795	-0.0795	1.248E-18	<250 µm Pb mussel bed
Yes	LAD	Fish host Percidae	0.08	0.0571	-91.43	0.9136	0.9230	0.9224	0.9126	0.9164	0.9287	0.5492	<2 mm Cd mussel bed
Yes	LAD	Redundancy	0.02	0.0572	24.43	-0.2472	-0.2479	-0.2246	-0.2467	-0.2446	-0.2291	-5.241E-05	<2 mm Pb gravel bar
Yes	LAD	Simpsons Index	0.29	0.0572	4.61	-0.0386	-0.0256	-0.0523	-0.0362	-0.0395	-0.0443	-0.0280	<2 mm Cd mussel bed
No	LAD	Equitability	0.05	0.0573	-18.15	0.2025	0.1915	0.1639	0.2008	0.1856	0.1810	0.0002	<2 mm Pb gravel bar
Yes	LAD	Brillouin Index	0.25	0.0575	72.33	-0.7059	-0.6144	-0.7469	-0.7041	-0.7194	-0.6844	-0.0748	<2 mm Cd mussel bed
No	LAD	Pielou's Evenness	0.01	0.0578	-23.43	0.2472	0.2479	0.2246	0.2467	0.2446	0.2291	5.241E-05	<2 mm Pb gravel bar
Yes	LAD	Fish host Fundulidae	0.19	0.0582	-8.19	0.0838	0.0886	0.0755	0.0829	0.0860	0.0808	-0.0003	Bulk Ba gravel bar
No	LAD	Density of *Ellipsaria lineolata*	0.00	0.0584	23.84	-0.2384	-0.2384	-0.2384	-0.2384	-0.2384	-0.2384	-6.125E-18	Bulk Pb mussel bed
Yes	LAD	Fish host Percidae	0.03	0.0589	-3.08	0.0344	0.0023	0.0630	0.0343	0.0439	0.0225	-0.0012	Bulk Pb mussel bed
No	LAD	Density of *Lampsilis brittsi*	0.22	0.0590	-38.76	0.3926	0.3950	0.3650	0.3956	0.3963	0.3843	-0.0885	Bulk PEQ gravel bar
No	LAD	Rarefy	0.11	0.0590	12.39	-0.1069	-0.0984	-0.1104	-0.1065	-0.1123	-0.0963	0.0320	<2 mm PEQ gravel bar
Yes	LAD	Fish host Fundulidae	0.09	0.0590	0.52	-0.0043	-0.0042	-0.0069	-0.0046	-0.0042	-0.0045	-4.544E-05	<250 µm Pb mussel bed
Yes	LAD	Density of *Strophitus undulatus*	0.09	0.0600	0.52	-0.0044	-0.0042	-0.0069	-0.0047	-0.0042	-0.0044	-4.493E-05	<250 µm Pb mussel bed

Yes	LAD	Density of *Strophitus undulatus*	0.19	0.0600	-8.22	0.0841	0.0888	0.0758	0.0831	0.0863	0.0810	-0.0003	Bulk Ba gravel bar
No	LAD	Brood guild bradytictic	0.13	0.0601	346.67	-3.4805	-3.3194	-3.4985	-3.4751	-3.4566	-3.4629	0.0012	<2 mm Ba gravel bar
Yes	LAD	Density of *Leptodea leptodon*	0.00	0.0610	2.65	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	-1.845E-17	<2 mm Cd mussel bed
Yes	LAD	Rarefy	0.31	0.0612	6.85	-0.0493	-0.0429	-0.0626	-0.0475	-0.0521	-0.0580	-0.0306	<2 mm Cd mussel bed
No	LAD	Density of *Actinonaias ligamentina*	0.00	0.0617	330.83	-3.3083	-3.3083	-3.3083	-3.3083	-3.3083	-3.3083	-8.5E-17	Bulk Pb mussel bed
No	LAD	Density of *Ligumia recta*	0.00	0.0621	18.54	-0.1854	-0.1854	-0.1854	-0.1854	-0.1854	-0.1854	-4.764E-18	Bulk Pb mussel bed
Yes	LAD	Density of *Lampsilis cardium*	0.21	0.0623	-24.38	0.2459	0.2377	0.2575	0.2461	0.2551	0.2351	-0.0007	Bulk Pb mussel bed
No	LAD	Density of *Ligumia recta*	0.00	0.0633	18.54	-0.1854	-0.1854	-0.1854	-0.1854	-0.1854	-0.1854	6.485E-16	<2 mm PEQ mussel bed
Yes	LAD	Inverse Simpsons Index	0.11	0.0638	127.29	-1.2412	-0.9052	-1.5343	-1.1934	-1.3489	-1.0698	0.2303	<2 mm PEQ gravel bar
No	LAD	Density of *Lampsilis brittsi*	0.25	0.0642	-47.97	0.4865	0.4920	0.4479	0.4903	0.4897	0.4776	-0.0011	Bulk Pb gravel bar
No	Logistic	Presence of *Actinonaias ligamentina*	0.31	0.0642	3346.68	-34.2538	-31.0200	-33.6851	-34.0220	-33.8588	-33.2892	2.2866	Bulk Cd mussel bed
No	Logistic	Presence of *Ellipsaria lineolata*	0.31	0.0642	3346.68	-34.2538	-31.0200	-33.6851	-34.0220	-33.8588	-33.2892	2.2866	Bulk Cd mussel bed
No	Logistic	Presence of *Ligumia recta*	0.31	0.0642	3346.68	-34.2538	-31.0200	-33.6851	-34.0220	-33.8588	-33.2892	2.2866	Bulk Cd mussel bed
No	LAD	Density of *Actinonaias ligamentina*	0.00	0.0643	330.83	-3.3083	-3.3083	-3.3083	-3.3083	-3.3083	-3.3083	1.157E-14	<2 mm PEQ mussel bed

No	LAD	Density of *Ellipsaria lineolata*	0.00	0.0643	23.84	-0.2384	-0.2384	-0.2384	-0.2384	-0.2384	-0.2384	8.338E-16	<2 mm PEQ mussel bed
Yes	LAD	Infestation displays lure	0.15	0.0644	-46.11	0.4711	0.4810	0.4308	0.4742	0.4788	0.4582	-0.0017	Bulk Pb gravel bar
No	LAD	Density of all species	0.16	0.0664	620.77	-6.1939	-6.0566	-6.0458	-6.2417	-6.1161	-6.2283	-0.0016	<250 µm Ba mussel bed
Yes	LAD	Shannon-Wiener Index Log10	0.11	0.0664	80.20	-0.7929	-0.6884	-0.8229	-0.7785	-0.8105	-0.7265	0.0693	<2 mm PEQ gravel bar
Yes	LAD	Shannon-Wiener Index ln	0.11	0.0670	55.59	-0.5496	-0.4772	-0.5704	-0.5396	-0.5618	-0.5036	0.0480	<2 mm PEQ gravel bar
No	LAD	Fisher's Alpha	0.38	0.0671	282.01	-2.7516	-2.6572	-2.8583	-2.7773	-2.8725	-2.6475	-0.1541	<2 mm Cd mussel bed
No	LAD	Shannon-Wiener Index ln	0.19	0.0674	50.50	-0.5057	-0.4293	-0.5129	-0.4931	-0.5073	-0.4607	0.0005	<250 µm Ba gravel bar
No	LAD	Density of *Venustaconcha ellipsiformis*	0.00	0.0678	13.35	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	2.201E-16	<250 µm PEQ mussel bed
No	LAD	Density of *Fusconaia flava*	0.22	0.0685	-15.73	0.1600	0.1578	0.1518	0.1607	0.1611	0.1560	-0.0150	<250 µm PEQ mussel bed
Yes	LAD	Redundancy	0.02	0.0685	23.83	-0.2410	-0.2408	-0.2196	-0.2406	-0.2385	-0.2233	-0.0049	<2 mm PEQ gravel bar
No	LAD	Simpsons Index	0.34	0.0686	4.61	-0.0386	-0.0256	-0.0523	-0.0362	-0.0395	-0.0443	-0.0280	<2 mm Cd mussel bed
No	LAD	Density of *Leptodea leptodon*	0.00	0.0687	2.65	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	-0.0265	2.556E-20	<250 µm Pb gravel bar
Yes	LAD	Fish host Fundulidae	0.09	0.0691	-0.06	0.0015	0.0027	-0.0024	0.0012	0.0016	0.0009	-0.0051	<250 µm PEQ mussel bed
No	LAD	Redundancy	0.01	0.0693	24.43	-0.2472	-0.2479	-0.2246	-0.2467	-0.2446	-0.2291	-5.241E-05	<2 mm Pb gravel bar
No	LAD	Inverse Simpsons Index	0.30	0.0697	117.84	-1.2084	-0.9562	-1.2339	-1.1564	-1.2071	-1.0287	0.0030	<250 µm Ba gravel bar
Yes	LAD	Pielou's Evenness	0.02	0.0698	-22.83	0.2410	0.2408	0.2196	0.2406	0.2385	0.2233	0.0049	<2 mm PEQ gravel bar

No	LAD	Density of all species	0.21	0.0700	697.79	-6.9747	-7.0162	-6.6419	-7.0200	-6.8770	-6.9846	-0.1815	<250 µm PEQ gravel bar
Yes	LAD	Fisher's Alpha	0.36	0.0701	314.32	-3.0779	-3.0222	-3.1522	-3.0936	-3.1992	-2.9752	-0.1876	<2 mm Cd mussel bed
No	LAD	Fish host Cyprinidae	0.02	0.0702	157.88	-1.5791	-1.5733	-1.5795	-1.5790	-1.5792	-1.5780	0.0008	<250 µm PEQ mussel bed
No	LAD	Fish host Fundulidae	0.06	0.0704	0.22	-0.0013	-0.0012	-0.0043	-0.0016	-0.0012	-0.0021	-5.237E-05	<250 µm Pb mussel bed
No	LAD	Density of *Lampsilis cardium*	0.30	0.0706	-35.43	0.3537	0.3749	0.3486	0.3533	0.3622	0.3506	0.0118	<2 mm Cd mussel bed
No	LAD	Density of *Pleurobema sintoxia*	0.19	0.0707	86.31	-0.8632	-0.8615	-0.8633	-0.8632	-0.8633	-0.8628	6.224E-06	<2 mm Ba mussel bed
Yes	LAD	Brood guild bradytictic	0.01	0.0707	344.01	-3.4448	-3.2972	-3.4651	-3.4453	-3.4235	-3.4385	-1.161E-05	<250 µm Ba mussel bed
No	LAD	Brillouin Index	0.40	0.0709	71.46	-0.7167	-0.5983	-0.7339	-0.6982	-0.7071	-0.6689	-0.0969	<2 mm Cd mussel bed
No	LAD	Density of *Elliptio dilatata*	0.02	0.0709	354.30	-3.5436	-3.5575	-3.5031	-3.5473	-3.5416	-3.5411	-7.574E-05	<250 µm Pb gravel bar
No	LAD	Equitability	0.04	0.0710	-15.79	0.1786	0.1639	0.1447	0.1769	0.1619	0.1586	0.0195	<2 mm PEQ gravel bar
Yes	Logistic	Presence of *Actinonaias ligamentina*	0.30	0.0710	3340.95	-34.2052	-30.9457	-33.6270	-33.9687	-33.8014	-33.2276	4.6871	Bulk Cd mussel bed
Yes	Logistic	Presence of *Ellipsaria lineolata*	0.30	0.0710	3340.95	-34.2052	-30.9457	-33.6270	-33.9687	-33.8014	-33.2276	4.6871	Bulk Cd mussel bed
Yes	Logistic	Presence of *Ligumia recta*	0.30	0.0710	3340.95	-34.2052	-30.9457	-33.6270	-33.9687	-33.8014	-33.2276	4.6871	Bulk Cd mussel bed
Yes	Logistic	Presence of *Truncilla donaciformis*	0.30	0.0710	-1772.26	16.9269	20.1864	17.5051	17.1635	17.3307	17.9046	4.6871	Bulk Cd mussel bed
No	LAD	Rarefy	0.35	0.0715	6.85	-0.0493	-0.0429	-0.0626	-0.0475	-0.0521	-0.0580	-0.0306	<2 mm Cd mussel bed

No	LAD	Shannon-Wiener Index Log10	0.19	0.0715	72.86	-0.7296	-0.6193	-0.7400	-0.7114	-0.7318	-0.6646	0.0007	<250 μm Ba gravel bar
Yes	LAD	Density of *Strophitus undulatus*	0.09	0.0719	0.00	0.0009	0.0020	-0.0030	0.0006	0.0009	0.0002	-0.0050	<250 μm PEQ mussel bed
Yes	LAD	Equitability	0.06	0.0728	-15.79	0.1786	0.1639	0.1447	0.1769	0.1619	0.1586	0.0195	<2 mm PEQ gravel bar
Yes	LAD	Fish host Percidae	0.02	0.0730	-0.38	0.0053	-0.0029	0.0245	0.0031	0.0069	0.0167	-0.0001	<250 μm Pb mussel bed
Yes	LAD	Tribe Anodontini	0.15	0.0735	33.03	-0.3281	-0.3113	-0.3553	-0.3238	-0.3297	-0.3272	-0.0003	Bulk Pb gravel bar
No	LAD	Pielou's Evenness	0.01	0.0736	-22.83	0.2410	0.2408	0.2196	0.2406	0.2385	0.2233	0.0049	<2 mm PEQ gravel bar
No	LAD	Density of *Lampsilis brittsi*	0.12	0.0739	-26.70	0.2681	0.2709	0.2598	0.2695	0.2746	0.2608	-0.0250	Bulk PEQ mussel bed
No	LAD	Tribe Anodontini	0.10	0.0743	30.52	-0.3033	-0.2828	-0.3263	-0.3018	-0.3033	-0.2973	-0.0003	Bulk Ba gravel bar
No	LAD	Fish host Centrarchidae	0.16	0.0749	632.03	-6.3210	-6.4037	-6.1015	-6.3430	-6.2870	-6.3182	-0.0007	<250 μm Pb gravel bar
No	LAD	Density of *Strophitus undulatus*	0.06	0.0749	0.12	-0.0003	0.0008	-0.0041	-0.0006	-0.0003	-0.0009	-0.0049	<250 μm PEQ mussel bed
No	Logistic	Presence of *Venustaconcha ellipsiformis*	0.39	0.0749	1882.34	-18.9886	-15.7899	-21.3070	-20.2950	-17.9261	-18.9521	-0.1137	Bulk Pb mussel bed
Yes	Logistic	Presence of *Venustaconcha ellipsiformis*	0.39	0.0749	1882.34	-18.9886	-15.7899	-21.3070	-20.2950	-17.9261	-18.9521	-0.1137	Bulk Pb mussel bed
No	LAD	Redundancy	0.01	0.0753	23.83	-0.2410	-0.2408	-0.2196	-0.2406	-0.2385	-0.2233	-0.0049	<2 mm PEQ gravel bar
Yes	LAD	Tribe Anodontini	0.15	0.0755	32.71	-0.3249	-0.3077	-0.3525	-0.3206	-0.3265	-0.3241	-0.0398	Bulk PEQ gravel bar
No	LAD	Fish host Fundulidae	0.06	0.0756	0.16	-0.0008	0.0003	-0.0045	-0.0010	-0.0007	-0.0014	-0.0048	<250 μm PEQ mussel bed

Yes	LAD	Fish host Catostomidae	0.10	0.0757	6.43	-0.0650	-0.0541	-0.0728	-0.0617	-0.0649	-0.0644	-0.0060	Bulk Cd mussel bed
No	LAD	Fish host Percidae	0.08	0.0759	-109.14	1.0907	1.1076	1.0950	1.0871	1.0993	1.0977	0.5607	<2 mm Cd mussel bed
Yes	LAD	Density of *Fusconaia flava*	0.21	0.0764	-7.16	0.0691	0.0809	0.0637	0.0708	0.0718	0.0724	0.0002	<250 µm Ba gravel bar
Yes	LAD	Density of *Lampsilis cardium*	0.14	0.0770	-25.35	0.2543	0.2634	0.2567	0.2537	0.2594	0.2541	-8.374E-05	<250 µm Pb mussel bed
No	LAD	Density of *Strophitus undulatus*	0.06	0.0774	0.18	-0.0008	-0.0007	-0.0039	-0.0011	-0.0007	-0.0017	-5.296E-05	<250 µm Pb mussel bed
No	LAD	Density of *Venustaconcha ellipsiformis*	0.00	0.0774	13.35	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	1.329E-17	<250 µm Pb mussel bed
No	LAD	Fish host Percidae	0.02	0.0777	-15.60	0.1582	0.1545	0.1696	0.1553	0.1656	0.1561	-0.0005	<2 mm Pb gravel bar
No	LAD	Fish host Cyprinidae	0.02	0.0782	157.88	-1.5791	-1.5734	-1.5795	-1.5790	-1.5793	-1.5780	7.252E-06	<250 µm Pb mussel bed
No	LAD	Density of *Fusconaia flava*	0.22	0.0786	-15.79	0.1602	0.1589	0.1521	0.1614	0.1617	0.1567	-0.0001	<250 µm Pb mussel bed
Yes	LAD	Infestation displays lure	0.14	0.0791	-43.26	0.4425	0.4492	0.4054	0.4457	0.4500	0.4289	-0.1943	Bulk PEQ gravel bar
No	LAD	Brood guild tachytictic	0.12	0.0810	518.72	-5.1932	-5.2401	-4.9365	-5.2146	-5.1825	-5.1684	-0.0004	<250 µm Pb gravel bar
No	LAD	Fish host Sciaenidae	0.62	0.0813	54.98	-0.5566	-0.5345	-0.5512	-0.5549	-0.5517	-0.5506	0.0009	Bulk Ba gravel bar
Yes	LAD	Density of *Alasmidonta marginata*	0.10	0.0813	6.45	-0.0652	-0.0544	-0.0730	-0.0619	-0.0651	-0.0646	-0.0060	Bulk Cd mussel bed
Yes	LAD	Shannon-Wiener Index ln	0.34	0.0829	49.15	-0.4831	-0.4241	-0.5024	-0.4727	-0.4860	-0.4691	-0.0671	<2 mm Cd mussel bed
No	LAD	Density of *Lampsilis cardium*	0.27	0.0831	-35.65	0.3582	0.3626	0.3629	0.3558	0.3681	0.3503	-0.0004	<2 mm Pb gravel bar

No	LAD	Density of *Lampsilis cardium*	0.21	0.0837	-40.00	0.4008	0.4148	0.3989	0.3987	0.4119	0.3913	-0.0268	<2 mm PEQ gravel bar
Yes	LAD	Density of *Lampsilis brittsi*	0.08	0.0837	-25.36	0.2536	0.2615	0.2455	0.2550	0.2594	0.2492	-0.0071	Bulk Cd mussel bed
No	LAD	Tribe Pleurobemini	0.06	0.0841	417.92	-4.1933	-4.1537	-4.1786	-4.1896	-4.1815	-4.1850	0.0018	Bulk Ba gravel bar
No	LAD	Inverse Simpsons Index	0.23	0.0844	102.85	-1.0082	-0.8915	-1.0469	-0.9774	-1.0124	-0.9897	-0.1638	<2 mm Cd mussel bed
No	LAD	Fish host Percidae	0.02	0.0856	-26.74	0.2682	0.2823	0.2664	0.2661	0.2793	0.2588	-0.0267	<2 mm PEQ gravel bar
Yes	LAD	Inverse Simpsons Index	0.13	0.0870	102.85	-1.0082	-0.8915	-1.0469	-0.9774	-1.0124	-0.9897	-0.1638	<2 mm Cd mussel bed
Yes	LAD	Fish host Percidae	0.07	0.0870	-20.50	0.2136	0.1949	0.2066	0.2160	0.2144	0.2052	-0.0016	Bulk Pb gravel bar
Yes	LAD	Infestation Releases	0.06	0.0874	38.11	-0.3763	-0.4160	-0.3060	-0.3782	-0.3828	-0.3806	-0.0005	<250 µm Pb mussel bed
Yes	LAD	Tribe Pleurobemini	0.05	0.0879	431.41	-4.3105	-4.3250	-4.2880	-4.3098	-4.3128	-4.3148	-0.0003	<250 µm Pb mussel bed
No	LAD	Shannon-Wiener Index Log10	0.45	0.0880	70.90	-0.6970	-0.6118	-0.7248	-0.6820	-0.7012	-0.6768	-0.0969	<2 mm Cd mussel bed
No	LAD	Infestation Releases	0.06	0.0881	-3.66	0.0209	0.0890	0.0383	0.0248	0.0318	0.0348	0.0019	Bulk Ba gravel bar
Yes	LAD	Density of *Lampsilis cardium*	0.33	0.0882	-34.66	0.3510	0.3553	0.3417	0.3514	0.3551	0.3459	-0.0009	Bulk Pb gravel bar
No	LAD	Fish host Ictaluridae	0.28	0.0883	43.76	-0.4441	-0.3761	-0.4429	-0.4415	-0.4437	-0.4311	0.0005	<2 mm Ba gravel bar
No	LAD	Density of *Strophitus undulatus*	0.16	0.0885	-8.09	0.0829	0.0877	0.0741	0.0821	0.0849	0.0799	-0.0003	Bulk Ba gravel bar
No	LAD	Tribe Quadrulini	0.28	0.0896	43.76	-0.4441	-0.3761	-0.4429	-0.4415	-0.4437	-0.4311	0.0005	<2 mm Ba gravel bar
Yes	LAD	Fish host Percidae	0.02	0.0899	-0.37	0.0057	-0.0032	0.0245	0.0030	0.0067	0.0166	-0.0161	<250 µm PEQ mussel bed

No	LAD	Shannon-Wiener Index ln	0.45	0.0900	49.15	-0.4831	-0.4241	-0.5024	-0.4727	-0.4860	-0.4691	-0.0671	<2 mm Cd mussel bed
Yes	LAD	Density of *Lampsilis cardium*	0.14	0.0901	-25.33	0.2544	0.2629	0.2566	0.2534	0.2592	0.2539	-0.0100	<250 μm PEQ mussel bed
Yes	LAD	Shannon-Wiener Index Log10	0.34	0.0902	70.90	-0.6970	-0.6118	-0.7248	-0.6820	-0.7012	-0.6768	-0.0969	<2 mm Cd mussel bed
No	LAD	Fish host Fundulidae	0.16	0.0908	-8.08	0.0828	0.0876	0.0739	0.0820	0.0848	0.0798	-0.0003	Bulk Ba gravel bar
No	LAD	Density of *Fusconaia flava*	0.23	0.0928	-6.03	0.0627	0.0412	0.0677	0.0677	0.0645	0.0568	-0.0783	<2 mm PEQ mussel bed
No	LAD	Infestation Storage	0.28	0.0932	37.96	-0.3861	-0.3181	-0.3849	-0.3835	-0.3857	-0.3731	0.0005	<2 mm Ba gravel bar
No	LAD	Fish host Catostomidae	0.09	0.0942	6.69	-0.0670	-0.0571	-0.0753	-0.0643	-0.0677	-0.0669	-0.0062	Bulk Cd mussel bed
Yes	LAD	Brood guild bradytictic	0.06	0.0958	341.89	-3.4221	-3.2747	-3.4448	-3.4239	-3.4056	-3.3968	-0.0315	<250 μm Cd gravel bar
Yes	LAD	Density of *Strophitus undulatus*	0.09	0.0968	-8.29	0.0836	0.0822	0.0837	0.0828	0.0872	0.0825	-0.0065	<250 μm PEQ gravel bar
Yes	Logistic	Presence of *Venustaconcha ellipsiformis*	0.38	0.0981	2122.96	-21.3277	-18.3380	-26.4119	-21.9087	-20.2228	-22.2179	-2.5615	<250 μm Cd gravel bar
Yes	LAD	Density of *Venustaconcha ellipsiformis*	0.00	0.0985	13.35	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	8.34E-17	<2 mm Cd mussel bed
No	LAD	Density of *Leptodea fragilis*	0.04	0.0989	2.07	-0.0208	-0.0157	-0.0217	-0.0209	-0.0203	-0.0204	-0.0005	<2 mm Cd mussel bed
No	LAD	Tribe Amblemini	0.02	0.0992	7.00	-0.0715	-0.0603	-0.0703	-0.0712	-0.0708	-0.0697	0.0001	Bulk Ba gravel bar
Yes	LAD	Density of *Venustaconcha ellipsiformis*	0.00	0.0996	13.35	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	-0.1335	-3.878E-18	Bulk Ba mussel bed

No	LAD	Density of *Fusconaia flava*	0.19	0.0999	-15.69	0.1581	0.1605	0.1507	0.1606	0.1607	0.1556	-0.0298	Bulk PEQ mussel bed
No	LAD	Density of *Leptodea fragilis*	0.10	0.0999	0.04	-0.0013	0.0093	-0.0021	-0.0009	-0.0013	0.0008	7.656E-05	<2 mm Ba gravel bar

Appendix 2. Summary of model results where forward selection was applied (alpha less than or equal to 0.01). Results are sorted by the P-value, which is the significance for the entire model. Results are sorted from smallest to largest P-value; PEQ, Probable Effects Quotient.

Reference site included	Model type	Response variable	Predictor variable	Intercept	Coefficient	R^2 or r^2	*P*-value
Yes	LAD	Infestation broadcast	<2 mm PEQ mussel bed	0.419665	-0.088662279	0.15283	0.0002
Yes	LAD	Density of *Quadrula pustulosa*	Bulk PEQ gravel bar	0.210814	-0.059848498	0.076567	0.0004
Yes	LAD	Infestation Storage	Bulk PEQ gravel bar	0.210462	-0.059748572	0.058042	0.0004
Yes	LAD	Tribe Quadrulini	Bulk PEQ gravel bar	0.210462	-0.059748572	0.046862	0.0004
Yes	LAD	Density of all species	<250 µm Pb mussel bed	3.961958	-0.003664796	0.247424	0.0005
Yes	LAD	Infestation broadcast	Bulk Pb mussel bed	0.418292	-0.000974359	0.149722	0.0006
Yes	LAD	Fish host Ictaluridae	Bulk PEQ gravel bar	0.210462	-0.059748572	0.056637	0.0006
No	LAD	Density of all species	<250 µm PEQ mussel bed	3.914079	-0.408319085	0.171606	0.0008
Yes	LAD	Brood guild bradytictic	<250 µm PEQ mussel bed	1.150569	-0.079963159	0.157315	0.0008
Yes	Logistic	Presence of *Amblema plicata*	<2 mm Ba mussel bed	-6.56826	-0.04846962	0.65582	0.0008
Yes	LAD	Brood guild bradytictic	<250 µm Pb mussel bed	1.168453	-0.000789229	0.16666	0.0009
No	LAD	Density of all species	<250 µm Pb mussel bed	3.823494	-0.003524479	0.195791	0.001
No	LAD	Fish host Centrarchidae	<250 µm PEQ mussel bed	1.067167	-0.070986181	0.096412	0.001
Yes	LAD	Density of all species	<250 µm PEQ mussel bed	4.073101	-0.426514649	0.225914	0.001
Yes	Logistic	Presence of *Quadrula pustulosa*	<2 mm Ba mussel bed	-2.37266	-0.023322388	0.473559	0.0012
No	LAD	Fish host Centrarchidae	<250 µm Pb mussel bed	1.060124	-0.000817455	0.103707	0.0014
Yes	LAD	Fish host Fundulidae	<250 µm Pb mussel bed	0.042193	-2.84991E-05	0.158603	0.0019
No	Logistic	Presence of *Amblema plicata*	<2 mm Ba mussel bed	-7.19769	-0.047668111	0.617513	0.0019
No	LAD	Brood guild bradytictic	<250 µm PEQ mussel bed	1.091169	-0.072582776	0.14316	0.002
Yes	Logistic	Presence of *Strophitus undulatus*	<250 µm Pb mussel bed	-0.65018	-0.00768094	0.474411	0.0021
Yes	Logistic	Presence of *Obliquaria reflexa*	<2 mm Ba mussel bed	-16.3476	-0.097370235	0.673337	0.0025
No	LAD	Brood guild bradytictic	<250 µm Pb mussel bed	1.168453	-0.000789229	0.151255	0.0026

Yes	Logistic	Presence of *Strophitus undulatus*	<250 µm PEQ mussel bed	-0.76801	-0.89241359	0.473047	0.0027
Yes	LAD	Fish host Centrarchidae	<250 µm Pb mussel bed	2.257123	-0.002123149	0.130437	0.003
Yes	LAD	Fish host Centrarchidae	<250 µm PEQ mussel bed	1.067167	-0.070986181	0.114911	0.0031
Yes	Logistic	Presence of *Leptodea fragilis*	<2 mm Ba mussel bed	-1.45535	-0.019942687	0.439108	0.0031
Yes	LAD	Density of *Strophitus undulatus*	<250 µm Pb mussel bed	0.041672	-2.81472E-05	0.156779	0.0032
Yes	LAD	Fish host Fundulidae	<250 µm PEQ mussel bed	0.041486	-0.003045766	0.150413	0.0033
Yes	LAD	Density of *Strophitus undulatus*	<250 µm PEQ mussel bed	0.040974	-0.003008164	0.148683	0.0037
No	Logistic	Presence of *Strophitus undulatus*	<250 µm Pb mussel bed	-0.93879	-0.007525463	0.436931	0.0038
No	LAD	Fish host Fundulidae	<250 µm Pb mussel bed	0.06719	-6.80884E-05	0.173144	0.0039
No	LAD	Density of *Strophitus undulatus*	<250 µm Pb mussel bed	0.067164	-6.80629E-05	0.17114	0.004
No	LAD	Density of *Strophitus undulatus*	<250 µm PEQ mussel bed	0.059964	-0.006861179	0.149145	0.004
No	LAD	Fish host Fundulidae	<250 µm PEQ mussel bed	0.060713	-0.006946944	0.150953	0.004
No	Logistic	Presence of *Strophitus undulatus*	<250 µm PEQ mussel bed	-1.05789	-0.87359873	0.435571	0.004
No	Logistic	Presence of *Quadrula pustulosa*	<2 mm Ba mussel bed	-2.66891	-0.022587433	0.430449	0.0042
Yes	Logistic	Presence of *Pleurobema sintoxia*	Fine Gravel	-1.91475	0.460521751	0.521199	0.0043
No	LAD	Infestation broadcast	Fine Gravel	-0.0828	0.0679	0.355381	0.0046
Yes	Logistic	Presence of *Truncilla truncata*	<250 µm Ba gravel bar	-2.67959	-0.013541644	0.532091	0.0046
Yes	LAD	Tribe Quadrulini	Bulk Pb gravel bar	-0.0227	-0.000350142	0.315386	0.0048
			Fine Gravel		0.052561401		
No	LAD	Brillouin's Index	<250 µm PEQ mussel bed	2.256003	-0.103290591	0.154661	0.005
No	Logistic	Presence of *Leptodea fragilis*	<2 mm Ba mussel bed	-1.73125	-0.019382701	0.401133	0.005
Yes	LAD	Infestation Storage	Bulk Pb gravel bar	-0.0227	-0.000350142	0.395308	0.0051
			Fine Gravel		0.052561401		
Yes	Logistic	Presence of *Actinonaias ligamentina*	<250 µm Ba gravel bar	-8.86148	-0.032700654	0.739387	0.0053
Yes	Logistic	Presence of *Ellipsaria lineolata*	<250 µm Ba gravel bar	-8.86148	-0.032700654	0.739387	0.0053
Yes	Logistic	Presence of *Ligumia recta*	<250 µm Ba gravel bar	-8.86148	-0.032700654	0.739387	0.0053
Yes	Logistic	Presence of *Pleurobema sintoxia*	<250 µm PEQ gravel bar	-3.04743	-0.484458951	0.488404	0.0055
Yes	Logistic	Presence of *Lampsilis brittsi*	Boulder	2.707499	0.489074423	0.417879	0.0056
Yes	LAD	Density of *Quadrula pustulosa*	Bulk Pb gravel bar	-0.07564	-0.000159476	0.346216	0.0058
			Fine Gravel		0.043584846		

Yes	LAD	Tribe Pleurobemini	<250 µm PEQ mussel bed	0.449533	-0.031021086	0.068491	0.0061
Yes	Logistic	Presence of *Obliquaria reflexa*	Bulk PEQ gravel bar	-2.68687	-2.060271561	0.502958	0.0062
No	LAD	Brillouin's Index	<250 µm Pb mussel bed	2.233539	-0.000893364	0.145644	0.0063
Yes	LAD	Infestation Releases	<250 µm PEQ mussel bed	0.449712	-0.031058447	0.075552	0.0065
Yes	LAD	Equitability	<250 µm Ba gravel bar	0.699005	0.000441106	0.156217	0.0066
Yes	LAD	Infestation Releases	<250 µm Pb mussel bed	0.660372	-0.000611191	0.08308	0.0066
Yes	LAD	Brood guild tachytictic	<250 µm Pb mussel bed	2.929122	-0.002968304	0.045667	0.0067
Yes	LAD	Equitability	<250 µm PEQ mussel bed	0.833984	0.024669491	0.170683	0.0067
Yes	LAD	Fish host Ictaluridae	Bulk Pb gravel bar	-0.0227	-0.000350142	0.385158	0.0068
			Fine Gravel		0.052561401		
Yes	LAD	Variety	<2 mm PEQ mussel bed	2.444929	-0.286541837	0.228866	0.0069
Yes	LAD	Density of *Potamilus alatus*	<250 µm PEQ mussel bed	0.033362	-0.002449301	0.146163	0.0071
Yes	LAD	Density of *Potamilus alatus*	<250 µm Pb mussel bed	0.033432	-2.25816E-05	0.151132	0.0072
Yes	LAD	Tribe Pleurobemini	<250 µm Pb mussel bed	0.583517	-0.000517336	0.074448	0.0072
Yes	LAD	Fish host Sciaenidae	<250 µm Pb mussel bed	0.106349	-7.1833E-05	0.072273	0.0075
Yes	LAD	Fish host Sciaenidae	<250 µm PEQ mussel bed	0.106538	-0.007821694	0.068216	0.0077
No	LAD	Infestation broadcast	<2 mm PEQ mussel bed	0.32042	-0.064664102	0.130273	0.008
No	LAD	Density of *Leptodea fragilis*	<2 mm Ba mussel bed	0.025078	-3.14412E-05	0.045612	0.0084
Yes	Logistic	Presence of *Amblema plicata*	<2 mm PEQ mussel bed	-1.50724	-1.903580409	0.403587	0.0088
Yes	LAD	Brillouin's Index	<250 µm PEQ mussel bed	2.454441	-0.127946427	0.18548	0.0091
Yes	Logistic	Presence of *Actinonaias ligamentina*	<250 µm PEQ gravel bar	-5.1451	-0.65920269	0.574573	0.0094
Yes	Logistic	Presence of *Ellipsaria lineolata*	<250 µm PEQ gravel bar	-5.1451	-0.65920269	0.574573	0.0094
Yes	Logistic	Presence of *Ligumia recta*	<250 µm PEQ gravel bar	-5.1451	-0.65920269	0.574573	0.0094
Yes	LAD	Brillouin's Index	<250 µm Pb mussel bed	2.460456	-0.00114399	0.17734	0.0098
Yes	LAD	Variety	Fine Gravel	1.076812	0.091558595	0.326129	0.0099

Appendix 3. Results of acute and chronic aquatic toxicity tests with barium, obtained from search of U.S. Environmental Protection Agency 'ECOTOX' database (http://cfpub.epa.gov/ecotox/) on February 8, 2016. Results were filtered to select freshwater tests with endpoints of mortality, growth, or reproduction. Chronic tests (gray highlights) were defined as tests with greater than 10 day duration (micrograms per liter [µg/L]).

CAS	Ref Number	Species	Duration (day)	Endpoint	Trend	Effect	Conc µg/L	Signif	Author	Year	Source	Title
									Barium			
7440393	5184	*Daphnia magna*	2	NOEC	NR	MORT	68000	NO-SIG	LeBlanc,G.A.	1980	Bull. Environ. Contam. Toxicol.24(5): 684-691	Acute toxicity of priority pollutants to water flea (*Daphnia magna*)
7440393	9607	*Lepomis macrochirus*	4	LC50	INC	MORT	198000	NA	U.S. EPA	1978	U.S.EPA Contract No.68-01-4646, Duluth, MN:9 p.	In-depth studies on health and environmental impacts of selected water pollutants
7440393	5184	*Daphnia magna*	2	LC50	INC	MORT	410000	NA	LeBlanc,G.A.	1980	Bull. Environ. Contam. Toxicol.24(5): 684-691	Acute toxicity of priority pollutants to water flea (*Daphnia magna*)
									Barium Chloride			
10361372	2022	*Daphnia magna*	21	EC50	NR	GREP	8900	NA	Biesinger,K.E., and G.M. Christensen	1972	J. Fish. Res. Board Can.29(12): 1691-1700	Effects of various metals on survival, growth, reproduction and metabolism of *Daphnia magna*
10361372	5421	*Austropotamobius pallipes* ssp. *pallipes*	30	LC50	NR	MORT	39000	NA	Boutet,C., and C. Chaisemartin	1973	Comptes Rendus Seances Soc. Biol. Fil.167(12): 1933-1938	Specific toxic properties of metallic salts in *Austro-potamobius pallipes pallipes* and *Orconectes limosus*

10361372	11838	*Onco-rhynchus mykiss*	28	LC50	NR	MORT	42700	NA	Birge,W.J., J.A. Black, A.G. Westerman, and J.E. Hudson	1980	In: C.Gale (Ed.), EPA-600/9-80-022, Oil Shale Symposium: Sampling, Analysis and Quality Assurance, March 1979, U.S.EPA, Cincinnati, OH:519-534	Aquatic toxicity tests on inorganic elements occurring in oil shale
10361372	5421	*Austro-potam-obius pallipes* ssp. *pallipes*	30	LC50	NR	MORT	43000	NA	Boutet,C., and C. Chaisemartin	1973	Comptes Rendus Seances Soc. Biol. Fil.167(12): 1933-1938	Specific toxic properties of metallic salts in *Austro-potamobius pallipes pallipes* and *Orconectes limosus*
10361372	5421	*Orco-nectes limosus*	30	LC50	NR	MORT	59000	NA	Boutet,C., and C. Chaisemartin	1973	Comptes Rendus Seances Soc. Biol. Fil.167(12): 1933-1938	Specific toxic properties of metallic salts in *Austro-potamobius pallipes pallipes* and *Orconectes limosus*
10361372	5421	*Orco-nectes limosus*	30	LC50	NR	MORT	61000	NA	Boutet,C., and C. Chaisemartin	1973	Comptes Rendus Seances Soc. Biol. Fil.167(12): 1933-1938	Specific toxic properties of metallic salts in *Austro-potamobius pallipes pallipes* and *Orconectes limosus*
10361372	5421	*Austro-pota-mobius pallipes* ssp. *pallipes*	4	LC50	NR	MORT	46000	NA	Boutet,C., and C. Chaisemartin	1973	Comptes Rendus Seances Soc. Biol. Fil.167(12): 1933-1938	Specific toxic properties of metallic salts in *Austro-potamobius pallipes pallipes* and *Orconectes limosus*
10361372	5421	*Orco-nectes limosus*	4	LC50	NR	MORT	78000	NA	Boutet,C., and C. Chaisemartin	1973	Comptes Rendus Seances Soc. Biol. Fil.167(12): 1933-1938	Specific toxic properties of metallic salts in *Austro-potamobius pallipes pallipes* and *Orconectes limosus*

10361372	14397	*Oncor-hynchus kisutch*	3	NOEC	INC	MORT	88800	NO-SIG	Holland,G.A., J.E. Lasater, E.D. Neumann, and W.E. Eldridge	1960	Res. Bull. No. 5, State of Washington Dept. Fish., Seattle, WA:263 p.	Toxic effects of organic and inorganic pollutants on young salmon and trout
10361372	12420	*Echino-gamm-arus berilloni*	4	LC50	NR	MORT	122000	NA	Vincent,M., B. Penicaut, and J. Debord	1986	Ann. Rech. Vet.17(4): 441-446	Comparative studies on the toxicity of metal chlorides and of a synthetic organic molluscicide, N-Trityl-Morpholine, upon two aquatic amphipod crustaceans, *Gammarus pulex* and *Echinogammarus berilloni*
10361372	2742	*Spongilla lacustris*	3	NR	NR	GREP	137000	NR	Ostrom,K.M., and T.L. Simpson	1978	Dev. Biol.64:332-338	Calcium and the release from dormancy of freshwater sponge gemmules
10361372	448	*Salmo trutta*	2	LC50	INC	MORT	150000	NA	Woodiwiss,F.S. , and G. Fretwell	1974	Water Pollut. Control73:396-405	The toxicities of sewage effluents, industrial discharges and some chemical substances to brown trout (*Salmo trutta*) in the Trent River authority area
10361372	14397	*Onco-rhynchus kisutch*	5	NOEC	INC	MORT	158000	NO-SIG	Holland,G.A., J.E. Lasater, E.D. Neumann, and W.E. Eldridge	1960	Res. Bull. No. 5, State of Washington Dept. Fish., Seattle, WA:263 p.	Toxic effects of organic and inorganic pollutants on young salmon and trout

10361372	14397	*Onco-rhynchus kisutch*	3	LOEC	INC	MORT	158000	SIG	Holland,G.A., J.E. Lasater, E.D. Neumann, and W.E. Eldridge	1960	Res. Bull. No. 5, State of Washington Dept. Fish., Seattle, WA:263 p.	Toxic effects of organic and inorganic pollutants on young salmon and trout
10361372	12420	*Echino-gamm-arus berilloni*	3	LC50	NR	MORT	162000	NA	Vincent,M., B. Penicaut, and J. Debord	1986	Ann. Rech. Vet.17(4): 441-446	Comparative studies on the toxicity of metal chlorides and of a synthetic organic molluscicide, N-Trityl-Morpholine, upon two aquatic amphipod crustaceans, *Gammarus pulex* and *Echinogammarus berilloni*
10361372	12420	*Gam-marus pulex*	4	LC50	NR	MORT	238000	NA	Vincent,M., B. Penicaut, and J. Debord	1986	Ann. Rech. Vet.17(4): 441-446	Comparative studies on the toxicity of metal chlorides and of a synthetic organic molluscicide, N-Trityl-Morpholine, upon two aquatic amphipod crustaceans, *Gammarus pulex* and *Echinogammarus berilloni*
10361372	12420	*Gam-marus pulex*	3	LC50	NR	MORT	255000	NA	Vincent,M., B. Penicaut, and J. Debord	1986	Ann. Rech. Vet.17(4): 441-446	Comparative studies on the toxicity of metal chlorides and of a synthetic organic molluscicide, N-Trityl-Morpholine, upon two aquatic amphipod crustaceans, *Gammarus pulex* and *Echinogammarus berilloni*
10361372	12420	*Echino-gamm-arus berilloni*	2	LC50	NR	MORT	258000	NA	Vincent,M., B. Penicaut, and J. Debord	1986	Ann. Rech. Vet.17(4): 441-446	Comparative studies on the toxicity of metal chlorides and of a synthetic organic molluscicide, N-Trityl-Morpholine, upon two aquatic amphipod crustaceans, *Gammarus pulex* and *Echinogammarus berilloni*

10361372	14397	*Oncorhynchus kisutch*	6	NOEC	INC	MORT	282000	NO-SIG	Holland,G.A., J.E. Lasater, E.D. Neumann, and W.E. Eldridge	1960	Res. Bull. No. 5, State of Washington Dept. Fish., Seattle, WA:263 p.	Toxic effects of organic and inorganic pollutants on young salmon and trout
10361372	14397	*Oncorhynchus kisutch*	5	LOEC	INC	MORT	282000	SIG	Holland,G.A., J.E. Lasater, E.D. Neumann, and W.E. Eldridge	1960	Res. Bull. No. 5, State of Washington Dept. Fish., Seattle, WA:263 p.	Toxic effects of organic and inorganic pollutants on young salmon and trout
10361372	14397	*Oncorhynchus kisutch*	0.96	NR-LETH	INC	MORT	282000	NA	Holland,G.A., J.E. Lasater, E.D. Neumann, and W.E. Eldridge	1960	Res. Bull. No. 5, State of Washington Dept. Fish., Seattle, WA:263 p.	Toxic effects of organic and inorganic pollutants on young salmon and trout
10361372	12420	*Echinogammarus berilloni*	1	LC50	NR	MORT	336000	NA	Vincent,M., B. Penicaut, and J. Debord	1986	Ann. Rech. Vet.17(4): 441-446	Comparative studies on the toxicity of metal chlorides and of a synthetic organic molluscicide, N-Trityl-Morpholine, upon two aquatic amphipod crustaceans, *Gammarus pulex* and *Echinogammarus berilloni*

10361372	118584	*Lepomis macrochirus*	0.04	NOEC	DEC	MOTL	342500	ANO-SIG	Zuccarelli,M.D., and R.L. Ingermann	2007	J. Exp. Zool.307:590-599	Calcium-induced quiescence of sperm motility in the bluegill (*Lepomis macrochirus*)
10361372	118584	*Lepomis macrochirus*	0	NOEC	DEC	MOTL	342500	ANO-SIG	Zuccarelli,M.D., and R.L. Ingermann	2007	J. Exp. Zool.307:590-599	Calcium-induced quiescence of sperm motility in the bluegill (*Lepomis macrochirus*)
10361372	12420	*Gammarus pulex*	2	LC50	NR	MORT	395000	NA	Vincent,M., B. Penicaut, and J. Debord	1986	Ann. Rech. Vet.17(4): 441-446	Comparative studies on the toxicity of metal chlorides and of a synthetic organic molluscicide, N-Trityl-Morpholine, upon two aquatic amphipod crustaceans, *Gammarus pulex* and *Echinogammarus berilloni*
10361372	547	*Leuciscus idus ssp. Melanotus*	2	LC0	INC	MORT	450000	NA	Juhnke,I., and D. Luedemann	1978	Z. Wasser-Abwasser-Forsch.11(5): 161-164	Results of the investigation of 200 chemical compounds for acute fish toxicity with the Golden Orfe test
10361372	14397	*Oncorhynchus kisutch*	6	LOEC	INC	MORT	500000	SIG	Holland,G.A., J.E. Lasater, E.D. Neumann, and W.E. Eldridge	1960	Res. Bull. No. 5, State of Washington Dept. Fish., Seattle, WA:263 p.	Toxic effects of organic and inorganic pollutants on young salmon and trout
10361372	547	*Leuciscus idus* ssp. *Melanotus*	2	LC50	INC	MORT	870000	NA	Juhnke,I., and D. Luedemann	1978	Z. Wasser-Abwasser-Forsch.11(5): 161-164	Results of the investigation of 200 chemical compounds for acute fish toxicity with the Golden Orfe test

10361372	547	*Leuciscus idus* ssp. *Melanotus*	2	LC100	INC	MORT	955000	NA	Juhnke,I., and D. Luedemann	1978	Z. Wasser-Abwasser-Forsch.11(5): 161-164	Results of the investigation of 200 chemical compounds for acute fish toxicity with the Golden Orfe test
10361372	508	*Gambusia affinis*	4	LC50	INC	MORT	1640000	NA	Wallen,I.E., W.C. Greer, and R. Lasater	1957	Sewage Ind. Wastes29(6): 695-711	Toxicity to *Gambusia affinis* of certain pure chemicals in turbid waters
10361372	508	*Gambusia affinis*	2	LC50	INC	MORT	3200000	NA	Wallen,I.E., W.C. Greer, and R. Lasater	1957	Sewage Ind. Wastes29(6): 695-711	Toxicity to *Gambusia affinis* of certain pure chemicals in turbid waters
10361372	12420	*Gammarus pulex*	1	LC50	NR	MORT	3980000	NA	Vincent,M., B. Penicaut, and J. Debord	1986	Ann. Rech. Vet.17(4): 441-446	Comparative studies on the toxicity of metal chlorides and of a synthetic organic molluscicide, N-Trityl-Morpholine, upon two aquatic amphipod crustaceans, *Gammarus pulex* and *Echinogammarus berilloni*
10361372	508	*Gambusia affinis*	1	LC50	INC	MORT	4400000	NA	Wallen,I.E., W.C. Greer, and R. Lasater	1957	Sewage Ind. Wastes29(6): 695-711	Toxicity to *Gambusia affinis* of certain pure chemicals in turbid waters

Barium nitrate

10022318	2851	*Gasterosteus aculeatus*	10	NR-LETH	NR	MORT	400000	NA	Jones,J.R.E.	1939	J. Exp. Biol.16(4): 425-437	The telation between the electrolytic solution pressures of the metals and their toxicity to the stickleback (*Gasterosteus aculeatus* L.)

100223 18	13669	*Strepto-cephalus probo-scideus*	1	LC50	INC	MORT	371270	NA	Calleja,M.C., G. Persoone, and P. Geladi	1994	Arch. Environ. Contam. Toxicol.26(1): 69-78	Comparative acute toxicity of the first 50 multicentre evaluation of in vitro cytotoxicity chemicals to aquatic non-vertebrates
100223 18	13669	*Brach-ionus calyc-iflorus*	1	LC50	INC	MORT	371270	NA	Calleja,M.C., G. Persoone, and P. Geladi	1994	Arch. Environ. Contam. Toxicol.26(1): 69-78	Comparative acute toxicity of the first 50 multicentre evaluation of in vitro cytotoxicity chemicals to aquatic non-vertebrates
									Barium sulfate			
772743 7**	2942	*Poecilia sp.*	4	LC0	NR	MORT	59000000	NA	Grantham,C.K., and J.P. Sloan	1975	EPA 560/1-75-004, Conf.Proc.on Environ.Aspects of Chemical Use in Well-Drilling Operations, Research Triangle Inst., NC:	Toxicity study drilling fluid chemicals on aquatic life

** did not report medium for tests.