Effects of Lead-Zinc Mining on Benthic Fish Density in Riffles of the Big River (Southeast Missouri)

Final Report

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USE OF DATA

The data were collected in accordance with the Missouri Department of Conservation (MDC) study plan and the analytical data conform to the intent of standard good laboratory practices. Data collection was focused on MDC objectives outlined in the study plan. The data may be used for other objectives; however, suitability of the data for those objectives will be the responsibility of the user. The purpose of this report is to communicate information within MDC to other state and federal agencies. However, the data are considered publically available and can be provided upon request.

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Summary

Mining activity in southeast Missouri has resulted in releases of metals into the environment, especially lead (Pb), cadmium (Cd), and zinc (Zn). Surface water and sediments from the Big River in St. Francois, Jefferson, and Washington Counties are contaminated with metals from near the town of Leadwood to the confluence with the Meramec River. The purpose of this study was to determine if the density of benthic riffle fish is impacted by mining-related heavy metals. Riffle fish density was measured at eight sites on the Big River. Two reference sites were located upstream of known mining activity. Six other sampling sites were distributed at or below mine locations. Sites were located along a gradient with two sites each in areas with high, moderate and low levels of metals in sediments. Each sampling site consisted of three consecutive riffles. Density of benthic riffle fish was determined in three randomly selected quadrats (approximately 32 m^2) for each site. Sediment and water concentrations were measured as part of companion studies conducted for mussels and crayfish during the same time period. Ten species of benthic riffle fish including darters, sculpins, madtoms and stonecats were collected during the study. Densities of adult benthic riffle fish at the two sites with high levels of metals in sediments were 1.2 and 1.3 fish/m^2 and were significantly lower than density at all other sites which ranged from 3.9 to 8.8 fish/m^2. Density of adult benthic riffle fish had a significant negative correlation with surface water and sediment Pb, Cd and Zn, with the exception of a similar but non-significant correlation with sediment Pb from the crayfish study. Lead and Cd concentrations were also measured in whole-body samples of Missouri saddled darter (*Etheostoma tetrazonum*) from each site. Density of benthic riffle fish was not correlated with whole-body concentrations of Pb and Cd in Missouri saddled darters. Whole-body concentrations of Pb in Missouri saddled darters were sufficiently high to trigger toxicological concern for wildlife, such as herons, that may feed on these animals. Findings from this study indicate that benthic riffle fish density is negatively correlated with mining-related heavy metals in sediments and surface water from the Big River.

Introduction

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) as amended, 42 U.S.C. 9601 et seq., and the Federal Water Pollution Control Act (FWPCA), as amended, 33 U.S.C. 1251 et seq., authorize the Federal Government and States to recover damages for injuries to natural resources and their supporting ecosystems, belonging to, managed by, appertaining to, or otherwise controlled by them.

The Secretary of the Department of the Interior (DOI) is designated under 40 CFR § 300.600(2) to act on behalf of the public as trustee for natural resources and their supporting ecosystems, managed or controlled by DOI. Pursuant to CERCLA, the Governor of the state of Missouri has designated the Director of the Missouri Department of Natural Resources as the Trustee for the state's natural resources. The Department and the Missouri Department of Conservation (MDC) entered into a Memorandum of Agreement whereby the two state agencies agreed to cooperate and coordinate in assessing injuries to natural resources and obtaining damages for those injuries. The Department of Conservation also serves as an expert on the flora and fauna of the state for the Department.

The DOI published regulations setting forth procedures by which a natural resource trustee can determine compensation for injuries to natural resources that have not been nor are expected to be addressed by response actions in 43 CFR Part 11. As part of the Natural Resource Damage Assessment (NRDA) process, the Trustees requested the assistance of the MDC in investigating impacts of mining activities on fish populations in the Big River.

Mining activity in southeast Missouri has resulted in release of metals into the environment, especially Pb, Cd, and Zn. Surface water and sediments from Big River in St. Francois, Jefferson and Washington Counties are contaminated with metals from near Leadwood to the confluence with the Meramec River (MDNR, 2003; Gale et al., 2004; Madden, 2006). Lead is known to accumulate in certain fish species (Czarneski, 1985; Schmitt and Finger, 1987; Gale et al., 2004). These fish contaminant data along with data from other studies resulted in the issuance of fish consumption advisories by the Missouri Department of Health and Senior Services (DHSS) for several fish species (DHSS, 2009).

Previous work in an adjacent basin (the Black River basin), indicates that density of benthic riffle fish may be sensitive to metal exposure in the sediment and water (Allert et al., 2009). The Big River basin has similar mining in its watershed, so it was hypothesized that density of benthic riffle fish in the Big River may also be affected by metal contamination of sediments associated with mining. Benthic-dwelling riffle fish species for the current study are defined as sculpins, darters, and *Noturus* spp. (madtoms and stonecats) based partially on the observations by Pflieger (1997).

The purpose of this study is to determine the density of benthic riffle fish in the Big River and evaluate whether the density is associated with metal concentrations in sediments and whole-body fish.

Material and Methods

Site Selection

Benthic riffle fish were sampled in riffle habitats at eight sites in the Big River (Table 1 and Figure 1). Two reference sites (R-1 and R-2) were upstream of the known mining areas (Figure 1) and therefore were expected to have low levels of metals in sediments (McKee, 2008). Two sites each were identified in areas with high (TH-1 and TH-2), moderate (TM-1 and TM-2) and low (TL-1 and TL-2) levels of metals in sediments based on previously collected information (McKee, 2008). Sites TH-1 and TH-2 are the closest sites downstream of the mine waste sites (Figure 1).

The sites selected to investigate benthic riffle fish densities were the same sites included in companion studies with crayfish (Allert et al., 2010) and mussels (Roberts et al., 2009). Allert et al. (2010) used the same site names as those described above. However, Roberts et al. (2009) used different site names and those names are included here to facilitate comparison between the studies: R-1= ID; R-2 = LG; TH-1 = 67D; TH-2 = HK; TM-1 = 67C; TM-2 = MA; TL-1 = WSP; TL-2 = BC. All sites were at the same locations with the exception of site LG in Roberts et al. (2009). Site LG was located approximately 8.4 km downstream from site R-2, but was still upstream of known mining areas. Site locations used in this study were documented by a hand-held global positioning system (GPS) receiver [±10 m, datum = World Geodetic System (WGS) 84].

Sampling Design

Each site consisted of three consecutive riffles. Benthic riffle fish density was estimated in three randomly placed 32 m^2 quadrats (targeted dimensions were 4 m by 8 m) at each site. The precise location of the quadrats within a site was determined prior to going to the field. Possible quadrat locations were determined for a particular site by plotting the three riffles on graph paper and then visually placing the maximum number of 32 m^2 quadrats into each riffle. From the total number of potential quadrats available, three quadrat locations were randomly selected to be sampled at that site.

Based on preliminary efforts, the block-net enclosure functioned most efficiently in riffles with water equal to or less than 30 cm deep at the corners and with flow rates equal to or less than 0.8 m/sec. To avoid too shallow of a riffle, a quadrat was required to have flow over the entire area and be at least 6 cm deep at the corners. Upon arriving at the sampling site, the field staff evaluated the riffles selected in the pre-field selection process to determine if each met the criteria established above. If a selected quadrat did not meet the criteria, then the next quadrat identified in the pre-field random assessment was selected and evaluated. The process was continued until three replicates were identified per site. Using this process and criteria resulted in some of the deepest and shallowest portions of the riffle not being sampled. However, the method provides for an accurate sampling of the intermediate depths and flow.

Site Characterization

Individual quadrats were characterized for water chemistry, flow, and substrate. Temperature, pH, conductivity, and dissolved oxygen were measured at the middle of the quadrat using a Hydrolab Quanta meter (Hach Industries, Loveland, CO). Water depth and velocity were measured at the four corners and middle of the quadrat. Water velocity was measured at a depth 60% above the bottom using a Marsh-McBirney Inc., Flo-Mate, Model 2000 Portable Flow Meter, (Hach Industries, Loveland, CO). Substrate was visually assigned to the following categories described by Allert et al. (2010): sand (<2 mm); gravel (2-16 mm); pebble (17-64 mm); cobble (65-250 mm); boulder (>250 mm); and bedrock.

Sediments and water metal concentrations were characterized in companion studies with crayfish (Allert et al., 2010) and mussels (Roberts et al., 2009), also conducted in the summer of 2008. Sediments were collected in depositional areas near to the riffle areas. Allert et al. (2010) report metal concentrations for sediments with particle size < 2 mm and for filtered stream water samples for each site. Roberts et al. (2009) report bulk sediment (maximum particle size) and the <0.25 mm fraction in separately collected and analyzed samples.

Riffle Fish Density Sampling Method

The density of benthic riffle fish species (i.e., sculpins, darters, and *Noturus* spp.) was determined for each of the three quadrats per site. Density was estimated using the depletion sampling method (Hillborn and Walters, 1992) whereby a quadrat was subject to repeated passes of a backpack electrofisher to achieve three sequentially decreasing catch values. Upon arriving at the acceptable quadrat location, t-posts were driven in each corner. A block-net (0.32 cm mesh by 122 cm continuous lead line seine) was anchored to each corner post. Additional posts were driven, if necessary. The bottom lead line was buried by hand into the substrate so that fish could not escape. The exact dimension of each final quadrat was recorded.

After each electrofishing pass for a particular quadrat, fish were collected into a bucket labeled for that particular pass and set outside the enclosure for evaluation. For each bucket, individual fish were identified to species (darters, sculpins, and *Noturus spp.*) counted, measured and recorded on the field datasheet. Fish were identified as adults or juveniles by using a species-specific juvenile threshold length (Appendix 1).

Fish Whole-Body Contaminant Sample

A sample of fish collected from each quadrat was retained for metals analysis. Based on expected abundance and desire not to mix species in the analytical sample, the Missouri saddled darter (*Etheostoma tetrazonum*) was selected as the species to use for whole-body analysis. A composite sample of 10 fish was targeted for each quadrat (one composite sample per quadrat). In a few cases, less than 10 fish were collected and these occasions are noted in the raw data.

Metal Analysis of Whole-Body Fish Samples

Fish were minced with a titanium meat cleaver and lyophilized. Dried samples were stored at room temperature in a 40-mL glass vial in a desiccator. To prepare fish samples for analysis, a dried sample of approximately 0.25 g was heated with nitric acid in a sealed low-pressure Teflon vessel in a laboratory microwave oven and dissolved in 6% nitric acid. Cadmium and Pb analyses were conducted using a PE/SCIEX Elan 6000® inductively coupled plasma-mass spectrometer (ICP-MS).   The analyses were conducted at the USGS Columbia Environmental Research Center, Columbia, MO.

Data Analysis

The number of all benthic riffle fish (adults + juveniles) and the number of adult benthic riffle fish in each quadrat were estimated using a closed–population removal estimator from the repeated sampling passes (Otis et al., 1978). Density estimates were made using the program MARK (White, 2008). Program MARK is a statistical package that allows parameter estimates for repeated samplings of closed populations. The program provided a population estimate, standard error and confidence interval for each quadrat. The number of fish per quadrat was converted to density by dividing the estimated number of fish by the area of the quadrat.

Non-transformed and $\log_{10}$-transformed density data were evaluated for normality using the Levene test. A one-way ANOVA was used to analyze for differences among sites. The Least Significant Difference (LSD) method was used for multiple mean comparisons. All statistical analyses were conducted using SAS (SAS institute, 2004).

Results

Benthic riffle fish sites were sampled between August 14, 2008 and September 12, 2008 (Table 1). Quadrats sampled had average depths between 0.08 and 0.28 m (Table 2). All corners of the quadrats were greater than 0.06 m (Appendix 2), as specified in the work plan (McKee, 2008). The mean velocities ranged from 0.07 to 0.78 m/sec and were below the 0.8 m/sec maximum identified in the work plan. Temperature, dissolved oxygen, conductivity, and pH for each quadrat are given in Table 2. No differences in chemical or physical characteristics were apparent among quadrats from visual examination of data (Table 2). Appendix 2 includes the substrate characterization data for each quadrat.

Concentration of metals in sediment and surface water were measured at the eight Big River sites as part of a companion study with crayfish (Allert et al., 2010), also conducted during the summer of 2008 (Table 3).   In addition, sediment metal concentrations were measured at or near these same Big River sites as part of a mussel field study (Roberts et al., 2009) during the same time period (Table 3).   In general, the levels of Pb, Cd, and Zn in sediment and surface water were highest at sites closest to the large mining waste piles

(TH-1 and TH-2) and lowest at the reference sites located upstream of the waste piles (R-1 and R-2).

Whole-body concentrations of Pb and Cd were measured in Missouri saddled darters from each of the sites (Table 4). Whole-body concentrations of Pb in darters were low at the reference sites [0.17 to 0.20 μg/g wet weight (ww)] compared to downstream sites in the mining area (7.99 to 19.57 μg/g ww). Similar differences were observed for whole-body concentrations of Cd.

Ten species of benthic riffle fish were collected during the study (Table 5). The number of species collected at each site ranged from 7 to 10. Gilt darter (*percina evides*) was absent at the most upstream sites (R-1 and R-2) and the slender madtom (*Noturus exilis*) was absent from the two sites furthest downstream (TM-2 and TL-2). No trend in species occurrence was apparent in relation to sediment metal concentrations, or upstream to downstream relationships.

The mean density of adult benthic riffle fish ranged from a low of 1.2 adults/m^2 at TH-2 to a high value of 8.8 adults/m^2 at the reference site, R-1 (Table 6 and Appendix 3). Density of all fish (adults+juveniles) ranged from 1.9 fish/ m^2 at TH-2 to a high of 15.2 fish/ m^2 at TL-2. The adults, as a percentage of total fish, ranged from 38 to 79%. Densities of benthic riffle fish at TH-1 and TH-2 were significantly lower than densities of fish at other sites (Figure 2) as determined by ANOVA and LSD mean separation tests using log $base_{10}$-transformed densities. Transformed values were used based on results of homogeneity testing (Appendix 2).

Comparison between benthic riffle fish density and Pb concentrations in sediments and Missouri saddled darters is shown in Figure 3. The Pb concentration in sediments and fish tissue was low at the reference locations but was increased at site TH-1 and remained elevated at sites further downstream. Benthic riffle fish density was lowest at sites highest in metals (TH-1 and TH-2). Conversely, riffle fish densities were highest upstream (at R-1) and downstream of TH-1 and TH-2.

Log_{10}-transformed densities of benthic riffle fish were negatively correlated with Pb, Cd, and Zn for surface water data from the crayfish study (Allert et al, 2010) and for sediment data from the mussel study (Roberts et al., 2009) (Table 7). Benthic riffle fish densities were significantly correlated with sediment Cd and Zn from the crayfish study (Allert et al., 2010), but not significantly correlated to their sediment Pb values (Table 7). The general negative correlation between benthic riffle fish densities and surface water metals is graphically presented in Figure 4.

Whole-body concentrations of Pb and Cd in Missouri saddled darters were not significantly correlated with benthic riffle fish density (Table 7 and Figure 5). The benthic riffle fish densities remained similar for Pb concentrations up to 17-18 μg/g ww wet weight but dropped sharply at the highest level of 19.54 μg/g ww. A similar trend was seen with Cd.

Discussion

Mining activity in southeast Missouri is extensive in the Old Lead Belt (Big River drainage and others) and the Viburnum Trend (Black River drainage and others) (Allert et al., 2009 and 2010). Previous studies have reported impacts on benthic riffle fish communities, especially madtoms and sculpins, downstream of mining sites (Wildhaber et al., 2000; Maret and MacCoy, 2002). Recently, Allert et al. (2009) found that sculpins and other benthic fish species in tributaries to the Black River were affected by metal exposure associated with mining activities. Sculpin were rare in our study with a few individuals (banded sculpin, *Cottus carolinae*) caught at sites TH-2, TM-1 and TM-2 (data not shown). Ozark sculpin (*Cottus hypselurus*), were collected in the Black River tributaries (Allert et al. 2009), but none were observed in the Big River. Based on habitat requirements, we expected to see more sculpin, especially banded sculpin. The reason that sculpin were rare in Big River could not be determined in this study. However, it may be due to different habitat requirements (e.g., water temperatures), long-term exposure to mining metals in sediment and water, or other unknown factors.

There was a considerable difference between the benthic riffle fish densities observed at the two reference sites. Adult benthic riffle fish density at site R-1 was highest among all sites at 8.8 fish/m^2,whereas site R-2 had a density (3.9 fish/m^2), lower than most other sites. The LSD mean separation test indicated a significant difference between riffle fish densities at R-1 and R-2 (reference sites) and sites TH-1 and TH-2, however, other post-hoc tests may not detect these same differences. For example, we evaluated Tukey's HSD post-hoc test, which is less likely to detect differences, and found that the densities of benthic riffle fish at site R-1 were significantly higher than the densities at TH-1 and TH-2; however, densities of benthic riffle fish at site R-2 were not significantly greater. Allert et al. (2010) did not see similar differences for crayfish density between these same two reference sites as we observe for the benthic riffle fish. Examination of the water chemistry, depth, flow and substrate did not reveal any reasons for the observed differences in benthic riffle fish density at the reference sites. R-2 is near a highway bridge and is utilized extensively by the public, whereas R-1 is isolated and much less visited. It is not known if this or some other unknown factor is responsible for the observed differences between the reference sites. Given the magnitude of decline in the benthic riffle fish density at TH-1 and TH-2, relative to the most upstream reference site (R-1) and information discussed below on specific toxicological information, we infer that the reduced densities observed at TH-1 and TH-2 are associated with mining-related heavy metals in the area.

Densities of benthic riffle fish were negatively correlated to surface water and sediment concentrations of Pb, Cd, and Zn, with one exception. No significant ($p>0.05$) correlation was observed between benthic fish density and sediment Pb from the crayfish study (Allert et al., 2010). Close examination of the data indicate that the lack of significance was likely related to differences in sediment lead observed at site TM-2. Sediment lead for site TM-2 in the crayfish study was 1100 μg/g (Allert et al., 2010) compared to 257 μg/g in the field mussel study (Roberts et al., 2009). Heterogeneity of samples within a site is not unexpected. A repeated correlation analysis of the combined sediment values from both studies yields a significant correlation for the sediments. This information, along with the significant correlation to surface water metals, supports the conclusion that benthic riffle fish density is associated with the level of these metals in water and sediment. Allert et al. (2009) measured pore water metals in the Black River sites, but did not have sediment or surface water concentrations. They observed significant negative correlations between sculpin density and concentrations of Pb, Cd, and Zn in pore water, but no significant correlation for these metals in pore water and in benthic riffle fish.

Whole-fish concentration of Pb and Cd in Missouri saddled darters did not correlate with benthic riffle fish density. Visual examination of the Missouri saddled darter capture data indicate that density of this species was lowest at the reference sites and highest at sites downstream (data not shown) with no apparent relationship to the whole body fish metals. However, these data do not warrant a conclusion that benthic riffle fish density is not related to whole-body metal concentrations. To adequately test this relationship would require determination of metal concentration in each species at each location which was beyond the resourcing of the project.

The Missouri saddled darter whole-fish data does provide information on how much Pb or Cd may be passed up the foodchain, potentially posing risk to higher trophic levels. Herons are common aquatic birds that feed on small aquatic organisms. For Pb, The no-effect hazard concentration (NEHC) for herons is 45.3 μg Pb/g dry weight (dwt) in food items as derived in Allert et al. (2009). Lead concentrations in Missouri saddled darters, when converted to dwt (based on measured moisture content) yield an average of 66.8 μg/g dwt for sites TH-1 and TH-2. Since 66.8 μg Pb/g fish is greater than the heron NEHC (45.3 μg Pb/g), there is potential risk to herons feeding in this area. This assessment assumes that 100% of the diet is made up of organisms with a similar Pb concentration. Allert et al. (2010) measured Pb concentrations in largescale stonerollers from the same area and found higher concentrations (175 μg/g dwt) than observed for Missouri saddled darter. Therefore, a continuous exposure to high Pb or other metals in this area is possible. Similar concerns were identified for wildlife feeding on crayfish from Big River (Allert et al., 2010)

Collectively these data indicate that riffle fish density is lowest in the areas closest to mining waste sites and with highest concentrations of mining-related metals in the sediment, water and organisms. Specific *in situ* toxicity information was not collected as part of this study. However, laboratory studies with fish exposed to Pb identified reduced growth at a whole-body concentration of 4.0 ppm Pb ww for brook trout (Holcombe et al, 1976) and reduced feeding at a whole-body concentration of 26.2 ppm Pb ww for fathead minnows (Weber et al., 1991) which are similar in range to whole-body Pb concentrations observed for Missouri saddled darter at sites TH-1 and TH-2 (17.73 to 19.57 ppm ww). Similarly, laboratory studies with fish exposed to cadmium identified decreased growth at whole-body concentrations of 0.25 ppm Cd ww for brook trout (Benoit et al. 1976) and 0.48 ppm Cd ww for Atlantic salmon (Peterson et al., 1983) which were in the range of whole-body Cd concentrations at sites TH-1 and TH-2 (0.38 to 0.5 ppm ww). The sediment levels of lead at TH-1 and TH-2 (680 to 2420 ppm dw) are well above 128 ppm dw which is the consensus probable effects concentration for Pb in sediment-dwelling organisms (MacDonald et al., 2000). Water concentrations of Pb at TH-1 and TH-2 (6.52 to 9.12 ppb) are in a range similar to the U.S. EPA's chronic water quality criteria for Pb of 5 to 9 ppb (USEPA, 2006). These observations provide a weight of evidence that that the low densities of benthic riffle fish at TH-1 and TH-2 are likely due to toxicological effects of mining metals in environmental media.

Conclusions

- Benthic riffle fish density is negatively correlated with concentration of Pb, Cd, and Zn in the surface water and sediments. These data support the hypothesis that biological effects associated with mining metals occur in the area nearest the mine waste areas and dissipate at distances downstream of this area.
- Whole-body concentrations of Pb and Cd in Missouri Saddled darters is not correlated to benthic riffle fish density or with density of Missouri saddled darters. However, more whole-body concentration data are needed for other species to conclude that benthic fish density is not related to body burdens.
- Whole-body concentrations of Pb and Cd of Missouri saddled darters near the mining waste sites may pose a risk to riffle fish consumers, such as herons, that feed on these or similar species.

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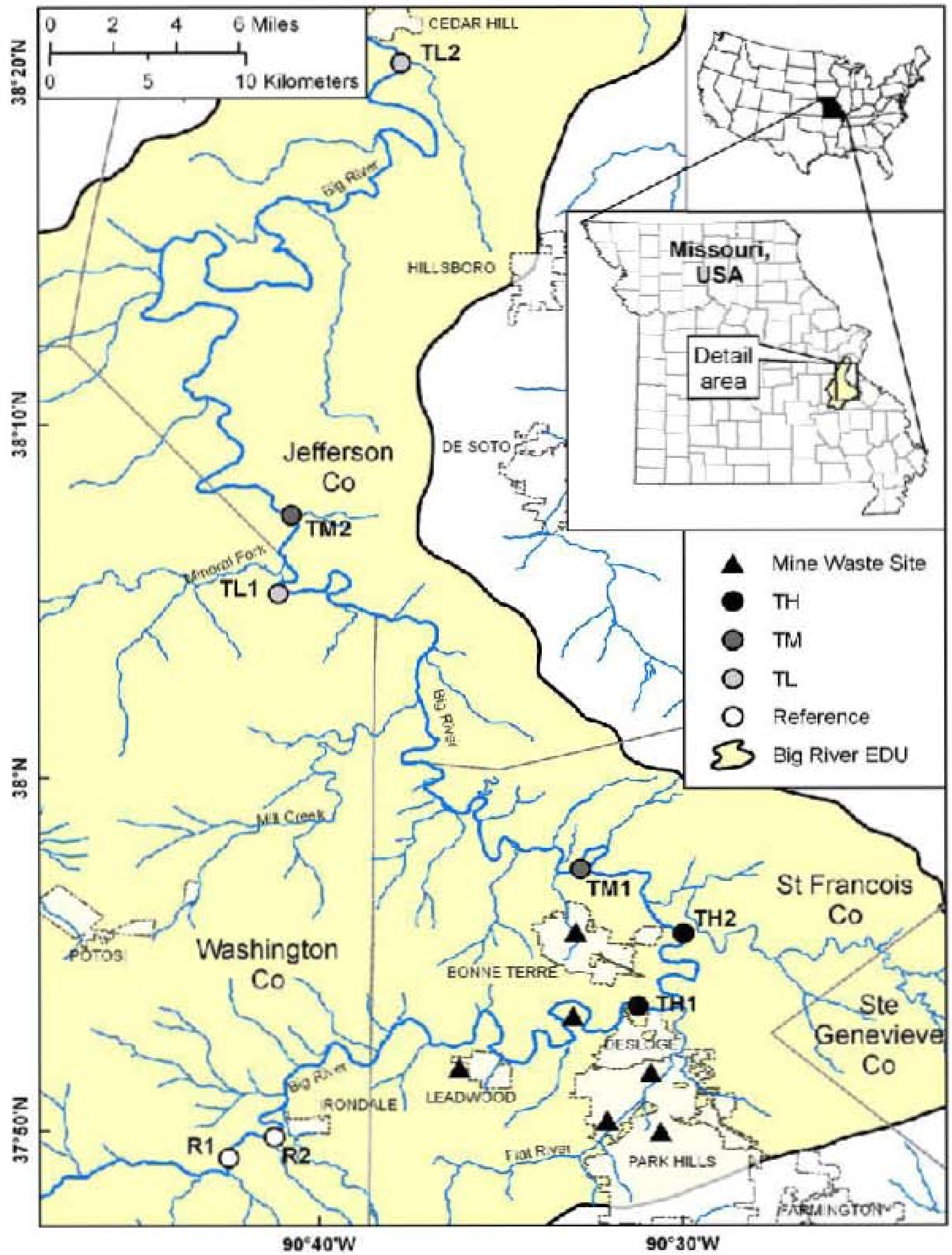

Figure 1. Map of riffle fish density sampling sites in the Big River, Missouri.

Table 1. Site locations for the benthic riffle fish density study in the Big River, Missouri.

Site	Type of site	Site name	Data Sampled	Approximate km (mi) from Desloge	North Latitude (m)	West Longitude (m)
R1	Reference	Irondale	8/25/2008	-34 (-21)	49.207	42.460
R2	Reference	Hwy U	8/22/2008	-31 (-15)	49.812	41.222
TH1	Mining	Desloge	9/10/2008	5 (3)	53.421	30.602
TH2	Mining	Hwy K	9/11-12/2008	10 (6)	55.757	30.398
TM1	Downstream	Cherokee Landing	8/26/2008	23 (14)	57.393	32.781
TL1	Downstream	Washington State Park	8/27/2008	55 (33)	5.524	40.920
TM2	Downstream	Mammoth Access	8/14/2008	61 (38)	7.427	40.720
TL2	Downstream	Cedar Hill	8/18/2008	119 (74)	20.715	38.058

Table 2. Quadrat water chemistry and flow data for the benthic riffle fish density study in the Big River, Missouri.

						Mean (n=5)	
		pH	Conductivity (umhos/cm)	Dissolved oxygen (ppm)	Temperature (C)	Depth (m)	Velocity (m/sec)
Site	Rep						
R-1	1	*	280.00	8.20	25.80	0.14	0.56
	2	*	260.00	8.60	27.00	0.26	0.36
	3	*	200.00	8.30	26.20	0.23	0.25
R-2	1	*	370.00	7.80	25.30	0.25	0.51
	2	*	380.00	7.20	24.70	0.28	0.51
	3	*	388.00	7.20	24.40	0.11	0.31
TH-1	1	8.90	406.00	8.50	20.30	0.27	0.49
	2	7.90	405.00	8.90	21.10	0.24	0.39
	3	8.00	405.00	9.50	21.70	0.25	0.65
TH-2	1	8.70	690.00	9.50	26.70	0.08	0.07
	2	9.10	740.00	9.90	25.60	0.20	0.75
	3	9.00	800.00	8.50	23.60	0.14	0.36
TM-1	1	8.20	666.00	8.30	24.60	0.26	0.78
	2	8.30	671.00	8.70	24.90	0.24	0.78
	3	8.20	667.00	7.40	23.80	0.17	0.52
TM-2	1	8.40	410.00	9.00	25.60	0.17	0.49
	2	8.50	410.00	8.50	24.90	0.21	0.70
	3	8.60	440.00	8.30	24.70	0.17	0.57
TL-1	1	7.80	500.00	9.91	26.40	0.28	0.54
	2	7.70	490.00	8.80	25.30	0.22	0.60
	3	7.90	520.00	8.10	24.60	0.15	0.48
TL-2	1	8.70	390.00	7.50	25.20	0.22	0.45
	2	9.10	425.00	7.50	25.70	0.26	0.48
	3	9.10	370.00	7.90	25.10	0.18	0.50

* pH was not collected at the reference site due to equipment malfunction

Table 3. Lead, cadmium and zinc concentrations in depositional sediments and surface water in the Big River, Missouri.

	Sediment Concentration, (µg/g dry weight (dw))						Surface Water Metals, µg/L		
	USFWS, 2008*			USGS, 2008**			USGS, 2008**		
Site	Lead	Zinc	Cadmium	Lead	Zinc	Cadmium	Lead	Zinc	Cadmium
R-1	15.0	17.0	0.04	7.9	5.7	0.04	0.05	0.62	0.01
R-2	17.0	37.0	0.04	10.5	5.4	0.05	0.06	0.45	0.01
TH-1	1500	1110	20.0	680	510	11.0	6.52	120	1.00
TH-2	2420	740	12.0	1040	350	5.8	9.18	94.8	0.72
TM-1	361	320	8.0	562	287	4.6	3.42	35.3	0.32
TM-2	257	532	3.0	1100	197	3.2	3.04	5.12	0.17
TL-1	559	196	2.4	209	75.5	1.0	3.08	5.46	0.19
TL-2	35.0	17.0	0.04	238	67.4	1.0	3.10	3.88	0.11

*Data from Big River mussel report (See Appendix B, Table 1 in Roberts et al., 2009). All sites are from the same sites used in the riffle fish and crayfish studies except for R-2 and TL-1. For these sites, we selected the nearest mussel study site.

**Sediments metals are simultaneous extractable metals from Big River crayfish report (Allert et al., 2010: Table 24). Pore water metals are from Allert et al. (2010: Table 18).

Table 4. Lead and cadmium whole-body tissue concentrations (μg/g wet weight) in Missouri saddled darter from the Big River, Missouri (n=3 per site*).

Site	Length (cm)		Lead (μg/g wet weight)		Cadmium (μg/g ww)	
	Mean	Std	Mean	Std	Mean	Std
R-1	7.87	0.60	0.17	0.04	0.02	0.00
R-2	6.47	1.22	0.20	0.04	0.07	0.04
TH-1	4.00	0.62	17.73	6.25	0.50	0.11
TH-2	6.67	0.31	19.57	1.17	0.38	0.04
TM-1	6.63	0.15	17.43	2.64	0.37	0.10
TM-2	6.00	0.36	14.23	1.94	0.39	0.02
TL-1	6.60	0.79	15.60	2.35	0.42	0.08
TL-2	6.33	0.35	7.99	1.38	0.23	0.01

*Three replicates per site, but each replicate was a composite of about 10 fish.

Table 5. Species occurrence of benthic riffle fish from Big River, Missouri.

	Site (X indicates presence of species at site)							
Species Name	R-1	R-2	TH-1	TH-2	TM-1	TM-2	TL-1	TL-2
Banded darter, *Etheostoma zonale*	X	X	X	X	X	X	X	X
Fantail darter, , *Etheostoma flabellare*	X	X	X	X	X	X	X	X
Greenside darter, *Etheostoma blennioides*	X	X	X	X	X	X	X	X
Orangethroat darter, *Etheostoma spectabile*	X	X	X	X	X	X	X	X
Rainbow darter, *Etheostoma caeruleum*	X	X		X	X	X	X	X
Missouri saddled darter, *Etheostoma tetrazonum*	X	X	X	X	X	X	X	X
Gilt darter, *Percina evides*			X	X	X	X	X	X
Banded sculpin, *Cottus carolinae*				X	X	X		
Stonecat, *Noturus flavus*					X			X
Slender madtom, *Noturus exilis*	X	X	X	X	X	X		
Total occurrence per site	7	7	7	9	10	9	7	8

Table 6. Density of adult benthic riffle fishes and all benthic riffle fishes (juveniles and adults) in the Big River, Missouri.

	Density of Adult Benthic Riffle Fishes (fish/m^2)			Density of All Benthic Riffle Fishes (fish/ m^2)			
Site	n	Mean	Standard Deviation	n	Mean	Standard Deviation	Percent Adults
R-1	3	8.8	1.8	3	11.8	1.2	74
R-2	3	3.9	2.1	3	6.4	3.6	61
TH-1	3	1.3	0.9	3	2.6	1.8	71
TH-2	3	1.2	1.2	3	1.9	1.6	64
TM-1	3	5.1	0.9	3	6.4	1.0	79
TM-2	3	8.5	8.6	3	14.0	9.4	60
TL-1	3	6.6	1.8	3	10.2	2.0	65
TL-2	3	5.7	0.9	3	15.2	4.3	38

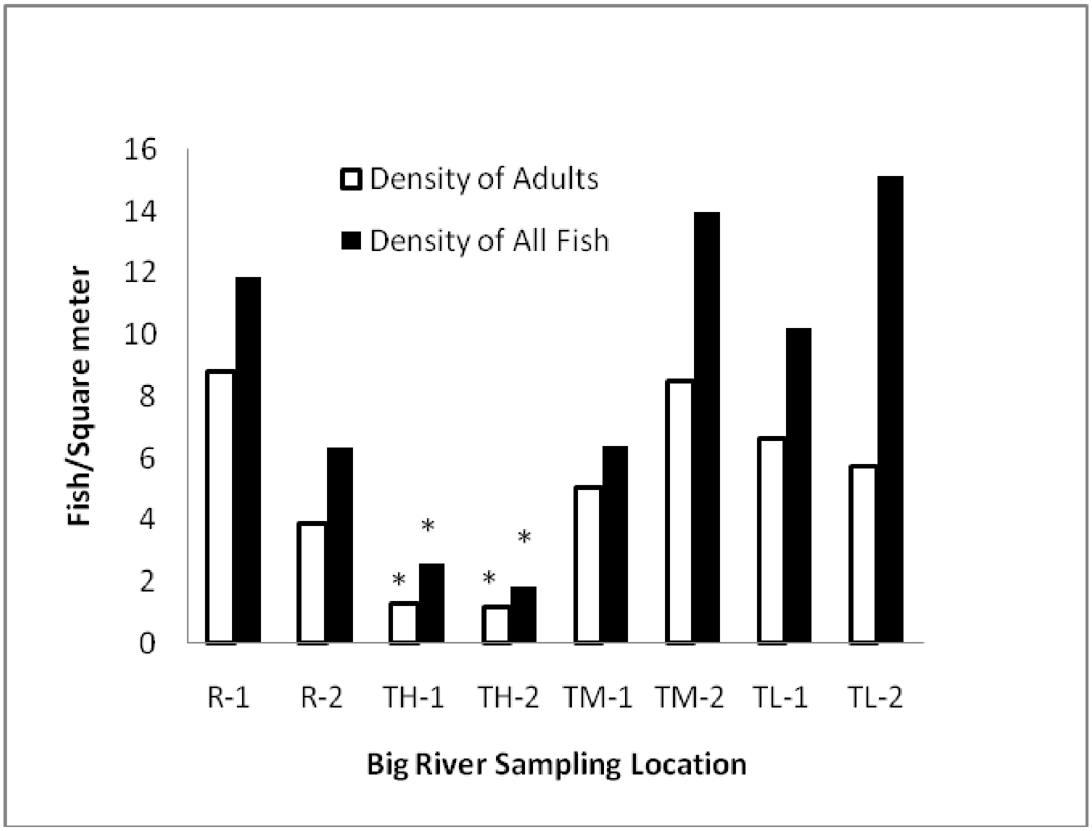

Figure 2. Density (fish/m^2) for benthic riffle fish collected at sites on the Big River. Asterisk (*) denotes significant difference from other means using the least significant difference multiple means comparison.

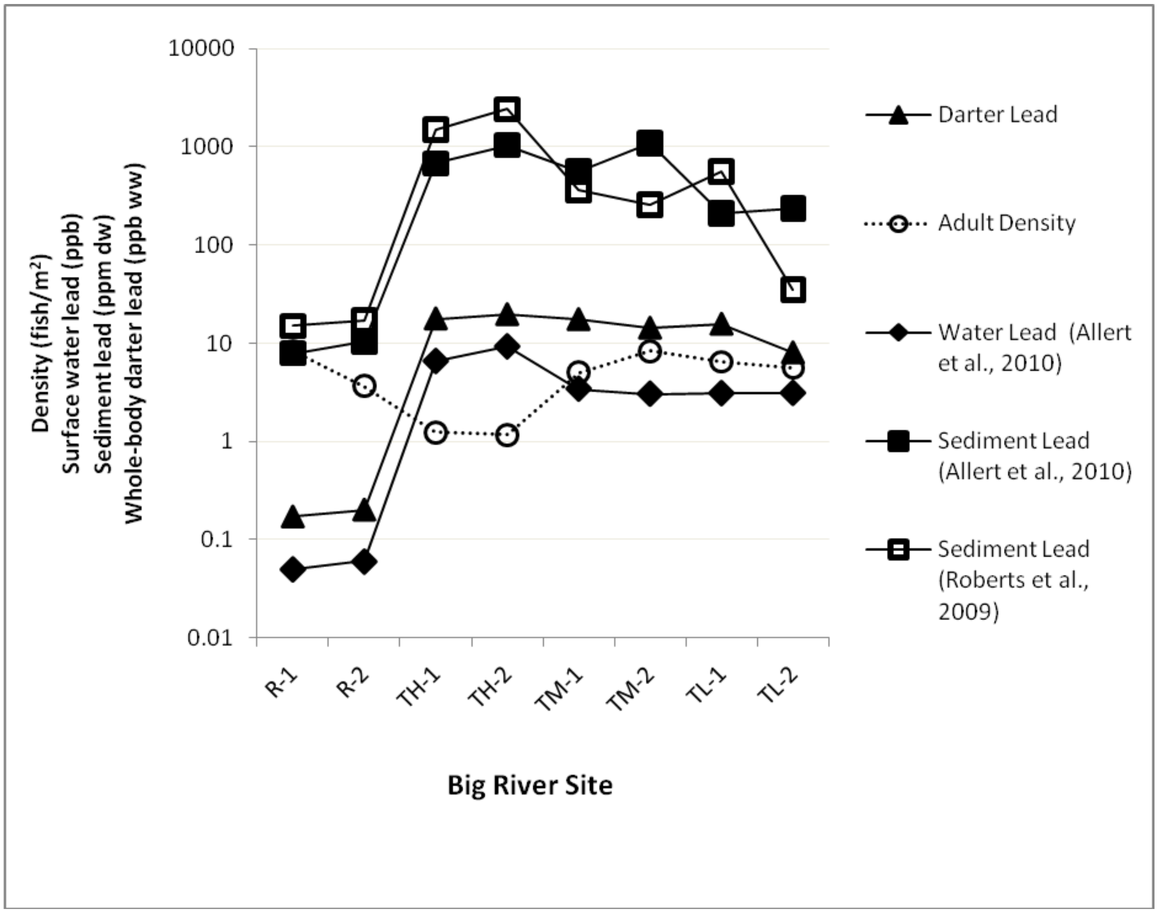

Figure 3. Comparisons of adult benthic fish density and lead concentrations in sediment, surface water and whole-body Missouri saddled darter at sampling sites in the Big River, Missouri.

Table 7. Pearson's corrrelation coefficients for benthic riffle fish density compared to metal concentrations in sediment and Missouri saddled darters.

Metal	Matrix*	Pearson Correlation Coefficient**	
		Log_{10}-transformed Adult Density	Log_{10}-transformed Density of All Fish
Lead	Mo. Saddled Darter (whole body)	-0.51 0.201	-0.49 0.21
	Sediment (Big River mussel study)	**-0.90** **0.003**	**-0.90** **0.002**
	Sediment (Big River crayfish study)	-0.51 0.191	-0.46 0.256
	Surface water (Big River crayfish study	**-0.82** **0.012**	**-0.77** **0.024**
Cadmium	Mo. Saddled Darter (whole body)	-0.46 0.249	-0.40 0.324
	Sediment (Big River mussel study)	**-0.83** **0.012**	**-0.81** **0.015**
	Sediment (Big River crayfish study)	**-0.78** **0.023**	**-0.73** **0.031**
	Surface water (Big River crayfish study	**-0.88** **0.004**	**-0.84** **0.009**
Zinc	Mo. Saddled Darter (whole body)	NA	NA
	Sediment (Big River mussel study)	**-0.80** **0.017**	**-0.75** **0.033**
	Sediment (Big River crayfish study)	**-0.77** **0.024**	**-0.75** **0.032**
	Surface water (Big River crayfish study	**-0.92** **0.001**	**-0.90** **0.003**

*Big River mussel study refers to sediment data from Roberts et al. (2009); Big River crayfish study refers to sediment data from Allert *et al.* (2009).

**In each cell the top number is the Pearson Correlation Coefficient and the lower number is the p-value. Bold cells are significant at $p<0.05$.

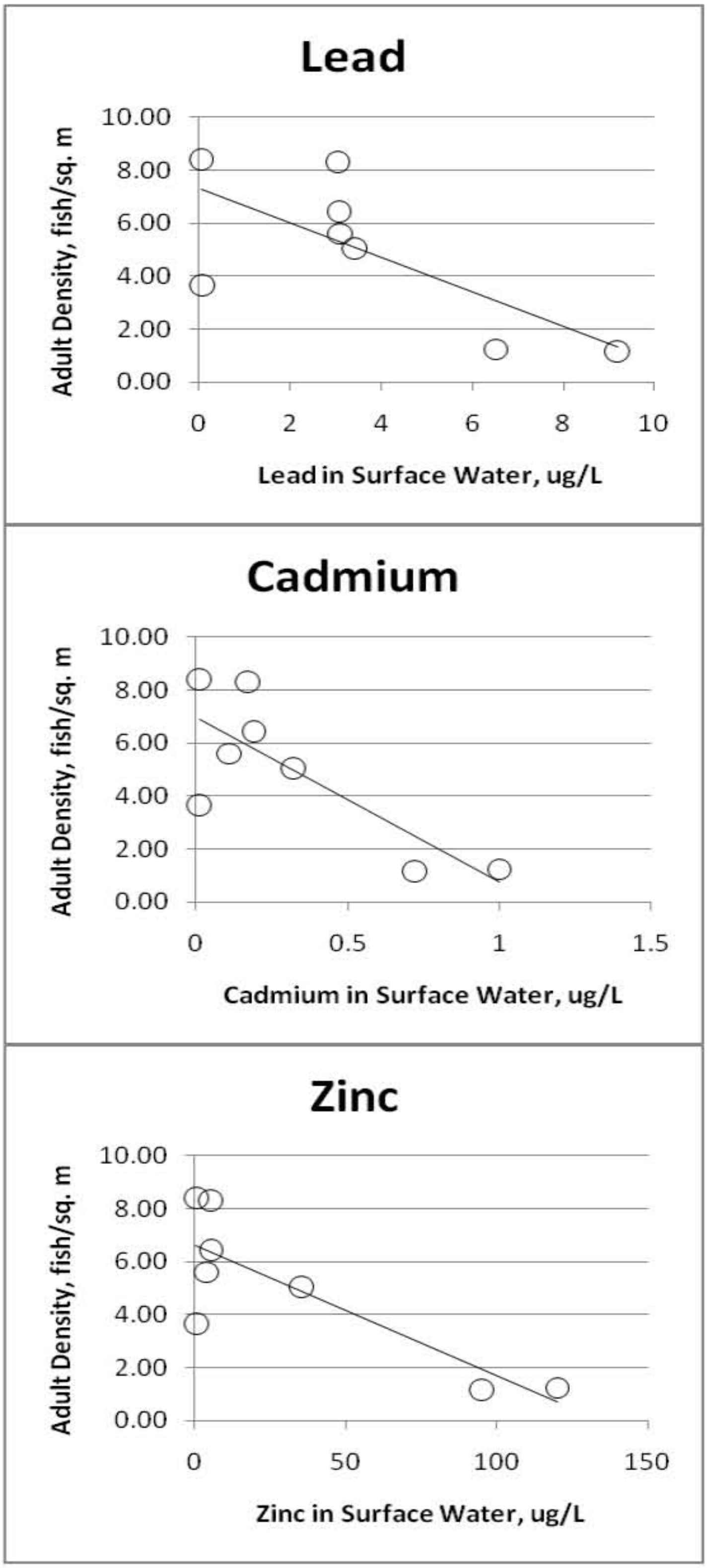

Figure 4. Scatter plot for average density of adult riffle fish versus surface water metal concentrations from Allert et al. (2010).

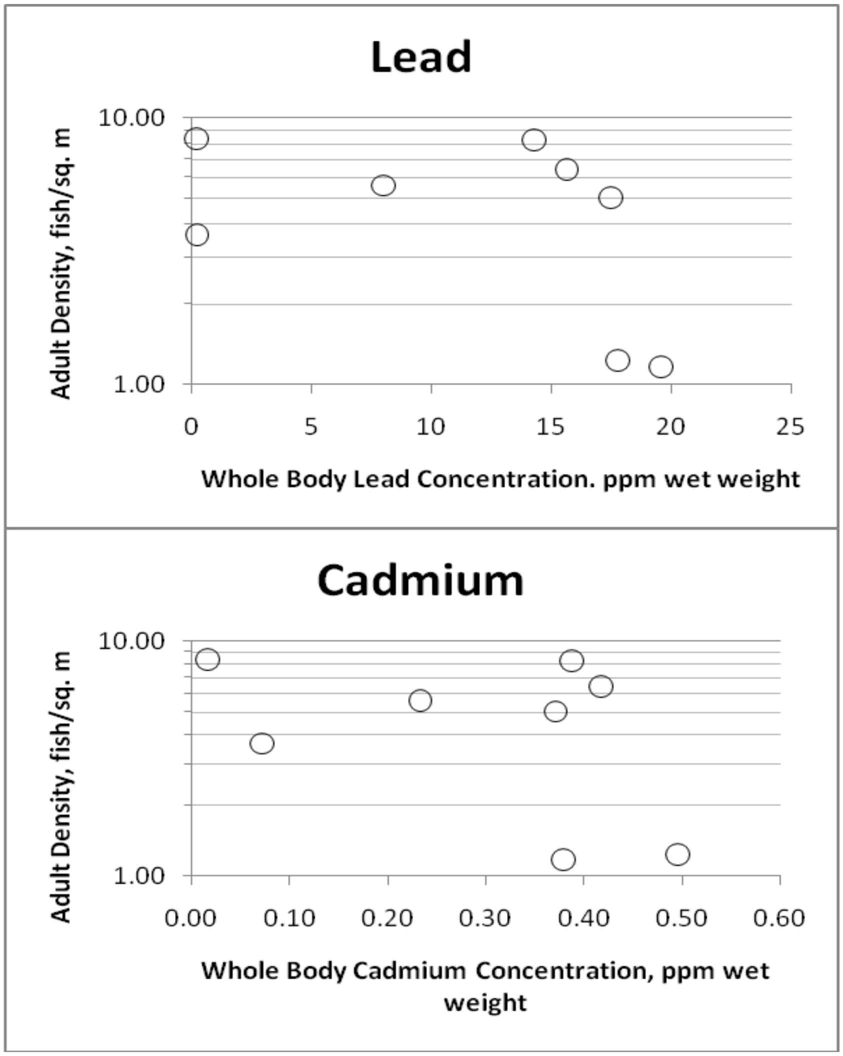

Figure 5. Scatter plot for average density of adult riffle fish versus whole-body concentrations of lead and cadmium in Missouri saddled darter.

Appendix 1

Total length thresholds between juvenile and adults lifestages of fish observed in the benthic riffle fish study*

Fish species	Adult threshold total length
Orangethroat darter, *Etheostoma spectabile*	28 mm
Rainbow darter, *Etheostoma caeruleum*	28 mm
Greenside darter, *Etheostoma blennioides*	64 mm
Fantail darter, , *Etheostoma flabellare*	33 mm
Banded darter, *Etheostoma zonale*	36 mm
Gilt darter, *Percina evides*	58 mm
Missouri saddled darter, *Etheostoma tetrazonum*	51 mm
Slender madtom, *Noturus exilis*	76 mm
Stonecat, *Noturus flavus*	76 mm
Banded darter, *Etheostoma zonale*	Not specified

*Information from Danny Brown, electronic communication, 9/17/08. Data based on Pflieger (1997) and other MDC expert opinions.

Appendix 2

Water Chemistry and Riffle Characteristics for Quadrats Sampled in Benthic Riffle Fish Study in the Big River, Missouri.

Water Chemistry and Riffle Characteristics for Quadrats Sampled in Benthic Riffle Fish Study in the Big River, Missouri.

Site	Replicate	Riffle #	Quadrat #	Date Sampled	pH	Conductivity (umhos/cm)	Dissolved oxygen (ppm)	Temperature (Centigrade)	Time	Width (m)	Length (m)	Sample point within riffle	Depth (m)	Velocity (m/sec)	Substrate*
R-1	1	R1	Q1	8/25/08	.	280	8.2	25.8	1140	4	8	Upper left	0.16	0.80	C
												Upper right	0.10	0.38	C
												Center	0.16	0.64	B
												Lower left	0.18	0.51	B
												Lower right	0.12	0.46	C
R-1	2	R2	Q3	8/25/08	.	260	8.6	27	1315	3	8	Upper left	0.26	0.30	C
												Upper right	0.30	0.46	B
												Center	0.28	0.77	B
												Lower left	0.23	0.28	C
												Lower right	0.24	0.01	B
R-1	3	R3	Q4	8/25/08	.	200	8.3	26.2	1445	4	8	Upper left	0.20	0.07	B
												Upper right	0.30	0.46	B
												Center	0.23	0.20	B
												Lower left	0.19	0.40	C
												Lower right	0.23	0.10	C
R-2	1	R3	Q7	8/22/08	.	370	7.8	25.3	1316	4	8	Upper left	0.16	0.67	B
												Upper right	0.28	0.80	C
												Center	0.20	0.80	B
												Lower left	0.30	0.08	C
												Lower right	0.30	0.21	C
R-2	2	R2	Q5	8/22/08	.	380	7.2	24.7	1202	4	8	Upper left	0.26	0.42	B
												Upper right	0.23	0.38	B/C
												Center	0.30	0.58	B/C
												Lower left	0.45	0.54	C
												Lower right	0.15	0.63	C
R-2	3	R1	Q2	8/22/08	.	388	7.2	24.4	1043	4	8	Upper left	0.09	0.26	C
												Upper right	0.10	0.24	BR
												Center	0.20	0.38	C
												Lower left	0.08	0.32	G
												Lower right	0.09	0.34	BR
TH-1	1	R3	Q9	9/10/08	8.9	406	8.5	20.3	1203	3	8	Upper left	0.30	0.86	G**
												Upper right	0.21	0.22	S**
												Center	0.30	0.53	G**
												Lower left	0.32	0.62	G**
												Lower right	0.21	0.20	S**
TH-1	2	R1	Q1	9/10/08	7.9	405	8.9	21.1	1336	5	5	Upper left	0.18	0.53	G**
												Upper right	0.30	0.23	S**
												Center	0.20	0.46	G**
												Lower left	0.22	0.24	G**
												Lower right	0.30	0.50	S**
TH-1	3	R1	Q2	9/10/08	8	405	9.5	21.7	Not Recorded	4	8	Upper left	0.30	0.38	NR
												Upper right	0.30	0.43	NR
												Center	0.27	0.80	NR
												Lower left	0.20	0.82	NR
												Lower right	0.19	0.80	NR

TL-1	1	R2	Q11	8/27/08	7.8	500	9.91	26.4	1410	3	8	Upper left	0.29	0.65	B/C
												Upper right	0.26	0.44	B/C
												Center	0.27	0.68	B/C
												Lower left	0.30	0.55	B/C
												Lower right	0.28	0.37	B/C
TL-1	2	R1	Q4	8/27/08	7.7	490	8.8	25.3	1250	3	8	Upper left	0.13	0.30	G/S
												Upper right	0.30	0.79	G
												Center	0.26	0.80	G
												Lower left	0.12	0.32	G/S
												Lower right	0.29	0.80	G
TL-1	3	R1	Q9	8/27/2008	7.9	520	8.1	24.6	1130	4	8	Upper left	0.11	0.24	G/S
												Upper right	0.21	0.64	G/S
												Center	0.12	0.52	G/S
												Lower left	0.13	0.51	G/S
												Lower right	0.17	0.47	G/S
TL-2	1	R3	Q19	8/18/08	8.7	390	7.5	25.2	1300	3	8	Upper left	0.30	0.58	G
												Upper right	0.10	0.28	G
												Center	0.28	0.56	C
												Lower left	0.30	0.61	C
												Lower right	0.12	0.20	G
TL-2	2	R3	Q18	8/18/08	9.1	425	7.5	25.7	1409	3	8	Upper left	0.27	0.42	C/G
												Upper right	0.24	0.33	G
												Center	0.24	0.86	G
												Lower left	0.28	0.33	G
												Lower right	0.26	0.47	G
TL-2	3	R2	Q15	8/18/08	9.1	370	7.9	25.1	1123	3	10	Upper left	0.08	0.02	C/G
												Upper right	0.25	0.51	C/G
												Center	0.20	0.53	C/G
												Lower left	0.08	0.53	G
												Lower right	0.30	0.90	C/G
TM-1	1	R2	Q3	8/26/08	8.2	666	8.3	24.6	1220	3	8	Upper left	0.21	0.60	G
												Upper right	0.24	0.80	G/S
												Center	0.23	0.78	G
												Lower left	0.17	0.61	G
												Lower right	0.46	1.10	G
TM-1	2	R3	Q4	8/26/08	8.3	671	8.7	24.9	1328	3	8	Upper left	0.10	0.80	NR
												Upper right	0.29	0.72	NR
												Center	0.30	0.80	NR
												Lower left	0.22	0.78	NR
												Lower right	0.30	0.80	NR
TM-1	3	R1	Q1	8/26/08	8.2	667	7.4	23.8	1058	4***	8***	Upper left	0.06	0.30	G/S
												Upper right	0.28	0.65	G/S
												Center	0.16	0.53	G/S
												Lower left	0.13	0.45	G
												Lower right	0.20	0.65	G
TM-2	1	R2	Q1	8/14/08	8.4	410	9	25.6	1510	4	6	Upper left	0.20	0.54	C
												Upper right	0.18	0.33	C
												Center	0.18	0.63	C
												Lower left	0.20	0.60	C
												Lower right	0.10	0.37	C

TM-2	2	R1	Q4	8/14/08	8.5	410	8.5	24.9	1330	4	8	Upper left	0.21	0.47	G
												Upper right	0.28	0.87	G
												Center	0.19	0.78	G
												Lower left	0.22	0.71	G
												Lower right	0.14	0.67	G
TM-2	3	R1	Q8	8/14/08	8.6	440	8.3	24.7	1240	3	10	Upper left	0.21	0.78	G
												Upper right	0.26	0.72	G
												Center	0.12	0.50	G
												Lower left	0.14	0.40	G
												Lower right	0.11	0.46	G
TH-2	1	R2	Q10	8/11/08	8.7	690	9.5	26.7	1450	4***	6***	Upper left	0.08	0.04	G
												Upper right	0.10	0.03	G
												Center	0.06	0.17	G
												Lower left	0.08	0.09	G
												Lower right	0.06	0.02	G
TH-2	2	R1	Q4	8/11/08	9.1	740	9.9	25.6	1320	4	8	Upper left	0.18	0.72	G
												Upper right	0.23	1.02	G
												Center	0.20	0.96	G
												Lower left	0.12	0.27	G
												Lower right	0.27	0.80	G
TH-2	3	R1	Q3	8/12/08	9	800	8.5	23.6	1000	4	8	Upper left	0.06	0.18	G
												Upper right	0.24	0.45	G
												Center	0.11	0.48	G
												Lower left	0.06	0.01	G
												Lower right	0.23	0.70	G

* S=sand (<2 mm); G=gravel (2-16 mm); P=pebble (17-64 mm); C=cobble (65-250 mm); B=boulder (>250 mm) and BR=bedrock (Allert et al., 2009)

** These samples also were noted to contain chat from mining operations

*** These quadrats were irregular in shape. The final size of the quadrat is the product of these two numbers.

Appendix 3

Benthic Riffle Fish Density Data

Site	Replicate	Area (sq. m)	Total Number of Adult Fish	Density (Adult Fish/sq. m)	Total Number of Fish (all sizes)	Density of All Fish (All Fish /sq. m)
R-1	1	32.0	349.20	10.91	422.85	13.21
R-1	2	24.0	187.17	7.80	270.48	11.27
R-1	3	32.0	246.14	7.69	353.59	11.05
R-2	1	32.0	119.78	3.74	201.87	6.31
R-2	2	32.0	191.91	6.00	318.48	9.95
R-2	3	32.0	60.99	1.91	90.11	2.82
TL-1	1	24.0	174.00	7.25	300.94	12.54
TL-1	2	32.0	146.53	4.58	299.22	9.35
TL-1	3	24.0	191.92	8.00	211.33	8.81
TL-2	1	30.0	157.04	5.23	336.81	11.23
TL-2	2	24.0	122.59	5.11	347.74	14.49
TL-2	3	24.0	162.24	6.76	473.69	19.74
TM-1	1	32.0	129.26	4.04	185.36	5.79
TM-1	2	24.0	125.28	5.22	140.47	5.85
TM-1	3	24.0	141.46	5.89	180.90	7.54
TM-2	1	32.0	134.66	4.21	325.90	10.18
TM-2	2	30.0	87.33	2.91	214.12	7.14
TM-2	3	32.0	586.65	18.33	789.42	24.67
TH-1	1	25.0	13.00	0.52	33.00	1.32
TH-1	2	32.0	30.00	0.94	58.00	1.81
TH-1	3	24.0	55.00	2.29	110.00	4.58
TH-2	1	32.0	14.00	0.44	25.00	0.78
TH-2	2	32.0	81.23	2.54	116.05	3.63
TH-2	3	25.4	14.00	0.55	29.00	1.14

Appendix 4

SAS Output for ANOVA, Levene homogeneity test and Least Significant Difference mean separation test

Levene Test for Homogeneity:

Density of Adults Benthic Riffle Fish (non-transformed)

Levene's Test for Homogeneity of DenAdt Variance ANOVA of Squared Deviations from Group Means					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Site	7	5908.4	844.1	3.75	0.0135
Error	16	3598.4	224.9		

Density of Adults Benthic Riffle Fish (log transformed)

Levene's Test for Homogeneity of LogDenAdt Variance ANOVA of Squared Deviations from Group Means					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Site	7	0.0514	0.00734	2.29	0.0806
Error	16	0.0513	0.00321		

Density of All Benthic Riffle Fish (non-transformed)

Levene's Test for Homogeneity of DenAll Variance ANOVA of Squared Deviations from Group Means					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Site	7	8126.7	1161.0	3.38	0.0206
Error	16	5490.6	343.2		

Density of All Benthic Riffle Fish (log transformed)

Levene's Test for Homogeneity of LogDenAll Variance ANOVA of Squared Deviations from Group Means					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Site	7	0.0197	0.00281	2.05	0.1114
Error	16	0.0220	0.00137		

The SAS System

The ANOVA Procedure

Dependent Variable: Log Transformation of Adult Density

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	3.01563798	0.43080543	6.22	0.0012
Error	16	1.10862783	0.06928924		
Corrected Total	23	4.12426582			

R-Square	Coeff Var	Root MSE	LogDenAdt Mean
0.731194	47.11132	0.263228	0.558737

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Site	7	3.01563798	0.43080543	6.22	0.0012

The SAS System

The ANOVA Procedure

Dependent Variable: Log Transformation of Density for All Fish

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2.81384578	0.40197797	8.43	0.0002
Error	16	0.76275436	0.04767215		
Corrected Total	23	3.57660014			

R-Square	Coeff Var	Root MSE	LogDenAll Mean
0.786738	27.29912	0.218340	0.799804

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Site	7	2.81384578	0.40197797	8.43	0.0002

The SAS System

The ANOVA Procedure

t Tests (LSD) for Log transformed Density of Adults

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.069289
Critical Value of t	2.11991
Least Significant Difference	0.4556

Means with the same letter are not significantly different.			
t Grouping	**Mean**	**N**	**Site**
A	0.9387	3	R-1
A			
A	0.8080	3	TL-1
A			
A	0.7838	3	TM-2
A			
A	0.7524	3	TL-2
A			
A	0.6981	3	TM-1
A			
A	0.5438	3	R-2
B	0.0161	3	TH-1
B			
B	-0.0710	3	TH-2

The SAS System

The ANOVA Procedure

t Tests (LSD) for Log transformed Density of All Fish

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	16
Error Mean Square	0.047672
Critical Value of t	2.11991
Least Significant Difference	0.3779

Means with the same letter are not significantly different.				
t Grouping		**Mean**	**N**	**Site**
	A	1.1689	3	TL-2
	A			
B	A	1.0845	3	TM-2
B	A			
B	A	1.0721	3	R-1
B	A			
B	A	1.0046	3	TL-1
B	A			
B	A	0.8025	3	TM-1
B				
B		0.7491	3	R-2
	C	0.3467	3	TH-1
	C			
	C	0.1700	3	TH-2