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# Effects of historical lead–zinc mining on riffle-dwelling benthic fish and crayfish in the Big River of southeastern Missouri, USA

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**Abstract** The Big River (BGR) drains much of the Old Lead Belt mining district (OLB) in southeastern Missouri, USA, which was historically among the largest producers of lead–zinc (Pb–Zn) ore in the world. We sampled benthic fish and crayfish in riffle habitats at eight sites in the BGR and conducted 56-day in situ exposures to the woodland crayfish (*Orconectes hylas*) and golden crayfish (*Orconectes luteus*) in cages at four sites affected to differing degrees by mining. Densities of fish and crayfish, physical habitat and water quality, and the survival and growth of caged crayfish were examined at sites with no known upstream mining activities (i.e., reference sites) and at sites downstream of mining areas (i.e., mining and downstream sites). Lead, zinc, and cadmium were analyzed in surface and pore water, sediment, detritus, fish, crayfish, and other benthic macro-invertebrates. Metals concentrations in all materials analyzed were greater at mining and downstream sites than at reference sites. Ten species of fish and four species of crayfish were collected. Fish and crayfish densities were significantly greater at reference than mining or downstream sites, and densities were greater at downstream than mining sites. Survival of caged crayfish was significantly lower at mining sites than reference sites; downstream sites were not tested. Chronic toxic-unit scores and sediment probable effects quotients indicated significant risk of toxicity to fish and crayfish, and metals concentrations in crayfish were sufficiently high to represent a risk to wildlife at mining and downstream sites. Collectively, the results provided direct evidence that metals associated with historical mining activities in the OLB continue to affect aquatic life in the BGR.

**Keywords** Lead–zinc mining · Benthic fish · Crayfish · *Orconectes hylas* · *Orconectes luteus* · In situ toxicity

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## Introduction

Lead–zinc (Pb–Zn) mining in Missouri has occurred since the 1700s. In the 1800s, deep-shaft mining and improved beneficiation methods facilitated increased exploration and mining of Pb–Zn ore in the Old Lead Belt mining district (OLB; Table 1) in southeastern Missouri. Mining in the OLB preceded environmental regulation and utilized comparatively inefficient extraction technologies. Extensive deposits of mine tailings are located throughout the OLB and represent substantial and continuing sources of metals to the Big River (BGR) and its tributary, Flat River (Fig. 1). The tailings pile located upstream of Desloge is situated along the river and represents a substantial source, as do those located near Leadwood and along Flat River (Fig. 1). Consequently, although mining ceased in the 1970s, contamination of lands, surface water, and ground water is extensive, and portions of the OLB have been designated as U.S. Environmental Protection Agency Superfund sites. Elevated concentrations of metals in surface water, sediment, fish, and benthic macro-invertebrates

**Table 1** List of abbreviations

| Variable abbreviations | Definition | Application | Reference |
|---|---|---|---|
| OLB | Old Lead Belt | Lead mining district where study occurs | – |
| BGR | Big River | River where study occurs; tailings within watershed have contaminated surface water, ground water, and lands | – |
| R | Reference site | Sites without upstream mining activity (R1, R2) | – |
| TH | Mining site | Sites downstream from mines and with large amounts of tailings and high metals concentrations (TH1, TH2) | – |
| TM | Downstream site | Sites downstream from mines and with intermediate amounts of tailings and metals concentrations (TM1, TM2) | – |
| TL | Downstream site | Sites downstream from mines and with low amounts of tailings and metals concentrations (TL1, TL2) | – |
| TR | Total recoverable metals (Pb, Zn, Cd) in sediment | Metals concentrations as determined by inductively-coupled plasma-mass spectrometry | – |
| ICP-MS | Inductively-coupled plasma-mass spectrometry | Method used to analyze environmental samples | Brumbaugh et al. (2007), May et al. (1997) |
| PEC | Probable effects concentration | Represents the concentration above which adverse effects on survival or growth are expected to occur | MacDonald et al. (2000) |
| PEQ | Probable effects quotients | PEQ = PEC/TR for each metal, with PEQs of 1.0 or greater associated with increased probability of toxic effects | Ingersoll et al. (2001), Besser et al. (2009a) |
| $\sum$PEQs | Sum of individual PEQs | Individual PEQs for each metal (Pb, Cd, Zn) summed to estimate risks from metal mixtures | Ingersoll et al. (2001) Besser et al. (2009a) |
| TU | Toxic unit | Measured concentration of metal divided by its chronic water-quality criterion, adjusted for hardness and the dissolved fraction of the metal | Wildhaber and Schmitt (1996), United States Environmental Protection Agency (2006) |
| $\sum$TUs | Sum of individual TUs | Toxic units normalize differences in toxicity among metals (Pb, Cd, Zn) so they can be summed to estimate cumulative risks from metal mixtures, with sums less than 1.0 predicted to be non-toxic | Wildhaber and Schmitt (1996) |
| HQs | Hazard quotients | The daily contaminant intake rate divided by the toxicity reference value | Schmitt et al. (2008) |
| $\sum$HQs | Sum of individual HQs | Individual HQs for each metal (Pb, Cd, Zn) summed to estimate risks from metal mixtures. $\sum$HQs that exceed 1.0 indicate risk. All assume a diet of 100 % crayfish. | Schmitt et al. (2008) |

from the BGR and other OLB streams have been documented, and metals concentrations in fish and crayfish have been determined to pose a risk to wildlife and humans (Buchanan 1979; MDHSS 2012; MDNR 2003, 2007; Schmitt et al. 2006 and references cited therein; Schmitt et al. 2007a, b, c, 2008 and references cited therein; Whelan 1983).

Field studies conducted in other mining districts have documented reduced population densities of riffle-dwelling crayfish downstream from Pb–Zn mines (Allert et al. 2008, 2012), and the sensitivity of crayfish to mining-related metals has been documented through both laboratory and in situ toxicity tests (Allert et al. 2009a; J.M. Besser, unpublished USGS data; Knowlton et al. 1983; Wigginton and Birge 2007). Crayfish are also vectors for the transfer of metals to higher trophic levels (Schmitt et al. 2011). In addition to crayfish, previous studies have shown that the riffle-dwelling fish community of Ozark streams is also sensitive to metals from Pb–Zn mining (Allert et al. 2009b; Besser and Rabeni 1987). Crayfish (*Orconectes* spp.) are important components of Ozark stream ecosystems; they are omnivorous primary consumers that often constitute a large percentage of the invertebrate biomass in streams (DiStefano 2005; Hobbs 1993; Momot 1995; Rabeni et al. 1995; Whitledge and Rabeni 1997). Crayfish feed primarily on coarse particulate organic matter augmented with varying proportions of periphyton (i.e., the assemblage of organisms such as bacteria, algae, and small fauna that form on underwater surfaces), aquatic invertebrates, and fish (Parkyn et al. 2001; Stenroth et al. 2006; Whitledge and Rabeni 1997). They process large quantities of organic matter and represent a significant food source for smallmouth bass (*Micropterus dolomieu*), other fishes, and riparian wildlife in the Ozarks (DiStefano 2005; Probst

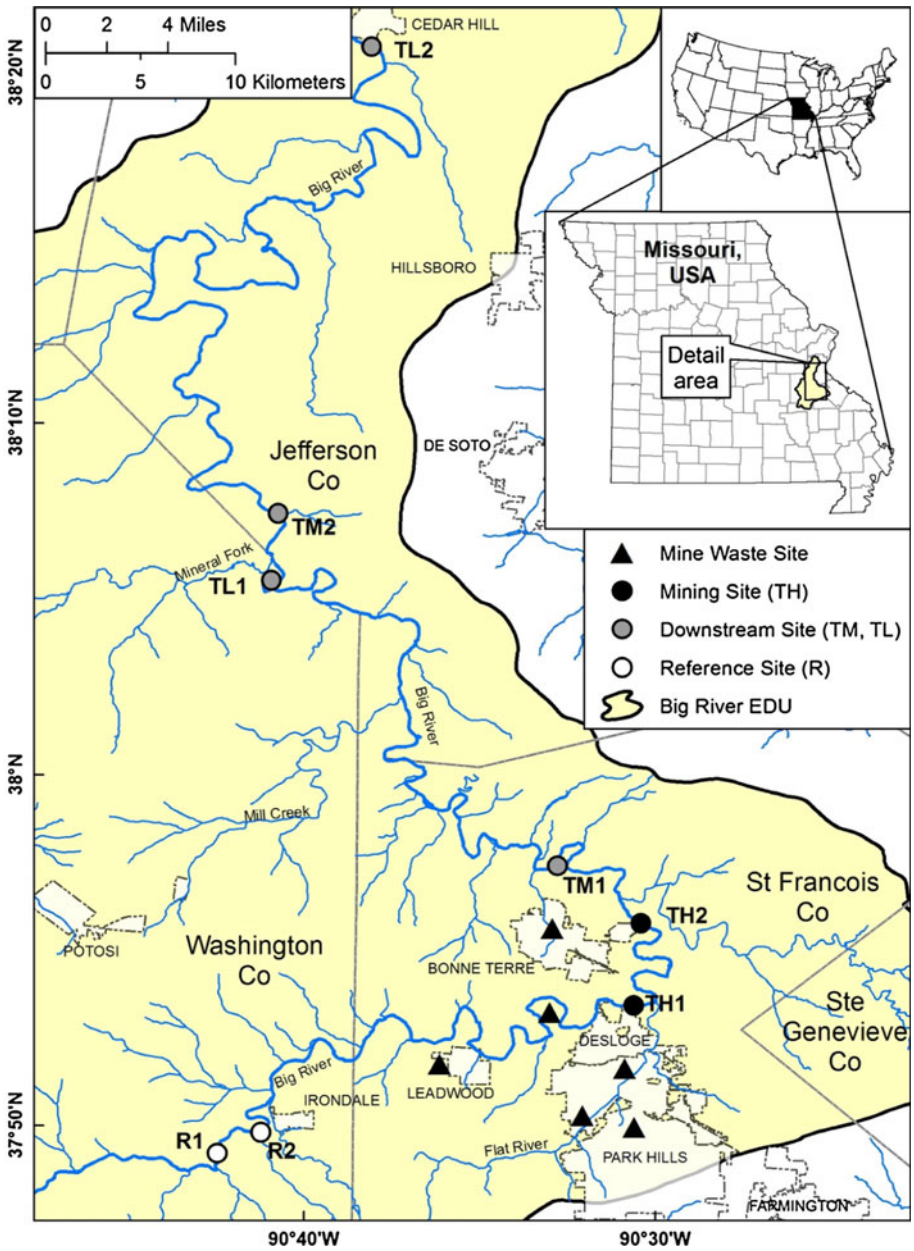


**Fig. 1** Study sites for in situ assessment and toxicity tests, Big River, Missouri, USA. Mine waste sites (i.e., tailings piles) are also shown. *Shaded area* outlines the Big River ecological drainage unit (EDU). See Table 1 for site definitions. Sites: *R*, reference; *M*, mining or *TH*, tailings high; *D*, downstream or *TM*, tailings medium, *TL*, tailings low

et al. 1984; Whitledge and Rabeni1997). Small fish are also important in the diets of smallmouth bass, a species that is popular among recreational anglers in the Ozarks (Mayers 2003; Weithman 1991). Collectively, these characteristics make crayfish and small fish important to the ecology and economies of the Ozarks.

Despite the well documented importance and sensitivity of riffle-dwelling fish and crayfish communities to metals and the equally well documented pollution history of the BGR, we are aware of no previous investigations that have evaluated the effect of mining-derived metals on its riffle-dwelling fish and crayfish communities. The objectives of this study were therefore (1) to evaluate riffle-dwelling benthic fish and crayfish densities in the BGR relative to concentrations of mining-derived metals by conducting a field survey; and (2) to evaluate the effects of metals released to the BGR from historical mining on survival and growth of juvenile crayfish as determined by in situ toxicity testing.

## Methods

### In situ assessments

Riffles were sampled for fish (August 14–September 12, 2008) and crayfish (July 7–July 17, 2008) at eight sites in the BGR (Fig. 1; Table 2). Sites were classified into three

**Table 2** Sampling locations for in situ assessments and in situ toxicity tests, Big River, Missouri, USA

| Site | Site type | Quantity of tailings | Site description | River km (mi) from Desloge | Latitude (°) | Longitude (°) | Discharge ($m^3$ $s^{-1}$) | Cage location |
|---|---|---|---|---|---|---|---|---|
| R1 | Reference | None | Upstream of Irondale | −34 (−21) | 37.4921 | 90.4246 | 26.8 | Upstream of riffle 3 |
| R2 | Reference | None | Hwy U (Irondale) | −31 (−15) | 37.4981 | 90.4122 | 34.1 | Upstream of riffle 1 |
| TH1 | Mining | High | Hwy 67 (Desloge) | 5 (3) | 37.5342 | 90.3060 | 55.1 | Downstream of riffle 3 |
| TH2 | Mining | High | Hwy K (Bonne Terre) | 10 (6) | 37.5576 | 90.3040 | 80.8 | Downstream of riffle 1 |
| TM1 | Downstream | Medium | Hwy 67 North of Bonne Terre (Cherokee Landing) | 23 (14) | 37.5739 | 90.3278 | 71.1 | Not tested |
| TL1 | Downstream | Low | Washington State Park | 55 (33) | 38.5524 | 90.4092 | 101.5 | Not tested |
| TM2 | Downstream | Medium | Missouri Department of Conservation Mammoth Access | 61 (38) | 38.7427 | 90.4072 | 65.4 | Not tested |
| TL2 | Downstream | Low | Upstream of Cedar Hill Mill Dam | 119 (74) | 38.2072 | 90.3806 | 178.1 | Not tested |

All sites were surveyed for crayfish and benthic riffle fish; toxicity tests were conducted at reference and tailing high sites. Riffle number refers to the closest riffle where quadrat sampling occurred; riffle 1 = most downstream riffle at a site

*R* reference, *M* mining, *TH* tailings high, *D* downstream, *TM* tailings medium, *TL* tailings low

groups based on proximity to mining areas and metals data: upstream reference sites, where no mining activities were reported and previous studies indicated low concentrations of metals (R1 and R2; Fig. 1); mining sites, which were directly downstream (<10 km) from the Desloge tailings pile (i.e., river km; determined by geographic information system; henceforth distance downstream from Desloge), and where metals concentrations were high (TH1 and TH2); and downstream sites, which were further downstream (>10 km) and where metals concentrations were intermediate (TM1, TM2, TL1, and TL2). Each site comprised a reach containing three riffles.

Riffle-dwelling benthic fish (henceforth fish) were sampled once in randomly selected 32-$m^2$ quadrats (targeted dimensions were 4 m × 8 m; maximum water depth = 30 cm; minimum water depth = 6 cm; maximum current velocity 0.8 m $s^{-1}$) at each site using a backpack electrofishing unit. Quadrats were blocked off with seines. Three quadrat samples were randomly located in each site (total $n = 3$ quadrats per site). All fish were identified to species (Pfleiger 1997), enumerated, and measured (total length to the nearest millimeter). Densities of sculpins (*Cottus* spp.), darters (*Etheostoma* spp., *Percina* spp.), and madtoms (*Noturus* spp.) were estimated for each quadrat using the depletion sampling method (Hilborn and Walters 1992). The number of fish in each quadrat was estimated using a closed population removal estimator from the repeated sampling passes (Otis et al. 1978). Density was estimated using the program MARK (White 2008), which estimates parameters from repeated samplings of closed populations. A population estimate, standard error, and confidence interval were computed for each quadrat. The fish density in each quadrat was estimated by dividing the estimated number of fish by the area of the quadrat. Three composite samples of ten Missouri saddled darters (*Etheostoma tetrazonum*) from each site were retained for metals analyses, except for one sample at R1 ($n = 5$); one sample at R2 ($n = 6$); and one sample at TM1 ($n = 12$). All fish except those retained for metals analyses were released alive to the stream.

Riffle-dwelling crayfish (henceforth crayfish) were sampled once at each site by disturbing the area within a 1-$m^2$ quadrat sampler (1 m long × 1 m wide × 1.5 m high) covered with 3-mm stretch delta mesh (DiStefano et al. 2003a; Larson et al. 2008). Seven quadrat samples were randomly located in each riffle (total $n = 21$ per site). Crayfish were identified to species (Pfleiger 1996) and sex and measured [carapace length (CL) to the nearest 0.1 mm (from the tip of rostrum to the posterior edge of the cephalothorax)]. The crayfish density in each quadrat was estimated by dividing the number of golden crayfish (*O. luteus*) in the quadrat by its area. *O. luteus* was used because it was the only species found at all sites and because *Orconectes* spp. have been shown to use habitats differentially (DiStefano et al. 2003b). All crayfish except those retained for metals analyses were released alive to the stream.

## In situ toxicity test

Juvenile woodland crayfish (*Orconectes hylas*) were obtained for the in situ toxicity test from ovigerous females

collected in April 2008 at the Bootleg Access on the BGR (Washington County, MO, USA), upstream of the study area. Additional juvenile *O. hylas* were collected from the Bootleg Access and at R1 in May 2008. Juvenile *O. luteus* were also obtained in May 2008 from the same river locations. Juvenile crayfish were reared at the Columbia Environmental Research Center (CERC) in Columbia, MO, USA in flow-through fiberglass tanks filled with well water (temperature 18 °C, pH 7.7, alkalinity 254 mg $l^{-1}$ as $CaCO_3$, hardness 286 mg $l^{-1}$ as $CaCO_3$) and fed flake food (Ziegler Brothers, Inc., Gardner, PA, USA) ad libitum daily until their body width was >2 mm.

A 56-day in situ toxicity test using juvenile *O. hylas* and *O. luteus* was conducted from July 24 to September 17, 2008 at the reference sites (R1, R2) and mining sites (TH1, TH2; henceforth cage sites; Fig. 1; Table 2) due to a limited number of test organisms. Crayfish were exposed in 0.28-$m^2$ hemicylindrical stainless-steel wire-mesh (2.7-mm diagonal opening) cages containing pebbles and cobbles (25–75 mm particle size) along with approximately 10 g of weathered organic material (henceforth detritus) collected from each cage site as food and shelter for caged crayfish (Allert et al. 2009a). Before stocking the cages, individuals of each species were measured (CL) and weighed [wet weight (ww) to nearest 0.1 g] and three composite samples of ten crayfish were retained for metals analyses. Mean CL of *O. hylas* (9.9 ± 0.13 mm, $n = 125$) was significantly greater than mean CL of *O. luteus* (5.9 ± 0.14 mm, $n = 261$; $F_{(1,814)} = 96.5$; $r^2 = 0.11$; $p < 0.0001$; Tukey's critical value of studentized range = 2.78) at the start of the in situ toxicity test, as was the mean ww of *O. hylas* (0.32 ± 0.01 g, $n = 125$) relative to *O. luteus* (0.09 ± 0.01 g, $n = 260$; $F_{(1,813)} = 104$; $r^2 = 0.11$; $p < 0.0001$; Tukey's critical value of studentized range = 2.78). At the beginning of the test, ten crayfish were placed in cages (six per species) at each site; mean density of crayfish in cages (about 36 $m^{-2}$) was comparable to densities found in riffles of Ozark streams (DiStefano et al. 2003a, b; Rabeni 1985; Riggert et al. 1999). Minced fish (largescale stonerollers, *Campostoma oligolepis*; henceforth stonerollers) caught at each cage site were added weekly to each cage in increasing increments to maintain dietary rations proportional (0.5 % of biomass) to anticipated crayfish biomass. Six cages at each site were sampled on day 28 and day 56 of the test. Sex of surviving crayfish was determined and CL and ww were measured. Surviving crayfish were frozen for metals analyses. A sub-sample of benthic macro-invertebrates collected off the material in the cages and a subsample of detritus at days 28 and 56 were frozen for metals analyses. Test endpoints included survival and growth (CL or ww relative to initial measurements).

## Habitat, water quality, sediment, and biotic assessment

Physical habitat characteristics [water depth (cm), current velocity (m $s^{-1}$), substrate classification, Allert et al. 2012] were measured adjacent to or within quadrat samples in each of the three riffles at all sites using methods of Bain et al. (1985) and Bain and Stevenson (1999). We used a Hydrolab® (Quanta meter; Hach-Hydromet, Loveland, CO, USA) to measure temperature, pH, conductivity, dissolved oxygen (DO), and turbidity in each riffle concurrent with fish and crayfish sampling and weekly adjacent to cages during the in situ toxicity test. A sub-surface grab sample of surface water from each riffle was also collected on the day of crayfish sampling for laboratory determinations of alkalinity, hardness, and sulfate (APHA et al. 2005) at each site and at cage sites on days 0, 28, and 56 of the in situ toxicity test. A subsample of the surface-water samples was collected for metals analyses. Samples for metals analyses were filtered on-site into pre-cleaned polyethylene bottles using a polypropylene syringe and filter cartridge (0.45-μm pore size) and placed on ice. Filtered water samples were subsequently acidified to 1 % (v/v) with nitric acid within four days of collection.

Concentrations of metals in sediment pore water (henceforth pore water) were measured because of the close association of fish and crayfish with sediment. Pore waters were collected at the four cage sites using passive pore-water samplers (peepers) fabricated from 50-ml polypropylene snap-cap vials and dialysis membranes (Brumbaugh et al. 2007). Six peepers were deployed in sediments adjacent to cages on days −1 and 20 of the 56-d in situ toxicity test and removed on days 13 and 33 of the test, respectively. Three peepers were used for water-quality analyses and three were sealed for metals analyses. All samples were placed on ice and refrigerated upon return to the laboratory. The contents of each peeper for metals analyses were acidified to an effective concentration of 0.16 M $HNO_3$ within 6 h of collection.

Composite samples of stream sediments were collected from depositional areas in close proximity to the sampled riffles at each site as part of a companion study (Besser et al. 2009b). Surficial sediments (about the top 10 cm) were collected within the wetted stream channel using PVC scoops and were wet-sieved (2-mm stainless-steel mesh) in the field to remove coarse particles using a minimum quantity of site water (Besser et al. 2009b). Sediments were analyzed to characterize metals concentrations, percent total organic carbon, percent water, and particle size distribution (Besser et al. 2009b).

## Metals analyses

Surface and pore water, sediments, detritus, benthic macro-invertebrates, fish, and crayfish samples were analyzed for

total recoverable (TR) metals (Zn, Cd, and Pb) by inductively-coupled plasma-mass spectrometry (ICP-MS; Brumbaugh et al. 2007; May et al. 1997). Animal tissues, sediment, and organic material were lyophilized and reduced to a coarse powder by mechanical crushing in a glass vial with a glass rod. Neither exoskeletons nor gut contents of any of the biota were removed before analysis to better represent potential risk of metals to predators. A dry mass of 0.25 g from each composited sample was digested using concentrated nitric acid and microwave heating. Moisture loss was determined gravimetrically for all biota samples. Concentrations were reported as $\mu g\ g^{-1}$ dw, but were also converted to ww using moisture content for some comparisons.

Method detection limits for metals analyses are listed in Allert et al. 2010. Of the 51 field-collected surface and pore-water samples analyzed, measured concentrations did not exceed the method detection limits in seven samples for Zn and 17 samples for Cd, all of which were from reference sites. Quality control measures incorporated at the digestion stage included digestion blanks, certified reference materials, replicates, and spikes. A calibration blank and an independent calibration verification standard were analyzed with every ten samples to confirm the calibration status of the ICP-MS during instrumental analyses of digestates. Percent recovery of calibration verification standards ranged from 93 to 97 %. Percent recovery of reference solutions used as laboratory control samples ranged from 76 to 129 %. Average percent recovery of analytical spikes averaged 94 %. Relative percent differences between duplicate analyses of samples were <23 %. As a check for potential interferences, dilution percent differences based on 5× dilutions of the biota sample digestates were determined; dilution percent differences were within the targeted range of 80–100 % except for Zn (129 %). Blank-equivalent concentrations for digestion blanks were less than corresponding method detection limits; therefore sample results were not corrected for blank-equivalent concentrations. Overall, quality assurance results indicated that the methods used provided acceptable accuracy and precision. The analytical results were not corrected for recovery.

## Exposure assessment

Metals concentrations in the sediment were converted to probable effects quotients (PEQs) by dividing TR metals concentrations by probable effects concentrations (PECs; MacDonald et al. 2000) for each metal. Individual PEQs for Pb, Cd, and Zn were summed ($\sum$PEQs) to estimate risks from the metal mixtures (Besser et al. 2009a; Ingersoll et al. 2001). We assumed that individual PEQs and $\sum$PEQs greater than one indicate potential toxicity (Besser et al. 2009a) and that effects are additive; i.e., neither synergistic nor antagonistic effects are accounted for.

The risk of toxic effects from metals in surface and in situ pore water to aquatic organisms was also assessed using a toxic unit (TU) approach (Wildhaber and Schmitt 1996). A TU is defined as the measured concentration of a metal divided by its chronic water-quality criterion (WQC), adjusted for hardness and the dissolved fraction of the metal (United States Environmental Protection Agency 2006). Although the WQC were developed for surface water, they are also reasonable estimates of the potential toxicity of pore waters to aquatic organisms (Wildhaber and Schmitt 1996). Toxic units for Pb, Cd, and Zn were summed to produce a total toxicity estimate for the mixture (i.e., a toxic-unit score, ΣTU) for each sample, with values greater than 1.0 indicating potential toxicity to aquatic biota. This approach also assumes additive effects.

We used the screening-level criteria developed by Schmitt et al. (2006) to evaluate potential adverse effects in wildlife from metals in crayfish. Toxicity thresholds of metals in receptor wildlife species were determined through food-chain analysis using procedures developed for ecological-risk assessment (United States Environmental Protection Agency (1992, 1993, 1997, 1999, 2007). This assessment used representative bird and mammal species based on body weight such as the American robin (*Turdus migratorius*) and short-tailed shrew (*Blarina brevicauda*), as surrogates for species of similar mass that consume crayfish. Hazard quotients (HQs) were calculated using site-mean concentrations of Pb, Zn, and Cd in crayfish and no-effect hazard concentrations (NEHCs) to estimate daily contaminant intake rates; NEHCs are consensus-based no-adverse effect level-based toxic reference values normalized for estimated daily food-ingestion rates (Schmitt et al. 2008). All assume a diet of 100 percent crayfish.

## Statistical analyses

Statistical analyses were conducted with Statistical Analysis System (SAS) for Windows (Release 9.2; SAS Institute, Cary, NC, USA). Censored values (< method detection limit) for metals concentrations in water collected from reference sites were replaced with 50 % of the method detection limit for statistical computations, figures, and tables. Site-means for fish and crayfish densities; physical habitat- and water quality; day-56 survival, CL, and ww of crayfish; and metals concentrations were used in statistical analyses. Prior to analyses, data were tested for normality and homogeneity of variance and found to be non-normally distributed. Data were therefore rank- or $\log_{10}$- transformed prior to analysis. Differences among groups of sites were tested using nested analysis-of-variance (ANOVA; cages nested within site), with site considered a fixed effect. Differences were tested as planned

non-orthogonal contrasts using single degree-of-freedom $F$ tests. The within-site mean squares for ranked variables were used in all tests. Differences in sediment PEQs among site groups were evaluated with Tukey's standard range test due to limited sample size. Associations between fish or crayfish density and metals concentrations were examined with Pearson correlation (r; $\log_{10}$-transformed fish density) or Spearman's correlation (ρ; crayfish density). A $p$ value of 0.05 was used to judge the significance of all tests unless otherwise indicated.

Separate linear regressions of fish and crayfish densities against physical and chemical habitat variables were performed with PROC REG with variable selection based on Akaike's information criterion (AIC; Burnham and Anderson 2002). In these analyses, models were evaluated relative to each other based on corrected AIC values (AICc). The AICc values are adjusted upward for sample size relative to the number of independent variables, which protects against over-fitting models due to small sample size (Burnham and Anderson 2002). Models with the smallest AICc were judged "most parsimonious" (i.e., most efficient), and those with AICc values that differed by <2.0 were considered equivalent (Burnham and Anderson 2002).

## Results

### Habitat and water quality measurements

Habitat quality differed significantly among site groups (Allert et al. 2010). Generally, mean substrate coarseness was significantly greater at reference sites (upstream) than at mining or downstream sites, whereas mean substrate homogeneity, water depth, and current velocity were significantly greater at downstream sites than at mining or reference sites (Allert et al. 2010). In situ water quality also differed significantly among site groups in both surface and pore waters due to the effects of mining and longitudinal changes from upstream to downstream. Mean surface-water temperature, specific conductance, turbidity, alkalinity, hardness, and sulfate were significantly greater at mining and downstream sites than at reference sites (Allert et al. 2010).

### Metals in environmental media

Concentrations of Pb, Zn, and Cd in fish and crayfish were significantly greater at mining sites than at reference or downstream sites, and were significantly greater at downstream sites (TM and TL) than at reference sites (Table 3; Allert et al. 2010). Metals concentrations in pore water, detritus, benthic macro-invertebrates, caged crayfish, and stonerollers at cage sites (i.e., reference and mining sites) also were significantly greater at mining sites than at reference sites (Table 3). Although not statistically analyzed due to small sample size and high within-site variation, concentrations of Pb, Zn, and Cd in sediment at mining and downstream sites were about 12–90 times greater than at reference sites and except for Cd at downstream sites, all were well above consensus PECs for sediment-dwelling organisms (Table 3; MacDonald et al. 2000).

### Density of fish and crayfish

Seven to ten species of fish were caught within each site group (McKee et al. 2010). Densities of fish at mining sites were significantly lower than at reference or downstream sites (Table 4). $\text{Log}_{10}$-transformed fish densities were negatively correlated with Cd and Zn concentrations in sediment and with Pb, Cd, and Zn concentrations in surface water (Table 4). Concentrations of Pb and Cd in Missouri saddled darters were not significantly correlated with riffle fish density (Table 5).

Four species of crayfish were caught at reference sites and downstream sites whereas only two species were caught at mining sites (Allert et al. 2010). Densities of crayfish at mining sites were significantly lower than at reference or downstream sites (Table 4). Mean CL of crayfish at reference and downstream sites were significantly greater than mining sites; there was no significant difference in mean CL between reference and downstream sites (Table 4). Mean crayfish density was significantly negatively correlated with Pb, Zn, and Cd concentrations in crayfish, surface water, and sediment, as was CL with Pb and Cd concentrations in all matrices analyzed (Table 5).

### In situ toxicity test

Survival of both crayfish species at day 56 was significantly lower at mining sites than at reference sites (Table 4). Conversely, mean CL and ww of both species was significantly greater at mining sites than reference sites (Table 4). Concentrations of metals in caged *O. hylas* and *O. luteus* were significantly greater at mining sites than at reference sites (Table 3). Riffle crayfish densities were significantly correlated with survival of caged *O. hylas* ($\rho = 1.0$, $p < 0.0001$), but not *O. luteus* ($\rho = 0.74$; $p = 0.26$; analysis not shown).

### Exposure assessment

Concentrations of Pb, Zn, and Cd in sediment at the mining and downstream sites were well above consensus probable-effects concentrations for sediment-dwelling organisms (Table 3). Sediment $\sum$PEQs were greater than one at mining sites and significantly greater than reference sites (Fig. 2). Crayfish (*O. luteus*) densities were reduced at

**Table 3** Mean (standard deviation) metal concentrations in environmental samples collected in riffles or cages in the Big River

| Sample type[a]/site group/criteria | *n* | Lead | Zinc | Cadmium |
|---|---|---|---|---|
| Golden crayfish (*Orconectes luteus*, μg $g^{-1}$ dry weight) | | | | |
| Reference (R) | 6 | 0.77 (0.08) | 80.8 (3.9) | 0.35 (0.05) |
| Mining (M) | 6 | 122 (44) | 308 (103) | 18.8 (4.24) |
| Downstream (D) | 12 | 58.1 (40.7) | 109 (44) | 8.81 (3.75) |
| R vs M | | $F_{(7,16)} = 132$** | $F_{(7,16)} = 97.8$** | $F_{(7,16)} = 173$** |
| R vs D | | $F_{(7,16)} = 59.4$** | $F_{(7,16)} = 12.5$** | $F_{(7,16)} = 62.1$** |
| D vs M | | $F_{(7,16)} = 31.2$** | $F_{(7,16)} = 62.3$** | $F_{(7,16)} = 53.4$** |
| Missouri saddled darter (*Etheostoma tetrazonum*, μg $g^{-1}$ dry weight) | | | | |
| R | 5 | 0.55 (0.14) | n.d.[f] | 0.14 (0.13) |
| M | 6 | 66.8 (7.3) | n.d. | 1.61 (0.39) |
| D | 12 | 44,7 (14.4) | n.d. | 1.13 (0.29) |
| R vs M | | $F_{(7,15)} = 119$** | n.d. | $F_{(7,15)} = 67.1$** |
| R vs D | | $F_{(7,15)} = 46.3$** | n.d. | $F_{(7,15)} = 33.1$** |
| D vs M | | $F_{(7,15)} = 36.2$** | n.d. | $F_{(7,15)} = 14.6$** |
| Caged *O. luteus* (μg $g^{-1}$ dry weight) | | | | |
| R | 6 | 1.12 (0.28) | 82.4 (2.6) | 0.34 (0.07) |
| M | 6 | 137 (47) | 237 (23) | 3.85 (1.09) |
| R vs M | | $F_{(1,10)} = 108$** | $F_{(1,10)} = 27.0$** | $F_{(1,10)} = 52.9$** |
| Caged woodland crayfish (*O. hylas*, μg $g^{-1}$ dry weight) | | | | |
| R | 6 | 1.29 (0.26) | 86.9 (4.2) | 0.28 (0.02) |
| M | 6 | 228 (100) | 317 (94) | 4.80 (1.45) |
| R vs M | | $F_{(1,10)} = 32.4$** | $F_{(1,10)} = 32.4$** | $F_{(1,10)} = 29.0$** |
| Detritus (μg $g^{-1}$ dry weight) | | | | |
| R | 4 | 15.9 (1.9) | 44.0 (1.9) | 0.33 (0.02) |
| M | 4 | 2318 (929) | 3084 (249) | 44.2 (23.8) |
| R vs M | | $F_{(3,4)} = 25.6$** | $F_{(3,4)} = 24.4$** | $F_{(3,4)} = 64.0$** |
| Macro-invertebrates (μg $g^{-1}$ dry weight) | | | | |
| R | 4 | 12.7 (4.4) | 105 (6) | 0.48 (0.16) |
| M | 4 | 720 (276) | 808 (142) | 12.2 (2.2) |
| R vs M | | $F_{(3,4)} = 64.0$** | $F_{(3,4)} = 12.8$** | $F_{(3,4)} = 25.6$** |
| Stonerollers (*Campostoma oligolepis*, μg $g^{-1}$ dry weight) | | | | |
| R | 4 | 2.39 (0.9) | 136 (15) | 0.08 (0.03) |
| M | 4 | 175 (36) | 519 (64) | 2.94 (0.38) |
| R vs M | | $F_{(3,4)} = 64.0$** | $F_{(3,4)} = 64.0$** | $F_{(3,4)} = 21.3$** |
| Surface water (μg $l^{-1}$) | | | | |
| R | 6 | 0.06 (0.01) | 0.54 (0.21) | 0.06 (0.01) |
| M | 6 | 7.85 (1.63) | 107 (16) | 0.86 (0.17) |
| D | 12 | 3.17 (0.49) | 12.3 (14.4) | 0.19 (0.09) |
| R vs M | | $F_{(7,16)} = 114$** | $F_{(7,16)} = 236$** | $F_{(7,16)} = 236$** |
| R vs D | | $F_{(7,16)} = 38.0$** | $F_{(7,16)} = 78.6$** | $F_{(7,16)} = 78.4$** |
| D vs M | | $F_{(7,16)} = 38.0$** | $F_{(7,16)} = 78.6$** | $F_{(7,16)} = 78.4$** |
| CWQC[b] | | 5–9 | 93–145 | 0.4–0.6 |
| Pore water (μg $l^{-1}$) | | | | |
| R | 6 | 0.08 (0.01) | 2.2 (0.9) | 0.01 (0.00) |
| M | 6 | 13.8 (10.6) | 104 (39) | 1.10 (0.36) |
| R vs M | | $F_{(3,8)} = 49.9$** | $F_{(3,8)} = 49.9$** | $F_{(3,8)} = 37.0$** |
| <2-mm sediment fraction (μg $g^{-1}$ dry weight)[c] | | | | |
| R | 2 | 12.5 (2.1) | 20.5 (0.7) | 0.06 (0.03) |

**Table 3** continued

| Sample type[a]/site group/criteria | *n* | Lead | Zinc | Cadmium |
|---|---|---|---|---|
| M | 2 | 1170 (467) | 860 (170) | 15.5 (3.54) |
| D | 4 | 710 (530) | 258 (163) | 3.25 (3.30) |
| PEC[d] | | 128 | 459 | 4.98 |

Shown are arithmetic site groups mean ± 1 standard deviation. Also shown are results of one-way analysis-of-variance (ANOVA) as *F*-values and degrees-of-freedom for differences among site groups (**$p \leq 0.001$). Ranked means within sites were used in all tests

[a] Reference sites = R1, R2; mining sites = TH1, TH2; downstream sites = TM1, TM2, TL1, TL2

[b] CWQC = federal chronic water-quality criteria adjusted for hardness and the dissolved fraction of the metal (United States Environmental Protection Agency 2006)

[c] Sediments collected in depositional zones near riffles (data from Besser et al. 2009b); due to small sample size ($n = 2$) no statistical comparison made

[d] PEC = probable effects concentration (MacDonald et al. 2000)

lower PEQs than fish densities; however, both densities were significantly reduced at mining sites compared to the reference sites (Fig. 2). Crayfish densities were significantly negatively correlated with ∑PEQ ($\rho = -0.95$; $p = 0.0004$); however, fish densities were not ($\rho = -0.56$; $p = 0.14$).

Surface- and pore-water ∑TUs were significantly greater at mining sites than at downstream or reference sites and exceeded one at mining sites, indicating potential risk to aquatic biota (Table 3; Allert et al. 2010). Densities of fish and crayfish were lower at sites where the toxicity threshold was exceeded (i.e., ∑TUs = 1) for surface water (Fig. 2). Surface- and pore-water ∑TUs were significantly negatively correlated with crayfish densities (surface water, $\rho = -0.97$; $p < 0.0001$; pore water, $\rho = -1.00$; $p < 0.0001$); however, they were not significantly correlated with fish densities (surface water, $r = -0.62$; $p = 0.099$; pore water, $\rho = -0.80$; $p = 0.200$).

Criteria used to evaluate risks of Pb, Zn, and Cd concentrations in crayfish to wildlife indicated that metals concentrations at mining and downstream sites are potentially hazardous to carnivorous wildlife (Fig. 3). Hazard quotients were greater for birds than mammals in their respective size category. Hazard quotients exceeded one

**Table 4** Mean densities (standard deviation) of riffle-dwelling benthic fishes and crayfish (*Orconectes luteus*), probable-effects quotient (∑PEQ), chronic surface- and pore-water toxic-unit score (∑TU), and percent survival and carapace length of caged *O. luteus* and woodland crayfish (*Orconectes hylas*)

| Variable | Site group[a] | | | | | |
|---|---|---|---|---|---|---|
| | Reference (R) | Mining (M) | Downstream (D) | R vs M | R vs D | D vs M |
| Benthic fish density (number $m^{-2}$) | 9.10 (3.83) | 2.21 (1.53) | 11.4 (5.8) | $F_{(7,16)} = 25.1$** | $F_{(7,16)} = 0.52$n.s. | $F_{(7,16)} = 42.3$** |
| *O. luteus* density (number $m^{-2}$) | 10.9 (2.8) | 1.1 (0.3) | 2.4 (1.1) | $F_{(7,16)} = 67.8$** | $F_{(7,16)} = 28.8$** | $F_{(7,16)} = 17.2$** |
| *O. luteus* carapace length (mm) | 14.3 (4.1) | 10.9 (0.4) | 13.9 (2.1) | $F_{(7,13)} = 6.39$** | $F_{(7,13)} = 0.31$n.s. | $F_{(7,13)} = 10.7$** |
| Sediment ∑PEQ[b] | 0.15 (0.02) | 14.1 (2.6) | 6.76 (4.7) | $F_{(2,5)} = 15.0$* | $F_{(2,5)} = 15.0$n.s. | $F_{(2,5)} = 15.0$n.s. |
| Chronic surface-water ∑TU | 0.03 (0.01) | 3.46 (0.44) | 0.87 (0.26) | $F_{(7,19)} = 189$** | $F_{(7,19)}$ 73.6** | $F_{(7,19)} = 55.5$** |
| Caged *O. luteus* percent survival | 100 (0.0) | 66.7 (22.5) | n.t | $F_{(3,8)} = 51.3$** | n.t. | n.t. – |
| Caged *O. luteus* carapace length | 15.8 (0.3) | 17.7 (0.4) | n.t. | $F_{(3,8)} = 10.1$* | n.t. | n.t. – |
| Caged *O. luteus* wet weight | 1.10 (0.1) | 1.61 (0.3) | n.t. | $F_{(3,8)} = 6.04$* | n.t. | n.t. – |
| Caged *O. hylas* percent survival | 98.3(4.1) | 73.3 (16.3) | n.t. | $F_{(3,8)} = 26.3$** | n.t. | n.t. – |
| Caged *O. hylas* carapace length | 15.1 (0.2) | 16.0 (0.4) | n.t. | $F_{(3,8)} = 6.05$* | n.t. | n.t. – |
| Caged *O. hylas* wet weight | 1.04 (0.1) | 1.32 (0.3) | n.t. | $F_{(3,8)} = 3.45$n.s. | n.t. | n.t. – |
| Chronic pore-water ∑TU | 0.05 (0.02) | 4.56 (1.91) | n.t. | $F_{(3,20)} = 75.2$** | n.t. | n.t. – |

Shown are arithmetic site groups means ± 1 standard deviation. Also shown are results of one-way analysis-of-variance (ANOVA) as *F*-values and degrees-of-freedom for differences among site groups (**$p \leq 0.01$; *$0.01 \leq p \leq 0.05$; *ns* $\geq 0.05$). Ranked mean square for riffles within sites were used

*n.t.* not tested

[a] Reference sites = R1, R2; mining sites = TH1, TH2; downstream sites = TM1, TM2, TL1, TL2

[b] Number of individuals per cage per species per site on day 0 of toxicity test = 10; number of cages sampled per species per site per day = 3

**Table 5** Pearson's coefficient of corrrelation (riffle-dwelling benthic fish density) and Spearman coefficients for correlation (riffle-dwelling crayfish density and carapace length) among density, carapace length, whole-body metal concentrations in Missouri saddled darters (*Etheostoma tetrazonum*) or golden crayfish (*Orconectes luteus*), <2-mm sediment, and surface water, Big River, Missouri, USA

| Metal/matrix | Pearson correlation coefficient $Log_{10}$-Transformed fish density (#/$m^{-2}$) | Spearman correlation coefficient Crayfish density (#/$m^{-2}$) | Spearman correlation coefficient Crayfish carapace length (mm) |
|---|---|---|---|
| Lead | | | |
| Missouri saddled darter | −0.49 | n.d. | n.d. |
| Sediment | −0.49 | **−0.90** | **−0.74** |
| Surface water | **−0.77** | **−0.83** | **−0.95** |
| Golden crayfish | n.d. | **−0.93** | **−0.90** |
| Cadmium | | | |
| Missouri saddled darter | −0.40 | n.d. | n.d. |
| Sediment | **−0.85** | **−0.98** | **−0.98** |
| Surface water | **−0.84** | **−0.88** | **−0.90** |
| Golden crayfish | n.d. | **−0.86** | **−0.88** |
| Zinc | | | |
| Missouri saddled darter | n.d. | n.d. | n.d. |
| Sediment | **−0.81** | **−0.74** | −0.62 |
| Surface water | **−0.90** | **−0.86** | **−0.88** |
| Golden crayfish | n.d. | −0.76 | −0.74 |

$n = 3$

*n.d.* not determined

[a] Bold coefficients are significant at $p < 0.05$

for Zn in robin-sized birds (mining, 1.41); for Cd in robin-sized birds (mining, 3.89; downstream, 1.82) and in shrew-sized mammals (mining, 3.03; downstream, 1.42); and for Pb in robin-sized birds (mining, 22.8; downstream, 10.8), and heron-sized birds (mining, 2.71; downstream, 1.28), and shrew-size mammals (mining, 3.23; downstream, 1.53).

## Regression analyses

Five regression models met the minimal AICc criterion of differing by two or less for fish (Table 6). Models contained 2–5 independent variables and best models (lowest AICc) included the variables Pb in Missouri saddled darters, distance downstream, riffle substrate coarseness, Zn in <2-mm sediment, riffle substrate homogeneity, and Pb in <2-mm sediment. Six regression models containing 2–5 variables met the minimal AICc for crayfish (Table 6). Models contained the variables surface-water ∑TU, distance downstream, quadrat substrate homogeneity, quadrat current velocity, carapace length, Pb in crayfish, Pb in <2-mm sediment, quadrat water depth, and Zn in <2-mm sediment.

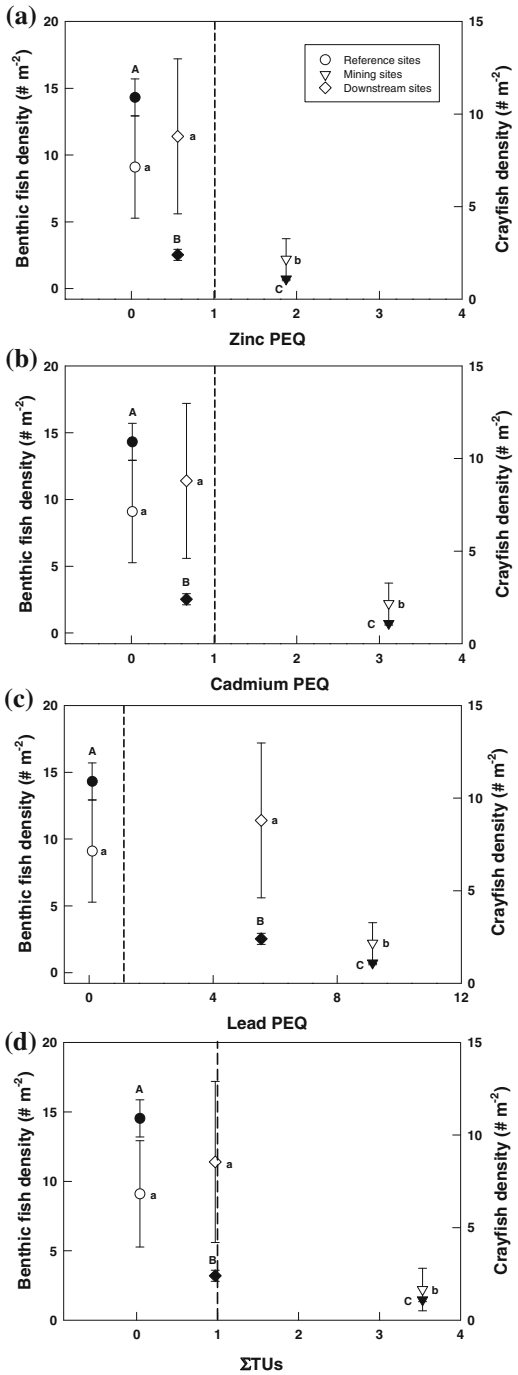


**Fig. 2** Riffle-dwelling benthic fish (*open symbol*) and crayfish (*Orconectes luteus;* (*closed symbol*) densities versus metal toxicity indices: **a** zinc probable effect quotient (PEQ); **b** cadmium PEQ; **c** lead PEQ (Ingersoll et al. 2001), and **d** sum of surface-water toxic unit (∑TU). Values for PEQ and ∑TU greater than one (*hashed line*) indicate potential toxicity. *Lower case letters* indicate site means for benthic fish densities that differ significantly; *capital letters* indicate site means for crayfish that differ significantly

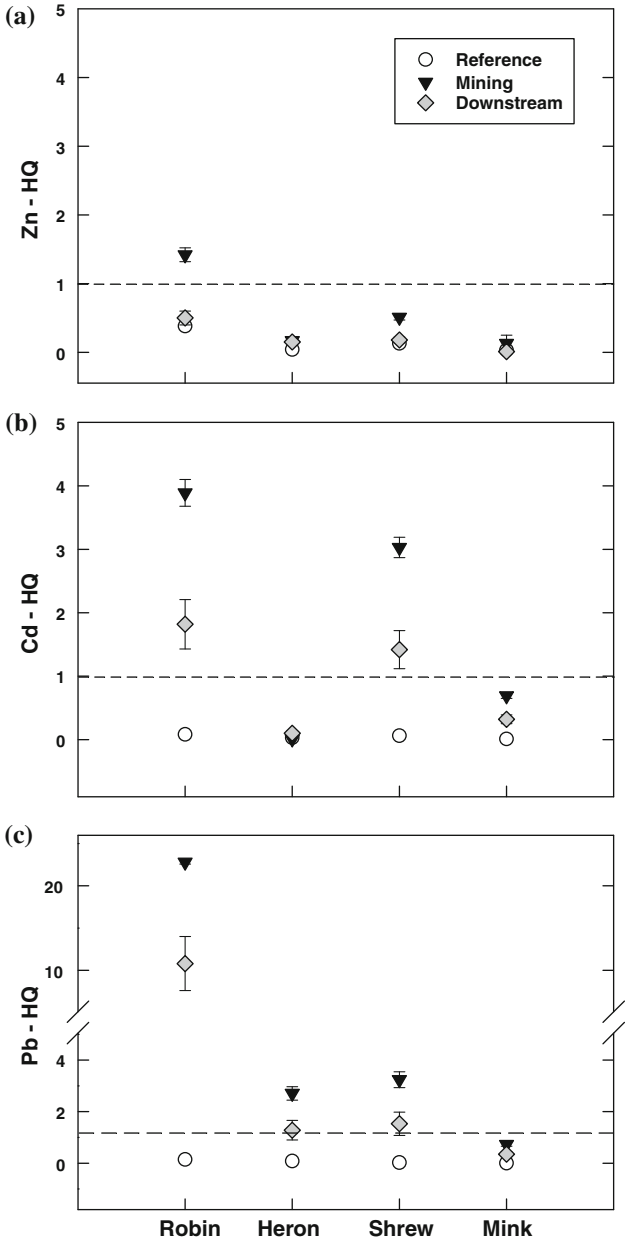


**Fig. 3** Hazard quotient (HQ) for dietary **a** zinc (Zn), **b** cadmium (Cd), and **c** lead (Pb) in *Orconectes luteus* for each representative species. *Hashed line* represents risk threshold

**Table 6** Linear regression of riffle-dwelling benthic fish and crayfish community densities against physical and chemical habitat variables was performed with PROC REG with variable selection based on Akaike's information criterion (AIC) corrected for small sample size (AICc)

| Population/model[b] | K[a] | AICc | ΔAICc |
|---|---|---|---|
| Benthic fish | | | |
| MSDPb distance | 2 | 31.658 | 0.000 |
| MSDPb, rif substr coarse, distance | 3 | 16.977 | 0.000 |
| MSDPb, sedZn, rif substr coarse, distance | 4 | −7.975 | 0.000 |
| MSDPb, sedZn, rif substr homogen, distance | 4 | −6.336 | 1.639 |
| MSDPb, sedZn, Pbsed, rif substr homogen, distance | 5 | −76.149 | 0.000 |
| Crayfish | | | |
| SW ∑TU, distance | 2 | −32.049 | 0.000 |
| quad substr homogen, quad cv, CL | 3 | −54.614 | 0.000 |
| crayPb, sedPb, quad substr coarse, quad depth | 4 | −171.900 | 0.000 |
| sedZn, sedPb, quad substr coarse, quad depth | 4 | −171.969 | −0.067 |
| sedZn, sedPb, distance, quad substr coarse, quad depth | 5 | −199.501 | 0.000 |
| crayPb, sedPb, distance, quad velocity, quad depth | 5 | −199.601 | −0.100 |

Also shown for each model is the AICs difference relative to the best model (Δ AICc). Ranked mean square for riffles within sites were used in all tests

[a] number of parameters

[b] MSDPb = lead in Missouri saddled darter (*Etheostoma tetrazonum*); distance = longitudinal distance downstream from Desloge tailings pile; substr coarse = riffle substrate coarseness; sedZn = zinc in <2-mm sediment; subst homogen = riffle substrate homogeneity; Pbsed = lead in <2-mm sediment; SW ∑TU = surface-water chronic sum of toxic units (∑TU); quad subst homogen = crayfish quadrat substrate homogeneity; quad cv = crayfish quadrat current velocity; CL = carapace length in riffle crayfish (*Orconectus luteus*); crayPb = lead in riffle crayfish (*O. luteus*); quad substr coarse = crayfish quadrat substrate coarseness, quad depth = crayfish quadrat water depth; quad velocity = quadrat current velocity

## Discussion

We conducted an in situ toxicity test using standardized methods to determine survival and growth of crayfish at four sites to isolate the effects of metals on crayfish. Results indicated that mortality of caged crayfish at mining sites was significantly higher than at reference sites. Metal concentrations in surviving caged crayfish were significantly higher at mining sites compared to reference sites. Elevated metal concentrations in caged crayfish at mining sites were highly correlated with elevated metal concentrations in pore water, detritus, other benthic macro-invertebrates, and stonerollers, which were sampled near the cages; and in Missouri saddled darters collected in the same riffles where wild crayfish were sampled. The results of the in situ toxicity test offer direct evidence that mining-derived metals are toxic to crayfish in the BGR. The effects of metals on wild populations of crayfish were evaluated at eight sites in the BGR to examine the ecological and toxicological significance of the in situ toxicity test. Results of crayfish population studies corroborated those of the cage studies and determined that densities of wild riffle crayfish were significantly lower at mining and

downstream sites compared to reference sites. Crayfish densities were negatively correlated with mining-derived metals measured in surface water, sediment, and crayfish (wild *O. luteus*). In addition, mean CL of wild *O. luteus* was negatively correlated with Pb, Cd, and Zn in crayfish. Although correlation analysis does not necessarily imply causality, we observed that metals explained a higher proportion of variance in riffle crayfish density compared to other possible chemical (e.g., DO and ammonia) or physical habitat variables.

Analyses of metals concentrations, an in situ toxicity test, and an assessment of riffle-dwelling benthic fish and crayfish densities provided multiple lines of evidence indicating that mining-derived metals negatively affect aquatic organisms in the BGR. Elevated concentrations of metals in fish and crayfish and other benthic macro-invertebrates in the BGR represent a risk to carnivorous fish and riparian wildlife because they are a food source for many species. Comparative high metals concentrations in centrarchids (MDHSS 2012) and other fishes in the BGR (e.g., Schmitt et al. 2006, 2007b) probably reflect metals concentrations in prey organisms (e.g., stonerollers and crayfish; Schmitt et al. 2007b, c). These elevated concentrations represent a hazard to wildlife and humans (MDHSS 2012). Although diet was the only exposure pathway considered in our screening-level assessment, significant waterborne exposure to metals would not be expected under the hard, carbonate-dominated water conditions of the BGR. Under these conditions, food chain transfer and dietary exposure are more important than waterborne exposure of Pb, Cd, and Zn (Besser et al. 2005, 2007a; Farag et al. 1999; Woodward et al. 1994). Dietary intake of metals in crayfish and fish include their exposure to metals from sediments since it is highly likely that crayfish and fish ingest contaminated sediment during feeding. Our analyses of whole-body metal concentrations inclusive of gut contents reflect this assumption.

In situ toxicity tests were not conducted with fish; however, there are several lines of evidence indicating that lower fish densities at the mining sites are due to metal toxicity. The greatest likelihood of effects of metals would result from toxic effects on larval and juveniles due to their sensitivity (Woltering 1984); lower densities at mining sites may be due to the loss of small size classes of fish (McKee et al. 2010). Densities may also be lower due to changes in reproduction, which could result from decreased feeding or growth. Water concentrations of Pb at mining sites averaged 7.85 μg $l^{-1}$, which is in the range of the U.S. Environmental Protection Agency's chronic water-quality criterion for Pb (5–9 μg $l^{-1}$; United States Environmental Protection Agency 2006). Sediment (<2 mm fraction) concentrations at the mining sites averaged 1170 μg Pb $g^{-1}$ which is considerably greater than the PEC of 128 μg Pb $g^{-1}$ for sediment. In the present study, mean whole-body concentration of Pb in Missouri saddled darters at the mining sites was 66.8 μg Pb $g^{-1}$ dry weight (dw), which is equivalent to about 18.7 μg Pb $g^{-1}$ wet weight (ww; 72 % moisture; McKee et al. 2010). The mean concentration of Pb in stonerollers at mining sites was 175 μg Pb $g^{-1}$ ww (Allert et al. 2010). Laboratory studies with fish exposed to Pb have identified reduced growth of brook trout (*Salvelinus fontinalis*) at a whole-body concentration of 4.0 μg Pb $g^{-1}$ ww (Holcombe et al. 1976) and reduced feeding of fathead minnows (*Pimpehales promelas*) at 26.2 μg Pb $g^{-1}$ ww (Weber et al. 1991), concentrations that were exceeded in our fish. Similarly, laboratory studies with fish exposed to Cd identified decreased growth in brook trout at whole-body concentrations of 0.25 μg Cd $g^{-1}$ ww (Benoit et al. 1976) and at 0.48 μg Cd $g^{-1}$ ww in Atlantic salmon (*Salmo salar*; Peterson et al. 1983); Cd concentrations in Missouri saddled darter (0.38–0.5 μg Cd $g^{-1}$ ww) and stonerollers (3.03–2.84 μg Cd $g^{-1}$ ww) at mining sites were within this range.

Exposure to heavy metals has resulted in the disappearance of crayfish downstream of mining inputs elsewhere (Allert et al. 2008, 2012; Kossakowski 1973; Thorp et al. 1979), possibly due to effects on molting and reproduction (Viikinkoski et al. 1995). In our study, growth of crayfish in cages was significantly greater at mining sites than at reference sites. The growth increase probably reflects the higher mortality in cages at mining sites, which resulted in lower densities and less competition for food relative to reference sites. We hypothesize that greater growth of surviving crayfish at mining sites compared to reference sites also occurred due to significantly higher nutrient (i.e., DOC, TN, TP) concentrations and periphyton (estimated by chlorophyll *a*; Allert et al. 2010) observed at mining sites in addition to lower density in the cages due to mortality (Allert et al. 2010). Increased nutrient concentrations and algal mats have been observed below tailings piles in the OLB and other mining districts in Missouri (Besser et al. 2007a; Gale et al. 1973; Jennett and Wixson 1972). Periphyton degrades organic matter and is a critical nutritional component for shredders such as crayfish (Cummins 1977). Significant growth in CL and ww of crayfish occurs only with molting (Reynolds 2002), when there is an intensive uptake of water into the hemolymph following ecdysis and an increase in $Ca^{2+}$ regulation (Holdich 2002 and references therein). Early instar and juvenile (first-year) crayfish are more sensitive than adults to metals and other toxicants (Eversole and Seller 1997; Knowlton et al. 1983; Wigginton and Birge 2007), possibly due to the high number of molts that occur during the first summer (Holdich 2002). The resulting rapid growth at mining sites would have resulted in more frequent molting, thereby further increasing mortality (Knowlton et al. 1983; Wigginton and Birge 2007). Increased sensitivity could

result in part from the influx of water and competition between metals (i.e., Cd) and $Ca^{2+}$ ions as the cuticle re-calcifies (Wigginton and Birge 2007 and references within).

Muck et al. (2002) showed that the loss of first-year reproductively mature females may limit the sustainability of *O. luteus* populations. Both lethal and sublethal effects of metals on juvenile crayfish may be at least partly responsible for reduced population densities at mining-affected sites. For example, sublethal exposure can cause changes in crayfish behavior that make them more vulnerable to predation. Alberstadt et al. (1999) determined that there was a significant decrease in shelter use by rusty crayfish (*Orconectes rusticus*) at concentrations of 1–3 mg Cd $l^{-1}$ and sustained hyperactivity at 3 mg Cd $l^{-1}$. Decreased use of shelter by crayfish and hyperactivity may increase the risk of predation, displacement, or dislodgement, all of which can reduce survival (Clark et al. 2008). As noted previously, crayfish are important for nutrient cycling and energy flow in stream ecosystems (Hobbs 1993; Whitledge and Rabeni 1997), represent a pathway for the transfer of metals and other contaminants to higher trophic levels, and are important in the diets of smallmouth bass and other fishes (DiStefano 2005; Probst et al. 1984; Rabeni et al. 1995). Effects on crayfish density and their accumulation of metals can therefore have far-reaching community-level consequences.

Stream communities, in the absence of elevated metals concentrations, vary predictably with distance downstream along gradients in abiotic factors such as stream width, stream gradient, and substrate size (Vannote et al. 1980). Fish species richness generally tends to increase with stream size due to several factors including increased average water depth, habitat heterogeneity, and the influence of immigration from tributaries (Vannote et al. 1980). The number of fish species in the BGR did increase with distance downstream (McKee et al. 2010), but sculpin (*Cottus* spp.) were unexpectedly rare in our study; although two species are present in the BGR system (Pfleiger 1997), we caught only a few banded sculpin (*Cottus carolinae*) at TH2, TM1, and TM2. Recent studies have indicated that sculpins (*Cottus* spp.) are sensitive to metals (Allert et al. 2009b; Besser et al. 2007b; Woodling et al. 2002). Nevertheless, it is unclear why they were rare in BGR. Further research would be needed to determine if other factors such as water temperature, in addition to or in lieu of long-term exposure to mining-derived metals, are involved.

## Conclusions

Our results indicate that metals and mining-related tailings adversely affect riffle-dwelling benthic fish and crayfish in the BGR. Our findings are similar to previous studies in the BGR that report effects on aquatic life and for benthic fish and crayfish in the Viburnum Trend, where mining-derived metals have also negatively affected benthic fish and crayfish populations. Elevated concentrations in crayfish and fish represent a hazard to wildlife and humans (MDHSS 2012; Schmitt et al. 2006). Because of their predominance in aquatic food webs and comparative sensitivity to metals, crayfish are effective sentinel organisms for assessing the bioavailability and ecological effects of metals. Our results also indicate that both the structure and function of the BGR ecosystem have been compromised as a result of contamination from historical Pb–Zn mining that has reduced the population densities of crayfish and fishes—the foundations of Ozark stream ecosystems (e.g., Whitledge and Rabeni 1997). Our study provides direct evidence that metals associated with historical mining activities in the OLB continue to affect aquatic life in the BGR. Worthwhile topics for future research include investigating relations between metals and the sustainability of crayfish populations; between substrate characteristics and crayfish densities; determining reasons for low sculpin densities in the BGR; and determining whether lower crayfish densities together with elevated metals concentrations in the BGR are negatively affecting high-value sport fish populations through reductions in growth rates, survival, and condition.

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**Disclaimer** Crayfish and fish were collected by MDC personnel; however, all procedures conformed to USGS guidelines for the humane treatment of test organisms during culture and experimentation and with American Fisheries Society, American Institute of Fishery Research Biologists, American Society of Ichthyologists and Herpetologists (2004) guidelines for the use of fish in research. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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