Prepared in cooperation with the Missouri Department of Conservation

Effects of mining-derived metals on riffle-dwelling crayfish and in-situ toxicity to juvenile *Orconectes hylas* and *Orconectes luteus* in the Big River of southeast Missouri, USA

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Administrative Report

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Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
	Area	
square meter (m^2)	0.0002471	acre
hectare (ha)	2.471	acre
hectare (ha)	0.003861	square mile (mi^2)
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
liter (L)	0.2642	gallon (gal)
cubic meter (m^3)	264.2	gallon (gal)
liter (L)	61.02	cubic inch (in^3)
	Flow rate	
cubic meter per second (m^3/s)	70.07	acre-foot per day (acre-ft/d)
meter per second (m/s)	3.281	foot per second (ft/s)
millimeter per year (mm/yr)	0.03937	inch per year (in/yr)
kilometer per hour (km/h)	0.6214	mile per hour (mi/h)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)
	Pressure	
kilopascal (kPa)	0.009869	atmosphere, standard (atm)
kilopascal (kPa)	0.01	bar
kilopascal (kPa)	0.2961	inch of mercury at 60°F (in Hg)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$°F=(1.8\times°C)+32$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

°C=(°F-32)/1.8

Vertical coordinate information is referenced to the insert datum name (and abbreviation) here, for instance, “North American Vertical Datum of 1988 (NAVD 88)”

Horizontal coordinate information is referenced to the insert datum name (and abbreviation) here, for instance, “North American Datum of 1983 (NAD 83)”

Altitude, as used in this report, refers to distance above the vertical datum.

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

Effects of mining-derived metals on riffle-dwelling crayfish and in-situ toxicity to juvenile *Orconectes hylas* and *Orconectes luteus* in the Big River of southeast Missouri, USA

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Natural Resource Damage Assessment and Restoration Administrative Report:

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Abstract

The Old Lead Belt mining sub-district in southeast Missouri, USA was one of the largest producers of lead-zinc ore in the world. Previous stream surveys found evidence of metal exposure to fish and crayfish. We conducted a 56-d in-situ test to assess toxicity and bioavailability of mining-derived metals to the woodland crayfish (*Orconectes hylas*) and golden crayfish (*Orconectes luteus*) at four sites in the Big River, which drains much of the Old Lead Belt. We also sampled crayfish populations in riffle habitats at eight sites in the Big River. Survival and growth of caged crayfish, riffle crayfish density, physical habitat, and water quality were examined at sites with no known upstream mining activities and at sites downstream of mining areas to assess the ecological effects of mining-derived metals. Metals (Co, Ni, Cu, Zn, Cd, Pb) were analyzed in surface water, pore water, sediment, detritus, fish, caged and riffle crayfish, and other macro-invertebrates. Mortality of caged crayfish was significantly higher at mining sites than at reference sites. Metal concentrations in caged crayfish, detritus, macro-invertebrates, and fish were significantly higher at mining sites than at reference sites. Mean concentrations of Pb (1.1–1.3 µg Pb/L), Zn (82–87 µg Zn/L), and Cd (0.28–0.34 µg Cd/L) in surviving caged crayfish at day 56 of the in-situ toxicity test were 105 to 207 times, 2.7 to 3.6 times, and 11.5 to 17.1 times lower than mean concentrations of Pb (137–228 µg Pb/L), Zn (237–317 µg Zn/L), and Cd (3.9–4.8 µg Cd/L) in surviving caged crayfish at mining sites. Mean concentrations of Pb (0.08 µg Pb/L), Zn (2.20 µg Zn/L), and Cd (0.01 µg Cd/L) in pore water at reference sites were significantly lower than mean concentrations Pb (13.8 µg Pb/L), Zn (104 µg Zn/L), and Cd (1.10 µg Cd/L) in pore water at mining sites. Mean concentrations of Pb (2317 µg Pb/L), Zn (3084 µg Zn/L), and Cd (44.2 µg Cd/L) in detritus at mining sites were 145-fold, 70-fold, and 134-fold higher than mean concentrations of Pb (15.9 µg Pb/L), Zn (44.0 µg Zn/L), and Cd (0.33 µg Cd/L) in detritus at reference sites. Mean concentrations of Pb (12.7 µg Pb/L), Zn (105 µg Zn/L), and Cd (0.48 µg Cd/L) in macro-invertebrates

at reference sites were 57-fold, eight-fold, and 25-fold lower than mean concentrations of Pb (720 µg Pb/L), Zn (808 µg Zn/L), and Cd (12.2 µg Cd/L) in macro-invertebrates at mining sites. Mean concentrations of Pb (2.39 µg Pb/L), Zn (136 µg Zn/L), and Cd (0.08 µg Cd/L) in fish at reference sites were 73-fold, four-fold, and 37-fold lower than mean concentrations of Pb (175 µg Pb/L), Zn (519 µg Zn/L), and Cd (2.94 µg Cd/L) in fish at mining sites. Mean riffle crayfish densities in riffle habitats were significantly higher at reference sites than riffle crayfish densities at mining and downstream sites. Metal concentrations in surface water, sediment, and riffle crayfish were significantly higher at mining sites than at reference sites. Mean concentrations of Pb (0.05 µg Pb/L), Zn (0.52 µg Zn/L), and Cd (0.01 µg Cd/L) in surface water at reference sites were 157-fold, 206-fold, and 86-fold lower than mean concentrations of Pb (7.85 µg Pb/L), Zn (107 µg Zn/L), and Cd (0.86 µg Cd/L) in surface water at mining sites and were 63-fold, 22-fold, and 19-fold lower than mean concentrations of Pb (3.14 µg Pb/L), Zn (11.4 µg Zn/L), and Cd (0.19 µg Cd/L) in surface water at downstream sites. Mean concentrations of Pb (21 µg Pb/L), Zn (13 µg Zn/L), and Cd (0.06 µg Cd/L) in sediment at reference sites were 56-fold, 67-fold, and 258-fold lower than mean concentrations of Pb (1170 µg Pb/L), Zn (870 µg Zn/L), and Cd (15.5 µg Cd/L) in sediment at mining sites and were 34-fold, 20-fold, and 54-fold lower than mean concentrations Pb (710 µg Pb/L), Zn (258 µg Zn/L), and Cd (3.25 µg Cd/L) in sediment at downstream sites. Mean Pb (122 µg Pb/L), Zn (380 µg Zn/L), and Cd (18.8 µg Cd/L) concentrations in riffle crayfish were significantly higher at mining sites than mean concentrations of Pb (0.77 µg Pb/L), Zn (80.8 µg Zn/L), and Cd (0.35 µg Cd/L) in riffle crayfish at reference sites and mean concentrations Pb (58.1 µg Pb/L), Zn (109 µg Zn/L), and Cd (8.81 µg Cd/L) in wild crayfish at downstream sites. Chronic toxic unit scores for surface water and pore water were both greater than three at mining sites, indicating significant risk of toxicity to aquatic biota. Riffle crayfish densities were negatively correlated with metal concentrations in surface water, sediment, and crayfish. Principal

components analyses showed a separation of reference and mining sites as the result of an inverse relationship between riffle crayfish density and metals concentrations in surface water and riffle crayfish. An assessment of potential adverse effects from metals in crayfish to wildlife indicate that the consumption of crayfish would be hazardous for wildlife. These findings indicate that metals associated with previous mining activities in the Old Lead Belt are negatively affecting crayfish populations in the Big River and that in-situ toxicity testing offered direct evidence that mining-derived metals found in the Big River are toxic to crayfish.

Key words: lead-zinc mining, metals, water quality, *Orconectes hylas*, *Orconectes luteus*, in-situ toxicity

Introduction

Lead (Pb) mining in Missouri has occurred since the 1700s. In the early 1800s, deep-shaft mining and improved beneficiation methods increased exploration and mining of Pb ore in the Old Lead Belt (OLB) mining sub-district in southeast Missouri (fig. 1). Mining for Pb and zinc (Zn) as well as ore processing in the OLB began in the 1860s and lasted about 100 years (Seeger, 2008, and references within). Mining in the OLB preceded environmental regulation and utilized comparatively inefficient extraction technologies. Consequently, contamination of lands, surface water, and ground water is extensive, and portions of the mining district have been designated as U.S. Environmental Protection Agency Superfund sites. Elevated concentrations of metals in fish and benthic macro-invertebrates from the OLB have been documented (Buchanan, 1979; Dwyer and others, 1988; Schmitt and Finger, 1987; Schmitt and others, 1984, 1993, 2007a, 2007b; Whelan, 1983) and metal concentrations in fish and crayfish have been determined to pose a risk to wildlife and humans (Schmitt and others, 2006, 2008). Metals also pose a risk to two species of crayfish (*Orconectes harrisoni, Cambarus maculatus*) that have been designated as Species of Conservation Concern by the Missouri Department of Conservation (Missouri Natural Heritage Program, 2009).

Previous studies have documented that the release of metals from mining districts in Missouri has elevated metal concentrations in aquatic biota and affected aquatic organisms, particularly crayfish (Allert and others, 2008; 2009; Besser and Rabeni, 1987; Besser and others, 2006, 2009a; Brumbaugh and others, 2007; Schmitt and others, 2007b). Lower densities or absence of crayfish have been documented downstream of mining sites in the Viburnum Trend mining district in southeast Missouri (Allert and others, 2008, 2009). Because crayfish are an important prey item for fish, other aquatic and terrestrial invertebrates, birds, and mammals (DiStefano, 2005; Hobbs, 1993) and are critical to organic matter processing in streams and the cycling of nutrients and energy through stream food webs (Momot,

1995; Parkyn and others, 2001), effects of metals on crayfish will likely have negative effects on other components of stream and surrounding ecosystems.

This research was designed to determine the effects of metals released from historical mining into the Big River of southeast Missouri, which drains the OLB. The objectives of this study were: 1) to evaluate the effects of cobalt (Co), nickel (Ni), copper (Cu), Zn, cadmium (Cd), and Pb in pore water, detritus, fish, crayfish, and other macro-invertebrates on survival and growth of juvenile crayfish using in-situ cages, 2) to evaluate riffle crayfish densities and crayfish species composition in the Big River relative to concentrations of mining-derived metals in surface water, wild crayfish, and sediment, and 3) to evaluate the potential effects in wildlife from metals in crayfish.

Methods

Study area

Crayfish in riffle habitats were sampled at eight sites in the Big River and an in-situ toxicity test was conducted at four of these sites (table 1). Sites were classified into three groups based on exposure and chemical data (M. McKee, Missouri Department of Conservation, oral commun., May 29, 2009): reference sites without known upstream mining activities−R1 (SEMO-1; Besser and others, 2009b), R2 (SEMO-22); mining sites−TH1 (SEMO-3), TH2 (SEMO-4), and downstream sites−TM1 (SEMO-5), TM2 (SEMO-9), TL1 (SEMO-23), TL2 (SEMO-11). Site locations were documented by a hand-held global positioning system (GPS) receiver [±10 m, datum = World Geodetic System (WGS) 84].

In-situ toxicity test

1. Crayfish collection and culture

Ovigerous *O. hylas* females (Pfleiger, 1996) were collected from the Bootleg Access on the Big River (Washington County, MO, USA) in May of 2008 and returned to the Columbia Environmental Research Center (CERC) in Columbia, MO, USA. Six females were held in individual 2-L flow-through aquaria filled with CERC well water (temperature 18 °C, pH 7.7, alkalinity 254 mg/L as $CaCO_3$, hardness 286 mg/L as $CaCO_3$) and fed frozen brine shrimp (San Francisco Bay Brand, Inc., San Francisco, CA, USA) *ad libitum* daily. Upon hatching and detaching from the adult females, juvenile crayfish were placed in a flow-through 2-m x 1-m fiberglass tank filled with CERC well water and fed flake food (Ziegler Brothers, Inc., Gardner, PA, USA) *ad libitum* daily until their body width was >2 mm.

Additional juvenile crayfish were collected from the Bootleg Access and one of the reference sites (R1) for the in-situ toxicity test. Juvenile *O. hylas* and *O. luteus* were placed in separate flow-through 1.2-m (diameter) circular fiberglass tanks filled with CERC well water and fed flake food (Ziegler Brothers, Inc., Gardner, PA, USA) *ad libitum* daily for 30 days prior to the start of the test. Before stocking crayfish into cages, a sample of crayfish were measured for mean carapace length (CL; from the tip of rostrum to the posterior edge of the cephalothorax, to the nearest 0.1 mm) and wet weight (to the nearest 0.1 g), prior to being frozen for metal analyses. Gender of these crayfish was not determined. Mean CL (9.9±0.13 mm, n =125) and weight (0.32±0.01 g, n =125) of *O. hylas* were significantly greater than the mean CL (5.9±0.14 mm, n = 261) and weight (0.09±0.01 g, n =260) of *O. luteus* at the start of the in-situ toxicity test.

2. In-situ toxicity test

A 56-d in-situ toxicity test was conducted from July 24 to September 17, 2008 with juvenile *O. hylas* and *O. luteus* at four sites in the Big River (fig. 1, table 1). Crayfish were exposed in hemicylindrical (0.28-m^2) cages constructed of stainless steel wire mesh (2.7-mm diagonal opening) and polyethylene (LDPE) reinforcing strips (Allert and others, 2009). Pebble and cobble (25–75 mm particle size) substrate and approximately 10 g of weathered organic material (henceforth detritus) collected from each site provided food and shelter for caged crayfish. Substrate, detritus, and three polyethylene scour pads were placed in three polyethylene-mesh packs (15 cm long x 30 cm wide; 1.27-cm diagonal opening), which were closed using plastic cable ties and secured to the bottom of each cage with stainless steel wire (Allert and others, 2009). Prior to placing weathered detritus into the mesh packs, all predatory insects (that is, Odonata and Plecoptera larvae) were removed. Cages were deployed in run habitats near the riffles sampled for the assessment of riffle (wild) crayfish density to insure cages would not be exposed with declining water levels. Cage bottoms were buried 2 to 4 cm into the stream sediment which exposed crayfish to subsurface water and anchored the cages. Minced fish (largescale stonerollers, *Campostoma oligolepis*; henceforth stonerollers) from each site were added weekly to each cage at that site in increasing increments to maintain dietary rations proportional (0.5% of biomass) to anticipated crayfish biomass.

Ten juvenile crayfish of each species were placed in 12 cages at each of the four sites. Six cages were sampled at each site on day 28 and day 56 of the in-situ toxicity test. Gender of surviving crayfish was determined and CL and wet weight were measured. Surviving crayfish were frozen for metal analyses. Test endpoints included survival and growth. Biomass (that is, standing crop) at day 28 and day 56 was estimated by multiplying the number of survivors of each species by the mean wet weight of each species at each site.

Crayfish density and species richness

We collected crayfish once by disturbing about one square meter of substrate within a 1-m^2 quadrat sampler (1 m long x 1 m wide x 1.5 m high) with 3-mm delta mesh (DiStefano and others, 2003a; Larson and others, 2008) at the eight sites from July 7–17, 2008. Each site consisted of a reach containing three riffles. Seven quadrat samples were randomly located in each riffle (total n =21 per site). We identified crayfish to species (Pfleiger, 1996) and gender and measured CL. All crayfish except those retained for metal analyses were released alive to the stream.

Additional qualitative sampling of crayfish was conducted to ascertain the presence of species in additional habitats. Thirty wire-funnel traps baited with canned dog food (DiStefano and others, 2009) were set in slower-flowing habitats (for example, pools, backwaters, emergent vegetation patches) in 1- to 1.5-m deep water. Traps were set at least 10 m apart, and were deployed overnight and harvested the following morning. Crayfish were processed as described above for quadrat samples.

Physical habitat measurements

Physical habitat variables [depth (cm), velocity (m/s), substrate classification] were measured in each of the three riffles sampled for crayfish, adjacent to or within quadrat samples, and near cages at all sites using methods of American Public Health Association and others (2005), Bain and others (1985), Bain and Stevenson (1999), Barbour and others (1999), Bovee and Milhouse (1978), Hamilton and Bergersen (1984), and Platts and others (1983). Measurements were once taken on the day of crayfish sampling. Current velocities were measured using a Marsh McBirney® 2000 portable flow meter at the substrate surface and 6/10 depth (henceforth mid-water depth). Substrate classification codes based primarily on diameter are listed in table 2. Additional information about the habitat assessment methods are presented in Allert and others (2008).

In-situ water quality measurements

We used a Hydrolab® (Loveland, CO, USA) Quanta meter to measure temperature, pH, conductivity, dissolved oxygen (DO), and turbidity in each riffle sampled for crayfish. Measurements were also taken weekly adjacent to cages during the in-situ toxicity test. Detection and recoveries of water quality standards were within acceptable criteria (±20%), thus none of the sample results required correction. A surface water grab sample from each riffle was collected for additional water quality analyses (total nitrogen, ammonia, total phosphorous, chlorophyll *a*, dissolved organic carbon, total suspended solids, alkalinity, hardness, sulfate; American Public Health Association and others, 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 removed for metal analyses. Samples for metal analyses were filtered on-site into a pre-cleaned polyethylene bottle 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 (J.T. Baker Inc., Phillipsburg, NJ, USA) within four days of collection.

Concentrations of metals in pore water were measured because of the close association of crayfish with sediment. Pore waters were collected using passive pore-water samplers (peepers). Peepers were fabricated from 50-mL polypropylene snap-cap vials and dialysis membranes and were filled with ultra-pure water (Brumbaugh and others, 2007). Peepers were placed in a 2-L polyethylene bottle filled completely with de-oxygenated, ultra-pure water, which was capped and stored in a refrigerator for one to two days before transportation on ice to the field.

Six peepers were deployed in sediments adjacent to cages twice during the in-situ toxicity test. After about two weeks in the sediment, peepers were removed and gently agitated in the stream water to remove any attached particles. The lid and membrane were inspected for the presence of any visible

particles; if particles were present, they were removed using a de-ionized water stream. The membrane and perforated cap were then carefully removed and replaced with a pre-labeled non-perforated cap. Three peepers were individually sealed in a small zip-seal bag and placed on ice within 20 minutes of retrieval for metal analyses. Upon return to the laboratory, the contents of each peeper were acidified to an effective concentration of 0.16 M HNO_3.

Pore water from the three remaining peepers was composited into two 100-ml pre-cleaned high-density polyethylene bottles at each site. We measured temperature, pH (Orion® 290A), conductivity (YSI® 135), and dissolved oxygen (YSI® 95) in the composited sample of the pore water immediately upon retrieval from the substrate on day 13 and day 33 of the in-situ toxicity test. Detection and recoveries of water quality standards were within acceptable criteria (±20%), thus none of the sample results required correction. The pore-water samples were either stored at 4 °C (for example, alkalinity, hardness, and sulfate) or acidified and stored at 4 °C (for example, ammonia, and dissolved organic carbon) until the analyses were conducted (American Public Health Association and others, 2005).

Sediment collection

Composite samples of stream sediments were collected at each site from depositional areas near riffles containing fine sediments. Surficial sediments (about the top 10 cm) were collected from depositional areas within the wetted stream channel using PVC scoops (Brumbaugh and others, 2007). Two 19-L plastic buckets were filled to about two-thirds of their volume with sediment to obtain about 20-L of sediment from each sampling site. Sediment samples contained substantial fractions of particles greater than 2 mm diameter (that is, greater than about 10% by volume), so sediments were wet-sieved through a 2-mm diameter stainless steel sieve in the field to remove coarse particles using a minimum quantity of site water (Brumbaugh and others, 2007). The resulting composite sediment samples were stored in the dark at 4 ºC. In the laboratory, sediments from each site were combined and homogenized

using an electrically powered drill and stainless steel auger. Sub-samples of the composite sediments were analyzed to characterize metal concentrations, percent total organic carbon, percent water, and particle size distribution (Besser and others, 2009b).

Laboratory water quality analyses

Alkalinity and hardness were measured by titration (American Public Health Association and others, 2005). Sulfate was measured by colorimetric detection with a Hach® 2100 Spectrophotometer (Loveland, CO, USA). Water samples for particulate organic carbon (POC) were acidified with 2 N sulfuric acid to a pH of 2 then filtered on Gelman® Type A/E glass-fiber filters the day after collection and stored at -20 °C until analysis. Particulate organic carbon was determined using a Coulometrics® Model 5020 Carbon Analyzer (UIC, Inc., Joliet, IL, USA) according to American Society for Testing and Materials Method D4129-05 (American Socity for Testing and Materials, 2005). Water samples for chlorophyll *a* were filtered on Gelman® Type A/E glass-fiber filters on the same day as collection and stored at -20 °C until analysis. In-vitro chlorophyll *a* was determined following extraction in 90% buffered acetone using a Turner® Model AU-10 Fluorometer (Turner Designs Inc., Sunnyvale, CA, USA) according to Standard Method 10200 H (American Socity for Testing and Materials, 2005). Total suspended solids (TSS) were analyzed based on methods recommended by the American Public Health Association and others (2005). Samples were filtered through a glass fiber filter (ProWeigh® pre-washed and pre-weighed glass fiber filters, nominal pore size 1.5 µm; Environmental Express, Mt. Pleasant, SC, USA) within four days of collection and immediately dried and weighed.

Samples for dissolved nutrient analyses were filtered through 0.4 µm-polycarbonate filters under vacuum pressure on the same day as collection and frozen (-20 °C) until analyses. Nutrients were measured in surface water samples with a Technicon® Autoanalyzer (Tarrytown, NY, USA) using

colorimetric detection (American Public Health Association and others, 2005). Total ammonia (NH_3) was analyzed using a salicylate/nitroprusside colorimetric reaction. Samples for total phosphorous (TP) and total nitrogen (TN) were digested in sodium hydroxide and potassium persulfate then analyzed using the automated ascorbic acid method for phosphate and the automated Cd reduction method for nitrate/nitrite (American Public Health Association and others, 2005). Dissolved organic carbon (DOC) was analyzed using a persulfate/UV digestion followed by colorimetric analysis of CO_2 using a Technicon® Autoanalyzer.

Method detection limits (MDLs) for water quality variables are listed in table 3. Recovery of reference standards used as laboratory control samples for these water quality analyses ranged from 96–107%. Instrumental precision, estimated by relative percent differences (RPDs) for replicate sample analyses ranged from 0–35%, with the exception of one replicate for TN (100%), two replicates for TP (81%, 85%), and three replicates for NH_3 (-73%, 62%, and 62%). Overall, detection and recovery of reference standards used as laboratory control samples for surface water quality parameters were within acceptable criteria, thus none of the sample results were corrected.

Determination of metal concentrations

1. Water

Surface and in-situ (from peepers) pore-water samples were analyzed for Co, Ni, Cu, Zn, Cd, and Pb by inductively-coupled plasma-mass spectrometry (ICP-MS; Brumbaugh and others, 2007; May and others, 1997). Method detection limits for metal analyses in water samples are listed in table 3. Percent recovery of calibration verification standards ranged from 93% to 97%. Percent recovery of reference solutions used as laboratory control samples ranged from 93% to 100%. Percent recovery of analytical spikes ranged from 89% to 97%. Relative percent differences between duplicate analyses of

pore-water samples ranged from 0.1% to 1.6%. As a check for potential interferences, dilution percent differences (DPDs) based on 5X dilutions of the biota sample digestates were determined; DPDs were within the suggested acceptance tolerance of 80–100% except for Ni (148%) and Zn (129%). Blank-equivalent concentrations (BECs) for digestion blanks were less than corresponding MDLs; therefore sample results were not corrected for BECs. Of the 51 field-collected samples analyzed, measured concentrations did not exceed the MDLs in three (6%) samples for Cu, seven (14%) for Zn, and 17 (33%) for Cd. Overall, quality assurance results indicated that the methods used provided acceptable accuracy and precision, thus none of the sample results were corrected.

The risk of toxic effects from metals (Ni, Cu, Zn, Cd, Pb) in surface and in-situ pore water was estimated using the toxic unit approach (Wildhaber and Schmitt, 1996). A toxic unit (TU) is defined as the measured concentration of each dissolved metal in pore water divided by its chronic surface water quality criterion (WQC), adjusted for hardness and the dissolved fraction of metal (U.S. Environmental Protection Agency, 2006). Although the WQC were developed for surface water, they are also reasonable estimates of the toxicity of pore waters to aquatic organisms (Wildhaber and Schmitt, 1996). There is currently no WQC for Co. Toxic units for metals are summed to produce a total toxicity estimate of the mixture (that is, toxic unit score, $\sum$TUs) for each sample, with values greater than 1.0 indicating potential toxicity to aquatic biota.

2. Biota and detritus

Detritus, macro-invertebrates, stonerollers, and whole riffle (wild) and caged crayfish from each site were analyzed for Co, Ni, Cu, Zn, Cd, and Pb by ICP-MS (Besser and others, 2006; Brumbaugh and others, 2005). Animal tissues 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. A dry mass of 0.25 g from each composited sample was digested

using concentrated nitric acid and microwave heating. 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. Method detection limits for biota and detritus are listed in table 3 and all measured concentrations exceeded the MDLs.

Recoveries of the elements from reference materials (fish, mussel, oyster, plant, and plankton) ranged from 76% to 129%. Relative percent differences for replicate analyses were <23% for all elements. Recoveries of method spikes for all six metals in separate spiked samples of all the sample types analyzed averaged 94%. Overall, quality assurance results indicated that the methods used provided acceptable accuracy and precision; therefore none of the sample results were corrected for recovery.

3. Sediment

Sediments from composite samples were analyzed for total recoverable metals, acid-volatile sulfide (AVS), and simultaneously-extracted metals (SEM). Total recoverable metals in fine sediment were analyzed by semi-quantitative multi-element ICP-MS scans. Recoveries of the metals of concern (Co, Ni, Cu, Zn, Cd, Pb) from reference sediment as measured by ICP-MS semi-quantitative scan ranged from 104% to 112%. Instrumental precision estimated by RPDs for replicate analyses of sediment were <3% for all metals of concern. Percent recovery for metals of concern in sediment reference materials ranged from 78% to 104%. Recoveries of method spikes for all six metals in separate spiked sediment samples analyzed averaged 95%. Blank-equivalent concentrations for digestion blanks were less than corresponding MDLs; therefore sample results were not corrected for

BECs. Overall, quality assurance results indicated that the methods used provided acceptable accuracy and precision, thus none of the sample results were corrected.

The SEM extracts were analyzed for five metals (Ni, Cu, Zn, Cd, Pb) by quantitative ICP-MS. Method detection limits for AVS and SEM in sediment are listed in table 3. All measured concentrations exceeded the MDLs. Recoveries of elements from calibration standards as measured by SEM quantitative method ranged from 95% to 104%. Percent recovery of sulfide in 1 N HCl extracted sediment reference materials was 100%. Instrumental precision estimated by RPDs were <2% for elements and <13% for duplicate 1 N HCl extraction of a sediment sample. Percent recovery for elements in reference materials ranged from 78% to 104%. Percent recovery of sulfide in 1 N HCl extracted blanks ranged from 98% to 100%. Recoveries of method spikes for all five metals in separate spiked samples analyzed averaged 100%. Overall, quality assurance results indicated that the methods used provided acceptable accuracy and precision, thus none of the sample results were corrected.

Statistical analysis

Statistical analyses were conducted using Statistical Analysis System (SAS) for Windows (Release 9.1; SAS Institute, Cary, NC, USA). Censored values (< MDL) for metal concentrations in water were replaced with 50% of the MDL for statistical computations, figures, and tables. All censored data were in samples from reference sites. Two values (8%) for concentrations of Cu in surface water; six values (23%) for Zn in surface water, and six values (23%) for Cd in surface water were censored. Three values for Zn in pore water (13%) and 11 values for Cd in pore water (48%) were censored. Prior to analyses, data were tested for normality and homogeneity of variance. Data were not normally distributed; therefore data were rank transformed prior to statistical analyses.

Survival of caged crayfish on day 56, riffle crayfish density, and the overall means for water quality, physical habitat, and metal concentrations were used in the statistical analyses. Differences in

caged crayfish survival and riffle crayfish density among sites and groups of sites were tested using nested analysis-of-variance (ANOVA; cages nested within site), with site considered a fixed effect. Differences in caged crayfish survival and riffle crayfish density among groups of sites were tested as planned non-orthogonal contrasts using single degree-of-freedom *F*-tests. The mean squares for caged crayfish survival and riffle crayfish density within a site were used in all tests. Differences in caged crayfish survival and riffle crayfish density among individual sites were also evaluated with Duncan's multiple range test. Differences in water quality, physical habitat measurements, and metal concentrations among groups of sites were tested using the same procedures. Finally, associations among riffle crayfish density, selected water quality and physical habitat variables, and metal concentrations were examined with Spearman's correlation. We examined the relationship among riffle crayfish densities and selected water quality and physical habitat variables, and concentrations of Pb in crayfish, surface water, and sediment using principal component analyses (PCA). Lead concentrations were selected to be representative of all metals because concentrations of metals (except Cu) were highly correlated ($r > 0.75$; $P < 0.05$) in the materials analyzed. We also used PCA to examine the relationship among survival and CL of caged crayfish with selected water quality variables and Pb concentrations in crayfish, pore water, detritus, other macro-invertebrates, and fish. Data used for cage survival and CL were the combined means of both species on day 56. A significance level of $P < 0.05$ was used to judge all statistical tests.

Results

In-situ toxicity test

1. Number, sex ratio, and size of caged crayfish

Number of surviving *O. hylas* and *O. luteus*, sex ratio, mean CL, wet weight, and biomass at day 28 and day 56 are listed in table 4. Sex ratios were approximately 1:1 for both species on day 56; however, for those crayfish collected on day 28, there were 1.5–2.5 times more male crayfish present at reference sites than at mine sites. Crayfish survival on day 28 was >90% at all sites, implying that the sex ratio at the reference sites at the time of stocking, which was not determined, was skewed. Survival of both species at day 56 was significantly lower at mining sites than at reference sites (table 4). Day-56 survival of both species at R1 and R2 was >98%; however, survival at day 56 of both species at TH1 and TH2 was 65–73%.

Both mean CL and wet weight of surviving caged crayfish increased at all sites during the 56-d in-situ toxicity test. Mean CL and wet weight of *O. hylas and O. luteus* at day 56 were significantly greater at mining sites than at reference sites; however, biomass of either species at day 56 was not significantly different among reference and mining sites (table 4). Growth rates (change per day in CL and wet weight) for both species at all sites were higher during the first 28 days of the test than the second 28 days; however, the percent change in CL and wet weight for both species at all sites was greater at day 56 than at day 28 (table 5). After 56 days, the greatest change per day and percentage change in CL and wet weight of *O. hylas* occurred at TH1, whereas they were highest for *O. luteus* at TH2.

Growth of caged *O. luteus* was greater than that of caged *O. hylas*. The increase in CL of *O. luteus* was 2–3.5 times higher and the increase in wet weight of *O. luteus* was 3–6 times higher than that

of *O. hylas* (table 5). The difference in the increase in CL or wet weight between the two species at day 56 was not significantly different among sites except at TH1. The difference in the increase in CL between the two species was only twice as high at TH1, whereas it was three-fold higher at R1, R2, and TH2. The difference in weight was five-fold higher at RI, R2, and TH2, whereas it was only three-fold higher at TH1.

2. Concentration of metals in caged crayfish

Concentrations of metals in caged *O. hylas* (table 6) and *O. luteus* (table 7) were significantly higher at mining sites than at reference sites. Concentrations of metals in both species were generally higher at TH2. Concentrations of Pb (97–272 µg/g) in both species were 100 to 200 times higher at mining sites than at reference sites, whereas all other metals except Cu were three to six times higher in both species at mining sites than at reference sites. Metal concentrations in both species reached concentrations comparable to riffle (wild) crayfish by day 28 of the in-situ study for all metals except Cd (fig. 2).

Riffle (wild) crayfish density, species richness, sex ratio, and size

Crayfish were collected at all eight sites sampled (table 8). Riffle densities ranged from 1.0/m^2 at TH1 to 12.7/m^2 at R1 and were significantly lower at mining sites than at either reference or downstream sites (table 8). Riffle crayfish densities were inversely related with metal concentrations in riffle crayfish (fig. 3). Riffle crayfish densities decreased with increasing metal concentrations and proximately to mining inputs; however densities remained significantly lower at downstream sites than at reference sites.

One specimen of *Cambarus diogenes* and one unknown crayfish species were collected at R1; however, they were not included in data summaries or analyses. Four species of crayfish were collected

at both reference sites (*Orconectes harrisoni*, *O. hylas*, *O. luteus*, *Orconectes medius*); two species (*Orconectes harrisoni*, *O. luteus*) were collected at mining sites, and four species (*O. harrisoni*, *O. luteus*, *O. medius*, *Orconectes virilis*) were collected at downstream sites (table 9). *Orconectes luteus* were collected at all sites and were the most abundant (table 9). *Orconectes hylas* were only collected at reference sites, whereas *O. virilis* were only collected at downstream sites (table 9). Mean CL of *O. luteus* were also significantly greater at reference sites (group mean =14.3 mm) compared to mining sites (group mean =11.1 mm; table 9).

No additional species of crayfish were collected by trapping in other habitats (for example, pools, backwaters, emergent vegetation patches) present at the sites (table 10). Four species of crayfish were collected by trapping at reference sites (*O. harrisoni*, *O. hylas*, *O. luteus*, and *O. virilis*) and three species at downstream sites (*O. harrisoni*, *O. hylas*, and *O. virilis*). Only one species (*O. virilis*) was collected by trapping at mining sites. *Orconectes luteus* was the most abundant species collected in traps at reference sites; however *O. virilis* was the most common species collected in traps at mining and at downstream sites. Catch per unit effort (CPUE) of baited trapping at reference sites (group mean =0.17) was about twice that of downstream (group mean =0.10) and mining sites (group mean =0.07); however CPUE (0.23) was highest at TL1.

Number, sex ratio, size, and metal concentrations in riffle (wild) crayfish

Number, sex ratio, and mean CL of riffle (wild) *O. luteus* collected in riffles for the composite metal samples are listed in table 11. Sex ratios were approximately 1:1 at all sites. Carapace lengths of crayfish in composite metal samples were significantly greater at reference sites than at mining or downstream sites. The total number of crayfish in composite metal samples was higher at downstream sites due to their smaller size. Metal concentrations in wild *O. luteus* did not differ significantly between

genders; however Pb concentrations did differ significantly with CL of wild *O. luteus* collected for composite metal samples. Crayfish with shorter CL had higher Pb concentrations at each site.

Metal concentrations in wild *O. luteus* collected in riffle habitats differed significantly among sites. Concentrations of all six metals in wild *O. luteus* were significantly higher at mining sites than in wild *O. luteus* at reference sites (table 12). Concentrations of Co, Ni, and Zn in wild *O. luteus* were about three to four times higher at mining sites than at reference sites, whereas Cd concentrations in wild *O. luteus* were about 50 times higher at mining sites than at reference sites (table 12). Lead concentrations in wild *O. luteus* at mining sites were more than 130 times higher than in wild *O. luteus* at reference sites (table 12). Concentrations of Ni, Zn, and Pb were higher in wild *O. luteus* collected at TH1 than at TH2, but these differences were not statistically significant (table 12).

Concentrations of metals generally declined in wild *O. luteus* with increasing distance from mining inputs; however, concentrations of Cu in wild *O. luteus* at TL2 (river km 119/river mile 74) were significantly different than reference sites and were 1.5 to 1.6 times higher than concentrations of Cu in wild *O. luteus* at either reference site (fig. 3, table 12). Concentrations of Co, Ni, Zn, Cd, and Pb in wild *O. luteus* at TL2 were not significantly different than concentrations at either reference site (table 12); however, concentrations of Co, Ni, Zn, Cd, and Pb in wild *O. luteus* at other downstream sites were significantly different and about 1.2 to 69 times higher than at reference sites (table 12).

Physical habitat measurements

Mean substrate size in riffles was significantly greater at reference sites than at either downstream or mining sites, and mean substrate size class in riffles was significantly greater at downstream sites than at mining sites (table 13). However, substrate at all sites was classified as either gravel or pebble. Riffle substrate homogeneity (indicated by low standard deviations of substrate size class) in riffles was significantly higher at mining sites than at downstream or reference sites (table 13).

Mean depth and current velocities of riffles sampled were significantly greater at downstream sites than at reference or mining sites (table 13). Current velocities at mining sites were significantly greater than velocities at reference sites.

Substrate size within the 1-m^2 quadrat sampler (that is, quadrats) was similar to that in riffles. Mean substrate size was significantly greater in quadrats at reference sites than in quadrats at mining or downstream sites and was also significantly greater in quadrats at downstream sites than in quadrats at mining sites (table 13). Substrate in quadrats at all sites was classified as either gravel or pebble. Substrate homogeneity was significantly lower in quadrats at mining sites than in quadrats at downstream sites, but not in quadrats at reference sites (table 13). Depth was significantly higher in quadrats at downstream sites than in quadrats at mining or reference sites (table 13). Current velocities at both depths were significantly higher in quadrats at downstream sites than in quadrats at reference sites. Current velocities at mid-water were significantly higher in quadrats at downstream sites than in quadrats at mining sites; however current velocities near the substrate were not significantly higher in quadrats at downstream sites than in quadrats at mining sites.

Substrate size near cages was significantly greater at reference sites than at mining sites; however, substrate homogeneity was significantly lower at reference sites than at mining sites (table 13). Substrate near cages was classified as gravel or pebble at all sites except TH2, where it was classified as sand. Mean depth near cages at reference sites was not significantly different than at mining sites (table 13). Mean current velocities at mid-water were not significantly higher at mining sites than at reference sites; however current velocities near the substrate were significantly different among mining and reference sites (table 13). Current velocities were significantly lower near cages than in riffle habitats where quadrat samples were taken.

Water quality measurements

General in-situ water chemistry differed significantly between the types of sites in both surface and pore waters (table 14). Mean surface-water temperature was significantly greater at downstream sites than at reference or mining sites (table 14); however pore-water temperature did not differ significantly among sites. Surface-water temperature was highest at TL2 (29 °C), but was below the maximum Missouri water quality criterion (32 °C; Missouri Department of Natural Resources 2009) for warm-water fisheries. The pH of surface water and pore water were similar at all sites and were within the Missouri water quality criterion set for pH (6.5–9; Missouri Department of Natural Resources 2009) for warm-water fisheries (table 14). Conductivity of surface water and pore water were significantly higher at mining and at downstream sites than at reference sites (table 14). Surface-water conductivity was highest at TM1 (529 µS/cm), whereas pore-water conductivity was highest at TH2 (622 µS/cm). Dissolved oxygen in surface water was significantly higher at mining sites than at reference or downstream sites (table 14). Dissolved oxygen at all sites exceeded the minimum Missouri water quality criterion for warm-water fisheries of 5 mg/L (Missouri Department of Natural Resources 2009). Turbidity in surface water was significantly higher and more variable at mining sites, notably TH2, than at either downstream or reference sites (table 14). Turbidity at all sites was greater than the aggregate reference value (2.3 NTU; based on the 25th percentile) for streams in the ecoregion; however, only turbidities at TH2 (9.8 NTU), TM2 (6.1 NTU), TL1 (8.2 NTU), and TL2 (9.4 NTU) were outside the range of reference values (1–5.2 NTU; based on the 25th percentile) in streams of the ecoregion (Missouri Department of Natural Resources 2009).

Alkalinity in surface water was significantly higher at downstream sites than at either mining or at reference sites and significantly greater at mining sites than at reference sites. Pore-water alkalinity were not significantly different among sites (table 15). Alkalinity values at all sites and for both water

types exceeded the minimum Missouri water quality criterion of 20 mg $CaCO_3$/L (USEPA 2006). Hardness and sulfate concentrations in surface water and pore water were significantly higher at mining sites than at reference sites. Hardness in surface water was highest at TM1 (307 mg $CaCO_3$/L) and in pore water at TH2 (311 mg $CaCO_3$/L). Hardness values of surface water and pore water at mining and downstream sites was greater than the aggregate values (200 mg $CaCO_3$/L; based on the 25th percentile; Missouri Department of Natural Resources 2009) for measured samples. Sulfate concentrations in surface water were higher at downstream sites than at reference sites (table 15). Sulfate concentrations in surface water (96 mg SO_4/L) and pore water (114 mg SO_4/L) were highest at TH2. Sulfate concentrations in surface water and pore water at all sites were less than the maximum Missouri water quality criterion of 1000 mg SO_4/L (Missouri Department of Natural Resources 2009).

Nutrient, carbon, and suspended solids in surface water and pore water differed significantly among types of sites. Total nitrogen and TP in surface water were both significantly higher at mining and at downstream sites than at reference sites (table 16). Ammonia concentrations in surface water were below the detection limit (0.014 mg/L) and the detectable ammonia concentrations in pore water were not significantly different among sites. None of the measured values for TN, TP, or NH_3 exceeded the maximum Missouri water quality criteria (Missouri Department of Natural Resources 2006; Missouri Department of Natural Resources 2009). The highest TN concentration in surface water was measured at TM1 (0.32 mg N/L); however it was only 36% of the Missouri water quality criterion of 0.9 mg N/L. The highest TP concentration in surface water was measured at TH2 (58.8 µg P/L), which were 78% of the criterion. The highest concentration of NH_3 in pore water was measured at TH2 (0.27 mg N/L) and was <50% of the range of NH_3 concentrations calculated using the Missouri criterion.

Mean chlorophyll *a*, TSS, and POC in surface water were significantly higher at downstream sites than at reference sites; however, there were no significant differences among individual sites for

POC (table 17). Measured concentrations of chlorophyll *a* at all sites did not exceed the Missouri water quality criterion (81 mg C/L; Missouri Department of Natural Resources 2006). In contrast, measured concentrations of TSS at all sites exceeded the aggregate reference value (2.5 mg/L; based on the 25th percentile; Missouri Department of Natural Resources 2009). The highest concentrations of TSS, which were measured at TM2 (12 mg/L) and TL2 (12 (mg/L), were four-fold higher than the criterion. Surface-water DOC was significantly higher at mining sites than at reference or downstream sites; however there was no significant difference in pore-water DOC among reference and downstream sites (table 17).

Metal concentrations in water, detritus, biota, and sediment

1. Water

Metal concentrations in surface water and pore water differed significantly among sites (table 18). Concentrations of metals in surface water and pore water were similar; however metal concentrations were generally higher in pore water (table 18). Concentrations of all six metals in surface water and pore water were significantly higher at mining sites than at downstream and at reference sites (table 18). Metal concentrations in surface waters were generally highest at TH2; however, concentrations of metals in pore waters were generally highest at TH1. Metal concentrations in surface water and in pore water at mining sites were 0.4 to 100 times higher than at reference sites and were highest for Pb, Zn, and Cd. Crayfish densities were inversely related to metal concentrations in surface water (fig. 4).

Concentrations of Pb (7–20 µg/L) and Cd (0.7–1.2 µg/L) in both surface water and pore water exceed the Missouri (Missouri Department of Natural Resources 2009), Big River watershed (Missouri Department of Natural Resources 2007), and USEPA water quality criteria (U.S. Environmental

Protection Agency 2006); however concentrations of Ni, Cu, and Zn in surface and pore waters did not exceed those criteria. There are no water quality criteria for Co. The total toxicity estimate of the mixture or chronic toxic unit scores (∑TUs) for all six metals were higher in pore water than in surface water. Chronic toxic unit scores in pore water and in surface water were significantly higher at mining sites than at reference or at downstream sites (table 19). Chronic toxic unit scores for surface and for pore waters at mining sites and at downstream sites were greater than or near one, indicating potential risk to aquatic biota. Chronic toxic unit scores for both surface water and pore water were highest at TH1.

2. Detritus and biota

Concentrations of metals in detritus, macro-invertebrates, and stonerollers differed significantly among types of sites. Concentrations of all six metals in detritus and macro-invertebrates were significantly higher at mining sites than at reference sites (table 20) and were generally highest at TH2. Concentrations of metals in detritus were five to 204 times higher at mining sites than at reference sites, and were highest for Zn, Pb, and Cd. Concentrations of metals in macro-invertebrates were 1.2 to 96 times higher at mining sites than at reference sites, and were highest for Pb, Cd, Co, and Zn.

Mean total length of stonerollers were significantly greater at mining site than at reference sites (table 21). Mean concentrations of all six metals in stonerollers were significantly higher (three to 75 times) at mining sites than at reference sites (table 21). Concentrations of metals in stonerollers were generally highest at TH2; however, concentrations of Zn, Cd, and Pb were not significantly different between TH1 and TH2.

3. Sediment

The percent of total organic carbon (TOC) in sediments ranged from 0.13% to 4.1% and was higher at mining and at downstream sites than at reference sites, with the exception of sediment collected at TL1 (table 22). Sediments from TL1, TH2, and R2 had the highest percentage of sand, whereas sediments from TM2 had the highest percentage of silt., and R1 had the highest percentage of silt (table 22).

Concentrations of total recoverable (TR) elements in sediments were generally highest at TH2; however, concentrations of Ba (2000 µg/g) and Cu (30 µg/g) were highest at TM2 (table 23). Concentrations of Zn, Cd, and Pb were 35 to 450 times higher at mining sites than at reference sites, and six to 200 times higher at downstream sites than at reference sites.

Percent water, percent loss on ignition, and AVS in sediment were generally higher at downstream sites than at reference or mining sites (table 24). Concentrations of SEM were generally lower than TR metal concentrations; however, the relative ranking of concentrations of metals across sites was similar for both methods. Simultaneously-extracted metal concentrations were higher at mining and downstream sites than at reference sites. The ratio of ∑SEM to AVS was less than one and the ∑SEM-AVS was less than zero at reference sites, indicating that metals in these sediments should not cause direct toxicity to benthic organisms. However, the ratio of ∑SEM to AVS was greater than one and ∑SEM-AVS was greater than zero at all mining and downstream sites, except for TL2, indicating bioavailability of metals to aquatic biota. The proportional contribution of metals to the total SEM (∑SEM) was similar across sites, with Pb and Zn contributing the greatest percentages at all sites (table 24). Nickel and Cu each contributed 5–8% of ∑SEM at the two reference sites, but the contribution of these metals at mining and at downstream sites was negligible (table 24).

Associations among riffle (wild) crayfish density and size, and physical and chemical variables

Spearman correlation analyses were conducted with riffle crayfish density and CL, selected surface-water quality and physical habitat variables, and metals concentrations in riffle crayfish, surface water, and sediment (n =8). Mean CL and density of riffle crayfish were significantly correlated (r =0.93). Riffle crayfish density and mean CL of riffle crayfish were significantly negatively correlated with all metal concentrations in crayfish except Cu (table 25). All metal concentrations except for Cu in crayfish were significantly inter-correlated (table 25, n =8).

Metals concentrations except for Cu in surface water were significantly inter-correlated (r-values >0.71) and were also significantly correlated with the ∑TUs (r-values >0.88). Conversely, ∑TUs were significantly negatively correlated with riffle crayfish density (r =-0.95) and mean CL (r =-0.91). Riffle crayfish density (r =-0.83) and mean CL of riffle crayfish (r =-0.86) were significantly negatively correlated with sulfate concentrations. Sulfate concentrations were significantly correlated with conductivity (r =0.86), hardness (r =0.86), TN (r =0.71), TP (r =0.71), and surface-water DOC (r =0.73). Chlorophyll *a* concentrations were significantly correlated with TP (r =0.90) and were significantly negatively correlated with the ratio of TN/TP ratio (r =-0.74). Surface-water DOC was significantly correlated with TN (r =0.76).

Riffle crayfish density was significantly negatively correlated (r-values >-0.74) with concentrations of all five metals measured in sediment (table 25). Mean CL of riffle crayfish was also significantly negatively correlated (r-values >-0.74) with all metals except for Zn in sediment. Concentrations of all metals except for Zn in sediment were significantly inter-correlated (r-values >0.80). Metal concentrations in sediment were significantly correlated with TOC (r-values >0.76). Substrate homogeneity in riffles was significantly correlated (r-values >0.74) with all metal concentrations except Cu in sediment. Substrate size in quadrat samples was significantly negatively

correlated with all metal concentrations except Cu in sediment (r-values >-0.74). Riffle crayfish density was significantly correlated with substrate class size in quadrats (r =0.90), but significantly negatively correlated with substrate homogeneity (r =-0.83). Mean CL of riffle crayfish was significantly correlated with substrate size in riffles (r =0.79) and substrate size in quadrats (r =0.97), but significantly negatively correlated with riffle substrate homogeneity (r =-0.93).

Principal components analyses

An interpretable ordination of riffle crayfish density, selected water quality and physical habitat variables, and Pb concentrations in surface water, sediment, and crayfish was obtained by PCA, with the first two axes explaining more of the variability than expected by chance alone (fig. 5a). The plot showed that the reference sites (R1 and R2) and mining sites (TH1 and TH2) were grouped separately from all other sites. Axis 1 (48%) and axis 2 (30%) accounted for 78% of the total variance among sites (fig. 5a). The variables with high factor loadings (>±0.29) on axis 1 were substrate homogeneity (-0.32), substrate size (-0.31), density of riffle crayfish (-0.31), CL of riffle crayfish (-0.30), sulfate concentrations in surface water (0.31), Pb (metals) concentrations in surface water (0.30), and Pb (metals) concentrations in riffle (wild) crayfish (0.29). The variables with highest positive factor loadings on axis 2 (>0.35) were surface-water temperature (0.37), distance downstream of the Desloge pile (or river km/mile; 0.36), mid-water velocity (0.36), and TSS (0.35). The PCA plot grouped reference and mining sites separately, the result of an inverse relationship between riffle crayfish density and Pb (metals) concentrations in surface water and in riffle crayfish. Downstream sites were grouped separately from reference and mining sites based on surface-water temperature, distance downstream of the Desloge pile (or river km/mile), mid-water velocity, and TSS.

A second interpretable ordination of caged crayfish survival, selected water quality variables, and Pb (metals) concentrations in caged crayfish, pore water, detritus, fish, and other macro-

invertebrates was obtained by PCA, with the first two axes explaining more of the variability than expected by chance alone (fig. 5b). Axis 1 (84%) and axis 2 (13%) accounted for 97% of the total variance among sites (fig. 5b). The variables with high factor loadings on axis 1 (>±0.30) were caged crayfish survival (-0.30), Pb (metals) concentrations in detritus (0.31), Pb (metals) concentrations in other macro-invertebrates (0.31), Pb (metals) concentrations in fish (0.32), Pb (metals) concentrations in caged crayfish (0.31), distance downstream of the Desloge pile (or river km/mile; 0.31), sulfate concentrations in surface water (0.31), and CL of caged crayfish (0.30). The factor loadings for all variables were positive except survival of caged crayfish. The variables with highest factor loadings on axis 2 (>±0.46) were pore-water temperature (0.52), Pb (metals) concentrations in pore water (0.50), and TP (-0.46). The PCA plot showed a separation between reference and mining sites, the result of an inverse relationship between caged crayfish survival and distance downstream of the Desloge pile (or river km/mile), Pb (metals) concentrations in detritus, fish, crayfish, and other macro-invertebrates, CL, and sulfate concentrations in pore water. Sites were further grouped relative to pore-water temperature, Pb (metals) concentrations in pore water, and TP.

Discussion

The biota of the Big River has been continuously exposed to mining-derived metals, including Pb, Zn, and Cd, for at least 20 years (Schmitt and Finger, 1987; Schmitt and others, 1984, 1993; Whelan, 1983). The results of this study which included an in-situ toxicity test, analyses of metals exposure, and a riffle (wild) crayfish population assessment, provided multiple lines of evidence that metals are negatively affecting crayfish in the Big River. Concentrations of mining-derived metals in all matrices evaluated at all sites downstream of mining areas, which included the Desloge tailings pile, were significantly higher than at sites upstream of mining areas. Our findings indicate that metals exposure and the uptake of metals by crayfish are still occurring in the Big River.

Our results are consistent with those of Besser and others (2009b) and Roberts and others (2009) who assessed the effects of metals concentrations on freshwater mussels populations in the Big River. Lead, Zn, and Cd concentrations in sediments collected from riffle-run habitats at sites directly downstream of mining areas were elevated and remained elevated through-out the length of the Big River. Concentrations of Pb, Zn, and Cd in riffle-run sediment were generally lower than concentrations of Pb, Zn, and Cd in our depositional sediments; however, sampling sites in both studies ranked the same for sediment metals concentrations. Variation in sediment metals concentrations between the two studies reflect spatial heterogeneity in metals in the Big River, collection techniques, and the size of the sediment fraction analyzed. Our results were also consistent with Besser and others (2006), who evaluated exposure of aquatic biota to Cd, Zn, and Pb in the Viburnum Trend mining district (VTMD). Although Pb concentrations in streams draining the VTMD were generally lower than those in the OLB, long-term Pb-mining activity in the VTMD has resulted in significantly elevated concentrations of Pb, Zn, and Cd in the food web. Concentrations of Pb, Zn, and Cd in plant biomass, invertebrates, and fish were significantly higher at sites in VTMD directly downstream of mining activities than those at reference sites.

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 macro-invertebrates, and stonerollers which were sampled in close proximity to the cages, and in Missouri saddled darters (*Etheostoma tetrazonum*) which were collected in the same riffles where wild crayfish were sampled (McKee and

others 2010). The results of the in-situ toxicity test offer direct evidence that mining-derived metals are toxic to crayfish in the Big River.

Growth of crayfish in cages was significantly greater at mining sites than at reference sites. Mean CL of caged crayfish at mining sites, especially TH2, was significantly larger than CL of caged crayfish at reference sites. Significant growth in CL and wet weight of crayfish occurs only with molting (Reynolds, 2002) when there is an intensive uptake of water into the blood after ecdysis and an increase in Ca^{2+} regulation (Holdich 2002, and references within). We hypothesize that greater growth of surviving crayfish at mining sites compared to reference sites occurred due to significantly higher nutrient (for example, DOC, TN, TP) concentrations and periphyton (estimated by chlorophyll *a*) observed at those sites. Periphyton (that is, the assemblage of organisms such as bacteria, algae, and small fauna that form on underwater surfaces) biodegrade organic matter and are a critical nutritional component for shredders such as crayfish (Cummins, 1977). The resulting growth and more frequent molting may have increased their exposure and sensitivity to metals (Knowlton and others, 1983; Wigginton and Birge, 2007), thus increasing mortality. Increased sensitivity could result in part from the influx of water and competition between metals (for example, Cd) and Ca^{2+} ions as the cuticle re-calcifies (Wigginton and Birge, 2007 and references within). The higher mortality in cages at mining sites suggest that the remaining caged crayfish would have experienced less competition for these food resources relative to caged crayfish at reference sites.

The effects of metals on free-ranging, wild populations of crayfish were evaluated at eight sites in the Big River 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, free-ranging riffle crayfish were significantly lower at mining and downstream sites compared to reference sites. Riffle crayfish densities were negatively correlated with mining-derived

metals measured in surface water, sediment, and riffle crayfish (wild *O. luteus*). In addition, mean CL of wild crayfish was negatively correlated with all metal concentrations in crayfish except Cu, which can be regulated by 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 (for example, dissolved oxygen and ammonia) or physical habitat variables.

Riffle crayfish densities at reference sites (10.9/m^2) were similar to those found in two Ozark streams where *O. luteus* was the predominant crayfish collected (DiStefano and others, 2003b). A large portion (>75%) of the crayfish populations found in those streams were first-year *O. luteus*. Early instar and juvenile (first-year) crayfish are more sensitive than adult crayfish to toxicants (Eversole and Seller, 1997; Wigginton and Birge, 2007) possibly due to the high number of molts during their first summer (Holdich, 2002). Heavy metals have been shown to affect populations of crayfish by the disturbance of the molting cycle and reproduction (Viikinkoski and others, 1995). Exposure to heavy metals has resulted in the disappearance of crayfish downstream of mining inputs (Allert and others, 2008; Kossakowski, 1973; Thorp and others, 1979), possibly due to the loss of first-year reproductively-mature females. Muck and others (2002) reported that first-year reproductively-mature females are critical to the sustainability of *O. luteus* populations.

Sublethal exposure to metals can cause changes in crayfish behavior including shelter-seeking or the escape response through tail-flipping. Alberstadt and others (1999) determined that there was a significant decrease in shelter use by *Orconectes rusticus* at concentrations of 1–3 mg Cd/L and sustained hyperactivity at 3 mg Cd/L. The reduced use of shelter by crayfish due to hyperactivity behavior such as tail-flipping may reduce survival because of the increased risk of predation, displacement or dislodgement (Clark and others, 2008).

Crayfish in streams have a vital role in nutrient cycling and can dominate energy flow (Whitledge and Rabeni 1997). Crayfish are omnivores that feed on detritus, macro-invertebrates, and fish depending on availability and life-stage (Hobbs, 1993; Whitledge and Rabeni, 1997). They are important processors of benthic algae in these streams (Rabeni et al. 1995) and coarse particulate organic matter (Momot, 1995; Parkyn and others, 2001; Whitledge and Rabeni, 1997). In many Ozark streams, they convert more coarse particulate organic matter into usable energy for other organisms than all other invertebrates combined (Rabeni et al. 1995; Whitledge and Rabeni 1997). The absence of crayfish due to metal contamination may have negative effects on organic material (for example, leaves, woody debris) processing, nutrient cycling, and energy transfer in Ozark streams. Crayfish are an important prey item for fish, other aquatic and terrestrial invertebrates, amphibians, reptiles, birds, and mammals (DiStefano, 2005; Hobbs, 1993), and are the predominant prey item of smallmouth bass (*Micropterus dolomieu*), an important species sought by recreational anglers (Mayers, 2003; Weithman, 1991) and of other centrarchid fishes (DiStefano, 2005; Probst and others, 1984; Rabeni and others, 1995). Reduced crayfish populations may therefore translate to less available prey for sport fishes.

Schmitt and others (2008) developed conservative screening-level criteria for the assessment of potential adverse effects in wildlife from metals in aquatic invertebrates, which we have adapted to assess the potential effects in wildlife from metals in crayfish. Toxicity thresholds were determined through food chain analysis using procedures developed for conducting ecological risk assessments (U.S. Environmental Protection Agency, 1992, 1997, 1999, 2007). Risk analysis for warm-blooded vertebrates is based on food chain analysis, where diet is the only exposure pathway considered in the screening-level assessments. The assessments assume that five metals (Co, Ni, Zn, Cd, Pb) act independently and that daily ingestion rates for this assessment comprise a diet of 100% crayfish. Food-chain analysis has not yet been extended to cold-blooded vertebrates. The no-effect hazard

concentrations (NEHC) for warm-blooded vertebrates are lower than published toxicity benchmarks for cold-blooded vertebrates (Schmitt and others, 2008 and references therein), with the exception of Cd. James and others (2004) reported reduced survival in American toads (*Bufo americanus*) fed a diet of earthworms with Cd concentrations as low as 4.7 µg/g dry-weight. This value is virtually identical to the lowest NEHC computed for homeotherms (4.8 µg/g dry-weight in crayfish, the robin model; table 26), indicating that a lower NEHC ultimately may need to be developed for Cd. The minimum and maximum concentrations of each metal measured in either wild or caged crayfish at all eight sites are listed in table 26. Hazard quotients that exceed 1.0 indicate risk to warm-blooded wildlife.

Our hazard quotients exceeded one for Zn in small birds (robin; *Turdus migratorius*; values =2); for Cd in small birds (robin; values =2–5) and in small mammals (shrew; *Blarina brevicauda*; values range from 1–4), and for Pb in both small (robin; values range from 32–64) and large birds (heron; *Ardea herodias*; values range from 4–8) and in small (shrew; values range from 5–9) and large mammals (American mink, *Mustela vison*; values range from 1–2). Hazard quotients were higher for birds than mammals in their respective size category. Hazard quotients indicate that small and large migratory birds such as heron, king rail (*Rallus elegans*), wood duck (*Aix sponsa*), belted kingfisher (*Megaceryle alcyon*), killdeer (*Charadrius vociferous*), brown thrasher (*Toxostoma rufum*), and great horned owl (*Bubo virginianus*) would be adversely affected due to the ingestion of crayfish, thus injuring trust wildlife species. Although hazard quotients were based on a 100% diet of crayfish, metals concentrations, particularly Pb, Cd, and Zn, in other macro-invertebrates and fish were similar to those in wild or caged crayfish. Therefore, hazard quotients based on a mixed diet would most probably indicate that birds and mammals are at similar risk of metal exposure.

Stream communities, in the absence of metals, have been shown to vary predictably with downstream gradients in abiotic factors such as stream width, stream gradient, and substrate size

(Vannote and others, 1980). In general, species richness tends to increase with increasing stream size due to several factors including increased average depth, habitat heterogeneity, and the influence of immigration from tributaries. However, longitudinal changes in crayfish species richness and density have not been adequately studied.

The relationship in streams between substrate size and crayfish is not clearly understood. Many species of crayfish have been associated with rubble or cobble-size substrate (adult *Orconectes punctimanus*−Rabeni 1985; young-of-year *Orconectes neglectus*−Gore and Bryant 1990); however, other species are reported to be associated with smaller-sized substrate or have no clear association (adult *O. luteus*−Rabeni 1985; adult *O. neglectus*−Gore and Bryant 1990). Crayfish abundance has also been shown to be positively associated with substrate size (Lodge and Hill, 1994; Olsson and Nyström, 2009; Parkyn and Collier, 2004) and particle size diversity (Flinders and Magoulick, 2007). Larger substrate particle size support higher numbers and species richness of crayfish due to greater microhabitat availability, retention of organic matter, and greater refugia to escape predators and moderate to high flow conditions (DiStefano and others, 2003b; Clark and others, 2008).

Substrates at sites immediately downstream of mining inputs in the Big River (TH1 and TH2) had statistically significantly smaller grain size and were more homogeneous than at reference sites; however substrate at all sampled sites was classified as either gravel or pebble. Average substrate size in the Big River might be comparatively small for Ozark streams based on previous studies of Ozark stream crayfish (Riggert et al. 1999; DiStefano 2003a; DiStefano et al. 2008) which indicated that pebble and cobble were the dominant substrate grain size present in other Ozark streams. Despite the need for additional research into the relationship of substrate particle size and stream crayfish populations, the results of our in-situ toxicity test offer direct evidence that it is the metals associated

with mining inputs that are adversely affecting wild crayfish populations in the Big River, not the smaller, more homogeneous substrate.

Roberts et al. (2009) showed significant positive correlations between both mussel species richness and mussel catch per unit effort (CPUE) with substrate embeddedness, sediment deposition, and channel flow status. These associations imply that mussel species richness and CPUE were higher at sites with less fine sediment, lower embeddedness, and lower channel aggradation. Roberts et al. (2009) noted the presence of mine tailings at sites directly downstream of mining areas; however these materials are generally similar in size to sand particles. Sand particles mixed with gravel have been shown to support diverse mussel poplulations unless contaminanted with metals (Buchanan 1979; Roberts and Bruenderman 2001), thus substrate particle size or habitat at sites directly downstream of mining areas is not limiting for mussel popuations in the Big River.

Conclusions

Previous studies (Czarnezki, 1985; Dwyer and others, 1988; Schmitt and Finger, 1987; Schmitt and others, 1984, 1993, 2007a, 2007b; Whelan, 1983) have documented elevated metal concentrations in aquatic biota in the OLB. Our results indicate that metals and mining-related wastes (that is, tailings) are adversely affecting crayfish in the Big River. Our findings are similar to those reported for crayfish in the VTMD, where mining-derived metals are also negatively affecting crayfish populations (Allert and others, 2008, 2009). High metal concentrations in centrarchids probably reflect high metal concentrations in crayfish and also may represent a hazard to wildlife (Schmitt and others, 2006, 2008). The loss of crayfish populations in the Big River have most likely impacted highly-valued sport fish populations (for example, changes in growth rates, survival, and condition of smallmouth bass). More broadly, our study indicates that the function of the Big River ecosystem has been compromised due to the loss of crayfish which are a key ecological component of Ozark streams and ecosystems.

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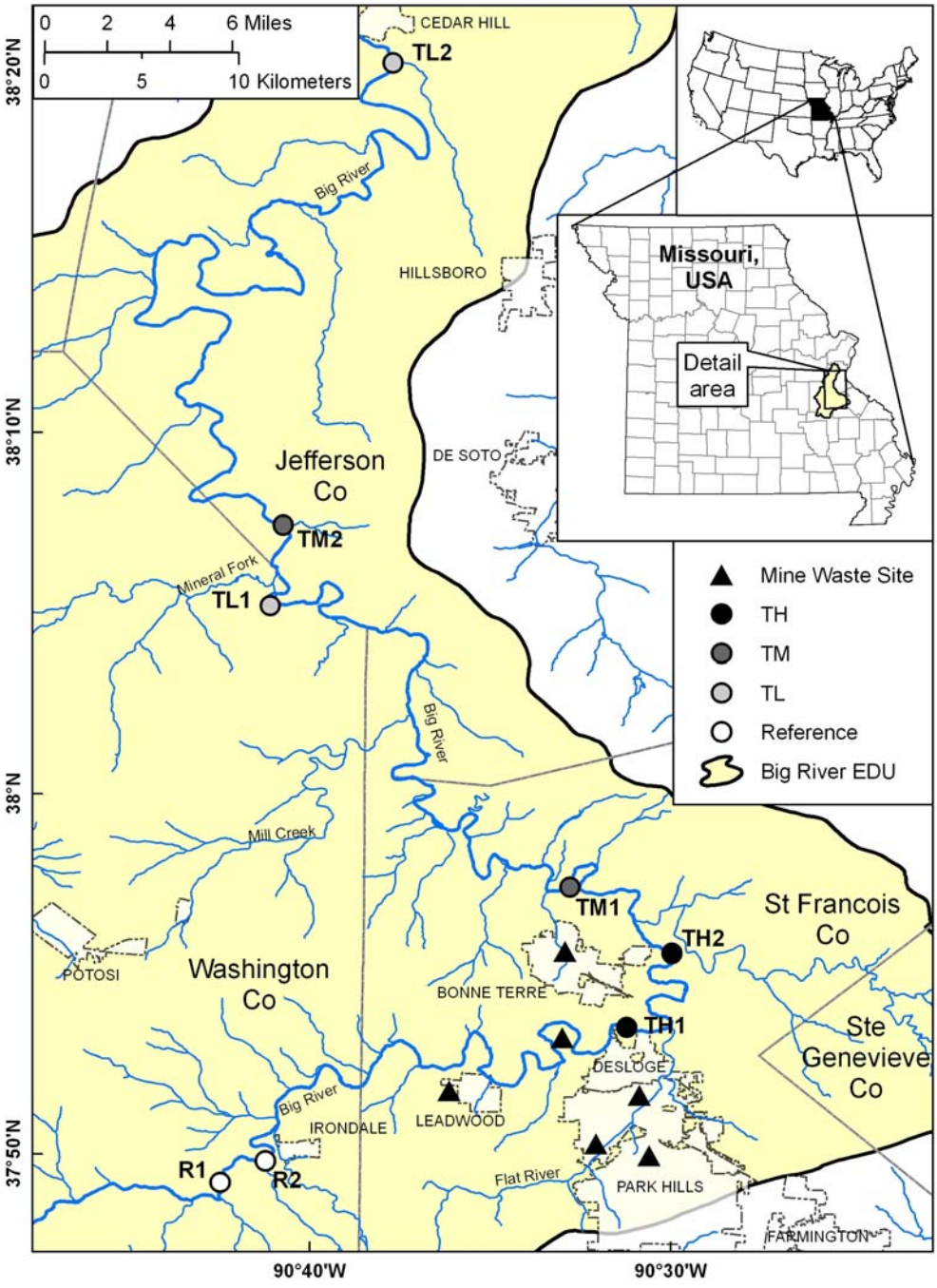

Figure 1. Map of in-situ toxicity study sites and crayfish density sampling sites, within the Big River EDU (Ecological Drainage Unit), Missouri.

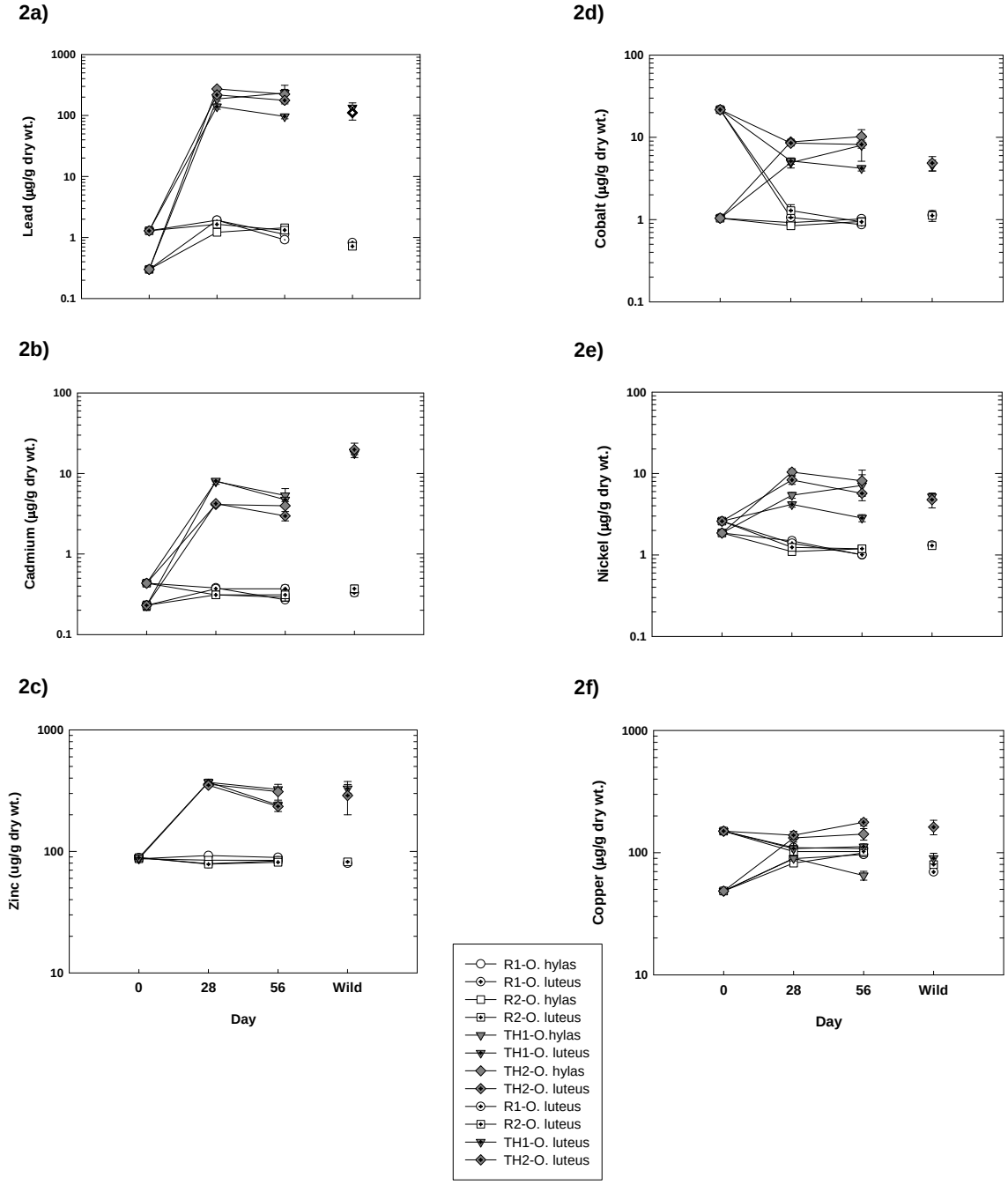

Figure 2. Mean metal concentrations (µg/g dry weight) in surviving *Orconectes hylas* and *Orconectes luteus* at days 0, 28, and 56 of the in-situ toxicity test, and wild *O. luteus* (day 28) at sampling sites, Big River, Missouri: (a) lead; (b) zinc; (c) cadmium; (d) cobalt; (e) nickel; and (f) copper.

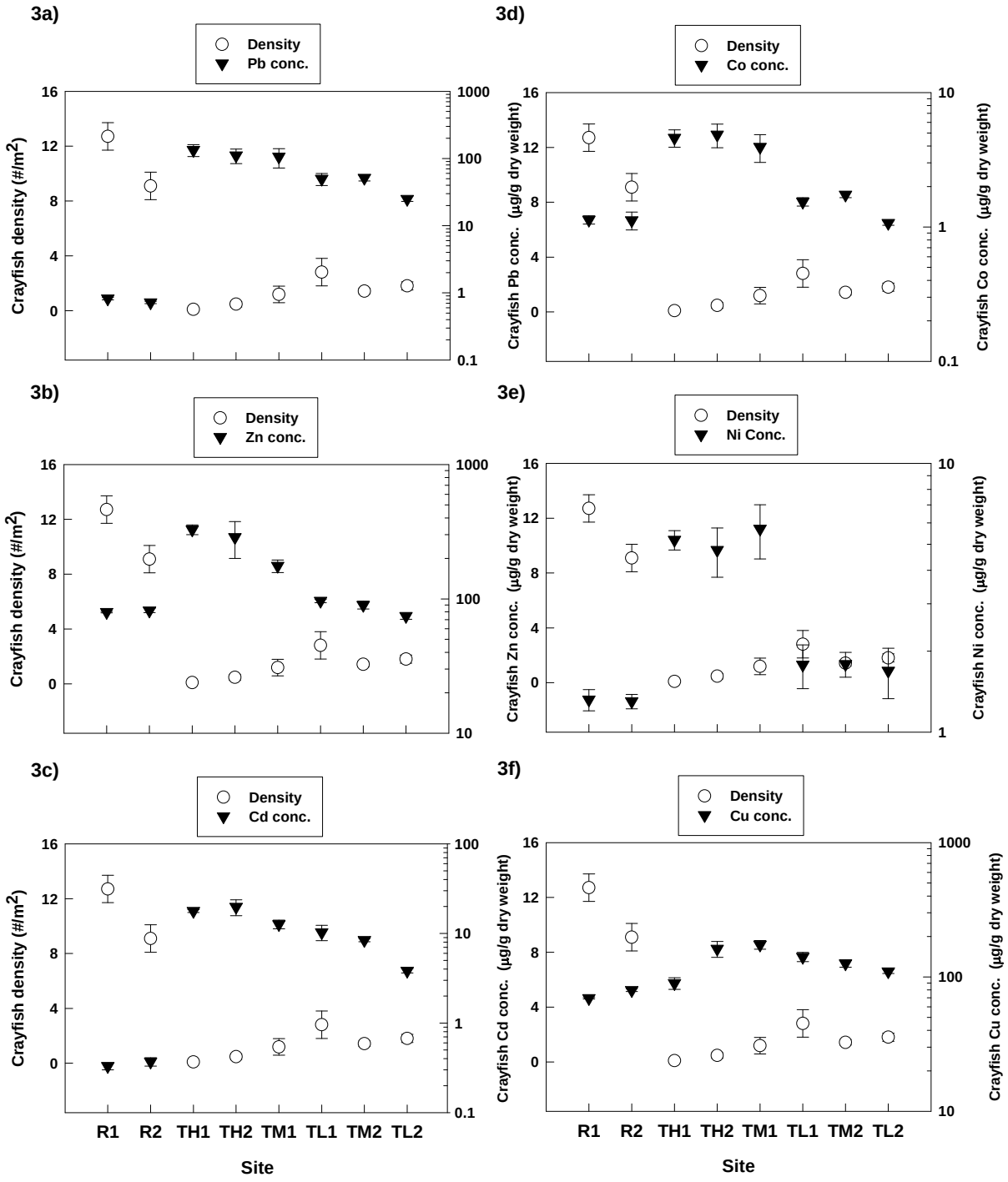

Figure 3. Riffle crayfish density and mean metal concentrations (µg/g dry weight) in wild *O. luteus* at sampling sites, Big River, Missouri: (a) lead; (b) zinc; (c) cadmium; (d) cobalt; (e) nickel; and (f) copper.

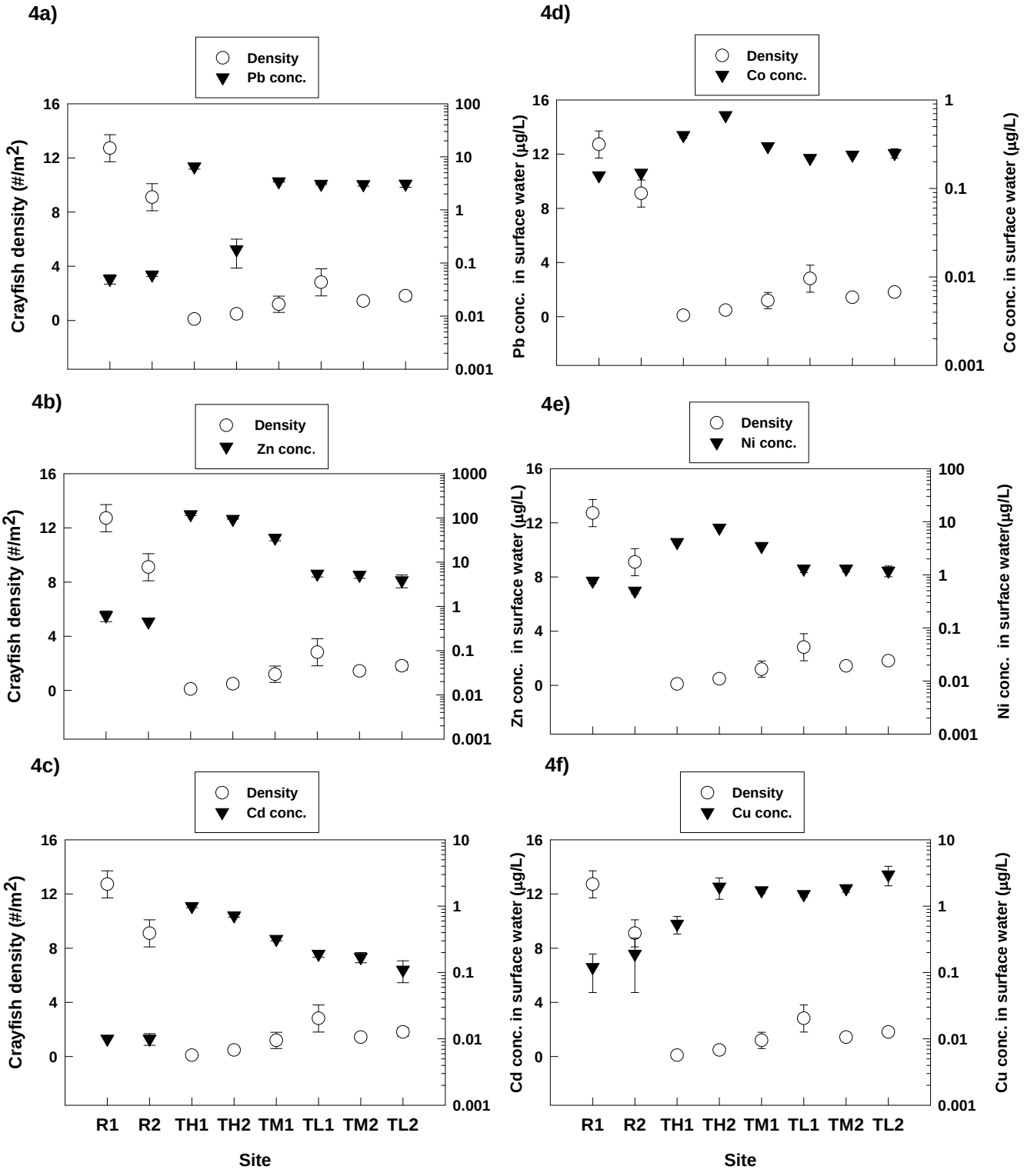

Figure 4. Riffle crayfish density and mean metal concentrations (µg/L) in surface water at sampling sites, Big River, Missouri: (a) lead; (b) zinc; (c) cadmium; (d) cobalt; (e) nickel; and (f) copper.

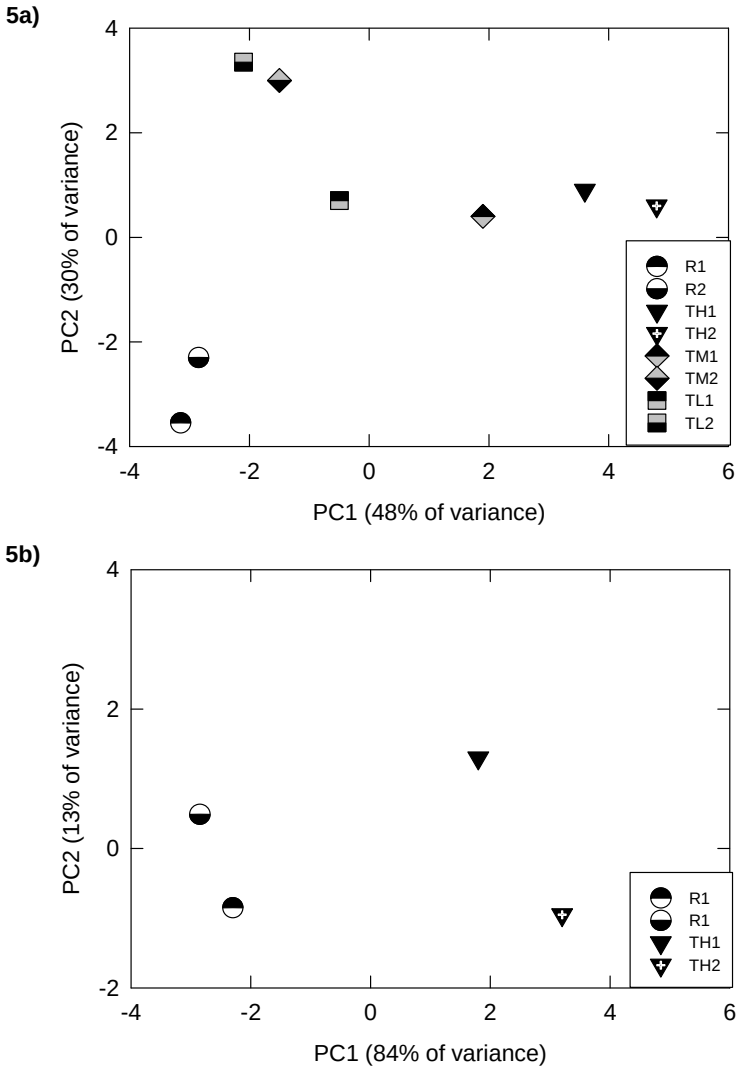

Figure 5. Principal components ordination of sites, Big River, Missouri: (a) eight sampling sites, based on physical and chemical variables [axis 1 (x-axis) and 2 (y-axis) account for 48% and 30% of the variance among sites]; (b) four cage sites based on physical and chemical variables [axis 1 (x-axis) and 2 (y-axis) account for 84% and 13% of the variance among sites].

Table 1. Site locations for the in-situ toxicity test and density study, Big River, Missouri.

[SEMO = Southeast Missouri; refers to site identifier of companion assessment of freshwater mussel populations (Besser and others, 2009b). Riffle number in 'Cage location' column refers to riffles where quadrat sampling occurred; riffle 1 = most downstream riffle at a site]

Site	SEMO reference	Type of site	Site name	River km (mi) from Desloge	N Lat (d)	N Lat (m)	W Long (d)	W Long (m)	Discharge (m^3/sec)	Cage location
R1	SEMO-1	Reference	Upstream Irondale	-34 (-21)	37	49.207	90	42.460	26.8	Upstream of riffle 3
R2	SEMO-22	Reference	Hwy U	-31 (-15)	37	49.812	90	41.222	34.1	Upstream of riffle 1
TH1	SEMO-3	Mining	Hwy 67 (Desloge)	5 (3)	37	53.421	90	30.602	55.1	Downstream of riffle 3
TH2	SEMO-4	Mining	Hwy K	10 (6)	37	55.757	90	30.398	80.8	Downstream of riffle 1
TM1	SEMO-5	Downstream	Hwy 67 North of Bonne Terre (Cherokee Landing)	23 (14)	37	57.393	90	32.781	71.1	--
TL1	SEMO-23	Downstream	Washington State Park	55 (33)	38	5.524	90	40.920	101.5	--
TM2	SEMO-9	Downstream	Mammoth MDC Access	61 (38)	38	7.427	90	40.720	65.4	--
TL2	SEMO-11	Downstream	Upstream Cedar Hill Mill Dam	119 (74)	38	20.715	90	38.058	178.1	--

Table 2. Substrate size class classification.

[modified from Bain and others, 1985]

Substrate type	Size class (mm)	Code
Smooth bedrock	--	1
Sand, silt	<2	1
Gravel	2–16	2
Pebble	17–64	3
Cobble	65–256	4
Boulder	>256	5
Irregular bedrock	--	6

Table 3. Method detection limit (MDL) for the analyses of water, biota, detritus, and sediment.

	MDL		
Analysis	**Water (µg/L)**	**Biota and detritus (µg/g dry weight)**	**Sediment (µg/g dry weight)**
Alkalinity	20,000	--	--
Hardness	5,000	--	--
Total nitrogen	21	--	--
Total phosphorous	13.3	--	--
Ammonia	14.5	--	--
Particulate organic carbon	65.9	--	--
Dissolved organic carbon	1220	--	--
Total suspended solids	11804	--	--
Chlorophyll *a*	0.90	--	--
Sulfate	--	--	--
Co	0.008	0.010−0.030	--
Ni	0.130	0.010−0.060	0.97
Cu	0.058	0.030−0.200	0.05
Zn	0.900	0.020−2.50	0.15
Cd	0.011	0.005−0.010	0.03
Pb	0.023	0.020−0.050	0.02
Mn	--	--	0.05
Fe	--	--	0.97
AVS	--	--	0.0001[1]

[1]µmol/g

Table 4. Number, sex ratio (F:M), mean carapace length, mean wet weight, and biomass (±1 standard error) of surviving *Orconectes hylas* and *O. luteus* exposed at day 28 and at day 56 of the in-situ toxicity test, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each test day (P <0.05). n =30 per species on day 0]

	Orconectes hylas					*Orconectes luteus*				
Day, site type, and site	***n***	**F:M**	**Carapace length (mm)**	**Wet weight (g)**	**Biomass (g/m²)**	***n***	**F:M**	**Carapace length (mm)**	**Wet weight (g)**	**Biomass (g/m²)**
Day 28										
Reference										
R1	29 a	11:18	14.0 (0.3) a	0.84 (0.06) a	8.14 (0.62) a	28 a	8:20	14.2 (0.4) b	0.88 (0.07) a	8.19 (0.96) a
R2	30 a	14:16	14.4 (0.4) a	0.95 (0.07) a	9.50 (0.89) a	27 a	8:19	13.3 (0.5) b	0.72 (0.08) a	6.50 (0.88) a
Group mean	59 A	25:34	14.2 (0.2) B	0.90 (0.05) A	8.82 (0.57) A	55 A	16:39	13.8 (0.3) B	0.80 (0.06) A	7.34 (0.69) A
Mining										
TH1	29 a	14:15	14.4 (0.3) a	0.84 (0.06) a	8.10 (0.30) a	28 a	17:11	14.7 (0.4) a	0.87 (0.07) a	8.11 (0.76) a
TH2	28 a	15:13	15.1 (0.4) a	1.03 (0.08) a	9.61 (0.52) a	29 a	14:15	15.3 (0.5) a	1.00 (0.11) a	9.78 (0.36) a
Group mean	57 A	29:28	14.7 (0.2) A	0.93 (0.05) A	8.85 (0.43) A	57 A	31:26	15.0 (0.3) A	0.94 (0.07) A	8.94 (0.53) A
Day 56										
Reference										
R1	30 a	15:15	15.4 (0.3) ab	1.12 (0.07) ab	11.6 (0.47) ab	30 a	13:17	16.0 (0.4) b	1.16 (0.09) b	11.6 (0.47) a
R2	29 a	16:13	14.8 (0.3) b	0.96 (0.08) b	10.4 (0.93) bc	30 a	16:14	15.6 (0.4) b	1.04 (0.08) b	10.4 (0.93) a
Group mean	59 A	31:28	15.1 (0.2) B	1.04 (0.05) B	11.0 (0.53) A	60 A	29:31	15.8 (0.3) B	1.09 (0.06) B	11.0 (0.53) A
Mining										
TH1	20 b	14:8	16.1 (0.6) ab	1.30 (0.18) ab	10.6 (2.64) a	20 b	8:10	16.6 (0.6) b	1.28 (0.19) b	8.98 (1.42) a
TH2	24 b	11:13	16.6 (0.5) a	1.50 (0.15) a	12:7 (0.92) c	19 b	12:7	17.9 (0.4) a	1.67 (0.12) a	10.6 (2.64) a
Group mean	44 B	25:21	16.4 (0.4) A	1.40 (0.12) A	9.79 (1.40) A	39 B	20:17	17.3 (0.4) A	1.48 (0.11) A	9.79 (1.39) A

Table 5. Growth rates [change per day in carapace length (CL) and wet weight (WT)], percent (%) increase in CL and WT from day 0, and % difference in increase of CL and WT (±1 standard error) between surviving *O. hylas* and *O. luteus*.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each test day (P <0.05)]

	O. hylas				*O. luteus*				% Difference between species	
Day, site type, and site	**Change in CL/ day**	**% Change in CL**	**Change in WT/ day**	**% Change in WT**	**Change in CL/ day**	**% Change in CL**	**Change in WT/ day**	**% Change in WT**	**CL**	**WT**
Day 28										
Reference										
R1	0.147 (0.01) b	41.3 (3) b	0.020 (0) a	162 (17) a	0.297 (0.02) ab	143 (10) ab	0.030 (0.01) a	925 (149) a	354 (52) a	604 (154) a
R2	0.163 (0.01) ab	45.3 (3) ab	0.023 (0003) a	195 (28) a	0.263 (0.02) b	126 (7) b	0.023 (0.003) a	727 (62) a	281 (33) a	396 (83) a
Group mean	0.155 (0.01) A	43.3 (2) A	0.022 (0.002) A	178 (16) A	0.280 (0.01) B	134 (7) B	0.027 (0.003) A	826 (85) A	318 (32) A	500 (91) A
Mining										
TH1	0.160 (0.01) ab	45.3 (2) ab	0.020 (0) a	160 (8) a	0.313 (0.02) ab	151 (9) ab	0.027 (0.003) a	904 (90) a	332 (9) a	564 (38) a
TH2	0.187 (0.01) a	52.3 (2) a	0.027 (0.003) a	220 (7) a	0.333 (0.01) a	160 (5) a	0.030 (0) a	1071 (34) a	306 (11) a	489 (28) a
Group mean	0.173 (0.01) A	48.8 (2) A	0.023 (0.002) A	190 (14) A	0.323 (0.01) A	155 (5) A	0.028 (0.001) A	988 (57) A	319 (9) A	526 (27) A
Day 56										
Reference										
R1	0.097 (0.003) ab	56.0 (2) ab	0.013 (0.003) ab	248 (15) ab	0.183 (0.003) ab	172 (3) b	0.020 (0) b	1237 (54) b	309 (14) a	502 (41) a
R2	0.090 (0.01) b	49.7 (3) b	0.010 (0) b	199 (19) b	0.173 (0.01) b	167 (9) b	0.017 (0.003) b	1105 (108) b	340 (37) a	573 (104) a
Group mean	0.093 (0.003) B	52.8 (2) B	0.012 (0.001) B	224 (15) B	0.178 (0.01) B	170 (4) B	0.018 (0.002) B	1171 (61) B	324 (19) A	538 (53) A
Mining										
TH1	0.164 (0.04) ab	91.8 (22) a	0.030 (0.01) ab	539 (185) ab	0.197 (0.01) ab	185 (11) ab	0.023 (0.003) ab	1424 (230) ab	217 (31) b	305 (75) b
TH2	0.120 (0.01) a	68.0 (4) ab	0.023 (0.003) a	376 (33) a	0.213 (0.003) a	205 (2) a	0.030 (0) a	1862 (58) a	304 (15) ab	500 (29) a
Group mean	0.148 (0.03) A	82.9 (14) A	0.028 (0.01) A	478 (115) A	0.205 (0.01) A	195 (7) A	0.027 (0.002) A	1643 (145) A	260 (25) B	407 (56) A

Table 6. Mean metal concentrations (µg/g; ±1 standard error) in surviving *Orconectes hylas* exposed during the 56-d in-situ toxicity test, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each test day (P <0.05). N = number of composite samples]

Day, site type, and site	*N*	Cobalt		Nickel		Copper		Zinc		Cadmium		Lead	
						Day 28							
Reference													
R1	3	0.92 (0.07)	c	1.49 (0.14)	c	89.2 (2.8)	b	92.3 (1.1)	b	0.38 (0.03)	c	1.91 (0.21)	c
R2	3	0.84 (0.03)	c	1.10 (0.01)	d	82.0 (2.4)	b	84.7 (3.3)	c	0.31 (0.003)	d	1.22 (0.04)	d
Group mean	6	0.88 (0.04)	B	1.31 (0.11)	B	85.6 (2.3)	B	88.5 (2.3)	B	0.35 (0.02)	B	1.57 (0.18)	B
Mining													
TH1	3	4.92 (0.68)	a	5.41 (0.46)	b	89.9 (4.2)	b	370 (9.6)	a	7.95 (0.62)	a	186 (14)	b
TH2	3	8.77 (0.69)	b	10.4 (1.0)	a	132 (17)	a	357 (10)	a	4.12 (0.13)	b	272 (21)	a
Group mean	6	6.85 (0.96)	A	7.89 (1.2)	A	111 (12)	A	363 (6.8)	A	6.03 (0.90)	A	229 (23)	A
						Day 56							
Reference													
R1	3	1.02 (0.08)	b	1.00 (0.03)	b	96.4 (2.4)	b	89.2 (2.3)	b	0.27 (0.01)	b	1.13 (0.03)	b
R2	3	0.95 (0.05)	b	1.19 (0.12)	b	99.6 (2.4)	c	84.4 (2.0)	b	0.29 (0.02)	b	1.45 (0.17)	b
Group mean	6	0.99 (0.04)	B	1.10 (0.07)	B	98.0 (1.7)	B	86.9 (1.7)	B	0.28 (0.01)	B	1.29 (0.11)	B
Mining													
TH1	3	8.01 (2.9)	a	7.13 (2.5)	a	64.9 (5.4)	b	324 (5.4)	a	5.32 (1.2)	a	234 (79)	a
TH2	3	10.2 (2.2)	a	8.13 (2.9)	a	142 (15)	a	310 (45)	a	4.22 (0.09)	a	223 (44)	a
Group mean	6	9.10 (1.7)	A	7.63 (1.7)	A	104 (19)	A	317 (38)	A	4.80 (0.59)	A	228 (41)	A

Table 7. Mean metal concentrations (µg/g; ±1 standard error) in surviving *Orconectes luteus* exposed during the 56-d in-situ toxicity test, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each test day (P <0.05). N = number of composite samples]

Day, site type, and site	*N*	Cobalt		Nickel		Copper		Zinc		Cadmium		Lead	
						Day 28							
Reference													
R1	3	1.06 (0.04)	c	1.39 (0.06)	c	110 (2)	ab	79.2 (4)	b	0.37 (0.02)	d	1.92 (0.06)	c
R2	3	1.29 (0.23)	c	1.24 (0.08)	c	102 (3)	b	78.4 (1)	b	0.31 (0.02)	c	1.65 (0.12)	d
Group mean	6	1.80 (0.12)	B	1.32 (0.06)	B	106 (2)	A	78.8 (2)	B	0.34 (0.02)	B	1.79 (0.09)	B
Mining													
TH1	3	5.16 (0.46)	b	4.18 (0.32)	b	108 (14)	b	371 (23)	a	8.10 (0.18)	a	140 (13)	b
TH2	3	8.52 (0.69)	a	8.33 (0.98)	a	139 (10)	a	350 (7)	a	3.96 (0.08)	b	218 (17)	a
Group mean	6	6.84 (0.84)	A	6.26 (1.04)	A	124 (10)	A	361 (12)	A	6.03 (0.93)	A	179 (20)	A
						Day 56							
Reference													
R1	3	0.87 (0.04)	b	1.01 (0.06)	c	108 (2)	b	83.4 (2)	b	0.37 (0.04)	c	0.92 (0.09)	d
R2	3	0.94 (0.03)	b	1.19 (0.04)	c	102 (2)	c	81.4 (1)	b	0.31 (0.05)	c	1.32 (0.12)	c
Group mean	6	0.91 (0.03)	B	1.10 (0.05)	B	105 (2)	B	82.4 (1)	B	0.34 (0.03)	B	1.12 (0.11)	B
Mining													
TH1	3	4.20 (0.3)	a	2.84 (0.28)	b	112 (6)	b	240 (3)	a	4.73 (0.25)	a	96.6 (8)	b
TH2	3	8.20 (0.8)	a	5.71 (0.31)	a	177 (11)	a	234 (21)	a	2.97 (0.39)	b	177 (13)	a
Group mean	6	6.22 (0.98)	A	4.28 (0.67)	A	145 (16)	A	237 (10)	A	3.85 (0.44)	A	137 (19)	A

Table 8. Number of quadrat samples, species richness, number of riffle crayfish, and rilffle density (±1 standard error) of *Orconectes luteus* collected by quadrat sampling, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different (P <0.05)]

Site type and site	No. of quadrats	Species richness	*N*	Density (#/m²)	
Reference					
R1	21	4	267	12.7 (1)	a
R2	21	4	191	9.1 (1)	ab
Group mean	42	4	458	10.9 (1)	A
Mining					
TH1	21	1	2	1.0 (0)	e
TH2	21	2	10	1.2 (0.2)	de
Group mean	42	2	12	1.1 (0.1)	C
Downstream					
TM1	21	1	25	1.9 (0.6)	de
TM2	21	4	30	1.9 (0.2)	cd
TL1	21	2	59	3.1 (1)	bc
TL2	21	1	38	2.6 (0.3)	bc
Group mean	84	4	152	2.4 (0.3)	B

Table 9. Number, species, and mean carapace length (±1 standard error) of crayfish collected by quadrat sampling, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different (P <0.05)]

	Species											
	O. harrisoni		*O. hylas*		*O. luteus*			*O. medius*		*O. virilis*		
Site type and site	*n*	Carapace length (mm)	*n*	Carapace length (mm)	*n*	Carapace length (mm)		*n*	Carapace length (mm)	*n*	Carapace length (mm)	
Reference												
R1	16	15.7 (1.9)	41	15.6 (0.8)	200	15.1 (0.6)	a	8	14.4 (3.0)	---	-- --	
R2	14	13.2 (1.1)	6	20.2 (3.1)	169	13.2 (0.5)	a	2	8.0 (0.1)	--	-- --	
Group mean	30	14.5 (1.2)	47	16.2 (0.8)	369	14.3 (0.4)	A	10	13.1 (2.5)	--	-- --	
Mining												
TH1	--	-- --	--	-- --	2	10.6 (0.2)	c	--	-- --	--	-- --	
TH2	1	9.6 --	--	-- --	9	11.2 (1.6)	c	--	-- --	--	-- --	
Group mean	1	9.6 --	--	-- --	11	11.1 (1.3)	B	--	-- --	--	-- --	
Downstream												
TM1	--	-- --	--	-- --	25	12.3 (1.0)	bc	--	-- --	--	-- --	
TM2	2	16.3 (1.3)	--	-- --	25	13.8 (1.0)	bc	1	19.7 --	2	23.8 (1.4)	
TL1	7	17.5 (0.8)	--	-- --	52	16.6 (1.0)	ab	--	-- --	--	-- --	
TL2	--	-- --	--	-- --	38	12.4 (0.3)	bc	--	-- --	--	-- --	
Group mean	9	17.2 (0.7)	--	-- --	140	14.2 (0.5)	AB	1	19.7 --	2	23.8 (1.4)	

Table 10. Number and species of crayfish collected by baited traps, Big River, Missouri.

[CPUE = catch per unit effort]

Site type and site	Number of traps	*Orconectes harrisoni*	*Orconectes hylas*	*Orconectes luteus*	*Orconectes medius*	*Orconectes virilis*	Total crayfish	CPUE
Reference								
R1	30	1	0	2	1	1	5	0.17
R2	30	0	0	5	0	0	5	0.17
Group total/mean	60	1	0	7	1	1	10	0.17
Mining								
TH1	26	0	0	0	0	2	2	0.08
TH2	30	0	0	0	0	2	2	0.06
Group total/mean	56	0	0	0	0	4	4	0.07
Downstream								
TM1	30	0	1	0	0	0	1	0.03
TM2	30	0	0	0	0	1	1	0.03
TL1	30	1	0	0	0	6	7	0.23
TL2	30	1	0	0	0	2	3	0.10
Group total/mean	120	2	1	0	0	9	12	0.10

Table 11. Number, sex ratio (F:M), and mean carapace length (±1 standard error) of wild *Orconectes luteus* collected by quadrat sampling for metal analyses, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different (P <0.05)]

Site type and site	*n*	F:M	Carapace length (mm)		
Reference					
R1	10	5:5	25.1	(0.8)	a
R2	14	7:7	23.4	(1.3)	a
Group mean	24	12:12	24.2	(0.9)	A
Mining					
TH1	1	M	10.4	--	c
TH2	8	5:3	11.3	(1.8)	c
Group mean	9	5:4	11.2	(1.6)	B
Downstream					
TM1	24	12:12	12.4	(1.0)	bc
TM2	24	14:10	13.7	(1.0)	bc
TL1	44	22:22	17.7	(1.1)	ab
TL2	38	12:26	12.4	(0.3)	bc
Group mean	130	60:70	14.5	(0.5)	B

Table 12. Riffle crayfish density of and mean metal concentrations (µg/g; ±1 standard error) in *Orconectes luteus* collected by quadrat sampling, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different (P <0.05). N = the number of composite samples]

Site type and site	Density (#/m²)		*N*	Cobalt		Nickel		Copper		Zinc		Cadmium		Lead	
Reference															
R1	12.7	a	3	1.13 (0.1)	c	1.32 (0.1)	b	69.5 (1)	f	79.7 (1)	e	0.33 (0.02)	f	0.82 (0.04)	cd
R2	9.1	ab	3	1.12 (0.2)	c	1.30 (0.1)	b	79.8 (2)	ef	81.9 (3)	de	0.37 (0.04)	ef	0.72 (0.03)	d
Group mean	10.9	A	6	1.13 (0.1)	C	1.31 (0.1)	C	74.6 (3)	B	80.8 (2)	C	0.35 (0.02)	C	0.77 (0.03)	C
Mining															
TH1	1.0	e	3	4.60 (0.7)	a	5.19 (0.4)	a	89.8 (9)	de	328 (27)	a	17.8 (0.66)	a	134 (27)	a
TH2	1.2	de	3	4.86 (1.0)	a	4.76 (1.0)	a	162 (22)	a	288 (88)	ab	19.8 (4.00)	a	111 (27)	a
Group mean	1.1	C	6	4.71 (0.5)	A	4.98 (0.5)	A	126 (19)	A	308 (42)	A	18.8 (1.70)	A	122 (18)	A
Downstream															
TM1	1.9	de	3	3.94 (0.9)	a	5.71 (1.3)	a	174 (13)	a	176 (19)	ab	12.7 (1.40)	b	106 (34)	a
TM2	1.9	cd	3	1.74 (0.1)	b	1.79 (0.2)	b	126 (8)	bc	90.0 (6)	cd	8.43 (0.36)	cd	51.3 (5)	b
TL1	3.1	bc	3	1.54 (0.1)	b	1.78 (0.3)	b	141 (11)	ab	96.5 (3)	bc	10.3 (2.00)	bc	49.7 (10)	b
TL2	2.6	bc	3	1.07 (0.0)	c	1.69 (0.4)	b	110 (4)	cd	74.5 (4)	e	3.82 (0.20)	de	25.0 (2)	c
Group mean	2.4	B	12	2.07 (0.4)	B	2.74 (0.6)	B	138 (8)	A	109 (13)	B	8.81 (1.08)	B	58.1 (12)	B

Table 13. Mean substrate size and homogeneity (standard deviation of substrate size), depth, and current velocity (±1 standard error) at two water depths in riffle habitats, in quadrat samples, and near cages, Big River, Missouri.

Sample type, site type, and site	*n*	Substrate class size	Substrate homogeneity	*n*	Depth (cm)	Velocity at substrate (m/sec)	Mid-water velocity (m/sec)
Riffles							
Reference							
R1	330	3.38 a	1.21 a	66	25.8 (0.69) c	0.28 (0.02) d	0.44 (0.02) d
R2	420	3.06 b	1.00 ab	84	32.8 (1.43) b	0.28 (0.02) d	0.42 (0.03) d
Group mean	750	3.20 A	1.11 A	150	29.1 (0.89) B	0.28 (0.02) C	0.43 (0.02) C
Mining							
TH1	425	3.04 b	0.58 d	85	27.6 (1.30) c	0.34 (0.01) bc	0.63 (0.03) bc
TH2	370	2.69 d	0.54 d	74	30.0 (1.70) b	0.31 (0.02) cd	0.58 (0.04) c
Group mean	795	2.88 C	0.56 C	159	28.6 (1.04) B	0.33 (0.01) B	0.61 (0.02) B
Downstream							
TM1	405	2.86 c	0.55 d	81	30.4 (1.4) b	0.45 (0.02) a	0.78 (0.26) a
TM2	350	3.12 b	0.70 bcd	70	29.1 (1.4) bc	0.83 (0.45) ab	0.72 (0.04) ab
TL1	420	3.35 a	0.83 abc	84	43.5 (1.60) a	0.31 (0.02) cd	0.77 (0.03) a
TL2	470	3.01 b	0.62 cd	94	40.9 (1.60) a	0.32 (0.02) cd	0.74 (0.03) a
Group mean	1645	3.08 B	0.68 B	329	36.5 (0.83) A	0.46 (0.10) A	0.76 (0.02) A
Quadrats							
Reference							
R1	101	3.61 a	0.85 a	21	23.0 (1) b	0.41 (0.05) a	0.28 (0.05) c
R2	105	3.44 a	0.76 ab	21	30.0 (3) b	0.5 (0.06) a	0.26 (0.04) bc
Group mean	206	3.52 A	0.80 AB	42	26.5 (2) B	0.45 (0.04) B	0.27 (0.03) B
Mining							
TH1	105	2.63 e	0.86 a	21	26.2 (3) b	0.48 (0.04) a	0.25 (0.02) bc
TH2	105	2.35 d	0.82 a	21	25.2 (3) b	0.49 (0.05) a	0.28 (0.03) bc
Group mean	210	2.49 C	0.84 A	42	25.7 (2) B	0.48 (0.03) AB	0.27 (0.02) B
Downstream							
TM1	105	2.82 cd	0.78 ab	21	30.0 (3) b	0.61 (0.05) a	0.33 (0.04) ab
TM2	105	3.15 b	0.65 ab	21	30.3 (3) b	0.70 (0.08) a	0.39 (0.04) a
TL1	105	3.20 b	0.90 a	21	40.7 (3) a	0.66 (0.05) a	0.35 (0.03) a
TL2	105	2.91 c	0.52 b	21	31.0 (3) b	0.65 (0.04) a	0.30 (0.04) a
Group mean	420	3.02 B	0.72 B	84	33.0 (1) A	0.66 (0.03) A	0.34 (0.02) A

Table 13. Mean substrate size and homogeneity (standard deviation of substrate size), depth, and current velocity (±1 standard error) at two water depths in riffle habitats, in quadrat samples, and near cages, Big River, Missouri—Continued

Sample type, site type, and site	*n*	Substrate class size	Substrate homogeneity	*n*	Depth (cm)	Velocity at substrate (m/sec)	Mid-water velocity (m/sec)
			Near cages				
Reference							
R1	120	2.96 a	1.54 a	30	52.7 (3) b	0.006 (0.01) b	0.12 (0.02) a
R2	120	3.00 a	0.70 a	30	61.9 (4) a	0.001 (0.01) b	0.18 (0.06) a
Group mean	240	2.98 A	1.19 A	60	57.3 (3) A	0.003 (0.01) B	0.15 (0.03) A
Mining							
TH1	120	2.43 b	0.59 b	30	52.9 (3) b	0.03 (0.01) a	0.12 (0.01) a
TH2	120	1.96 c	0.94 c	30	57.1 (3) ab	0.04 (0.01) a	0.16 (0.03) a
Group mean	240	2.19 B	0.82 B	60	55.0 (2) A	0.03 (0.01) A	0.14 (0.01) A

Table 14. Mean values (±1 standard error) for in-situ water quality at sampling sites in Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each sample type (P <0.05). na = not available]

Sample type, site type, and site	*n*	Temperature (°C)	*n*	pH	*n*	Conductivity (µS/Cm)	*n*	Dissolved Oxygen (mg/L)	*n*	Turbidity (NTU)
Surface water										
Reference										
R1	21	24.4 (0.66) bc	15	8.09 (0.04) bc	15	385 (0.01) c	21	7.46 (0.12) bc	14	2.6 (0.60 c
R2	19	24.8 (0.65) bc	13	8.14 (0.04) abc	13	386 (0.01) c	19	7.02 (0.24) cd	13	3.1 (1.0) c
Group mean	40	24.6 (0.50) B	28	8.11 (0.03) A	28	386 (0.01) B	40	7.25 (0.13) B	27	2.9 (0.6) C
Mining										
TH1	13	24.7 (0.67) bc	13	8.10 (0.03) bc	13	564 (0.02) ab	19	7.47 (0.17) bc	13	5.0 (0.6) abc
TH2	13	24.8 (0.72) bc	13	8.23 (0.04) ab	13	607 (0.03) ab	19	8.86 (0.39) ab	13	9.8 (7.6) c
Group mean	26	24.7 (0.48) B	26	8.17 (0.03) A	26	586 (0.02) A	38	8.17 (0.24) A	26	7.4 (3.7) A
Downstream										
TM1	3	24.7 (0.02) c	3	8.21 (0) a	3	613 (0) a	3	5.98 (0.12) d	3	2.9 (0.01) bc
TM2	3	27.7 (0.06) ab	3	8.16 (0.01) ab	3	529 (0) ab	3	9.19 (0.07) a	3	6.1 (0.6) ab
TL1	3	26.1 (0.07) bc	3	8.04 (0.01) c	3	576 (0) ab	3	5.95 (0.07) d	3	8.2 (1.6) ab
TL2	3	29.0 (0.16) a	3	8.13 (0.01) ab	3	504 (0) b	3	8.70 (0.05) a	3	9.4 (1.5) a
Group mean	12	26.9 (0.48) A	12	8.14 (0.02) A	12	556 (0.01) A	12	7.46 (0.45) B	12	6.6 (0.9) B
Pore water										
Reference										
R1	3	24.1 (0.19) a	3	7.58 (0.28) a	3	398 (0.01) b	3	6.82 (0.42 a	--	-- -- --
R2	3	25.8 (0.10) a	3	7.56 (0.38) a	3	385 (0.01) b	3	6.56 (1.00) a	--	-- -- --
Group mean	6	24.9 (0.39) A	6	7.57 (0.21) A	6	392 (0.01) B	6	6.69 (0.68) A	--	-- -- --
Mining										
TH1	3	24.8 (0.43) a	3	7.62 (0.28) a	3	576 (0.04) a	3	7.26 (0.56) a	--	-- -- --
TH2	3	23.3 (1.50) a	3	7.56 (0.46) a	3	622 (0.04) a	3	7.87 (2.00) a	--	-- -- --
Group mean	6	24.1 (0.78) A	6	7.59 (0.24) A	6	599 (0.03) A	6	7.57 (0.92) A	--	-- -- --
Criteria										
		32[1]		6.5–9[1]		na		5[1]		2.3[2]

[1] Missouri Department of Natural Resources, Code of Regulations (2009), Chapter 7, *http://www.sos.mo.gov/adrules/csr/current/10csr/10c20-7A-G.pdf* warm-water fisheries

[2] USEPA (2000), based on 25th percentile, range 1.0–5.2 NTU

Table 15. Mean alkalinity, hardness and sulfate concentrations (±1 standard error) at sampling sites in Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each sample type (P <0.05)]

Sample type, site type, and site	*n*	Alkalinity (mg $CaCO_3$/L)			Hardness (mg $CaCO_3$/L)			*n*	Sulfate (mg SO_4/L)		
Surface water											
Reference											
R1	11	188	(8)	b	198	(80	b	10	13	(2)	b
R2	11	186	(8)	b	197	(9)	b	10	13	(1)	b
Group mean	22	185	(7)	C	196	(7)	B	20	13	(1)	C
Mining											
TH1	11	199	(9)	ab	281	(18)	a	10	83	(14)	a
TH2	11	200	(9)	ab	297	(20)	a	10	96	(14)	a
Group mean	22	198	(7)	B	286	(16)	A	20	91	(10)	A
Downstream											
TM1	3	224	(0)	a	307	(3)	a	3	80	(3)	a
TM2	3	225	(1)	a	273	(1)	ab	3	50	(1)	a
TL1	5	220	(1)	ab	289	(1)	a	5	70	(1)	a
TL2	3	225	(2)	a	264	(1)	ab	3	38	(1)	a
Group mean	14	223	(1)	A	284	(4)	A	14	61	(4)	B
Pore water											
Reference											
R1	3	195	(5)	a	207	(6)	ab	4	12	(0.3)	b
R2	1	196	(0)	a	204	(0)	a	3	11	(1)	b
Group mean	4	196	(4)	A	206	(4)	B	7	11	(0.4)	B
Mining											
TH1	2	204	(8)	a	295	(25)	ab	3	97	(18)	a
TH2	2	206	(6)	a	311	(29)	a	3	114	(19)	a
Group mean	4	205	(4)	A	303	(16)	A	6	106	(12)	A
Criteria											
		20[1]			200[2]				1000[3]		

[1] USEPA (2006)

[2] Missouri Department of Natural Resources (2009), water quality standards; *http://www.dnr.mo.gov/env/wpp/rules/wpp-rule-dev.htm*; lower 25th percentile value of representative number of samples

[3] Missouri Department of Natural Resources (2009), water quality standards; *http://www.dnr.mo.gov/env/wpp/rules/wpp-rule-dev.htm*; sulfate plus chloride

Table 16. Mean total nitrogen (TN), total phosphorous (TP), ammonia (NH3) concentrations (±1 standard error), and TN/TP ratio at sampling sites in Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each sample type (P <0.05)]

Sample type, site type, and site	*n*	TN (mg N/L)		TP (µg P/L)		TN/TP		*n*	NH3 (mg N/L)		
Surface water											
Reference											
R1	13	0.08 (0.02)	dc	5.92 (1.2)	d	2.7 (0.9)	a	12	<0.014	--	--
R2	11	0.11 (0.03)	dc	7.20 (1.1)	d	1.8 (0.5)	a	11	<0.014	--	--
Group mean	24	0.09 (0.02)	B	6.49 (0.8)	B	2.3 (0.5)	A	23	<0.014	--	--
Mining											
TH1	12	0.14 (0.02)	cd	10.2 (2.3)	cd	2.3 (0.8)	a	12	<0.014	--	--
TH2	15	0.28 (0.03)	ab	58.8 (4.3)	a	0.57 (0.1)	a	14	<0.014	--	--
Group mean	27	0.22 (0.02)	A	37.2 (5.4)	A	1.4 (0.4)	A	26	<0.014	--	--
Downstream											
TM1	4	0.32 (0.02)	a	57.3 (2.6)	a	0.57 (0.1)	a	4	<0.014	--	--
TM2	3	0.10 (0.01)	dc	12.7 (0.8)	bc	0.81 90.1)	a	4	<0.014	--	--
TL1	5	0.17 (0.03)	bc	23.3 (1.6)	b	0.70 (0.1)	a	5	<0.014	--	--
TL2	4	0.06 (0.003)	d	14.0 (0.32)	b	0.39 (0.02)	a	4	<0.014	--	--
Group mean	16	0.17 (0.03)	A	27.5 (4.6)	A	0.61 (0.1)	A	19	<0.014	--	--
Pore water											
Reference											
R1	--	-- --	--	-- --	--	-- --	--	2	<0.014	--	--
R2	--	-- --	--	-- --	--	-- --	--	2	0.10	(0.003)	a
Group mean	--	-- --	--	-- --	--	-- --	--	4	0.13	(0.13)	A
Mining											
TH1	--	-- --	--	-- --	--	-- --	--	2	<0.014	--	--
TH2	--	-- --	--	-- --	--	-- --	--	2	0.27	(0.27)	a
Group mean	--	-- --	--	-- --	--	-- --	--	4	0.13	(0.13)	A
Criteria											
		0.90[1]		75[1]		--			0.55–2.4[2]		

[1] Missouri Department of Natural Resources (2006), Regional Technical Assistance Group, ambient water quality criteria recommendations for rivers and streams; *http://www.dnr.mo.gov/env/wpp/rules/wpp-rule-dev.htm*

[2] Missouri Department of Natural Resources (2009), Code of Regulations, Chapter 7, *http://www.sos.mo.gov/adrules/csr/current/10csr/10c20-7A-G.pdf* warm-water fisheries

Table 17. Mean chlorophyll a, total suspended solids (TSS), particulate organic carbon (POC), and dissolved organic carbon (DOC; ±1 standard error) at sampling sites in Big River, Missouri

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each sample type (P <0.05). na = not available]

Sample type, site type, and site	*n*	Chlorophyll *a* (mg C/L)		*n*	TSS (mg/L)		*n*	POC (mg C/L)		*n*	DOC (mg C/L)	
Surface water												
Reference												
R1	10	1.27 (0 .15)	bc	10	2.7 (0.1)	cd	13	292 (39)	a	15	1.19 (0.1)	ab
R2	12	2.44 (0.66)	c	9	2.8 (0.4)	d	13	292 (34)	a	17	1.23 (0.2)	ab
Group mean	22	1.91 (0.38)	B	19	2.7 (0.2)	C	26	292 (25)	B	32	1.21 (0.1)	B
Mining												
TH1	13	2.53 (0.39)	abc	10	4.0 (0.3)	bcd	10	306 (38)	a	15	1.56 (0.3)	ab
TH2	11	3.47 (0.53)	ab	12	3.5 (0.7)	cd	14	339 (41)	a	17	1.84 (0.2)	a
Group mean	24	2.96 (0.33)	A	22	3.7 (0.4)	B	24	325 (29)	AB	32	1.71 (0.2)	A
Downstream												
TM1	3	3.33 (0.14)	ab	3	3.9 (0.3)	bc	3	426 (12)	a	4	1.30 (0.1)	a
TM2	4	2.36 (0.52)	abc	5	12 (1.0)	a	5	430 (12)	a	3	0.91 (0.1)	b
TL1	6	3.41 (0.11)	a	3	5.3 (0.1)	ab	3	285 (22)	a	5	1.38 (0.1)	a
TL2	3	3.18 (0.14)	ab	4	12 (0.3)	a	3	461 (23)	a	4	0.93 (0.1)	b
Group mean	16	3.09 (0.17)	A	15	9.0 (1.0)	A	14	405 (19)	A	16	1.16 (0.1)	B
Pore water												
Reference												
R1	--	-- --	--	--	-- --	--	--	-- --	--	2	0.93 (0.3)	a
R2	--	-- --	--	--	-- --	--	--	-- --	--	2	0.91 (0.1)	a
Group mean	--	-- --	--	--	-- --	--	--	-- --	--	4	0.92 (0.1)	A
Mining												
TH1	--	-- --	--	--	-- --	--	--	-- --	--	4	1.17 (0)	a
TH2	--	-- --	--	--	-- --	--	--	-- --	--	2	1.65 (0.6)	a
Group mean	--	-- --	--	--	-- --	--	--	-- --	--	6	1.33 (0.2)	A
Criteria												
		81[1]			2.5[2]			na			na	

[1] Missouri Department of Natural Resources (2006), Regional Technical Assistance Group, ambient water quality criteria recommendations for rivers and streams, *http://www.dnr.mo.gov/env/wpp/rules/wpp-rule-dev.htm*

[2] Department of Natural Resources, water quality standards (2009); *http://www.dnr.mo.gov/env/wpp/rules/wpp-rule-dev.htm*; lower 25th percentile value of representative number of samples

Table 18. Mean metal concentrations (µg/L; ±1 standard error) in surface and pore waters, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each sample type (P <0.05)]

Sample type, site type, and site	*n*	Cobalt		Nickel		Copper		Zinc		Cadmium		Lead	
Surface water													
Reference													
R1	3	0.14 (0.003)	e	0.77 (0.09)	d	0.12 (0.07)	c	0.62 (0.2)	d	0.01 (0)	d	0.05 (0.01)	d
R2	4	0.15 (0.003)	e	0.50 (0.06)	d	0.19 (0.14)	c	0.45 (0)	d	0.01 (0.002)	d	0.06 (0.004)	d
Group mean	7	0.15 (0.003)	C	0.62 (0.07)	C	0.16 (0.08)	C	0.52 (0.1)	C	0.01 (0.001)	C	0.05 (0.004)	C
Mining													
TH1	3	0.40 (0.03)	ab	4.10 (0.26)	ab	0.54 (0.16)	c	120 (7)	ab	1.00 (0.05)	a	6.52 (0.65)	ab
TH2	3	0.67 (0.02)	a	7.68 (0.29)	a	1.96 (0.69)	ab	94.8 (0.5)	a	0.72 (0.04)	ab	9.18 (0.10)	a
Group mean	6	0.54 (0.06)	A	5.89 (0.82)	A	1.25 (0.45)	A	107 (6)	A	0.86 (0.07)	A	7.85 (0.66)	A
Downstream													
TM1	3	0.30 (0.01)	bc	3.47 (0.09)	b	1.73 (0.17)	ab	35.3 (4.8)	b	0.32 (0.02)	b	3.42 (0.11)	bc
TM2	5	0.24 (0.01)	cd	1.30 (0.17)	c	1.85 (0.23)	ab	5.12 (0.8)	c	0.17 (0.03)	c	3.04 (0.24)	c
TL1	3	0.22 (0.01)	d	1.30 (0.20)	c	1.51 (0.13)	a	5.46 (0.9)	c	0.19 (0.02)	c	3.08 (0.09)	c
TL2	3	0.25 (0.03)	d	1.19 (0.27)	c	3.00 (0.98)	b	3.88 (1.2)	c	0.11 (0.04)	c	3.10 (0.42)	c
Group mean	14	0.25 (0.01)	B	1.76 (0.26)	B	2.00 (0.25)	B	11.4 (3.6)	B	0.19 (0.02)	B	3.14 (0.12)	B
Pore water													
Reference													
R1	6	0.12 (0.01)	b	0.29 (0.05)	d	0.28 (0.02)	c	1.80 (0.6)	c	0.01 (0)	b	0.07 (0.01)	b
R2	6	0.46 (0.16)	a	0.51 (0.06)	c	0.27 (0.04)	c	2.61 (0.9)	c	0.01 (0)	b	0.08 (0.01)	b
Group mean	12	0.29 (0.09)	B	0.40 (0.05)	B	0.27 (0.02)	B	2.20 (0.5)	B	0.01 (0)	B	0.08 (0.004)	B
Mining													
TH1	6	0.82 (0.41)	a	3.50 (0.23)	b	0.51 (0.07)	b	138 (8)	a	1.17 (0.37)	a	19.6 (5)	a
TH2	6	0.44 (0.16)	a	4.93 (0.29)	a	1.55 (0.09)	a	71.1 (7)	b	1.03 (0.18)	a	7.10 (2)	a
Group mean	12	0.63 (0.22)	A	4.21 (0.28)	A	1.03 (0.16)	A	104 (11)	A	1.10 (0.20)	A	13.8 (3)	A
Missouri Criteria		--		94–113[1]		13–16[1]		193–233[1]		0.4–0.5[1]		5–7[1]	
Big River watershed		--		--		--		193[2]		--		5[2]	
USEPA Criteria		--		215–337[3]		16–26[3]		93–145[3]		0.4–0.6[3]		5–9[3]	

[1] Missouri Department of Natural Resources, Code of Regulations (2009), Chapter 7, *http://www.sos.mo.gov/adrules/csr/current/10csr/10c20-7A-G.pdf* warm-water fisheries

[2] Missouri Department of Natural Resources (2007), Total maximum daily load information sheet for Big River and Flat River Creek, *http://www.dnr.mo.gov/env/wpp/docs/2074-2080-2168-2170-big-r-tmdl.pdf*

[3] USEPA (2006)

Table 19. Chronic toxic unit scores (∑TUs) for surface and pore waters, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each sample type (P <0.05)]

Sample type, site type, and site	*n*	Nickel		Copper		Zinc		Cadmium		Lead		∑TUs	
Surface water													
Reference													
R1	3	0.003	bc	0.007	c	0.006	d	0.013	d	0.008	c	0.038	d
R2	4	0.002	c	0.012	c	0.005	d	0.019	d	0.011	c	0.048	d
Group mean	7	0.003	C	0.010	C	0.005	C	0.014	C	0.010	C	0.044	C
Mining													
TH1	3	0.014	a	0.0245	c	0.963	a	1.961	a	0.839	a	3.802	a
TH2	3	0.025	a	0.085	b	0.716	ab	1.341	ab	1.094	a	3.262	ab
Group mean	6	0.020	A	0.055	A	0.840	A	1.651	A	0.967	A	3.532	A
Downstream													
TM1	3	0.011	a	0.074	ab	0.266	b	0.597	b	0.405	b	1.353	b
TM2	5	0.005	b	0.088	ab	0.043	c	0.341	c	0.041	b	0.886	c
TL1	3	0.004	b	0.068	b	0.043	c	0.370	c	0.390	b	0.876	c
TL2	3	0.004	b	0.146	a	0.033	c	0.231	c	0.430	b	0.844	c
Group mean	14	0.006	B	0.093	B	0.089	B	0.379	B	0.409	B	0.975	B
Pore water													
Reference													
R1	6	0.002	d	0.016	b	0.028	c	0.018	b	0.015	c	0.079	b
R2	6	0.015	c	0.016	b	0.012	c	0.018	b	0.016	c	0.068	b
Group mean	12	0.002	B	0.016	B	0.023	B	0.016	B	0.014	B	0.071	B
Mining													
TH1	6	0.012	b	0.023	b	1.080	a	2.217	a	2.452	a	5.784	a
TH2	6	0.016	a	0.066	a	0.530	b	1.898	a	0.950	b	3.459	a
Group mean	12	0.014	A	0.044	A	0.805	A	2.057	A	1.701	A	4.622	A

Table 20. Mean metal concentrations (µg/g; ±1 standard error) in detritus and macro-invertebrates collected for 56-d in-situ toxicity study, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each sample type (P <0.05)]

Sample type, site type, and site	*n*	Cobalt	Nickel	Copper	Zinc	Cadmium	Lead
				Detritus			
Reference							
R1	6	8.13 (0.4) c	10.9 (0.8) b	12.4 (1) b	44.1 (3) b	0.35 (0.02) c	15.2 (2) c
R2	6	7.62 (0.5) c	11.1 (0.6) b	11.6 (0.4) b	44.0 (1) b	0.32 (0.02) c	16.6 (1) c
Group mean	12	7.87 (0.3) B	11.0 (0.5) B	12.0 (0.4) B	44.0 (2) B	0.33 (0.01) B	15.9 (1) B
Mining							
TH1	6	95.7 (8) a	104 (8) a	56.4 (4) a	2871 (302) a	23.8 (4) a	1535 (137) b
TH2	6	74.8 (4) b	81.8 (5) a	82.1 (8) a	3297 (200) a	64.6 (5) b	3100 (255) a
Group mean	12	85.3 (5) A	92.7 (6) A	69.2 (6) A	3084 (184) A	44.2 (7) A	2317 (273) A
				Macro-invertebrates			
Reference							
R1	6	2.83 (0.33) b	4.73 (1.6) b	19.2 (2.9) bc	104 (16) b	0.60 (0.18) b	16.3 (8.3) b
R2	6	2.78 (0.31) b	4.44 (1.7) b	17.1 (1.9) c	105 (12) b	0.35 (0.07) b	9.10 (2.4) b
Group mean	12	2.80 (0.22) B	4.58 (1.1) B	18.2 (1.7) B	105 (10) B	0.48 (0.10) B	12.7 (4.3) B
Mining							
TH1	6	16.7 (2.4) a	19.1 (3.1) a	23.2 (1.9) a	769 (90) a	11.3 (1.8) a	562 (63) a
TH2	6	21.6 (2.8) a	22.3 (4.6) a	48.8 (8.3) a	848 (145) a	13.1 (3.1) a	877 (180) a
Group mean	12	19.1 (1.9) A	20.7 (2.7) A	36.0 (5.6) A	808 (82) A	12.2 (1.7) A	720 (103) A

Table 21. Mean total length and metal concentrations (µg/g; ±1 standard error) in largescale stonerollers (*Campostoma oligolepis*) collected for 56-d in-situ toxicity study, Big River, Missouri.

[Sites with the same lower case letter and groups of sites with the same capital letter are not significantly different for each sample type (P <0.05). N = number of composite samples]

Site type and site	*n*	Total length (mm)		*N*	Cobalt		Nickel		Copper		Zinc		Cadmium		Lead	
Reference																
R1	37	78 (3)	b	4	0.63 (0.09)	c	1.2 (0.21)	c	3.6 (0.11)	c	148 (10)	b	0.05 (0.004)	c	1.62 (0.38)	b
R2	52	79.4 (1)	b	4	1.02 (0.11)	b	2.0 (0.11)	c	4.0 (0.30)	c	124 (8.1)	b	0.11 (0.02)	b	3.15 (0.31)	b
Group mean	89	78.9 (1)	B	8	0.82 (0.09)	B	1.6 (0.19)	B	3.8 (0.18)	B	136 (7.6)	B	0.08 (0.01)	B	2.39 (0.37)	B
Mining																
TH1	33	95.1 (3)	a	4	2.2 (0.81)	b	7.1 (3.8)	b	5.9 (1.1)	b	559 (98)	a	3.03 (1.1)	a	150 (51)	a
TH2	40	94.1 (3)	a	4	4.1 (0.60)	a	9.9 (2.0)	a	12 (2.6)	a	479 (73)	a	2.84 (0.51)	a	200 (30)	a
Group mean	73	94.5 (2)	A	8	3.1 (0.58)	A	8.5 (2.1)	A	8.9 (1.7)	A	519 (58)	A	2.94 (0.55)	A	175 (29)	A

Table 22. Percent total organic carbon (TOC) and particle size of sediments, Big River, Missouri.

Site type and site	Percent TOC	Percent sand (0.20–2 mm)	Percent silt (0.2–0.002 mm)	Percent clay (<0.002 mm)
Reference				
R1	0.27	83.3	0.93	15.8
R2	0.34	87.1	1.93	11.0
Mining				
TH1	2.40	75.6	12.5	11.9
TH2	4.07	89.3	-0.33	11.0
Downstream				
TM1	2.54	82.8	7.27	9.91
TM2	1.21	62.4	22.8	14.8
TL1	0.13	90.0	1.62	8.41
TL2	0.56	67.8	17.6	14.7

Table 23. Concentrations (µg/g dry weight) of selected total recoverable elements in sediment, Big River, Missouri as determined by ICP-MS semi-quantitative scan.

[MDL = method detection limit]

Site type and site	Ba	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
Reference									
R1	40	100	6000	2	5	3	20	0.04	11
R2	40	200	7000	4	6	4	21	0.07	14
Mining									
TH1	40	2000	16000	8	10	6	980	18	840
TH2	60	4000	24000	20	20	20	740	13	1500
Downstream									
TM1	40	3000	18000	10	10	20	470	8	850
TM2	2000	1000	15000	10	20	30	300	3	1400
TL1	300	900	11000	6	7	5	130	1	320
TL2	200	300	8000	6	10	10	130	1	270
MDL	0.40	0.04	4.00	0.04	0.40	0.40	0.40	0.04	0.04

Table 24. Percent water, loss on ignition, acid volatile sulfide (AVS; µmol/g dry weight), and simultaneously extracted metals (µg/g dry weight) in sediments.

					1-M HCl (simultaneously) extracted metals (µg/g dw)											
Site type and site	Water (%)	LOI (%)	AVS (µmol/g)	∑SEM	Ni	% of total SEM	Cu	% of total SEM	Zn	% of total SEM	Cd	% of total SEM	Pb	% of total SEM	∑SEM / AVS[a]	∑SEM - AVS[b]
Reference																
R1	19.1	0.91	1.20	15	1.00	6.5	0.72	4.7	5.70	37.1	0.042	0.3	7.90	51.4	0.13	-1.05
R2	17.5	1.05	0.73	18	1.41	7.7	0.94	5.1	5.44	29.7	0.046	0.3	10.5	57.3	0.24	-0.55
Mining																
TH1	23.6	1.20	3.52	1210	7.14	0.6	2.05	0.2	510	42.1	11.0	0.9	680	56.2	3.22	7.82
TH2	20.5	1.98	1.04	1413	10.3	0.7	6.53	0.5	350	24.8	5.76	0.4	1040	73.6	10.31	9.66
Downstream																
TM1	21.3	1.37	4.45	867	8.22	0.9	5.00	0.6	287	33.1	4.61	0.5	562	64.8	1.66	2.92
TM2	32.4	2.88	4.12	1320	7.17	0.5	12.9	1.0	197	14.9	3.16	0.2	1100	83.3	2.10	4.55
TL1	22.7	1.40	0.06	289	1.84	0.6	1.64	0.6	75.5	26.1	0.99	0.3	209	72.3	39.47	2.17
TL2	24.2	1.61	3.88	313	3.04	1.0	4.00	1.3	67.4	21.5	1.01	0.3	238	75.9	0.59	-1.58

[a] ∑ / AVS = ∑[Ni,Cu,Zn,Cd,Pb] mmol/g ÷ AVS mmol/g

[b] ∑ - AVS = ∑[Ni,Cu,Zn,Cd,Pb] mmol/g - AVS mmol/g

Table 25. Spearman coefficients for correlation among riffle crayfish density, mean carapace length (CL), and metal concentrations in crayfish, surface water, and sediment, Big River, Missouri.

[Values listed in boldface are significant (P <0.05). Cobalt was not measured in crayfish or sediment]

Metal	Matrix	Wild *Orconectes luteus* Co	Ni	Cu	Zn	Cd	Pb	Riffle crayfish density	Riffle crayfish CL
Cobalt	Surface water	**0.74**	**0.81**	0.62	**0.71**	**0.90**	**0.88**	**-0.90**	**-0.98**
	Sediment	--	--	--	--	--	--	na	na
	Crayfish	--	**0.83**	0.55	**0.93**	**0.88**	**0.90**	**-0.79**	**-0.71**
Nickel	Surface water	**0.93**	**0.90**	0.67	**0.86**	**0.95**	**0.98**	**-0.90**	**-0.88**
	Sediment	**0.76**	**0.83**	**0.81**	0.64	**0.83**	**0.81**	**-0.83**	**-0.81**
	Crayfish	**0.83**	--	**0.71**	**0.81**	**0.86**	**0.93**	**-0.81**	**-0.83**
Copper	Surface water	0.14	0.36	0.64	0.02	0.43	0.36	-0.50	-0.52
	Sediment	**0.90**	**0.93**	0.57	**0.88**	**0.93**	**1.00**	**-0.93**	**-0.90**
	Crayfish	0.55	**0.71**	--	0.50	**0.71**	0.57	-0.48	-0.52
Zinc	Surface water	**0.88**	**0.90**	0.60	**0.90**	**0.95**	**0.98**	**-0.86**	**-0.88**
	Sediment	0.52	0.64	**0.71**	0.36	0.59	0.62	**-0.74**	-0.62
	Crayfish	**0.93**	**0.81**	0.50	--	**0.90**	**0.88**	**-0.76**	**-0.74**
Cadmium	Surface water	**0.86**	**0.88**	0.62	**0.93**	**0.98**	**0.95**	**-0.88**	**-0.90**
	Sediment	**0.81**	**0.88**	0.54	**0.81**	**0.90**	**0.95**	**-0.98**	**-0.98**
	Crayfish	**0.88**	**0.86**	**0.71**	**0.90**	--	**0.93**	**-0.86**	**-0.88**
Lead	Surface water	**0.71**	**0.79**	0.64	**0.74**	**0.93**	**0.86**	**-0.83**	**-0.95**
	Sediment	0.69	**0.71**	0.57	0.57	**0.71**	**0.79**	**-0.90**	**-0.74**
	Crayfish	**0.90**	**0.93**	0.57	**0.88**	**0.93**	--	**-0.93**	**-0.90**

Table 26. No-effect hazard concentrations (NEHC) of metals and hazard quotient (HQ) of wild (*O. luteus*) and caged (*O. luteus* and *O. hylas*) crayfish for receptor wildlife species.

[dw = dry-weight; values in boldface exceed 1.0 indicating risk]

		Concentration				HQ			
		Wild *O. luteus*		Caged crayfish		Wild *O. luteus*		Caged crayfish	
Species	NEHC[1]	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
					Cobalt				
Robin[2]	25	0.79	6.19	0.78	14.3	0.032	0.248	0.031	0.572
Heron[3]	211	0.79	6.19	0.78	14.3	0.004	0.029	0.004	0.068
Shrew[4]	59.1	0.79	6.19	0.78	14.3	0.013	0.105	0.013	0.242
Mink[5]	262	0.79	6.19	0.78	14.3	0.003	0.024	0.003	0.055
					Nickel				
Robin[2]	22.1	1.14	7.96	0.94	13.8	0.052	0.360	0.043	0.624
Heron[3]	186	1.14	7.96	0.94	13.8	0.006	0.043	0.005	0.074
Shrew[4]	13.7	1.14	7.96	0.94	13.8	0.083	0.581	0.069	1.007
Mink[5]	60.7	1.14	7.96	0.94	13.8	0.019	0.131	0.015	0.227
					Zinc				
Robin[2]	217	0.67	462	71.4	417	0.003	**2.13**	0.329	**1.92**
Heron[3]	1836	0.67	462	71.4	417	0.0004	0.252	0.039	0.227
Shrew[4]	608	0.67	462	71.4	417	0.001	0.760	0.117	0.686
Mink[5]	2693	0.67	462	71.4	417	0.0002	0.172	0.027	0.155
					Cadmium				
Robin[2]	4.8	0.29	25.3	0.24	8.58	0.060	**5.27**	0.050	**1.79**
Heron[3]	40.8	0.29	25.3	0.24	8.58	0.007	0.620	0.006	0.210
Shrew[4]	6.2	0.29	25.3	0.24	8.58	0.047	**4.08**	0.039	**1.38**
Mink[5]	27.5	0.29	25.3	0.24	8.58	0.011	0.920	0.009	0.312
					Lead				
Robin[2]	5.4	0.67	173	0.77	350	0.124	**32.0**	0.143	**64.8**
Heron[3]	45.3	0.67	173	0.77	350	0.015	**3.82**	0.017	**7.73**
Shrew[4]	37.9	0.67	173	0.77	350	0.018	**4.56**	0.020	**9.23**
Mink[5]	168	0.67	173	0.77	350	0.004	**1.03**	0.005	**2.08**

[1] NEHC=Toxicity reference value (TRV)/daily food ingestion (DI); HQ= maximum concentration in crayfish/NEHC; all assuming a diet of 100 percent crayfish

[2] American robin, *Turdus migratorius;* DI=1.52 kg/kg/d (USEPA, 1993)

[3] Great blue heron, *Ardea herodias*; DI=0.18 kg/kg/d (USEPA, 1993)

[4] Short-tailed shrew, *Blarina brevicauda*; DI=0.62 kg/kg/d (USEPA, 1993)

[5] American mink, *Mustela vison*; DI=0.14 (USEPA, 1993)