Effects of mining-derived metals on riffle-dwelling crayfish in southwestern Missouri and southeastern Kansas of the Tri-State Mining District, USA

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Abstract

The Tri-State Mining District (TSMD) formerly was one of the largest producers of lead-zinc ore in the world. Riffle-dwelling crayfish populations were sampled at 16 sites in 4 tributaries of the Spring River located in southwestern Missouri and southeastern Kansas within the TSMD. Crayfish species richness, crayfish density, and physical-habitat and water quality were examined at each site to assess the ecological effects of mining-derived metals on crayfish. Metals (lead, zinc, cadmium, nickel, copper) were analyzed in samples of surface water, sediment, detritus, and whole crayfish. Three species of crayfish (*Orconectes neglectus neglectus; Orconectes macrus*; *Orconectes virilis*) were collected during the study; however only *O. n. neglectus* was collected at all sites. Mean crayfish densities differed significantly among sites and were significantly lower at mining sites than reference sites. Concentrations of metals in surface water, sediment, detritus, and whole crayfish were significantly correlated with a greater proportion of mine-waste within the drainage area and were significantly greater at sites downstream from mine-waste (e.g., chat piles). Principal components analyses indicated a separation of sites because of an inverse relationship among riffle crayfish density and mining-related (i.e., metals concentrations) and physical-habitat quality (i.e., water depth) variables. Sediment probable-effects concentrations quotients and surface-water toxic-unit scores were significantly correlated; both indicated risk of toxicity to aquatic biota at several sites. Metals concentrations in crayfish within the TSMD exceeded concentrations known to be toxic to carnivorous wildlife; this indicates that mining-derived metals in crayfish have the potential to impair carnivorous wildlife due to the transfer of metals through dietary pathways. Additionally, mining-derived metals in crayfish have the potential to impair ecosystem function through the reduction in organic-matter decomposition and nutrient cycling in streams due to reduced crayfish densities.

Key words: Tri-state mining district, lead-zinc mining, metals, crayfish, *Orconectes neglectus neglectus*, *Orconectes macrus*

Introduction

The Tri-State Mining District (TSMD) occupies an area of some 6,475 km^2 in southwestern Missouri, southeastern Kansas, and northeastern Oklahoma. The TSMD was mined for zinc (Zn) and lead (Pb) for more than 150 years, beginning in the mid-1800s and ending in the late 1960s, with peak production occurring during World War II (Stewart 1986). Sites contaminated to varying degrees by wastes from historical mining, ore processing, and smelting are widely distributed in the area. Previous studies have documented that the release of metals from mining activities has resulted in widespread environmental contamination (Barks 1977; Czarneski 1985; Davis and Schumacher 1992; Pope 2005), potential risk to humans (Schmitt et al. 2006), and effects on aquatic organisms including crayfish (Angelo et al. 2007; Brumbaugh et al. 2005; MacDonald et al. 2010; Schmitt et al. 2006; Wang et al. 2010; Wildhaber et al. 1997, 2000).

Crayfish are an important structural component of many aquatic systems including Ozark streams where they are the predominant macrionvertebrate (Rabeni 1995). Crayfish play an integral role in stream ecosystems by shredding organic matter and facilitating the cycling of nutrients and energy through stream food webs (Creed 1994; Momot 1978, 1995; Rabeni et al. 1995; Parkyn et al. 2001). Crayfish are an important prey item for fish, many other aquatic and terrestrial vertebrates (DiStefano 2005; Hobbs 1993; Probst et al. 1984; Rabeni et al. 1995; Whitledge and Rabeni 1997), and waterfowl (DiStefano 2005 and references therein). Recent research has demonstrated that crayfish significantly affect aquatic microhabitats via ecosystem engineering (Zhang et al. 2004), which may have indirect implications for federally-listed endangered mussels. Therefore, the effects of metals on crayfish can have significant direct and indirect effects on stream ecosystems. Crayfish have been used in environmental assessments of metals in aquatic ecosystems because they have limited home ranges, accumulate metals in a relatively short period, and are sensitive to mining-derived metals (Allert et al. 2008, 2009, 2010; Besser et al. 2007; Stinson and Eaton 1983; Wigginton and Birge 2007).

The objectives of this study were to determine crayfish species composition and densities in riffle habitats at selected stream sites in the TSMD; evaluate riffle crayfish densities relative to concentrations of mining-derived metals (Pb; Zn; cadmium, Cd; nickel, Ni; copper, Cu) in surface water, sediment, detritus, and whole crayfish collected in riffle habitats; characterize physical-habitat and water quality of riffle habitats at selected sites; and evaluate the potential effects of mining-derived metals in crayfish to secondary consumers.

Methods

Study area

Crayfish in riffle habitats were sampled in tributaries of the Spring River of southwestern Missouri and southeastern Kansas at 16 sites in Jenkins Creek, Center Creek, Turkey Creek, and Shoal Creek (Table 1; Figure 1). Sites were sampled once and selected based on stream order (Strahler 1952); proximity to designated areas (i.e., areas of mine-waste) within the U.S. Environmental Protection Agency (USEPA) National Priority List (NPL) Superfund site; and access to public or private lands where written permission could be obtained from landowners. Post-priori classification of sites was done based on downstream proximity to sources of mining-related contaminants (Table

1; Figure 1); metals concentrations in the materials analyzed from each site; and correlation analyses. Sites considered to be upstream from contaminated areas (e.g., reference sites) include J1, C1, T1, T2, S1, and S2 (Figure 1); sites considered to be affected by and directly downstream from contaminated areas (e.g., mining sites) include C2, C3, C4, T3, S3, and S6 (Figure 1); and sites considered to be further downstream from mining or less contaminated areas (e.g., downstream sites) include T4, C5, S4, and S5 (Figure 1). Site locations were documented by a hand-held global positioning system (GPS) receiver [±10 m, datum = World Geodetic System (WGS) 84]. The Shoal Creek waste-water treatment facility (WWTP; Apricot Drive, Joplin, Missouri) for the city of Joplin discharges effluent into Shoal Creek immediately downstream from S3. A grab sample of the effluent was collected July 23, 2008; data are included for the effluent in all water-quality tables, but not statistical comparisons. There are additional WWTPs located in proximity to sites on Center Creek (Webb City WWTP, downstream from C3); on Turkey Creek (Lone Elm WWTP, downstream from T3); and on Shoal Creek (Granby WWTP, upstream from S1), which were not sampled.

Drainage area was estimated using ArcMap® by manually drawing polygons around land areas that drained to the sampling point, using Digital Raster Graphics (DRGs) of U.S. Geological Survey 1:24,000 topographic maps as a base layer. The area of mine-waste within each drainage was estimated by manually drawing polygons around mine-waste identified in the same DRGs and calculating their combined area within ArcMap®. Stream gradient was estimated by measuring stream distance from one or two topographic lines upstream and downstream from the sampling point and then dividing by the elevation change. In-stream distance (km) to the nearest mine-waste source was estimated by measuring the stream length using DRGs within ArcMap®.

Field collections

Crayfish species composition and density

Crayfish species composition and densities were determined at each of the 16 sites by disturbing the substrate inside a 1-m^2 weighted-PVC quadrat frame placed on the stream bottom directly upstream of a kick seine (1.5 m length x 1.5 m height) with 3-mm delta mesh from July 13–29, 2009 (15 sites) and on September 3, 2009 (Site S6). Each site consisted of a stream reach containing three riffles. Eight kick-seine subsamples were randomly located in each riffle (total n =24 per site). Crayfish were identified to species (Pfleiger 1996) and gender (when possible), and carapace length (CL) measured (to the nearest 0.1 mm) from the tip of rostrum to the posterior edge of the cephalothorax. All crayfish except those retained for metals analyses from each riffle at each site were released alive to the stream.

Physical-habitat measurements

Physical-habitat variables (wetted width, water depth, velocity, substrate coarseness) were estimated for each of the three sampled riffles at each site by taking measurements at each 1-m^2 quadrat just prior to crayfish sampling and along multiple lateral transects using methods of Bain and Stevenson (1999), Barbour et al. (1999), Bovee and Milhouse (1978), Hamilton and Bergersen (1984), and Platts et al. (1983). Lateral transects were established to characterize wetted width, water depth (cm), and current velocity (m/s) of the riffles, and were located longitudinally every 10 m in riffles that were <50 m long; every 20 m for riffles 50–100 m long; and every 30 m for riffles >100 m long. Sampling intervals along transects were based on stream width (range =3–11 stations). Substrate coarseness was estimated visually within each 1-m^2 kick-seine quadrat and along transects (Bain et al. 1985). Substrate particles were assigned a value based on six coarseness categories at five random points within a 0.5 m x 0.5 m grid (Table 2; Bain et al. 1985) and mean substrate coarseness was calculated. Water depth and current velocities were measured within each 1-m^2 kick-seine quadrat and along transects using a Marsh-McBirney® 2000 portable flow meter at the substrate surface (transects only) and at sixth-tenths (0.6) measured depth (henceforth mid-water depth).

Three additional substrate samples were collected in an undisturbed location within each riffle using a 1.1-L cylindrical grab sampler. Substrate was wet-sieved with four sieves (38.1-, 19.0-, 9.5-, and 2.0-mm apertures) and wet-weighed to determine the percentage of total mass in each size class. For each substrate sample, sediment passing through the 2.0-mm sieve was transported to the laboratory for further processing and determination of particle size (American Public Health Association et al. 2005).

A reach-scale assessment of physical-habitat quality of each sampling site was conducted using the Qualitative Habitat Evaluation Index (QHEI; Ohio Environmental Protection Agency 2006). The QHEI uses six metrics to provide an evaluation of lotic macrohabitats. Although the QHEI was developed for fish, it has been used to evaluate macrohabitat condition for macroinvertebrate communities (Ohio Environmental Protection Agency 2006). Metrics used in the QHEI included substrate (maximum score =20); instream cover (maximum score =20); channel morphology (maximum score =20);

bank erosion and riparian zone (maximum score =10); pool-glide and riffle-run quality maximum score =20); and gradient of the drainage area (maximum score =10). The maximum possible QHEI score for a site is 100.

<u>In-situ water-quality measurements</u>

A Hydrolab® (Loveland, Colorado, USA) Quanta meter was used to measure temperature, pH, specific conductance, dissolved oxygen, and turbidity in each riffle sampled for crayfish. A surface-water grab sample was collected from each riffle for additional water-quality analyses. The calibration of the Quanta was checked daily with water-quality standards and against dissolved oxygen calibration tables. Detection and recoveries of water-quality standards for field-collected water-quality measurements were within study criteria (±20%); thus, none of the sample results required correction for accuracy.

Laboratory analyses

<u>Water-quality analyses</u>

Quality-control measures for all water samples included blanks, certified reference materials, and replicates. A calibration blank and an independent calibration verification standard were analyzed with every 10 samples to confirm the calibration status of the instrumental analyses; 10–20 standards were run per analysis.

Alkalinity and hardness were measured by titration (American Public Health Association 2005). Sulfate was measured by colorimetric detection with a Hach® 2100 Spectrophotometer (Loveland, Colorado, USA). Surface-water samples for particulate organic carbon (POC) were acidified to a pH of 2 with 2.0 N sulfuric acid on the same day of collection and then filtered with Gelman® Type A/E glass-fiber filters (nominal pore size =0.45 µm) the day after collection. Filters were stored frozen (-20 °C) until analyzed. Particulate organic carbon concentrations were determined using a Coulometrics® Model 5020 Carbon Analyzer (UIC, Inc., Joliet, Illinois, USA) according to American Society for Testing and Materials Method D4129-05 (American Society for Testing and Materials 2005). Water samples for chlorophyll *a* were filtered with Gelman® Type A/E glass-fiber filters (nominal pore size =0.45 µm) on the same day as collection, and filters were stored frozen until analyzed. In-vitro chlorophyll *a* concentrations were determined following extraction in 90% buffered acetone using a Turner® Model AU-10 Fluorometer (Turner Designs Inc., Sunnyvale, California, USA) according to USEPA Method 445.0 (Arar and Collins 1997). Water samples were analyzed for total suspended solids (TSS) using methods recommended by the American Public Health Association (2005). Samples were filtered with a glass-fiber filter (ProWeigh® pre-washed and pre-weighed glass-fiber filters; nominal pore size =1.5 µm; Environmental Express, Mt. Pleasant, South Carolina, USA) within 4 days of collection, dried at 105 °C, and then weighed for residue.

Nutrients concentrations were determined with a Technicon® Autoanalyzer (Tarrytown, New York, USA) using colorimetric detection (American Public Health Association 2005). Water samples were filtered with 0.4 µm-polycarbonate filters under vacuum pressure on the same day as collection and the filtrate was frozen until analyzed for dissolved nutrients. Total ammonia nitrogen (NH_3-N) concentrations were determined using a salicylate/nitroprusside colorimetric reaction. Nitrite/nitrate (NO_3-N) concentrations were determined using cadmium reduction (method adapted from Technicon® Industrial Method No. 158-71W). Samples for total phosphorous (TP) and total nitrogen (TN) were digested in sodium hydroxide and potassium persulfate; concentrations were determined using the automated ascorbic acid method for phosphate and the automated Cd-reduction method for nitrate/nitrite (American Public Health Association 2005). Dissolved organic carbon (DOC) concentrations were determined using a persulfate/UV digestion followed by colorimetric analysis of CO_2.

Method detection limits (MDLs) for water-quality variables are listed in Table 3. Recovery of reference standards used as laboratory control samples for water-quality analyses ranged from 82–115%, except for one replicate of NO_3-N (125%); four replicates for sulfate (67%, 122%, 133%, 133%); one replicate for TP (142%); and three replicates for DOC (123%, 126%, and 132%). Instrumental precision, estimated by relative percent differences (RPDs) for replicate sample analyses were within the study criteria of 80–120% except for one (8%) replicate for sulfate (39%); two (15%) replicates for TSS (21%, 27%); two (12%) replicates for POC (34%, 35%); one (8%) replicate for DOC (22%); two (14%) replicates for TN (200%, 50%); one (7%) replicate for TP (20%); and three (23%) replicates for NH_3-N (24%, 49%, 59%). Overall, detection and recovery of reference standards used as laboratory-control samples for surface-water-quality parameters were within the study criteria (±20%); thus none of the sample results were corrected.

Determination of metals concentrations in crayfish and detritus

Crayfish for metals analyses were subsampled from those collected during kick-seine sampling. Generally, five crayfish from each riffle at each site were composited for metals analyses. Additional crayfish for metals analysis samples were collected when necessary by additional kick seining or by hand at one riffle at five sites (C5, T3, T4, S3, S5; Figure 1). Composite samples from one riffle at three sites (T4, S1, S4; Figure 1) contained only three crayfish. Crayfish were rinsed with site water and placed in pre-cleaned high-density polyethylene (HDPE) containers. All containers (including those for detritus, sediment, and water) were pre-cleaned by submerging in a bath containing a solution of 4 M HNO_3 and 2 M HCl for 30 minutes, followed by triple rinsing with ultra-pure water (18 M-Ohm/cm) and drying in a laboratory oven equipped with filtered air supply. Detritus (e.g., weathered leaves) was collected with a kick net and by hand on the day of crayfish sampling at the stream margins (e.g., banks) of each riffle sampled. Detrital material was rinsed with site water and placed in pre-cleaned 125-ml HDPE containers for metals analyses. Samples were placed on ice; frozen (-20 °C) the same day of collection; and stored frozen until they were analyzed.

Animal tissues and organic material were lyophilized (i.e., freeze-dried) and reduced to a coarse powder by mechanical crushing in a glass vial with a glass rod. Neither exoskeletons nor gut contents of the crayfish were removed before analysis. A dry mass of 0.25 g from each composite sample was digested using concentrated nitric acid and microwave heating. Composite samples of whole crayfish and detritus from each site were analyzed for Pb, Zn, Cd, Ni, and Cu by inductively-coupled plasma-mass spectrometry (ICP-MS; Besser et al. 2007; Brumbaugh et al. 2005).

Determination of metals concentrations in sediment

Composite samples of stream sediments were collected in riffles at each site, composited, and analyzed. Surficial sediments (about the top 10 cm) were collected within the wetted stream channel using PVC scoops (Besser et al. 2009b). Sediments were wet-sieved through a 2-mm (2000-µm) stainless-steel mesh sieve in the field to remove coarse particles using a minimum quantity of site water (Besser et al. 2009b; Brumbaugh et al. 2007). Two pre-cleaned 125-ml glass containers were filled to about two-thirds of their volume from each sampling site. Samples were placed on ice and refrigerated (4 °C) until analyzed. In the laboratory, a subsample of the <2000-µm sediment sample was passed through a 250-µm stainless-steel mesh sieve. Total recoverable metals (Pb, Zn, Cd, Ni, and Cu) in the both (<250-µm and <2000-µm) fractions of sediment were analyzed by ICP-MS (Brumbaugh et al. 2007; May et al. 1997), because crayfish likely are to incidentally ingest both size fractions of sediment, which are associated with organic material. Total organic carbon (TOC) concentrations in both fractions were analyzed using a UIC® coulometer (American Society for Testing and Materials 2005).

Determination of metals concentrations in water

A subsample of a surface-water grab sample was removed for metals analyses. Samples for metals analyses were filtered on-site into a pre-cleaned 25-ml polyethylene bottle using a polypropylene syringe and filter cartridge (0.45-µm pore size) and placed on ice. Filtered water samples subsequently were acidified to 1% (v/v) with nitric acid

(J.T. Baker Inc., Phillipsburg, New Jersey, USA) within 4 days of collection. Surface-water samples were analyzed for Pb, Zn, Cd, Ni, and Cu by ICP-MS.

Analytical quality assurance

Quality-control measures for metals samples 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 10 samples to confirm the calibration status of the ICP-MS during instrumental analyses of digestates.

All measured concentrations in detritus, sediment, and whole crayfish exceeded the MDLs listed in Table 3. Recoveries of all five metals from certified standard reference materials (sediment, mussel, oyster, plant, and plankton) ranged from 79–100% for sediment (n =2), 78–124% in plankton or plant (n =3), and 90–101% in oyster or mussel tissue (n =4). Percent recovery of calibration verification standards was maintained within the study criteria of 90–110% during all analyses. Percent relative standard deviations for triplicate digestion and analysis (two each for detritus and <250-µm sediment; three each for crayfish and <2000-µm sediment) among the five metals ranged from 1.1–9.2% for detritus; 0.7–4.1% for <250-µm sediment; 0.9–17% for crayfish; and 4.4–28% for <2000-µm sediment. Recoveries of method spikes for all five metals in separate spiked samples (four each for detritus and <250-µm sediment; six each for crayfish and <2000-µm sediment) ranged from 74–115%.

Potential interferences were determined by analysis of five-fold dilutions of selected representative samples and by analysis of an interference check solution, which contained unusually high concentrations of interfering constituents. All dilution percent differences (DPDs) were within the study criteria of ±10%. Mean recovery from the interference check solution (n =8) was within the study criteria of 80–120% for Cu, Cd, and Pb, but was exceeded for Ni (128%) and Zn (138%). Blank-equivalent concentrations (BECs) for digestion blanks were less than corresponding MDLs for all elements; therefore, sample results were not corrected for BECs.

Method detection limits for analyses of metals in water samples are listed in Table 3. Of the 39 field-collected samples analyzed, measured concentrations did not exceed the MDLs in 4 (10%) samples for Cu; 3 (8%) for Zn; 5 (13%) for Ni; 8 (21%) for Pb; and 10 (26%) for Cd. Percent recovery of calibration verification standards during analyses of water samples ranged from 92–108%. Percent recovery of the five metals in reference solutions used as laboratory control samples ranged from 96–100%. Percent recovery of analytical spikes ranged from 92–110%. Relative percent differences between duplicate analyses of water samples ranged from 0–2.3%. As a check for potential interferences, DPDs based on five-fold dilutions of selected water samples ranged from 0.1–3.2%. Potential interferences were checked using an interference check solution; mean recovery from the interference check solution (n =2) was within or near the study criteria of 80–120% for Cu, Cd, and Pb, but was exceeded for Ni (163%) and Zn (144%). Although recovery for Ni and Zn was elevated in the interference check solution, it contained unusually high concentrations of interfering constituents that were not expected to be problematic in the water samples. Blank-equivalent concentrations for digestion blanks were less than corresponding MDLs for all elements except Zn (0.74–2.98 ng/ml); therefore, sample results were not corrected for BECs. Overall, quality assurance results indicated that the analytical methods had acceptable accuracy and precision for the study criteria.

Assessment of risk of metals concentrations

Hazard assessment

Elevated concentrations of metals (Pb, Cd, Zn) in fish and crayfish within the TSMD have been shown to represent ecological risk to fish and carnivorous wildlife (Schmitt et al. 2006, 2008). The screening-level criteria developed by Schmitt et al. (2006) were used to assess the potential hazards of metals in crayfish collected during this study. Toxicity thresholds of metals in target species were determined through food-chain analysis, using procedures developed for conducting ecological risk assessments (USEPA 1992, 1993, 1997, 1999a, 2007b). The assessments used representative bird and mammal consumer species based on body weight such as American robin (*Turdus migratorius*) and short-tailed shrew (*Blarina brevicauda*), which can be extrapolated to similar-sized species that consume crayfish. Hazard quotients (HQs) were calculated using site-mean concentrations of Pb, Zn, and Cd in crayfish and no-effect hazard concentrations (NEHC) to estimate daily contaminant intake rates; NEHC are consensus-based no-adverse effect level-based toxic reference values (TRVs) normalized for estimated daily food-ingestion rates (IRs; Schmitt et al. 2008). All assume a diet of 100% crayfish. To summarize best and worst case conditions for metals concentrations in crayfish collected during the study, HQs were calculated using minimum and maximum concentrations of Pb, Zn, and Cd in crayfish collected during the study. Hazard quotients were compared to those calculated in previous investigations in the

TSMD and other mining districts in Missouri, USA (Allert et al. 2010; Schmitt et al. 2006); data from Schmitt et al. (2006) were converted from wet to dry weight for comparison to data in this study. There is no analogous procedure to assess risk to predatory fish; however, measured concentrations were compared to benchmark values from scientific literature.

Sediment probable-effects concentrations

Site-mean total-recoverable (TR) metals concentrations in <250-µm fraction of sediment were converted to probable-effects concentrations quotients (PEQs) by dividing site-mean TR metal concentration by the probable-effect concentration (PEC; MacDonald et al. 2000) for each metal. Data were compared to general and TSMD-specific risk thresholds associated with a 10% (PEC_{10}) or 20% (PEC_{20}) reduction in a measured endpoint (MacDonald et al. 2000, 2010). The <250-µm fraction was used because metals concentrations were correlated between the two size fractions; therefore, using either sediment fraction in the assessments would yield similar results. Individual mean PEQs for the five metals were summed (∑PEQs) to estimate risks from the metals mixtures (Besser et al. 2009a; Ingersoll et al. 2001); ∑PEQs greater than 1 generally indicate potential toxic effects (Besser et al. 2009a). MacDonald et al. (2010) determined TSMD-specific ∑PEQs for Pb, Zn, and Cd and calculated low-risk (6.47) and high-risk (10.04) toxicity thresholds to better assess potential toxic effects or risk in the TSMD.

Surface-water toxic units

The cumulative risk of toxic effects from metals in surface water was estimated using the toxic unit approach as described by Wildhaber and Schmitt (1996). A toxic unit (TU) is defined as the measured concentration of each dissolved metal in surface water divided by the chronic ambient water-quality criterion (WQC) for the metal, adjusted for hardness and the dissolved fraction of metal (USEPA 2006); the hardness-based criterion was used for copper, not the biotic ligand model (BLM) because of the lack of necessary data for the BLM (USEPA 2007a). Toxic units for metals are summed to produce a total toxicity estimate of the mixture for each sample (i.e., toxic unit score = $\sum$TUs); values greater than 1.0 generally indicate potential toxicity to aquatic biota.

Statistical analysis

Statistical analyses were conducted using Statistical Analysis System (SAS) for Windows (Release 9.1; SAS Institute, Cary, North Carolina, USA). Before analyses, data were tested for normality and homogeneity of variance. Data were not normally distributed; therefore, all analyses were conducted using ranked-transformed data. Ranks for the site-means for riffle crayfish density, water quality, physical-habitat quality, and metals concentrations were used in the statistical analyses. Censored values (< MDLs) for metals concentrations in surface water were replaced with 50% of the MDL for statistical computations, figures, and tables. All censored data were in surface-water samples from reference sites.

Differences in the ranked site-means of measured variables among sites and selected variables among streams were tested using nested analysis-of-variance

(ANOVA; riffles nested within site), with site considered a fixed effect. Differences in the means of measured variables among individual sites were evaluated with Duncan's multiple range test. Differences in selected measured variables among groups of sites (i.e., reference, mining, downstream) were tested using planned non-orthogonal contrasts using single degree-of-freedom *F*-tests. The within-site-mean squares were used in all tests. Associations among site-means for riffle crayfish density and selected physical-habitat quality and water quality variables, and metals concentrations were examined using Spearman's rank correlation analyses. A significance level of $P < 0.05$ was used to judge all statistical tests.

The relationships among riffle crayfish densities and metals concentrations, and physical-habitat and water-quality variables were examined using principal component analyses (PCA). A single metal concentration (site-mean Pb concentration in detritus) was retained to control for colinearity of metals concentrations and water-quality variables. Lead concentrations in detritus was included because detritus is an important component of crayfish diet (i.e., exposure pathway) and concentrations were correlated (r-values >0.75) with many water-quality variables (i.e., specific conductance, hardness, sulfate concentrations) and metals concentrations (except Cu) in all materials analyzed. Water depth in 1-m^2 kick-seine quadrats was retained to control for colinearity of physical-habitat variables because it was strongly correlated with many physical-habitat variables (i.e., current velocity; substrate coarseness, drainage, gradient).

Results

Crayfish species composition and density

Crayfish were collected at all sites (Table 4). There was only one site (J1) which was upstream from most sources of mining-related contaminants (e.g., designated areas with USEPA NPL Superfund site), where crayfish were collected in all 24 kick-seine subsamples. There were four sites (T3, T4, S3, S6) directly downstream from mine-wastes where crayfish were collected in fewer than one-half the kick-seine subsamples (data not shown). Only one crayfish species, *Orconectes (Procericambarus) neglectus neglectus* Faxon (Ringed Crayfish), was collected at all sites. *Orconectes* (*Procericambarus*) *macrus* Williams (Neosho Midget Crayfish) was collected at four sites (J1, C1, S1, S2), which were all located in the eastern and upstream portion of the Spring River drainage. A third species, *Orconectes (Gremicambarus) virilis* Hagen (Virile Crayfish), was collected only at J1 and C1.

There were significant differences in mean densities of *O. n. neglectus* among the sites sampled (Table 4); mean densities were significantly lower at mining and downstream sites (Table 5). Mean densities of *O. n. neglectus* were significantly greater at J1 (28.5/m^2), T1 (34.2/m^2) and T2 (29.0/m^2) compared to all other sites. Mean densities at all other sites ranged from about 1/m^2 in lower Shoal Creek (S3 – S6) to about 9/m^2 in Center Creek (C3 – C4).

There were significant differences in mean densities of *O. macrus* among sites (Table 4). *Orconectes macrus* densities were significantly greater at J1 (5.3/m^2) and C1 (13.0/m^2) than at S1 (2.3/m^2) and S2 (0.8/m^2). Mean densities of *O. macrus* were three-fold greater than mean densities of *O. n. neglectus* at C1; however, mean densities of *O.*

n. neglectus were 1.5- to 5-fold greater than *O. macrus* at the three other sites (J1, S1, S2) where both species were collected.

Combined mean densities of *O. n. neglectus* and *O. macrus* were significantly lower at mining and downstream sites than reference sites (Table 5). Combined mean densities at J1 (33.8/m^2), C1 (17.0/m^2), and S1 (5.7/m^2) were not significantly different from mean densities of *O. n. neglectus* at T1 (34.2/m^2) or T2 (29.0/m^2; Table 4); combined mean densities at S2 (3.0/m^2) were significantly lower than the combined densities at J1 and C1. The combined mean density at S2 was not significantly different from mean densities of *O. n. neglectus* at C2 – C5 (3.8–9.4/m^2), T3 (2.3/m^2), S3 – S4 (1.2–1.3/m^2) or S6 (1.1/m^2), but was significantly greater than S5 (1.0/m^2).

There were significant differences in mean CL of *O. n. neglectus* among sites (Table 6). Carapace lengths of male *O. n. neglectus* were significantly larger than CL of female *O. n. neglectus* ($F_{(1,3195)}$ =42.2; $P < 0.0001$). Mean CL of *O. n. neglectus* was significantly larger at sites in Shoal Creek than those in other creeks except T4 (14.8 mm); mean CL was greatest at S2 (25.3 mm; Table 6). Most sites had a similar range in CL (as defined by the 25th and 75th percentiles); however, ranges were greater at J1, C1, S1 and S3 than the other sites (Figure 2). No small (≤10 mm) *O. n. neglectus* were collected at three sites in lower Shoal Creek (S4 – S6). No *O. n. neglectus* greater than 25 mm were collected at five sites (C2, C5, T4, S4, S6). *Orconectes n. neglectus* greater than 30 mm were collected at six sites (J1, C1, T2, S1 – S3). The median CL for *O. n. neglectus* at all sites was between 10–20 mm, except for S1 and S2, where median CL was >20 mm (Figure 2). The sex ratio of *O. n. neglectus* at most sites was about 1; with the exception at C1, where there was about twice the number of females than males and at S5, where there was about one-half the number of females than males (Table 6).

Mean CL of *O. macrus* was significantly smaller than *O. n. neglectus* at the four sites where the two species were collected ($F_{(1,1423)}$ =16.7; P <0.0001). There were significant differences in CL of *O. macrus* among sites (Table 6), but not between male and female *O. macrus* ($F_{(1,504)}$ =0.12; P =0.72). Mean CL of *O. macrus* was greatest at S2 (14.4 mm). The median CL of *O. macrus* was between 10–15 mm at the four sites where they were collected (Figure 2). There were approximately 25% more female than male *O. macrus* at all sites (Table 6). Mean CL of *O. virilis* collected at J1 and at C1 was <25 mm; therefore they were most likely juvenile crayfish (Table 6; Pfleiger 1996).

Metals concentrations

<u>Crayfish</u>

There were no significant differences in mean CL between sexes for either species (*O. n. neglectus*, $F_{(1,226)}$ =2.29; P =0.13; *O. macrus*, $F_{(1,54)}$ =3.37; P =0.06); however, there were significant differences in mean CL among sites for *O. n. neglectus* collected for metals analyses (Table 7). Mean CL of *O. n. neglectus* taken for metals analyses were greater at J1 (25.0 mm), C1 (25.9 mm), T1 (23.9 mm), S1 (30.0 mm), and S2 (26.4 mm), which were classified as reference sites. Because metals analyses in crayfish were done on composite samples, the gender or CL of crayfish in the composite samples could not be directly linked to a specific metal concentration; however, increasing Pb, Zn, and Cd concentrations in *O. n. neglectus* were significantly correlated with increasing CL of

O. n. neglectus. In contrast, metals concentrations in *O. macrus* were not significantly correlated with the size of *O. macrus*.

Metals concentrations of each of the five metals in *O. n. neglectus* differed significantly among sites (Table 8) and by several orders of magnitude (Figure 3). Metals concentrations in crayfish were significantly greater at sites downstream from mine-waste in Center Creek (C2 – C5) and Turkey Creek (T3, T4). Mean Pb concentrations in *O. n. neglectus* collected at sites C2 – C5 were 7- to 16-times greater than at C1, whereas mean Zn concentrations were 3- to 4-times greater and mean Cd concentrations were 8- to16-times greater. In Center Creek, mean concentrations of Pb (15.7 µg/g), Zn (417 µg/g), and Ni (2.06 µg/g) in *O. n. neglectus* were greatest at C5; however, mean concentrations of Cd (8.67 µg/g) and Cu (82.1 µg/g) were greatest at C2. Mean concentrations of Pb, Zn, and Cd in *O. n. neglectus* collected in Turkey Creek were about 2- to 4-times greater at T3 and T4 than at T1 and T2. Mean concentrations of Pb (20.8 µg/g), Zn (500 µg/g), Cd (10.1 µg/g), Ni (1.91 µg/g), and Cu (117 µg/g) in *O. n. neglectus* were greatest at T3. Although metals concentrations were elevated compared to J1 and C1, metals concentrations in *O. n. neglectus* collected in Shoal Creek generally were significantly lower than those collected at downstream sites in Center Creek or Turkey Creek. Mean concentrations of Zn (235 µg/g), Cd (2.16 µg/g), and Cu (91.8 µg/g) in *O. n. neglectus* were greatest at S6; mean concentrations of Pb (5.46 g/g) were greatest at S1; and mean concentrations of Ni (1.89 µg/g) were greatest at S3. Combined species mean concentrations of Pb and Zn were significantly greater at S1 and S2 than J1 and C1, and concentrations of Cd were significantly greater at S1 (Table 8). Mean concentrations of Pb, Zn, and Cd in *O. n. neglectus* were significantly greater at mining and downstream sites than at reference sites; concentrations of Zn and Cd in *O. n. neglectus* were significantly greater at mining sites than downstream sites (Table 9).

Mean concentrations of all metals in *O. macrus* were greatest at S1 (Table 8). There were no significant differences in Cu concentrations in *O. macrus* among sites; however, concentrations of Pb (10.4 µg/g), Zn (249 µg/g), and Cd (1.81 µg/g) in *O. macrus* were significantly greater at S1 than at sites J1 and C1. Nickel concentrations in *O. macrus* were significantly greater at S1 (2.08 µg/g), S2 (2.02 µg/g) than in *O. macrus* collected at J1 (1.33 µg/g) and C1 (1.45 µg/g).

Mean metals concentrations in *O. n. neglectus* and *O. macrus* were similar; however, mean concentrations in *O. macrus* were significantly greater than mean concentrations in *O. n. neglectus* for 11 of 20 comparisons (Table 8). There were significantly greater concentrations of all metals in *O. macrus* than in *O. n. neglectus* at S1; significantly greater concentrations of Zn, Cd, and Cu in *O. macrus* at C1; significantly greater concentrations of Pb and Cu in *O. macrus* at S2 ; and significantly greater concentrations of Cd in *O. macrus* at J1 (Table 8).

Mean Pb concentrations in *O. n. neglectus* were comparable to crayfish collected in Spring River tributaries in pre-2000 from the TSMD; however, Pb concentrations were about 4-times greater than those reported in crayfish collected in the mainstem Spring River in 2001–2002 (Table 10). Mean Zn concentrations in *O. n. neglectus* were about 1.6-times greater than those collected in crayfish in 2001–2002; however, they were 1.2-times lower than those collected pre-2000. Mean Cd concentrations in *O. n. neglectus* were about 5-times greater than concentrations in crayfish collected in 2001–2002, but they were similar to those collected in crayfish pre-2000. Mean concentrations for Pb in

O. n. neglectus were comparable to those in *Orconectes hylas* collected in the Viburnum Trend Mining District (VTMD), but about 6-times lower than *Orconectes luteus* collected in the Old Lead Belt Mining District (OLBMD; Table 10). Mean Zn concentrations in *O. n. neglectus* were 1.5- to 5-times greater than crayfish collected in the VTMD or OLBMD (Table 10). Mean Cd concentrations were about 5-times greater than those in crayfish collected in the VTMD, but 2-times lower than those collected in the OLBMD (Table 10). Mean Ni concentrations in *O. n. neglectus* were about one-third of the maximum concentrations previously reported in crayfish from the VTMD or OLBMD (Table 10).

Detritus

Mean metals concentrations in detritus differed significantly among sites (Table 11); concentrations of Pb, Zn, and Cd in detritus were significantly greater at mining and downstream sites than at reference sites (Table 9). Metals concentrations in detritus were lowest at J1, whereas C2 – C5 and T3 – T4 generally had the greatest metals concentrations in detritus. Sites in Shoal Creek generally had lower concentrations of metals in detritus except for S6, which were comparable to C2 – C5 and T3 – T4 (Table 9). Mean concentrations of Pb, Zn, and Cd in detritus collected at sites downstream from Center Creek and Turkey Creek were 100–1000-times greater than in detritus collected at J1 or C1. Mean Pb concentrations in detritus were greatest at C2 (1029 µg/g) and T4 (1021 µg/g); mean Zn concentrations were greatest at C2 (21967 µg/g) and T4 (12700 µg/g), and mean Cd concentrations were greatest at C2 (288 µg/g), T4 (153 µg/g), and C3 (152 µg/g). Mean Ni concentrations in detritus were greatest at C2 (38.7 µg/g), S6 (38.7

µg/g), and T4 (37.7 µg/g). Mean Cu concentrations were greatest at C2 (53.7 µg/g), S3 (53.7 µg/g), and T4 (51.7 µg/g). Metals concentrations in detritus were 10- to 100-times greater than metals concentrations in whole crayfish (Figure 3).

Sediment

Mean percent TOC in the <250-µm fraction of sediment ranged from 0.7% at T1 to 3.3% at S5 and generally was greater at downstream sites in all creeks, except Center Creek (Table 12). Mean percent TOC in the <2000-µm fraction of sediment ranged from 0.2% at T1 to 2.4% at T4, and generally were lower than those in <250-µm sediments (Table 12). No apparent longitudinal trend in mean percent TOC in the <2000-µm fraction of sediment was evident in the creeks (Table 12).

Mean metals concentrations in sediment differed significantly among sites (Table 12); mean concentrations of Pb, Zn, and Cd in the <250-µm fraction of sediment were significantly greater at mining and downstream sites than at reference sites (Table 9). Mean metals concentrations in the <250- and <2000-µm fractions were correlated, as were metals concentrations within each sediment fraction. Metals concentrations in the <250-µm fraction generally were greater than in the <2000-µm fraction (Table 12). Mean metals concentrations in the <2000-µm fraction were greater than in <250-µm fraction at C1 (Pb, Ni); C2 (Zn); C4 (Zn, Cd, Cu); T2 (Pb); T3 (Zn); and S2 (Zn, Cd). Metals concentrations in sediment generally were greatest at sites downstream from mine-waste in Turkey Creek and Center Creek. Mean concentrations of Pb, Zn, and Cd were 10- to 100-times greater at all sites than J1 or C1. Mean concentrations of Pb in the <250-µm fraction were greatest at T3 (2653 µg/g), T4 (1161 µg/g), and C4 (1155 µg/g).

Mean Zn concentrations in the <250-µm fraction were greatest at T3 (16467 µg/g), C4 (12690 µg/g), and C3 (10207 µg/g). Mean Cd concentrations in the <250-µm fraction were greatest at T3 (99.6 µg/g), C4 (87.8 µg/g), and C3 (68.2 µg/g). Mean concentrations of Pb, Zn, and Cd in the <250-µm fraction were similar to those in detritus (Figure 3) and were 10- to 100-times greater than mean concentrations in whole crayfish.

General PECs for Pb (128 µg/g), Zn (459 µg/g), and Cd (4.98 µg/g; MacDonald et al. 2000) generally were exceeded in both fractions of sediment at all sites. Mean metals concentrations in sediment from J1, C1 T1, S1, S2, S4, and S5 generally did not exceed TSMD-specific low-risk (PEC_{10}) and high-risk (PEC_{20}) thresholds for Pb, Zn, and Cd (Tabel 12; MacDonald et al. 2010). Mean Cu concentrations did not exceed the general PEC in either the <250- or <2000-µm fraction at any site (Table 12; MacDonald et al. 2000). Mean Ni concentrations in the <250-µm fraction collected at T3 (66.8 µg/g) exceeded the general PEC for Ni (48.6 µg/g); Ni concentrations at T4 (42.9 µg/g) approached the PEC (Table 12; MacDonald et al. 2000).

Surface water

Mean metals concentrations in surface water differed significantly among sites (Table 13); mean concentrations of Pb, Zn, and Cd in surface water were significantly greater at mining and downstream sites than at reference sites (Table 9). Mean metals concentrations in surface water were 10- to 1000-fold lower than concentrations in whole crayfish (Figure 3). Mean metals concentrations in surface water were generally greatest at sites downstream of mine-waste in Turkey Creek and Center Creek and lowest in Jenkins Creek. Mean Pb concentrations in surface water were greatest at T3 (1.63 µg/L);

mean Zn concentrations were greatest at C2 (421 µg/L); mean Cd concentrations were greatest at C2 (1.74 µg/L) and T3 (1.71 µg/L); mean Ni concentrations were greatest at T4 (2.13 µg/L); T3 (1.86 µg/L); and C2 (1.81 µg/L); and mean Cu concentrations were greatest at T4 (1.88 µg/L). Mean concentrations of Pb, Ni, or Cu in surface water did not exceed either the state of Missouri water-quality standard or USEPA water-quality criteria at any site. Estimated values for the BLM-based criterion for Cu are about 18 µg/L, which would not have been exceeded (Table 13). Mean concentrations of Zn at C2 (421 µg/L), C3, (255 µg/L), and C4 (261 µg/L) in Center Creek and at T1 (156 µg/L), T3 (344 µg/L), and T4 (230 µg/L) in Turkey Creek exceeded the State water-quality standard (193–223 µg/L) and Federal water-quality criteria (93–145 µg/L; Table 13). Mean concentrations of Zn in surface water at S6 (91.4 µg/L) approached the Federal criterion for Zn. Mean concentrations of Cd in surface water at C2 (1.74 µg/L), C4 (1.06 µg/L), T2 (0.42 µg/L), T3 (1.71 µg/L), and T4 (1.33 µg/L) exceeded the State water-quality standard (0.4–0.5 µg/L) and Federal water-quality criteria (0.4–0.6 µg/L).

Assessment of risk of metals concentrations

Hazard assessment for carnivorous wildlife

Criteria used to evaluate risks of Pb, Zn, and Cd in crayfish to wildlife indicated that metals concentrations at several sites are potentially hazardous to carnivorous wildlife. Hazard quotients were greater for birds than mammals in their respective size category. Hazard quotients did not exceed 1.0 for mean Pb, Zn, or Cd concentrations in *O. n. neglectus* for any representative wildlife species at J1, C1, S2, S3, S4, and S5 (Table 14). Hazard quotients exceeded 1.0 for mean Pb, Zn, and Cd concentrations for robin-sized birds (HQs =1.27–3.88) and for mean Cd concentrations in shrew-sized mammals (HQs =1.17–1.63) at C2, C4, T3, and T4. Hazard quotients exceeded 1.0 for mean Pb and Zn concentrations in *O. n. neglectus* for robin-sized birds (HQs =1.01–2.93) at C3, C5, and T2. Hazard quotients for mean Pb concentrations in *O. n. neglectus* exceeded 1.0 for robin-sized birds (HQs =1.02) at S1 and for mean Zn concentrations for robin-sized birds at T1 (HQs =1.08) and S6 (HQs =1.11). No HQs exceeded 1.0 for mean Pb, Zn, or Cd concentrations in *O. macrus* for any representative wildlife species at J1 or C1; however, HQs exceeded 1.0 for mean Pb concentrations for robin-sized birds (HQs =1.13–1.94) at S1 and S2 and for mean Zn concentrations for robin-sized birds (HQ =1.15) at S1 (Table 15).

No HQs for the minimum site-mean concentrations of Pb, Zn, and Cd in *O. n. neglectus* exceeded 1.0 (Table 16). Hazard quotients for the maximum site-mean concentrations of Zn, Cd, and Pb in *O. n. neglectus* did exceed 1.0 for robin-sized birds and shrew-sized mammals (HQs =2.09–3.88) and for Zn and Pb concentrations in *O. macrus* for robin-sized birds (HQs =1.13–1.94; Table 15), indicating the potential for adverse effects in carnivorous wildlife.

Hazard quotients calculated based on maximum site-mean metals concentrations in *O. n. neglectus* and *O. macrus* generally were lower than those calculated for crayfish collected in previous studies in the TSMD (Table 17). Hazard quotients for Zn in both species were about 3- to 5-times lower than those for crayfish previously collected in the TSMD; similar to HQs calculated for crayfish collected in the OLDMD; and about 3-times as great as those calculated for *O. hylas* collected in the VTMD (Table 17). Hazard quotients for Cd in *O. n. neglectus* were at least twice that of those calculated for crayfish collected in previous studies in the TSMD. Hazard quotients for Cd in *O. n. neglectus* were about one-half that of those calculated for crayfish collected in the OLBMD in 2008; twice as large as those calculated for crayfish collected in the OLDMB in 1982; and about 3- to 5-times greater than those calculated for *O. hylas* collected in the VTMD (Table 17). Hazard quotients for Pb in both species were greater than those for crayfish collected from the mainstem Spring River in the TSMD in 2001–2002, but lower than those in crayfish collected in the Spring River tributaries in pre-2001. Hazard quotients for Pb in both species collected from TSMD were similar to crayfish collected in *O. hylas* (VTMD); however, they were about 1/6 to 1/9 of those calculated for crayfish collected in the OLMBD.

<u>Sediment probable-effects concentrations quotients</u>

Sediment probable-effects concentrations quotients indicated risk of toxicity for one or more metals in the four creeks sampled (Table 18); ∑PEQs were greater than one at all sites (Table 18; MacDonald et al. 2000). TSMD-specific low-risk (6.47) toxicity thresholds for $\sum PEQ_{Pb,Zn,Cd}$ were exceeded at all sites, except J1 and C1 (Table 18; Figure 4; MacDonald et al. 2010). TSMD-specific high-risk (10.04) toxicity thresholds for $\sum PEQ_{Pb,Zn,Cd}$ were exceeded at C2 – C5; T2 – T4; S3; S5; and S6. Individual metal PEQs and ∑PEQs generally were greatest in Center Creek and Turkey Creek; $\sum PEQ_{Pb,Zn,Cd}$ were significantly greater at mining and downstream sites than at reference sites (Table 5). The PEQs for Zn and Cd were generally greatest at all sites and contributed the most to ∑PEQs. The sites with the greatest ∑PEQs were T3 (78), C4

(55), C3 (43), and T4 (42), which were significantly greater than all other sites (Table 18). The ∑PEQs for J1 and C1 were 5- to 40-fold lower than all other sites.

Plots of crayfish densities and TSMD-specific $\sum PEQ_{Pb,Zn,Cd}$ indicated that TSMD-specific $\sum PEQ_{Pb,Zn,Cd}$ reasonably predicted risk or the reduction in crayfish densities at the sites (Figure 5). Mean densities of *O. n. neglectus* and *O. macrus* (Figure 5a) and *O. n. neglectus* (Figure 5b) densities were reduced at sites that exceeded the TSMD-specific toxicity thresholds at all sites except T3 (Table 18). No *O. macrus* were collected at sites that exceeded the low-risk $\sum PEQ_{Pb,Zn,Cd}$ (Figure 5c).

Surface-water toxic units

The ∑TUs for surface waters at C2 – C4, T3, and T4 were greater than one, indicating potential risk to aquatic biota (Figure 4). The ∑TUs for surface waters were significantly greater at mining and downstream sites than at reference sites (Table 5; Table 19). Chronic toxic-unit scores were greatest for Zn at C2 (2.07), C3 (1.19), C4 (1.37), T3 (1.21), and T4 (1.34) indicating that the overall risk of toxicity primarily was the result of high Zn concentrations (Zn-TU was greater than 95% of ∑TUs; Table 19). Sites T1 (0.80) and S6 (0.69) had ∑TUs >0.50, which were primarily because of high Zn concentrations. Sites J1 (0.02) and C1 (0.01) had significantly lower ∑TUs than all other sites.

Physical habitat

Physical-habitat quality differed significantly among sites. Mean wetted width in Shoal Creek generally was twice the wetted width of the other creeks sampled; mean wetted width was widest at S3 (26.2 m) and narrowest at T1 (5.8 m; Table 20). Mean substrate coarseness (as determined by in-situ visual assessment) generally was greatest in Shoal Creek and smallest in Jenkins Creek and in Center Creek (Table 20). Substrate size at all sites predominately was gravel to pebble. Three sites (J1, T3, and S3) had significant amounts of irregular bedrock resulting in increased substrate homogeneity, whereas all other sites (C2 – C5, S4 – S5) with significantly greater embeddedness (low substrate homogeneity) were located downstream from mine-waste sites. Mean substrate coarseness was greatest at S2 (3.59 mm) and S5 (3.28 mm) and least at J1, C2, C5, T1, and T4 (2.14–2.77 mm). Mean substrate coarseness at C1 (3.13 mm) was significantly greater than all other sites in Center Creek, whereas mean substrate coarseness at S3 (2.60 mm) was significantly less (mostly bedrock) than all other sites in Shoal Creek. Mean substrate homogeneity was similar across all sites, but generally greatest in Center Creek. Mean substrate homogeneity was greatest at S3 (1.26), T3 (1.09), S1 (1.06), and J1 (1.05), and least at C5 (0.57) and S5 (0.59). Mean water depth and current velocities in Shoal Creek and Center Creek were similar and were about twice that measured in Jenkins Creek and Turkey Creek (Table 20). Mean water depth was greatest at S2 (38.9 cm) and shallowest at T2 (8.4 cm). Mean mid-water current velocity was greatest at S5 (0.82 cm/sec) and least at J1 (0.13 cm/sec). Mean current velocity at the substrate surface was greatest at S5 (0.37 cm/sec) and least at J1 (0.11 cm/sec).

Physical-habitat quality within the 1-m^2 kick-seine quadrats was similar to that measured along the lateral transects (Table 21). Substrate coarseness, water depth, and current velocity within kick-seine quadrats were generally greater at sites in Shoal Creek than all other sites. Mean substrate coarseness within kick-seine quadrats in Shoal Creek was significantly greater at S1 – S5 (3.27–3.68 mm) than S6 (3.04 mm). Mean substrate homogeneity ranged from 0.53–0.93 mm; it was generally lowest at C2 – C5. Mean water depth within kick-seine quadrats were significantly greater at mining and downstream sites than at reference sites (Table 5). Mean water depth within kick-seine quadrats were deepest at S2 (43 cm) and shallowest at T2 (10.5 cm; Table 21). Mean mid-water current velocities within kick-seine quadrats were significantly greater at S5 (1.07 cm/sec) and significantly lower at J1 (0.27 cm/sec), T1 (0.32 cm/sec), T2 (0.30 cm/sec), and T3 (0.28 cm/sec).

Substrate particle size as characterized by sediment-grab samples did not differ significantly among most sites and was predominantly larger-sized substrate (i.e., ≥19 mm; Table 22). No <2-mm sediment was collected in sediment-grab samples at S3, and only one of the sediment-grab samples collected at J1, C2, S1, S2, and S6 contained material that was <2 mm.

Habitat quality as indicated by QHEI scores generally was good at all of the sampling sites (Figure 6). Although narrative ratings (e.g., excellent, poor) for sites are not always predictive of aquatic assemblages, QHEI scores in headwater streams >70% and QHEI scores in larger streams >75% are given a narrative rating of excellent. The only site considered to be a headwater stream was T1 (drainage area <52 km^2; Ohio EPA 2010), and it had a QHEI score of 73, which is considered excellent. The QHEI scores at eight sites (J1, C3 – C5, T2 – T4, S3) were less than 75%; however, QHEI scores were within the narrative-rating range of good (60–74%; Ohio EPA 2006). Seven sites (C1, C2, S1, S2, S4 – S6) had QHEI scores >75%; three sites (S1, S2, and S5) had QHEI scores greater than 80%. Two sites (J1, C5) had the lowest scores for two or more of the metrics. The sites with the greatest QHEI scores (S1, S2, and S5) had the greatest scores for at least four of the metrics. Scores for substrate and channel morphology at all sites were greater than 70% of the maximum score allowed for each metric. Several sites scored the maximum allowed for substrate (S1, S2, S5) and gradient (C1 – C5; T2, T4, S1, S2, S4 – S6). This was because of the predominance of gravel and boulder, which are considered "best-type" substrate for the QHEI; well-developed channel sinuosity; and no stream channelization at all sites. The metrics with the greatest range in scores were gradient (40%); instream cover (35%), and pool-glide and riffle-run quality (30%). The gradient at T1 (8.98 m/km) scored the lowest (60%), while most sites had scores ≥90% for this metric. The range in instream cover scores was primarily due to the difference in the presence of pools, backwaters, woody debris, and macrophytes at some sites. Differences in pool-glide and riffle-run quality were primarily due to differences in substrate stability in riffles and the ratio of pool width to riffle width.

Water quality

There were significant differences in measured water-quality parameters among sites. Mean temperature generally was greatest in Turkey Creek and Center Creek and lowest in Jenkins Creek (Table 23). The lowest mean water temperature was measured at S6 (19.5 ºC); however, this site was sampled in September, whereas all other sites were sampled in July. The lowest mean water temperature during July was measured at J1 (21.3 ºC). The greatest mean water temperatures were measured at T4 (27.1 ºC), S4 (27.0 ºC), and C4 (26.9 ºC); however, all were below the Missouri water-quality standards for warm-water streams (32 °C; MDNR 2009). Although there were significant differences in mean pH values, the range of measured values (7.7–8.2) were within the Missouri water-quality standards (6.5–9.0; MDNR 2009). Mean specific conductance was significantly greater at T1 – T4 and C2 – C3 than all other sites; the greatest mean specific conductance measured at T3 (558 µS/cm) and T4 (496 µS/cm). The lowest mean specific conductance measured was at J1 (316 µS/cm). Mean specific conductance of the WWTP effluent (800 µS/cm) was twice the measured specific conductance measured at S3 (331 µS/cm; upstream from WWTP). Mean specific conductance at S4 (361 µS/cm; downstream from WWTP) was significantly greater than S3 (331 µS/cm); however, it was significantly lower than the specific conductance at S1 (365 µS/cm). Mean dissolved oxygen concentrations generally were lowest in Turkey Creek; however, concentrations at all sites were above the standard for dissolved oxygen (5.0 mg/L; MDNR 2009). Mean dissolved oxygen concentrations were greatest at S4 (9.4 mg/L) and lowest at T1 (6.4 mg/L). Mean turbidity at all but four sites was greater than the water-quality standard (5.6 NTU; USEPA 2000); however, the range in turbidity (0.4–19.8 NTU) was relatively small and the greatest measured value was only about four times more than the standard (Table 23). Greater values may reflect that several sites (C2 – C4; S2) were sampled after rain events. Although turbidity values measured in the WWTP effluent (26.3 NTU) were greater than any sampled site, turbidity values (9.5–12.5 NTU) at sites S4 – S6 (downstream from the WWTP) were lower than values (13.2–19.8 NTU) at S1 – S3 (upstream from the WWTP).

There were significant differences in mean alkalinity, hardness, and sulfate concentrations among sites (Table 24). Mean alkalinity concentrations were all greater than the Federal water-quality criterion (20 mg $CaCO_3$/L; USEPA 2006). Mean hardness concentrations at C2 (207 mg $CaCO_3$/L), C3 (215 mg $CaCO_3$/L), and T1 – T3 (203–270 mg $CaCO_3$/L) exceeded the State standard (200 mg $CaCO_3$/L). Mean sulfate concentrations were elevated at C2 – C5 (27–65 mg SO_4/L) in Center Creek and at all sites in Turkey Creek (25–100 mg SO_4/L) relative to J1 (2.8 mg SO_4/L), C1 (0.7 mg SO_4/L), and sites in Shoal Creek (5.2–9.1 mg SO_4/L). Although mean sulfate concentrations were about ten-fold greater in the WWTP effluent (41 mg SO_4/L) than sulfate concentrations at S1; concentrations at S4 (5.0 mg SO_4/L) and S5 (5.8 mg SO_4/L) were not significantly greater than sites upstream from the WWTP (Table 24). The greatest alkalinity (164 mg $CaCO_3$/L), hardness (270 mg $CaCO_3$/L), and sulfate concentrations (100 mg SO_4/L) were measured at T3.

There were significant differences in chl *a* concentrations among sites (Table 25). Downstream sites (C3 – C5; 2.27–2.88 µg C/L) in Center Creek and Shoal Creek (S4 – S6; 1.51–2.57 µg C/L) had the greatest mean chl *a* concentrations; however, concentrations in lower Shoal Creek may reflect nutrient contributions of WWTP effluent (8.96 mg C/L). Concentrations of chl *a* at all sites were about 20-times lower than the State water-quality standard (81 µg C/L; MDNR 2009). Mean DOC concentrations were greatest at T4 (2.52 mg C/L), T1 (1.45 mg C/L), and S6 (1.76 mg C/L); and lowest at S1 (0.74 mg C/L; Table 25). Mean POC concentrations differed significantly among sites creeks; the POC concentrations were significantly lower at J1 (0.33 mg C/L) and T2 – T4 (0.17–0.33 mg C/L). The greatest POC concentration was measured at S2 (0.95 mg C/L; Table 25). Mean POC concentrations in the effluent of the WWTP were more than 100-times greater than of any sampling sites; however, mean POC concentrations at S4 (0.74 mg C/L) were not significantly greater than S3 (0.72 mg C/L). Mean concentrations of

TSS were equal to or greater than the State water-quality standard (8.70 mg/L; MDNR 2009) at all sites in Shoal Creek and Center Creek (Table 25). Mean TSS concentrations were significantly higher at S2 (18.8 mg/L), C2 (16.4 mg/L), C3 (18.3 mg/L), and C4 (17.8 mg/L) than all other sites. These sites were all sampled following a rain event. The lowest TSS concentrations were measured at T4 (0.97 mg/L), T3 (1.93 mg/L), and J1 (3.47 mg/L).

Nutrient concentrations differed significantly among sites (Table 26). Nitrogen compounds generally were greatest in Jenkins Creek and lowest in Turkey Creek. Mean total NH_3-N concentrations at all sites were below the Missouri water-quality standard (0.7–2.2 mg N/L). Mean total NH_3-N concentration was greatest J1 (0.218 mg N/L), C3 (0.197 mg N/L), S2 (0.193 mg N/L), and C2 (0.139 mg N/L), which were about 100-fold more than all other sites. Mean NO_3-N concentrations were similar at all sites except T1 – T3, which were 10-fold lower than the other sites (Table 26). Mean TN concentrations at all sites except T1 – T3 (0.4–0.8 mg/L) were all greater than the State water-quality standard (0.90 mg N/L; MDNR 2009). Mean TN and NO_3-N concentrations measured in the effluent of the WWTP were 10-fold greater than any site; however, mean TN and NO_3-N concentrations were not significantly greater at the Shoal Creek sites (S4 – S5) downstream from the discharge. Mean TP concentrations generally were greatest in Shoal Creek; however, mean TP concentrations were significantly greater at T4 (741 µg P/L) than all other sites. Mean TP concentrations measured at S4 – S6 were greater than S1 – S3 and may have been elevated because of the high TP concentrations (3942 µg P/L) in the WWTP effluent. Mean TP concentrations at T4 (741 µg P/L), C3 (139 µg P/L), C5 (93 µg P/L), and all Shoal Creek sites (158–233 µg P/L) were greater than the

State water-quality standard (75µg P/L; MDNR 2009). The TN:TP ratios were from 11–17 in Shoal Creek; 6–37 in Turkey Creek; 17–53 in Center Creek; and 96 in Jenkins Creek.

Association among variables

The proportion of upstream tailings area to drainage area was significantly correlated with $\sum PEQ_{Pb,Zn,Cd}$ (r =0.88); $\sum TU_{Pb,Zn,Cd}$ (r =0.89); specific conductance (r =0.85); hardness (r =0.69); sulfate concentrations (r =0.88); all metals concentrations in surface water, detritus, and both size fractions of sediment (except Ni in <2000-µm sediment; e.g., r ≥0.80); and mean Pb, Zn, and Cd concentrations in *O. n. neglectus* (e.g., r ≥0.78). Surface water $\sum TU_{Pb,Zn,Cd}$ and sediment $\sum PEQ_{Pb,Zn,Cd}$ were significantly correlated (r =0.835); neither were significantly correlated with mean *O. n. neglectus* densities or combined densities of *O. n. neglectus* and *O. macrus*. Combined mean densities of *O. n. neglectus* and *O. macrus* were negatively correlated with upstream tailings area (r =-0.52).

Mean *O. n. neglectus* densities were not significantly correlated with metals concentrations in any of the materials analyzed (Table 27); however, mean *O. macrus* densities were negatively correlated with mean Pb concentrations in the <250-µm fraction of sediment (r =-1.00); and combined mean densities of crayfish were correlated with mean Ni concentrations in detritus (r =-0.52); and mean Cu concentrations in surface water (r =-0.54; Table 27). Metals concentrations in surface water, detritus, and both size fractions of sediment (<2000-µm sediment not shown) were significantly correlated (Table 27). Metals concentrations in whole crayfish were significantly correlated with metals concentrations in detritus (all significant except Cu); in surface water, and in the <250-µm fraction of sediment (all significant except Ni and Cu).

Mean *O. n. neglectus* densities were negatively correlated with mean *O. n. neglectus* CL (r =-0.71); drainage size (r =-0.66); mean wetted width of creeks (r =-0.72); mean water depth in kick-seine quadrats (r =-0.58); mean mid-water current velocity in kick-seine quadrats (r =-0.54), mean substrate coarseness in kick-seine quadrats (r =-0.55), and mean percent TOC in <250-µm sediment (r =-0.54).

Principal components analyses

A series of interpretable ordinations of crayfish density, water depth at kick-seine quadrats, and Pb concentrations in detritus was obtained by PCA for *O. n. neglectus* densities; the combined densities of *O. n. neglectus* and *O. macrus*; *O. n. neglectus* in Jenkins Creek, Center Creek, and Turkey Creek; and *O. n. neglectus* in Shoal Creek (Figure 7). Each ordination resulted in the first two PCA axes explaining more of the variation (90–92%) than expected by chance alone. Mean crayfish density, mean Pb concentration in detritus and mean water depth had high factor (e.g., eigenvectors) loadings (>±0.65) in all ordinations and indicated that measures of mining-related waste (e.g., Pb concentrations in detritus) and physical-habitat variables (e.g., water depth in kick-seine quadrats) are important for explaining the variation in *O. n. neglectus* (crayfish) densities. The ordinations for *O. n. neglectus* densities and for the combined densities of *O. n. neglectus* and *O. macrus* were similar (Figure 7); sites C1 was slightly more separated (e.g., closer to J1, T1, T2) from the groups of sites when the combined densities are used because of density factor loadings. Principal components analysis also was conducted for sites in Shoal Creek and for those in Jenkins Creek, Turkey Creek, and Center Creek, because Shoal Creek was significantly deeper (and the current velocity was greater) than the other creeks sampled (one-way ANOVA, NPAR1WAY; $F_{(2, 1916)}$, F =297; P <0.0001). In both ordinations, sites were separated based on high eigenvectors (>±0.70) on PC1 for densities of *O. n. neglectus* and Pb (metals) concentrations. Site C1 was placed nearer to J1, T1, and T2 because of low densities of *O. n. neglectus* (although mean densities of *O. macrus* were about 3-times greater), low Pb (metals) concentrations, and shallow water depth (PC2). Sites in Shoal Creek were strongly separated by metals concentrations and water depth along PC1 (Figure 7); sites S1 and S2 were separated based on greater densities of *O. n. neglectus*.

Discussion

Previous studies have used crayfish to assess the bioavailability of metals through the analysis of whole crayfish because of their important functional role in streams and limited home ranges (Allert et al. 2008, 2009, 2010; Besser et al. 2007; Schmitt et al. 2006, 2007). Our results indicate that concentrations of Pb, Zn, and Cd in whole crayfish, surface water, sediment, and detritus remain elevated at sites downstream from mine-waste in the TSMD and are consistent with previous investigations that have documented elevated metals concentrations in food-web components at sites downstream from mining activity or mine-waste in the TSMD (Angelo et al. 2007; Brumbaugh et al. 2005; MacDonald 2010; Schmitt et al. 2006; Wildhaber et al. 1997, 2000).

Metals have been shown to accumulate in crayfish in the laboratory and in aquatic ecosystems (Allert et al. 2008, 2009, 2010; Bagatto and Alikham 1987a, 1987b; Evans

1980; Gillespie et al. 1977; Stinson and Eaton 1983; Wigginton and Birge 2007); however, the utility of crayfish as indicators of exposure to metals may be species dependent, and for metals such as Zn and Cu, may be limited because of the rapid depuration of these metals (Kouba et al. 2010). Previous studies have been inconclusive regarding the relationship between crayfish size and metals concentrations (Bennet-Chambers and Knott 2002; Knowlton et al. 1983; Mirenda 1986a, 1986b). We determined that increasing metals concentrations were correlated with increasing size of *O. n. neglectus* (range = 15.0–30.0 mm; $F_{(15,222)}$ =17.6.9; R^2 =0.54). Mean CL of *O. n. neglectus* used in metal analyses was greatest at sites classified as reference sites, which may explain why there were no correlations among *O. n. neglectus* densities and metals concentrations in the materials analyzed. Similarly-sized crayfish (CL ±5 mm) should be taken in future studies to collect crayfish representative of those of the general population at a site (Schmitt et al. 2007). Although sex ratios in *O. n. neglectus* subsampled for metals analyses were skewed at more sites than the sex ratios for all of the crayfish collected by kick seining, metals concentrations in crayfish have not been found to differ between genders (Allert et al. 2010; Bagatto and Alikhan 1987b). Abiotic factors (e.g., water depth, current velocity) and biotic factors (e.g., molt frequency, predation) may affect metals concentrations in crayfish.

Metals sensitivity

Differences in metals sensitivity among crayfish species may be due to life stage; frequency of molts; and prior metals exposure (i.e., Cd), as demonstrated by calculated lethal concentrations (LC_{50}s) for Cd, which range from 0.037 mg Cd/L for juvenile

Orconectes placidus to 58.5 mg Cd/L for adult *Procambarus clarkii* (Chambers 1995; Del Ramo et al. 1987; Mirenda 1986a, 1986b; Wiggington and Birge 2007). Surface to volume ratio, increased metabolic rates, and greater permeability of exoskeleton in juvenile (<1 year-old) crayfish are possible reasons for the greater variability and increased sensitivity to metals concentrations in juveniles than subsequent age (or size) classes of crayfish (Bennet-Chambers and Knott 2002; Chambers 1995; Wigginton and Birge 2007).

Price and Payne (1984a) estimated five size (age) classes in riffle populations of *O. n. chaenodactylus* in the White River drainage; the two smallest size classes were approximately 5–18 mm (58% of the breeding population) and 13–23 mm (26% of the breeding population). Price and Payne (1984a, 1984b) estimated the size of juvenile *O. n. chaenodactylus* at the end of the first year of study to be 7.8–13.4 mm; therefore, the lack of small crayfish at several of our sites (S4 –S5) mostly likely is because of a lack of age-0 (juvenile) individuals. Age-0 *O. n. chaenodactylus* molt as many as eight times per year; however, molting in adult *O. n. chaenodactylus* varies from 1–2 to 3–4 molts per year (Price and Payne 1978, 1984b; Rabalais and Magoulick 2006a). Timing and frequency of molts (and therefore growth) vary within crayfish because of environmental variables such as water temperature, food availability, competition, and predation. Warmer surface-water temperatures at mining and downstream sites in Turkey Creek, Center Creek, and Shoal Creek may have resulted in more frequent molts and lower crayfish densities at sites with elevated metals concentrations because of increased susceptibility to metals during molt (Allert et al. 2009, 2010; Knowlton et al. 1983; Wigginton and Birge. 2007).

Previous exposure to metals may confer some protection because of metallothionein induction (MT) and/or through the transfer of maternal MT-mRNA (Chambers 1995); however, Naqvi and Howell (1993) documented significantly lower fecundity and hatching success in *P. clarkii* when exposed to either Cd or Pb. Size at sexual maturity in male and female *O. n. chaenodactylus* ranges from about 12 mm CL to greater than 20 mm CL (Flinders and Magoulick 2005; Larson and Magoulick 2008; Price and Payne 1984b). Although age-0 and age-1 individuals may attain sexual maturity (Magoulick and DiStefano 2007; Price and Payne 1984a), Larson and Magoulick (2008) determined that only about 9% of the total female crayfish <20 mm were ovigerous. Although first-year crayfish can contribute significantly to reproductive potential of a population (Muck et al. 2002), larger crayfish tend to have larger clutch sizes (Larson and Magoulick 2008); therefore, they may be more important to sustaining crayfish populations. Sites in lower Center Creek (C3 – C5), and Turkey Creek (T1 – T2) did not have crayfish CL >25 mm (Figure 2), which could affect density because of lower fecundity.

Mixtures of metals may be antagonistic; for example, high Zn concentrations confer some protection against the toxic effects of Cd (Leland and Kuwabara 1985, references therein). In-situ and laboratory toxicity tests are important tools for assessing metals exposure (metals concentrations), subsequent bioaccumulation of metals, and potential toxicity to known age-classes of crayfish because it is impossible to observe temporal changes and bioaccumulation in the environment. Risk assessments are more effective when contaminant (e.g., metals) concentrations are linked with the status of aquatic organisms (e.g., crayfish density) and toxicological information such as toxicity tests or other sub-lethal measurements.

Hazard assessment

Our results indicate that if consumed, present concentrations of metals in crayfish in the TSMD potentially are hazardous to carnivorous wildlife, including birds and mammals; therefore, it is appropriate to assume that they could impair Trust wildlife species, such as migratory waterfowl. Although hazard quotients were based on a 100% diet of crayfish, previous studies determined that metals concentrations, particularly Pb, Zn, and Cd, were elevated in other wildlife prey such as other macroinvertebrates and fish, as well as detritus and sediment in the TSMD (Angelo et al. 2007; Schmitt et al. 2006; Wildhaber et al. 1997, 2000). Concentrations of Pb, Zn, Cd, and Ni in *O. n. neglectus* collected in this study are similar to or greater than concentrations of metals in crayfish (Decapoda) collected at sites in Center Creek (C5), Turkey Creek (T4), and Shoal Creek (S2 and S6) reported by Wildhaber et al. (1997; Table 28). Concentrations of Cu in *O. n. neglectus* generally are 1/5 to 1/3 lower than those reported by Wildhaber et al. (1997). Metals concentrations in *O. n. neglectus* reinforce that there are continuing inputs of metals in the three tributaries of the Spring River.

Crayfish are omnivores and the primary shredders present in Ozark streams (Rabeni et al. 1995); however, incidental ingestion of sediment is likely as crayfish feed on organic material, which could result in food-chain transfer of metals from these materials to predatory wildlife. Beyer et al. (2004) reported several bird species including mallards (*Anas platyrhynchos*), ring-necked ducks (*Aythya collaris*), and brown thrashers (*Toxostoma rufum*) had elevated tissue metals concentrations when compared to reference sites within the TSMD; all of these species prey on crayfish (DiStefano 2005). Beyer et al. (2004) suggest that the most likely route of exposure is through the ingestion of sediment and plant material; however, the diets of laying and molting female ducks shift largely to animal foods (Drobney and Fredickson 1979; Heitmeyer 1984); therefore, crayfish may be an important contributor of metals for birds due to their large size despite making up only a small percentage of their diets.

Dietary pathways have been identified as important routes of metals exposure (Besser et al. 2005; Farag et al. 1999); therefore, it is reasonable to assume that important stream sport fishes are at risk from metals concentrations in crayfish. Crayfish are important prey organisms for several of Missouri's most important sport fish such as smallmouth bass (*Micropterus dolomieu*; Probst et al. 1984; Weithman 1994; DiStefano 2005), rock bass (*Ambloplites rupestris*; Probst et al. 1984), largemouth bass (*M. salmoides*; Miner 1978; Keast 1985; Wheeler and Allen 2003), spotted bass (*M. punctulatus*; Novinger 1988), flathead catfish (*Pylodictis olivaris*; Roell and Orth 1993), blue catfish (*Ictalurus furcatus*; Brown and Dendy 1961), and longear sunfish (Cooner and Bayne 1982). Schmitt et al. (2006) reported concentrations of Pb, Zn, and Cd in crayfish (*Orconectes* spp.), common carp (*Cyprinus carpio*); channel catfish (*Ictalurus punctatus*); and centrachids (*Micropterus salmoides*; *Micropterus punctulatus*; *Pomoxis annularis*) from TSMD mainstem sites in Oklahoma were significantly greater at sites affected by mining than reference sites, and that some concentrations in fish exceeded concentrations known to cause effects such as reduced hatchability and condition factors (Schmitt et al. 2006, references therein). Schmitt et al. (1993) also reported elevated metals concentrations in shortnose redhorse (*Moxostoma macrolepidotum*) from Center Creek.

TSMD-specific $\sum PEQ_{Pb,Zn,Cd}$ thresholds generally were predictive of reduced crayfish densities at sites with increased $\sum PEQ_{Pb,Zn,Cd}$. No *O. macrus* were collected at sites with $\sum PEQ_{Pb,Zn,Cd}$ above either threshold. Mean *O. n. neglectus* densities at sites with $\sum PEQ_{Pb,Zn,Cd}$ above threshold values were no more than one-third as great as those at sites below thresholds, except for T3 (Table 18). Mean densities of crayfish can be affected by dispersal because of flooding or the result of crayfish migration (Jacob Westhoff, oral communication), which may be a greater factor in smaller-order streams such as Turkey Creek.

Allert et al. (1997) collected pore water, surface water, and sediment samples and conducted *Ceriodaphnia dubia* 7-day toxicity test using pore waters at several of the same sites (C3, C5, S3, S6) sampled in this study. Metals concentrations in pore water and sediment were elevated at all of these sites (Table 29). Sediment metals concentrations were 1–10-times greater than concentrations in samples collected in this study (Table 29). Sediment Zn and Cd concentrations in Center Creek were greater than those in Shoal Creek. Pore-water $\sum$TUs were 2–100-times greater than surface-water $\sum$TUs calculated in this study, but were again based primarily on Zn concentrations (Table 28). Specific conductance and sulfate concentrations in surface water reported in this study were only slightly lower than those reported by Allert et al. (1997; Table 29). Survival and/or reproduction of *Ceriodaphnia* in pore waters were reduced at C3 and C5 (Table 29). Although 100% site water from S6 could not be tested because of the limited amount of pore water collected for the test (Allert et al. 1997), the pore-water $\sum$TUs and sediment metals concentrations were lower than those at S3, suggesting that survival and reproduction in 100% site water from S6 would have been at least as great as that in 100% site water from S3. Results from the toxicity test conducted by Allert et al. (1997) indicated that metals concentrations at sites C3 and C5 put aquatic organisms at an increased risk than those at sites S3 and S6, which were consistent with ∑PEQs and surface-water ∑TUs from this study.

Crayfish species composition

Although the diversity of crayfish in the Missouri Ozarks is large (>25 species; Pfleiger 1996), many crayfish are restricted to a single drainage and are further restricted within drainages because of macrohabitat partitioning among species (DiStefano et al. 2003; Flinders and Magoulick 2005; Rabeni 1985). The Spring River drainage includes five crayfish species; three species (*O. macrus, O. n. neglectus*, and *O. virilis*) often present in streams within the drainage (Pflieger 1996) were collected during this study. *Orconectes n. neglectus* inhabits small creeks to large streams, occurring in riffles and shallow pools, and grows to a larger size (total length =10 cm) than *O. macrus* (total length =5 cm; Pfleiger 1996), but a slightly smaller size than *O. virilis* (total length =12 cm). *Orconectes virilis* is a widely distributed species that is most abundant in habitats without strong flows and those with cover such as slab rock or organic matter (Pfleiger 1996), the likely reason for why many individuals of this species were not collected in riffles.

Orconectes macrus is endemic to the Ozark Highlands and primarily is present in the western-flowing rivers north of the Illinois River in Oklahoma (Dillman et al. 2010).

Dillman et al. (2010) confirmed species status for *O. macrus* and indicated that there are three clades of *O. macrus* found within the Ozark Highlands, two of which reside within the Spring River drainage, exclusive of North Fork Spring River and parts of Shoal Creek in Missouri (Pflieger 1996). It is unclear why *O. macrus* is not present at sites in the lower reaches of Center Creek and Shoal Creek, despite being in the lower Neosho River and Spring River. Dillman et al. (2010) confirmed the same pattern in distribution of *O. macrus* within the Spring River Basin, adding one additional site in Shoal Creek between S1 and S2. *Orconectes macrus* typically inhabits rocky substrates in swift, shallow water and is a relatively sedentary species that spends most of its time in cavities beneath rocks or in excavated tunnels in gravelly substrate (Pfleiger 1996). It could be that *O. macrus* is more sensitive to metals than *O. n. neglectus*, or that the sedentary nature of *O. macrus* within excavated tunnels exposes them to greater metals concentrations found in pore water and sediment (Allert et al. 1997; Brumbaugh et al. 2007), which may have eliminated *O. macrus* from the lower reaches in Center Creek and Shoal Creek.

Crayfish density and physical habitat

Crayfish are known to partition habitats based on size (Brewer et al. 2009; DiStefano et al. 2003; Lodge and Hill 1994). Allert et al. (2008 and references therein) and Riggert et al. (1999) reported densities (0.9–37.0/m^2) of comparable *Orconectes* species at references sites or at sites downstream (>7 km) from mining in streams of southeast Missouri. Rabalais and Magoulick (2006b) reported densities of *Orconectes neglectus chaenodactylus* in riffle habitats ranging from 2–3/m^2 for juveniles to 2–8/m^2

for adults, which are comparable to densities of *O. n. neglectus* at most sites in this study. Flinders and Magoulick (2005) reported densities of *O. n. chaenodactylus* in all habitats (e.g., riffles, runs, backwaters, macrophyte beds) of second- and third-order streams to range from 0–3.63/m^2; no *O. n. chaenodactylus* were collected in first-, fourth-, or fifth-order streams. Mean densities of *O. n. neglectus* at our reference sites (mean =16.9/m^2; range =0.6–54.5/m^2) were comparable to densities of *O. luteus* at reference sites (mean =10.9/m^2; range =9–12.7/m^2) in the Big River (Allert et al. 2010) and to densities of *O. hylas* (13.6–15.4/m^2) at reference sites in tributaries of the Black River (Allert et al. 2008); densities at reference sites were 10-fold greater than reported crayfish densities at mining sites in all studies.

Previous studies differed in their characterization of habitat selection by *O. neglectus*. Gore and Bryant (1990) determined that *O. neglectus* partition habitat, with young typically present in moderate-velocity (25–45 cm/sec) cobble habitats and adults present in low-velocity (0 cm/sec) macrophyte beds or high-velocity (>65 cm/sec) cobbled habitats. Other studies (Flinders and Magoulick 2005; Magoulick and DiStefano 2007) reported no significant difference in habitat selection by different size classes of *O. neglectus* or *O. n. chaenodactylus* (Williams 1952). The range of water depth, current velocities, and substrate measured at sites were comparable to those reported by Flinders and Magoulick (2005); Magoulick and DiStefano (2007), and Rabalais and Magoulick (2006b). Current velocities in kick-seine quadrats at sites in Center Creek and Shoal Creek (>0.60 cm/sec) were greater than previously reported ranges for young *O. neglectus* (≤25 cm; Gore and Bryant 1990), which may have limited densities in those creeks. Water depth and current velocity (significantly correlated in both quadrats and riffle habitats) were more important variables in explaining crayfish densities than in previous studies investigating the effects of mining-derived metals in crayfish (Allert et al. 2008, 2009). Several studies (Flinders and Magoulick 2005; Rabalais and Magoulick 2006b) have reported that *O. n. chaenodactylus* densities were correlated negatively with water depth in larger streams (stream order >3), which may explain the lower densities of *O. n. neglectus* in the significantly deeper Shoal Creek than the other streams sampled in the study.

There is limited information on the importance of reach- and watershed-scale variables on crayfish abundance; however, several studies (DiStefano et al. 2008; Burkey and Simon 2010; Lodge and Hill 1994; Westhoff et al. 2006) indicate that microhabitat not reach- or watershed-scale variables such as instream cover, substrate, and water depth are better predictors of local crayfish abundance than watershed-scale variables such as drainage area. In addition, several of these studies indicated that watershed-scale variables, such as increased watershed size and stream size, were related to lower local crayfish abundance. The inverse relationship between crayfish density and stream size (≥5 order) or watershed (drainage) size may be related to the decrease in allochthonous materials in larger streams (Vannote et al. 1980); loss of habitat heterogeneity (Allan 2004; Clark 2009; Mitchell and Smock 1991), smaller percentage of suitable habitat, or increased predation (Flinders and Magoulick 2003; Hill and Lodge 1995; Stein and Magnuson 1976). The ability of either reach- or watershed-scale variables to predict crayfish abundance may be species dependent; however, in-channel morphology, riparian cover, stream cover, and substrate have been repeatedly identified as important variables for predicting crayfish abundance, perhaps because these variables directly affect other abiotic factors (i.e., temperature, dissolved oxygen, current velocity) and biotic factors such as predation and food availability.

Water quality

Specific conductance and concentrations of hardness and sulfate were elevated at sites downstream from mine-waste in the TSMD; however, these concentrations generally did not exceed State water-quality standards or Federal water-quality criteria for protection of aquatic life. Elevations in concentrations of these constituents commonly occur in Pb-Zn mining areas of the Midwest because of the high-surface area of metal-ore particles in tailings piles and abandoned mine shafts where HCO_3^- ions in precipitation and groundwater increase the solubility of Ca^+, Mg^+, and HSO_4^- ions observed in surface waters. Although these mining constituents are distinct within mining districts, they are of less ecological significance than concentrations of Pb, Zn, and Cd described previously.

Ammonia is the nutrient of greatest concern to aquatic biota of streams. Mean total NH_3-N concentrations across all sites (0.019 mg N/L; range 0.001–0.218 mg N/L) were well below the national acute (3.68 mg N/L; temperature =22 ^{o}C; pH =8.0) and chronic (0.331 mg N/L; temperature =22 ^{o}C; pH =8.0) water-quality criteria for warm-water streams (USEPA 1999b), including concentrations (0.008 mg N/L) at the waste-water treatment plant on Shoal Creek, because of oxidative processes in the treatment plant operations. Total NH_3-N concentrations were elevated at Jenkins Creek (0.218 mg N/L) probably because of localized animal and/or septic inputs; however, concentrations likely were oxidized quickly downstream. Therefore, total NH_3-N would not be considered a chemical stressor for crayfish; rather, metals are the primary water-quality constituents of concern.

National synthesis studies have demonstrated that nutrient concentrations in streams draining urban and agricultural catchments are significantly elevated relative to nutrients measured in forested and less altered watersheds, and have been increasing across the United States during the past 20 years (Dubrovsky and Hamilton 2010). Concentrations of TN and TP typically are 10-fold greater in urban and agricultural watersheds across the Springfield Plateau in Missouri (Peterson et al. 1998). Justice et al. (2010) compared nutrient concentrations in 30 wadeable streams of the Ozarks to algal, macroinvertebrate, and fish assemblages and determined that median concentrations of TN and TP were 0.393 mg N/L (range 0.07–4.7 mg N/L) and 15 µg P/L (range 2–62 µg P/L), respectively.

Approximately 1.5-fold greater concentrations of TN (range 2.3–3.1 mg N/L) and 3-fold greater TP concentrations (range 158–233 µg P/L) were observed at sites in Shoal Creek compared to Justice et al. (2010). Measured site-mean concentrations in Shoal Creek exceed current Missouri water-quality standard for TN (0.90 mg N/L) and TP (75 µg P/L; MNDR 2009). The range in TN (2.0–2.6 mg N/L) and TP (49–139 µg P/L) concentrations in Center Creek also exceeded Missouri water-quality standards. Jenkins Creek, used as a metals reference site for this study, exceeded criteria for TN, but not for TP. Elevated TN at Jenkins Creek was largely composed of NO_2^-, NO_3^-, and NH_3^+, which probably reflects the rapid oxidation of NH_3^+ derived from localized cattle grazing and a localized single home-sewage inflow. Nutrient concentrations in Turkey Creek did not exceed Missouri standards for TN and TP, except at one site (T4), which likely was affected by row-crop agriculture and grazing and possible discharge from the Lone Elm WWTP as exhibited by elevated NO_2^- and NO_3^-. Justice et al. (2010) reported that Shoal Creek had an TN:TP ratio of 33, which was 3-fold greater than that observed in this study. An TN:TP ratio of 10–15 is considered optimum for primary productivity in aquatic environments, such as lakes (Redfield et al. 1963) and streams (Lohman et al. 1992; Van Nieuwenhyse and Jones 1996). Therefore, Shoal Creek nutrient conditions appear optimum for primary productivity, whereas Turkey Creek, Center Creek, and Jenkins Creek are phosphorus-limited.

Elevated concentrations of TN and TP in streams frequently can result in excessive primary production of benthic algae and diatoms, which are aesthetically unpleasing. In some situations, elevated levels of primary productivity can result in oxygen depletion because of organic matter decomposition and night-time respiration. Elevated nutrient concentrations were observed, but dissolved oxygen levels and water clarity remained high because of low suspended chl *a* and organic matter concentrations. Elevated concentrations of TN and TP did not result in increased turbidity or excessive benthic algal biomass (personal observation), because TN and TP were comprised largely of dissolved forms (NO_2^-, NO_3^-, and dissolved phosphorus). Unlike reservoirs and lakes, it is difficult to relate TN and TP concentrations to biological health of stream communities such as algal biomass because of multiple factors including shading, hydrologic conditions (e.g., discharge), and grazing rates of invertebrates and fishes (Dobbs et al. 1998; Dubrovsky and Hamilton 2010; Evans-White et al. 2001), which can reduce accumulation of benthic algae.

Conclusions

Results indicate that metals concentrations remain elevated in the environment and in crayfish in the TSMD. Crayfish densities at mining sites were significantly lower than at reference sites. Metals concentrations were significantly higher at mining sites than reference sites. Metals concentrations in crayfish may represent a hazard to fish and wildlife. Reduced crayfish densities imply loss of ecosystem function because crayfish are a key ecological component of Ozark streams and their surrounding ecosystems. Additional laboratory and field toxicity tests would provide additional information on the effects of metals and metals mixtures on crayfish, nutrient cycling, and ecosystem function. Genomic research also may provide further insight into the association between crayfish distribution and abundance patterns in the TSMD and environmental stressors such as heavy metals.

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Figure 1. Sample sites, mine-waste (i.e., chat piles), city of Joplin boundary, and designated areas within U.S. Environmental Protection Agency National Priority List Superfund site in the watersheds of the study. Shades of grey distinguish individual drainages.

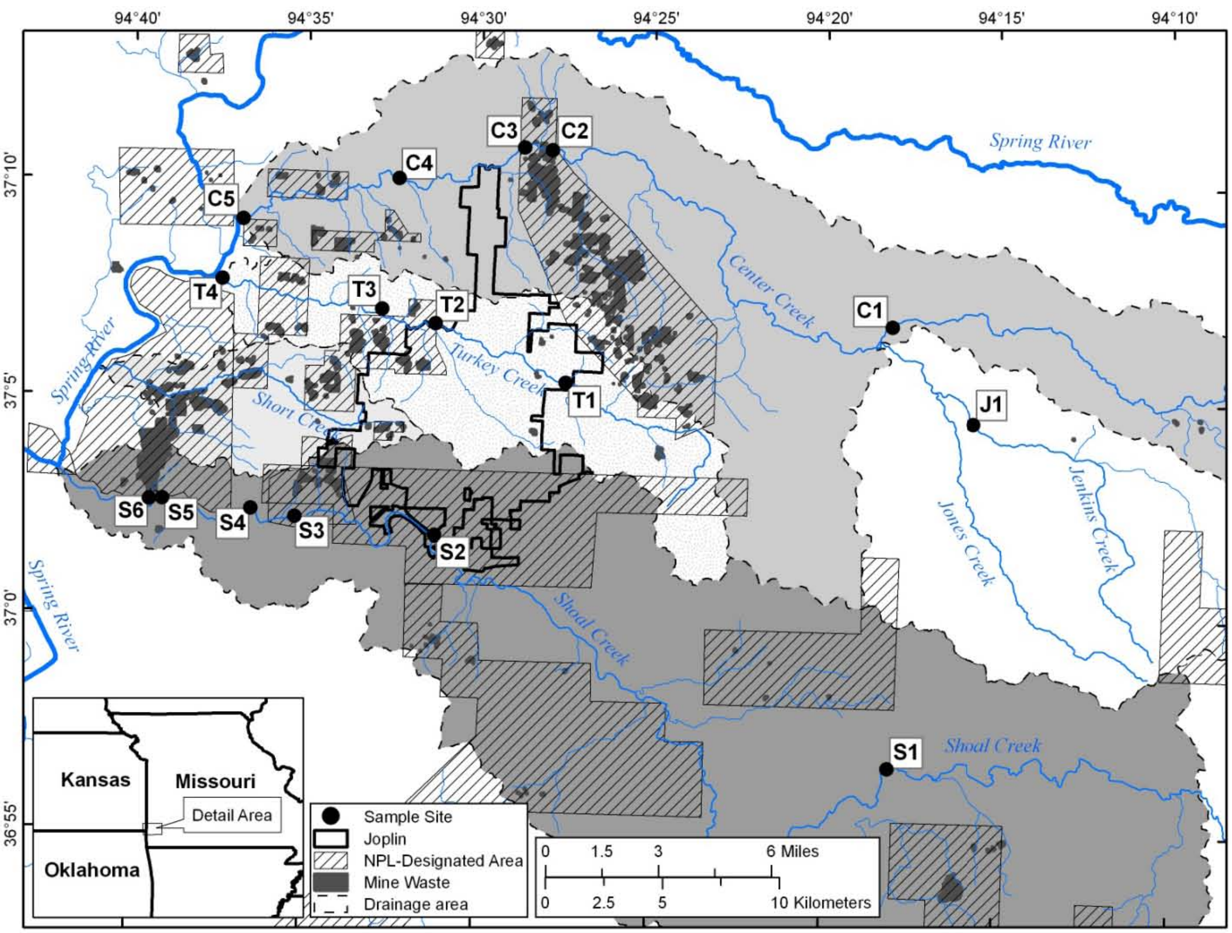

Figure 2. Carapace lengths (mm) of a) *Orconectes neglectus neglectus*; and b) *Orconectes macrus*. Lines within boxes represent 25th percentile (lower), median (middle), and 75th (upper) percentile.

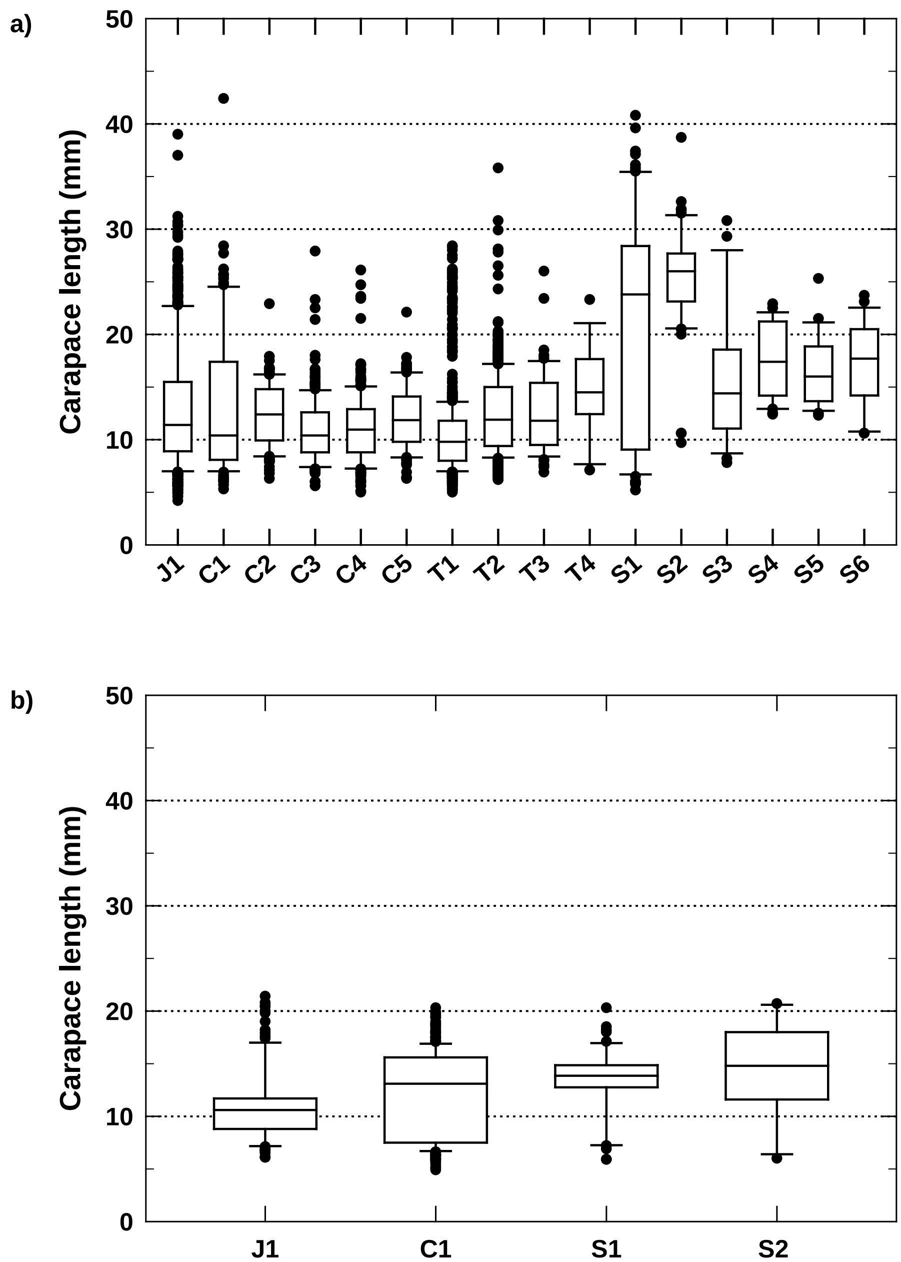

Figure 3. Mean concentrations of lead (Pb), zinc (Zn), and cadmium (Cd) in surface water, <250-µm fraction of sediment, detritus, and a) *Orconectes neglectus* and b) *Orconectes macrus*.

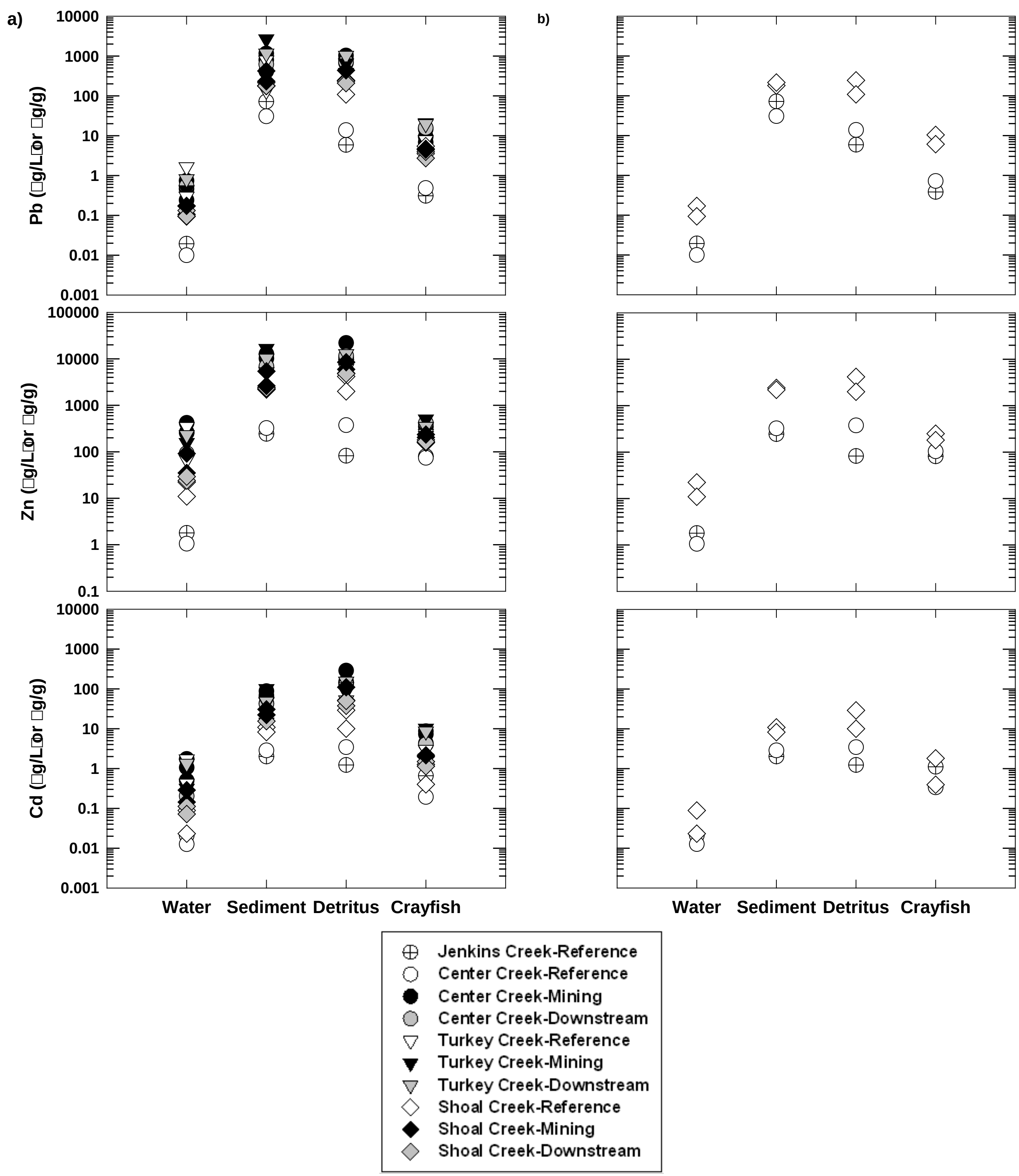

Figure 4. Assessment of risk of metals toxicity in a) <250-μm fraction of sediment; and b) surface water. $\sum PEQ_{Pb,Zn,Cd}$ = sediment probable-effect concentration quotient ($\sum$concentration/probable-effect concentration; MacDonald et al. 2000). Lines represent Tri-State Mining District low-risk (dashed) and high-risk (solid) toxicity thresholds (MacDonald et al., 2010. $\sum TU_{Pb,Zn,Cd}$ = surface-water toxic unit ($\sum$concentration/water-quality criterion; USEPA, 2005). Values <1.0 are predicted to be non-toxic.

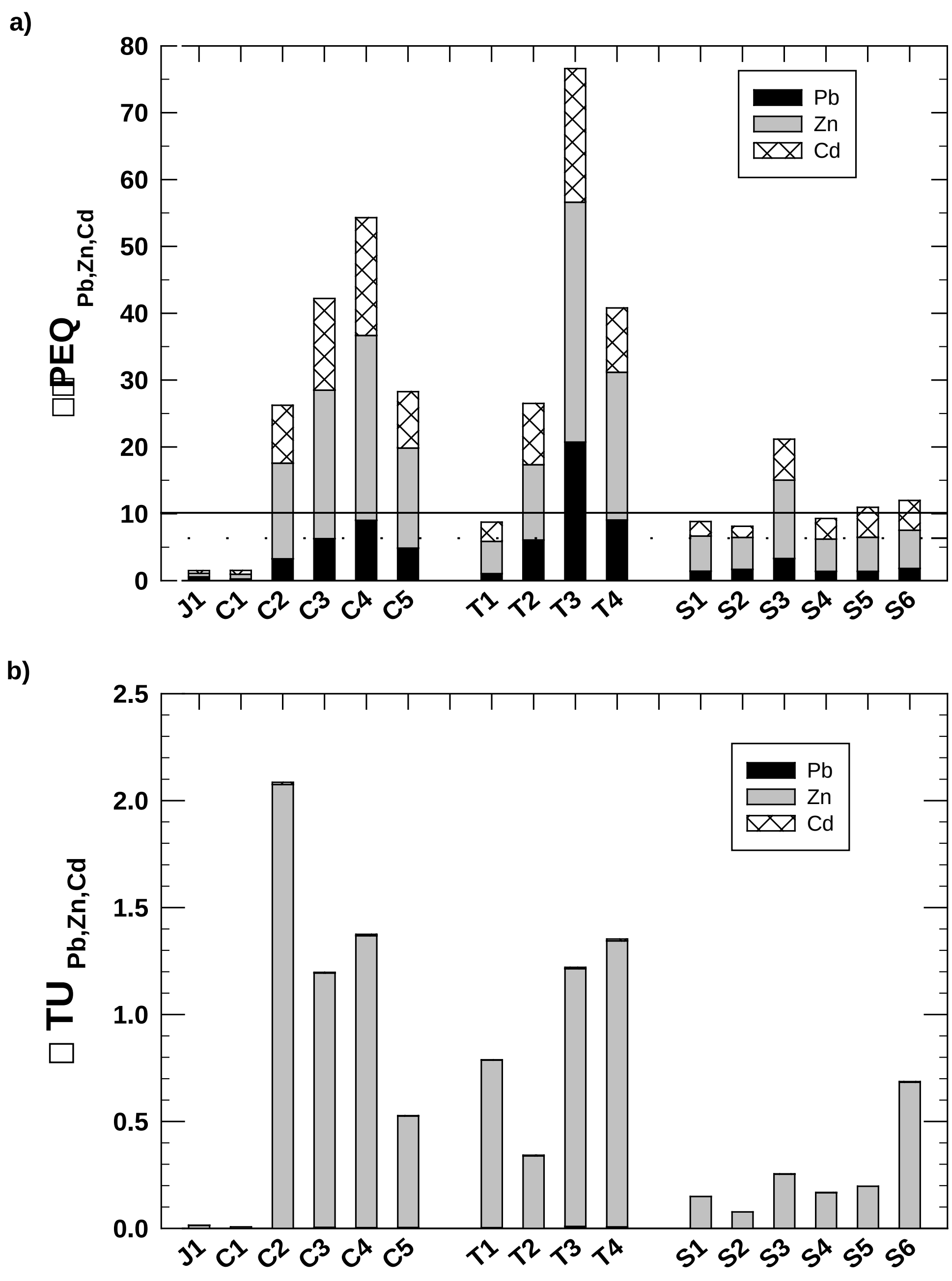

Figure 5. Relationship among sediment probable-effects concentrations quotients ($\sum PEQ_{Pb,Zn,Cd}$; MacDonald et al. 2010) and a) combined mean densities of *Orconectes neglectus neglectus* and *Orconectes macrus*; b) mean densities of *O. n. neglectus*; and c) mean densities of *O. macrus*. Lines represent Tri-State Mining District low-risk (dashed) and high-risk (solid) toxicity thresholds (MacDonald et al. 2010).

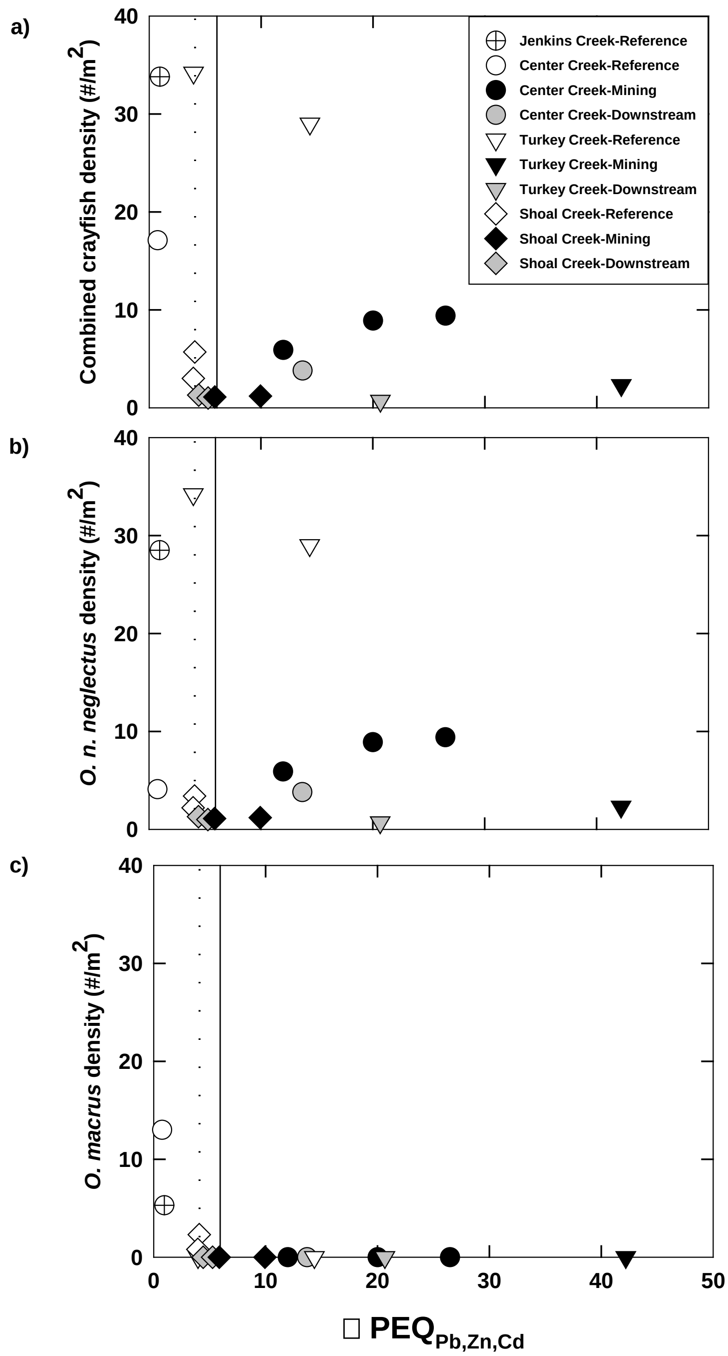

Figure 6. Quality Habitat Evaluation Index Score (QHEI; OHIO EPA 2006) for sample sites. QHEI scores in the range of 60–70% are considered "good"; scores >70% in headwater streams and scores >75% in larger streams are considered "excellent" (Ohio EPA 2006).

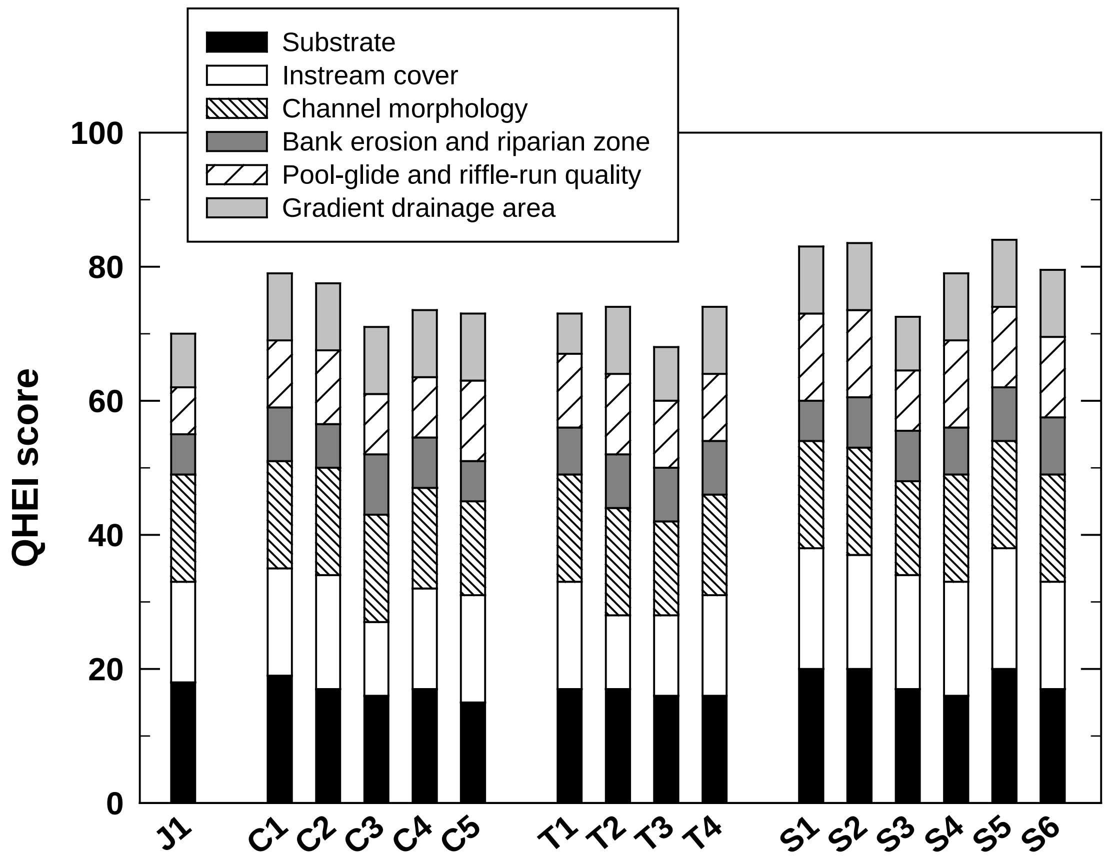

Figure 7. Principal components ordination based on metals concentrations (e.g., Pb concentrations in detritus) and physical-habitat quality (e.g., water depth) for a) mean densities of *Orconectes neglectus neglectus*; b) combined mean densities of *O. n. neglectus* and *O. macrus*; c) mean densities of *O. n. neglectus* at sites in Jenkins Creek, Center Creek, and Turkey Creek; d) mean densities of *O. n. neglectus* in Shoal Creek.

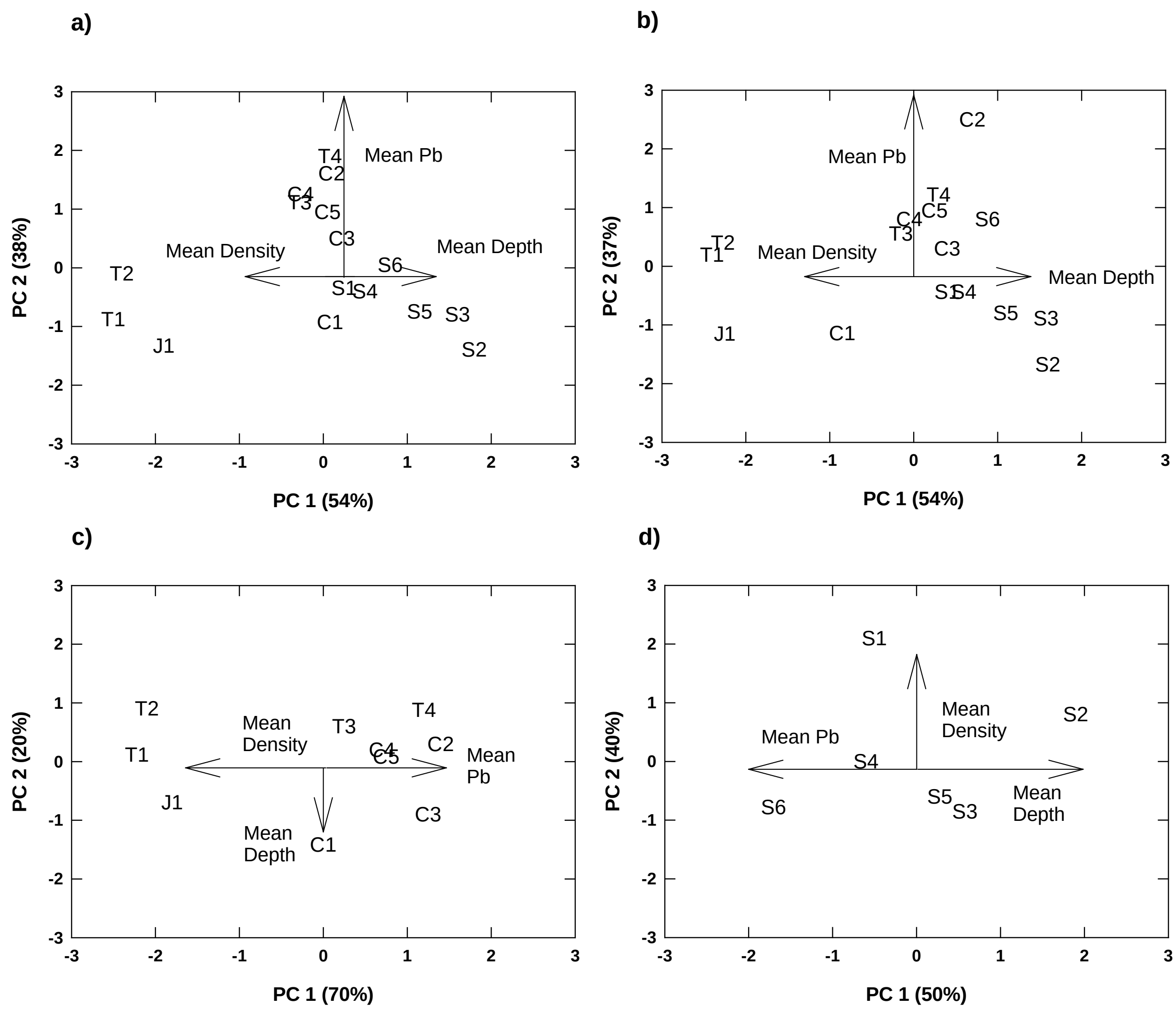

Table 1. Sampling locations in Jenkins Creek, Center Creek, Turkey Creek, and Shoal Creek in southwestern Missouri and southeastern Kansas, USA. Site type: R = reference site; M = mining site; D = downstream site.

Stream/site	Latitude	Longitude	Gradient (m/km)	Drainage area (ha)	Upstream tailings area (ha)	Tailings area/ drainage area	Distance downstream from tailings (km)	Stream order	Site type
Jenkins Creek									
J1	37º04’33.4”	94º15’40.1”	5.01	9324	0.70	0.00008	5.1	3	R
Center Creek									
C1	37º06’46.9”	94º18’02.9”	4.36	29526	27.8	0.00094	15.4	5	R
C2	37º10’46.6”	94º27’56.2”	2.52	66045	588	0.00891	3.4	5	M
C3	37º10’04.6”	94º32’21.3”	2.52	67340	785	0.01166	0.6	5	M
C4	37º10’45.8”	94º28’44.7”	2.46	73297	815	0.01112	3.9	5	M
C5	37º09’05.4”	94º36’50.7”	2.72	77182	908	0.01176	1.0	5	D
Turkey Creek									
T1	37º05’24.1”	94º27’27.9”	8.98	4144	81.7	0.01971	2.7	2	R
T2	37º06’38.4”	94º31’17.2”	3.66	6734	143	0.02128	4.1	3	R
T3	37º06’51.6”	94º32’44.9”	3.63	9324	225	0.02417	1.1	4	M
T4	37º07’46.9”	94º37’35.2”	4.79	11396	318	0.02790	4.5	4	D
Shoal Creek									
S1	36º56’34.7”	94º18’03.9”	3.36	69671	96.0	0.00138	6.8	5	R
S2	37º01’25.7”	94º31’11.0”	2.84	84952	120	0.00142	5.1	5	R
S3	37º02’07.7”	94º35’16.5”	1.52	113441	181	0.00160	2.6	5	M
S4	37º02’24.0”	94º36’30.1”	2.32	114736	209	0.00182	2.4	5	D
S5	37º02’31.2”	94º39’08.0”	3.08	116549	213	0.00183	7.1	5	D
S6	37º02’33.8”	94º39’23.6”	3.08	117585	345	0.00294	0.5	5	M

Table 2. Substrate coarseness classification (modified from Bain et al. 1985). '--' = not available.

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

Table 3. Method detection limits (MDL) for analytes in water, whole crayfish and detritus, and sediment. '--' = not available.

	MDL		
Analysis	**Water (μg/L)**	**Crayfish and detritus (μg/g dry weight)**	**Sediment (μg/g dry weight)**
Alkalinity	20000	--	--
Hardness	5000	--	--
Total nitrogen (TN)	110	--	--
Nitrite/nitrate (NO_3-N)	50	--	--
Ammonia (NH_3-N)	15	--	--
Total phosphorous (TP)	17	--	--
Particulate organic carbon	245	--	--
Dissolved organic carbon	280	--	--
Total suspended solids	1890	--	--
Chlorophyll *a*	0.41	--	--
Sulfate	1830	--	--
Nickel	0.12−0.13	0.04−0.13	0.06−0.13
Copper	0.04	0.02−0.37	0.04−0.07
Zinc	0.3−0.60	0.25−9.20	0.14−1.40
Cadmium	0.02−0.05	0.01−0.12	0.01−0.07
Lead	0.02−0.03	0.01−0.03	0.01−0.02

Table 4. Number and mean densities (±1 standard error) of *Orconectes neglectus neglectus*, *Orconectes macrus*, and combined densities of *O. n. neglectus* and *O. macrus*. Also shown are the results of one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05). '--' = not available.

	Orconectes neglectus neglectus			***Orconectes macrus***			**Combined**		
Site/ type	**No. collected**	**Mean density (#/m^2)**		**No. collected**	**Mean density (#/m^2)**		**No. collected**	**Mean density (#/m^2)**	
J1/R	684	28.5 (4.8)	a	126	5.3 (0.8)	a	810	33.8 (5.2)	a
C1/R	98	4.1 (2.0)	bc	311	13.0 (3.8)	a	409	17.1 (4.1)	abc
C2/M	141	5.9 (4.2)	bc	--	----		141	5.9 (4.2)	def
C3/M	213	8.9 (3.5)	b	--	----		213	8.9 (3.5)	bcd
C4/M	225	9.4 (4.9)	b	--	----		225	9.4 (4.9)	cd
C5/D	90	3.8 (0.9)	bc	--	----		90	3.8 (0.9)	de
T1/R	821	34.2 (8.5)	a	--	----		821	34.2 (8.5)	a
T2/R	695	29.0 (12.8)	a	--	----		695	29.0 (12.8)	ab
T3/M	55	2.3 (0.4)	bc	--	----		55	2.3 (0.4)	defgh
T4/D	16	0.7 (0.2)	c	--	----		16	0.7 (0.2)	i
S1/R	82	3.4 (1.0)	bc	54	2.3 (0.9)	b	136	5.7 (0.4)	bcd
S2/R	53	2.2 (0.8)	bc	19	0.8 (0.2)	b	72	3.0 (0.3)	defg
S3/M	29	1.2 (0.3)	c	--	----		29	1.2 (0.3)	fghi
S4/D	20	1.3 (0.1)	c	--	----		20	1.3 (0.1)	efghi
S5/D	25	1.0 (0.2)	c	--	----		25	1.0 (0.2)	hi
S6/M	27	1.1 (0.5)	c	--	----		27	1.1 (0.5)	ghi
ANOVA									
		$F_{(15,3181)}$ 6.25**			$F_{(3,506)}$ 17.8**			$F_{(15,3687)}$ 10.8**	
		R^2 0.75			R^2 0.89			R^2 0.83	

Table 5. Site-type mean densities (±1 standard error) of *Orconectes neglectus neglectus*, combined densities of *O. n. neglectus* and *Orconectes macrus*, probable-effects concentrations quotients (∑PEQs), toxic-unit scores (∑TUs), and water depth in 1-m^2 kick-seine quadrats. Also shown are the results of planned non-orthogonal contrasts among groups of sites as *F*-values among groups of sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site-type means with the same lower case letter are not significantly different for each sample type (P >0.05).

Site type	***Orconectes neglectus neglectus*** **density (#/m^2)**	**Combined density (#/m^2)**	**$\sum PEQ_{Pb,Zn,Cd}$**	**$\sum TU_{Pb,Zn,Cd}$**	**Water depth (cm)**
R[1]	16.9 (4.0) a	20.5 (3.86) a	9.2 (2.1) c	0.23 (0.07) c	21.8 (0.5) c
M[2]	4.79 (1.33) b	4.79 (1.33) b	38.7 (6.3) a	1.14 (0.14) a	26.4 (0.5) a
D[3]	1.68 (0.41) c	1.68 (0.41) c	22.3 (4.0) b	0.56 (0.14) b	24.8 (0.5) b
R vs M	$F_{(2,45)}$ 15.0**	$F_{(2,45)}$ 52.1**	$F_{(2,45)}$ 352**	$F_{(2,45)}$ 2029**	$F_{(2,45)}$ 86.8**
R vs D	$F_{(2,45)}$ 33.6**	$F_{(2,45)}$ 87.8**	$F_{(2,45)}$ 111**	$F_{(2,45)}$ 439**	$F_{(2,45)}$ 60.9**
D vs M	$F_{(2,45)}$ 5.44*	$F_{(2,45)}$ 8.49**	$F_{(2,45)}$ 39.3**	$F_{(2,45)}$ 374**	$F_{(2,45)}$ 0.28ns

[1]Reference sites: J1, C1, T1, T2, S1, S2
[2]Mining sites: C2, C3, C4, T3, S3, S6
[3]Downstream sites: T4, C5, S4, S6

Table 6. Number, species, mean carapace length (±1 standard error), and sex ratio (F:M) of crayfish collected by kick-seining. Unidentifiable crayfish were collected at C1 (n =1) and C2 (n =1). Also shown are the results of one-way analysis-of-variance (ANOVA) as *F*-values, coefficients of determination (R^2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each species (P >0.05). nd = not determined. '--' = not available.

	Orconectes neglectus neglectus				***Orconecetes macrus***				***Orconectes virilis***		
Site/ type	**No. collected**	**Carapace length (mm)**		**F:M**	**No. collected**	**Carapace length (mm)**		**F:M**	**No. collected**	**Carapace length (mm)**	**F:M**
J1/R	684	13.1 (0.2)	fg	355:291	126	11.1 (0.3)	c	66:57	3	18.8 (5)	1:2
C1/R	98	13.2 (0.7)	fgh	63:33	311	11.9 (0.2)	bc	174:136	2	14.3 (3)	0:2
C2/M	141	12.4 (0.3)	f	69:72	--	----		--	--	----	--
C3/M	213	10.9 (0.2)	gh	100:110	--	----		--	--	----	--
C4/M	225	11.1 (0.2)	fgh	106:115	--	----		--	--	----	--
C5/D	90	12.0 (0.3)	fg	38:52	--	----		--	--	----	--
T1/R	821	10.5 (0.1)	h	456:344	--	----		--	--	----	--
T2/R	695	12.5 (0.1)	fg	363:330	--	----		--	--	----	--
T3/M	55	12.6 (0.5)	f	27:28	--	----		--	--	----	--
T4/D	16	14.8 (1.0)	de	7:9	--	----		--	--	----	--
S1/R	82	21.2 (1.2)	cde	14:13	54	13.5 (0.4)	ab	31:23	--	----	--
S2/R	53	25.3 (0.7)	a	29:24	19	14.4 (1.1)	a	11:8	--	----	--
S3/M	29	15.7 (1.2)	e	16:13	--	----		--	--	----	--
S4/D	20	17.7 (0.6)	ab	17:13	--	----		--	--	----	--
S5/D	25	16.6 (0.7)	bc	8:17	--	----		--	--	----	--
S6/M	27	17.1 (0.7)	bcd	14:13	--	----		--	--	----	--
ANOVA											
		$F_{(15,3181)}$ 36.6**				$F_{(3,506)}$ 5.88**				nd	
		R^2 0.15				R^2 0.03				nd	

Table 7. Number, sex ratio (F:M), and mean carapace length (±1 standard error) of *Orconectes neglectus neglectus* and *Orconectes macrus* collected for metals analyses. Also shown are the results of one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05). '--' = not available.

	Orconectes neglectus neglectus				*Orconectes macrus*			
Site/ type	**n**	**F:M**	**Carapace length (mm)**		**n**	**F:M**	**Carapace length (mm)**	
J1/R	15	10:5	25.0 (1.2)	ab	16	9:7	15.1 (1.0)	a
C1/R	14	7:7	25.9 (1.3)	ab	14	8:6	16.5 (0.5)	a
C2/M	15	5:10	15.3 (0.4)	fg	--	--	-- --	
C3/M	16	9:7	15.0 (1.1)	g	--	--	-- --	
C4/M	17	7:10	16.5 (1.3)	defg	--	--	-- --	
C5/D	15	9:6	15.7 (0.6)	fg	--	--	-- --	
T1/R	14	8:6	23.9 (1.1)	b	--	--	-- --	
T2/R	16	9:7	20.5 (1.3)	c	--	--	-- --	
T3/M	15	8:7	16.7 (0.9)	defg	--	--	-- --	
T4/D	13	4:9	15.7 (1.1)	efg	--	--	-- --	
S1/R	15	8:7	30.0 (1.4)	a	13	7:6	15.9 (1.2)	a
S2/R	15	4:11	26.4 (1.3)	ab	13	7:6	15.8 (0.6)	a
S3/M	15	7:8	19.8 (1.6)	cd	--	--	-- --	
S4/D	15	11:4	18.8 (0.9)	cde	--	--	-- --	
S5/D	15	5:10	17.9 (0.9)	cdef	--	--	-- --	
S6/M	13	7:6	18.7 (1.0)	cde	--	--	-- --	
ANOVA								
			$F_{(15,212)}$ 17.3**				$F_{(3,52)}$ 0.38ns	
			R^2 0.54				R^2 0.02	

Table 8. Mean density and metals concentrations (±1 standard error) in *Orconectes neglectus neglectus*; *Orconectes macrus*; and species combined. Also shown are the results of one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site-mean concentrations with the same lower case letter (O. n. neglectus), same capital letter (O. macrus), same capital-italic letter (combined), and number (comparison among species at a site) are not significantly different (P >0.05). N = the number of composite samples.

Site/ type	**N**	**Species**	**Density (#/m^2)**	**Metals concentrations (µg/g dry weight)**									
				Lead		**Zinc**		**Cadmium**		**Nickel**		**Copper**	
J1/R	3	*O. macrus*	5.3	0.38 (0.06)	B,1	81.1 (2.50)	B,1	1.11 (0.12)	B,1	1.33 (0.11)	B,1	93.3 (21.4)	A,1
J1/R	3	*O. n. neglectus*	28.5	0.31 (0.04)	g,1	80.2 (5.90)	ef,1	0.66 (0.12)	jk,2	1.02 (0.08)	c,1	80.8 (4.8)	abc,1
J1/R	6	combined	33.8	0.34 (0.04)	*G*	80.6 (2.9)	*F*	0.89 (0.13)	*H*	1.18 (0.09)	*C*	87.1 (10.2)	*ABCD*
C1/R	3	*O. macrus*	13.0	0.72 (0.21)	B,1	104 (3.10)	B,1	0.34 (0.04)	D,1	1.45 (0.31)	B,1	111 (6)	A,1
C1/R	3	*O. n. neglectus*	4.1	0.48 (0.12)	g,1	74.4 (6.10)	f,2	0.19 (0.02)	l,2	1.09 (0.12)	bc,1	79.5 (10.6)	abc,2
C1/R	6	combined	17.1	0.60 (0.12)	*G*	89 (7)	*F*	0.27 (0.04)	*I*	1.27 (0.17)	*BC*	95.1 (8.9)	*ABC*
C2/M	3	*O. n. neglectus*	5.9	6.79 (2.39)	cde,*CDE*	356 (62.7)	b,*AB*	8.67 (0.19)	ab,*AB*	1.83 (0.35)	a,*ABC*	82.1 (5.2)	abc, *ABCD*
C3/M	3	*O. n. neglectus*	8.9	14.6 (4.55)	ab,*AB*	339 (0.60)	b,*B*	4.32 (0.11)	c,*CD*	1.39 (0.07)	abc,*ABC*	70.7 (4.5)	bcd, *CDE*
C4/M	3	*O. n. neglectus*	9.4	9.33 (0.48)	abc,*ABC*	399 (20.6)	ab,*AB*	7.29 (0.50)	b,*BC*	1.81 (0.14)	a,*AB*	73.5 (5.7)	bcd, *BCDE*
C5/D	3	*O. n. neglectus*	3.8	15.7 (3.46)	ab,*AB*	417 (45.2)	ab,*AB*	4.04 (0.38)	cd,*CD*	2.06 (0.22)	a,*A*	70.9 (3.7)	bcd, *CDE*
T1/R	3	*O. n. neglectus*	34.2	4.38 (0.71)	def,*EF*	241 (29)	c,*C*	1.80 (0.15)	fg,*FG*	1.50 (0.24)	abc,*ABC*	51.8 (6.7)	d, *E*
T2/R	3	*O. n. neglectus*	29.0	7.74 (2.25)	bcd,*BDC*	220 (24)	c,*C*	2.97 (0.25)	de,*DE*	1.30 (0.25)	abc,*BC*	88.9 (4.6)	ab, *ABCD*
T3/M	3	*O. n. neglectus*	2.3	20.8 (2.83)	a,*A*	500 (22)	a,*A*	10.1 (0.16)	a,*A*	1.91 (0.18)	a,*AB*	117 (7.2)	a,*A*
T4/D	3	*O. n. neglectus*	0.7	19.7 (5.77)	a,*A*	346 (15)	b,*AB*	8.58 (1.24)	ab,*AB*	1.50 (0.02)	abc,*ABC*	85.4 (4.8)	abc, *ABCD*
S1/R	3	*O. macrus*	2.3	10.4 (1.23)	A,1	249 (9.4)	A,1	1.81 (0.12)	A,1	2.08 (0.11)	A,1	116 (5.1)	A,1
S1/R	3	*O. n. neglectus*	3.4	5.46 (0.27)	cde,2	181 (8.4)	cd,2	1.27 (0.07)	hi,2	1.40 (0.04)	abc,2	84.4 (3.8)	abc,2
S1/R	6	combined	5.7	7.93 (1.24)	*BCD*	215 (16)	*CD*	1.54 (0.14)	*G*	1.74 (0.16)	*ABC*	100 (8)	*AB*
S2/R	3	*O. macrus*	0.8	6.08 (0.37)	A,1	181 (7)	A,1	0.39 (0.06)	C,1	2.02 (0.12)	A,1	115 (10)	A,1
S2/R	3	*O. n. neglectus*	2.2	3.54 (0.41)	ef,2	157 (32)	de,1	0.40 (0.12)	kl,1	1.56 (0.25)	abc,1	83.0 (6.7)	abc,2
S2/R	6	combined	3.0	4.81 (0.62)	*DE*	169 (16)	*DE*	0.40 (0.06	*I*	1.79 (0.16)	*ABC*	99.2 (9.1)	*ABC*
S3/M	3	*O. n. neglectus*	1.2	4.67 (1.06)	def,*DEF*	204 (35)	cd,*CDE*	1.99 (0.36)	fg,*FG*	1.89 (0.48)	ab,*ABC*	73.4 (6.4)	bcd, *BCDE*
S4/D	3	*O. n. neglectus*	1.3	2.72 (0.24)	gf,*FG*	160 (15)	de	1.49 (0.10)	gh,*G*	1.32 (0.23)	abc,*ABC*	65.9 (6.8)	ab, *DE*
S5/D	3	*O. n. neglectus*	1.0	3.96 (1.02)	ef,*EF*	166 (7)	de,*E*	1.14 (0.07)	ji,*H*	1.34 (0.14)	abc,*ABC*	88.1 (2.4)	cd, *ABCD*
S6/M	3	*O. n. neglectus*	1.1	4.46 (0.49)	def,*DEF*	235 (14)	c,*C*	2.16 (0.26)	ef,*EF*	1.46 (0.09)	abc,*ABC*	91.8 (14.1)	abc, *ABCD*

Table 8. Riffle crayfish density of and mean metals concentrations (±1 standard error)—Continued.

Site/ type	N	Species	Density (#/m^2)	Metals concentrations (µg/g dry weight) Lead	Zinc	Cadmium	Nickel	Copper
Orconectes neglectus neglectus among sites								
ANOVA								
$F_{(15,212)}$				13.1*	19.6**	77.4**	2.00*	2.99**
R^2				0.86	0.90	0.97	0.48	0.58
Orconectes neglectus neglectus among site type								
ANOVA								
$F_{(2,45)}$				6.41**	13.3**	18.4**	5.06*	0.12ns
R^2				0.22	0.37	0.45	0.18	0.01
Orocnectes macrus among sites								
ANOVA								
$F_{(3,52)}$				0.17ns	0.74ns	0.46ns	1.09ns	11.4**
R^2				0.01	0.03	0.02	0.04	0.34
Combined species among sites								
ANOVA								
$F_{(15,264)}$				18.2**	27.3**	71.9**	1.96*	3.02**
R^2				0.86	0.90	0.96	0.40	0.51
Combined species among site type								
ANOVA								
$F_{(2,45)}$				7.65**	18.8**	29.1**	1.80ns	1.80ns
R^2				0.21	0.40	0.51	0.06	0.06
Between species at sites where both collected								
ANOVA								
$F_{(3,1)}$				40.2*	28.0**	30.0**	9.54**	6.29**
R^2				0.89	0.85	0.89	0.67	0.57

Table 9. Site-type mean metals concentrations (±1 standard error) in *Orconectes neglectus neglectus*, detritus, <250-µm fraction of sediment, and surface water. Also shown are the results of planned non-orthogonal contrasts among groups of sites as *F*-values (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05); site-type means with the same lower case letter are not significantly different for each site type.

Site type	Lead		Zinc		Cadmium	
***Orconectes neglectus neglectus* (µg/g dry weight)**						
R[1]	3.65 (0.7)	b	159 (17)	c	1.22 (0.24)	c
M[2]	10.1 (1.7)	a	339 (26)	a	5.76 (0.77)	a
D[3]	10.5 (2.6)	a	272 (35)	b	3.81 (0.94)	b
R vs M	$F_{(2,45)}$ 47.3**		$F_{(2,45)}$ 120**		$F_{(2,45)}$ 531**	
R vs D	$F_{(2,45)}$ 22.3**		$F_{(2,45)}$ 32.4**		$F_{(2,45)}$ 155**	
D vs M	$F_{(2,45)}$ 2.05ns		$F_{(2,45)}$ 17.0**		$F_{(2,45)}$ 66.6**	
Detritus (µg/g dry weight)						
R	156 (36)	b	3281 (663)	c	34 (11)	c
M	643 (70)	a	10772 (1438)	a	138 (20)	a
D	543 (124)	a	8500 (1261)	b	92 (20)	b
R vs M	$F_{(2,45)}$ 168**		$F_{(2,45)}$ 312**		$F_{(2,45)}$ 129**	
R vs D	$F_{(2,45)}$ 71.9**		$F_{(2,45)}$ 132**		$F_{(2,45)}$ 41.6**	
D vs M	$F_{(2,45)}$ 9.59**		$F_{(2,45)}$ 18.8**		$F_{(2,45)}$ 13.6**	
<250-µm fraction of sediment (µg/g dry weight)						
R	235 (73)	c	2093 (409)	c	14.0 (3.7)	c
M	947 (231)	a	8991 (1435)	a	58.6 (8.7)	a
D	534 (128)	b	5393 (1011)	b	31.9 (4.2)	b
R vs M	$F_{(2,45)}$ 155**		$F_{(2,45)}$ 235**		$F_{(2,45)}$ 341**	
R vs D	$F_{(2,45)}$ 51.2**		$F_{(2,45)}$ 54.5**		$F_{(2,45)}$ 93.4**	
D vs M	$F_{(2,45)}$ 15.9**		$F_{(2,45)}$ 40.1**		$F_{(2,45)}$ 47.1**	
Surface water (µg/L)						
R	0.15 (0.03)	c	39.0 (12.4)	c	0.13 (0.04)	c
M	0.56 (0.11)	a	237 (29)	a	0.87 (0.14)	a
D	0.36 (0.08)	b	83.9 (22.4)	b	0.38 (0.14)	b
R vs M	$F_{(2,45)}$ 689**		$F_{(2,45)}$ 2105**		$F_{(2,45)}$ 1286**	
R vs D	$F_{(2,45)}$ 325**		$F_{(2,45)}$ 332**		$F_{(2,45)}$ 187**	
D vs M	$F_{(2,45)}$ 32.6**		$F_{(2,45)}$ 541**		$F_{(2,45)}$ 352**	

[1]Reference sites: J1, C1, T1, T2, S1, S2
[2]Mining sites: C2, C3, C4, T3, S3, S6
[3]Downstream sites: T4, C5, S4, S6

Table 10. Species, location, collection period, and maximum metal concentration (µg/g dry weight) for composite samples of crayfish collected in the Tri-State Mining District (TSMD); in the Old Lead Belt Mining District (OLBMD), and in the Viburnum Trend Mining District (VTMD). nd = not determined

Species, location, collection period	**Lead (µg/g)**	**Zinc (µg/g)**	**Cadmium (µg/g)**	**Nickel (µg/g)**
Orconectes neglectus neglectus (TSMD, current study)	20.8	500	10.1	2.06
Oconectes spp. (mainstem Spring River, TSMD, 2001–2002)[1]	5.05	313	1.9	nd
Oconectes spp. (mainstem and tributaries of Spring River, TSMD, pre-2000)[2]	18.0	597	8.2	nd
Orconectes spp. (OLBMD, 1982)[2]	193	276	2.05	nd
Orconectes luteus (OLBMD, 2008)[3]	134	328	19.8	5.71
Orconectes hylas (VTMD, 2004)[4]	18.0	100	2.20	6.60

[1]Schmitt et al. (2006), Table 3
[2]Schmitt et al. (2006), Table 5; originally reported as wet weight; dry weight values computed assuming moisture content of 80% for crayfish (Schmitt et al. 2006).
[3]Allert et al. (2010), Table 12
[4]Allert et al. (2008), Table 6

Table 11. Mean metals concentrations (±1 standard error) in detritus. Also shown are the results of one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each metal (P >0.05). n = number of samples.

Site/		Metals concentrations (µg/g dry weight)									
type	n	Lead		Zinc		Cadmium		Nickel		Copper	
J1/R	3	5.88 (0.75)	h	82.3 (8.3)	j	1.23 (0.21)	f	12.8 (0.8)	g	13.1 (0.6)	f
C1/R	3	14 (1)	gh	375 (41)	j	3.45 (0.63)	ef	19.2 (1.9)	fg	19.3 (2.5)	def
C2/M	3	1029 (181)	a	21967 (4625)	a	288 (56)	a	38.7 (7.1)	ab	53.7 (8.6)	a
C3/M	3	671 (49)	ab	9977 (825)	bc	152 (3)	ab	23.3 (0.8)	ef	28.7 (3.0)	bcd
C4/M	3	824 (133)	a	10653 (851)	bc	143 (16)	ab	33.1 (3.9)	abc	42.4 (8.4)	ab
C5/D	3	711 (247)	ab	11647 (2261)	b	127 (51)	b	28.6 (2.0)	cd	31.2 (9.6)	bcd
T1/R	3	183 (7)	efg	6973 (574)	ef	52.0 (7.9)	cd	25.3 (1.6)	def	18.9 (2.1)	def
T2/R	3	381 (104)	cd	6083 (371)	fg	110 (46)	b	27.5 (1.4)	cde	35.8 (7.4)	abc
T3/M	3	655 (48)	ab	7630 (326)	de	84.0 (10.4)	bc	32.3 (2.1)	abc	38.0 (2.6)	ab
T4/D	3	1021 (196)	a	12700 (1185)	ab	153 (30)	ab	37.7 (2.8)	a	51.7 (8.1)	a
S1/R	3	244 (20)	cde	4180 (333)	hi	29.1 (3.7)	de	18.4 (0.5)	fg	21.1 (1.6)	def
S2/R	3	108 (40)	de	1997 (705)	ij	10.0 (5.7)	ef	18.8 (1.4)	fg	16.0 (2.5)	abc
S3/M	3	243 (37)	fgh	5937 (547)	fg	51.6 (6.7)	cd	30.6 (1.6)	abc	53.7 (27.0)	ef
S4/D	3	233 (17)	de	4883 (362)	gh	37.7 (5.0)	de	27.5 (0.6)	cde	22.4 (1.2)	bcde
S5/D	3	209 (64)	ef	4773 (1044)	gh	50.0 (20.0)	d	28.3 (2.7)	bcd	26.2 (4.3)	cdef
S6/M	3	437 (30)	bc	8470 (308)	cd	110 (6.2)	b	38.7 (4.0)	a	37.7 (0.8)	ab
ANOVA											
$F_{(15,32)}$		22.6**		43.3**		17.4**		13.1**		6.98**	
R^2		0.91		0.95		0.89		0.86		0.77	

Table 12. Mean percent total organic carbon (%TOC; ±1 standard error) and mean metals concentrations (±1 standard error) in <250-µm and <2000-µm fractions of sediment. Also shown are the results of one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees-of-freedom for differences in each sediment fraction among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sediment fraction (P >0.05).

Site/		Fraction		Metals concentrations (µg/g)				
type	n	(µm)	%TOC	Lead	Zinc	Cadmium	Nickel	Copper
J1/R	3	<250	1.6 (0.1) def	72.1 (28.0) g	244 (52) f	2.01 (0.38) g	34.7 (2.2) abcd	16.1 (2.2) fg
	3	<2000	0.4 (0.2) cd	28.4 (1.9) g	162 (20) g	0.95 (0.18) g	27.1 (2.4) ab	9.7 (1.2) def
C1/R	3	<250	2.6 (0.1) ab	30.7 (0.4) g	323 (14) f	2.85 (0.10) fg	25.1 (1.8) cdef	13.8 (0.80) g
	3	<2000	1.0 (0.6) abc	32.5 (4.2) fg	268 (11) eg	1.51 (0.33) g	28.1 (2.3) a	10.0 (0.80) bcdef
C2/M	3	<250	1.5 (0.4) def	416 (50) cd	6567 (174) b	43.1 (3.2) bc	25.0 (2.8) cdef	17.5 (1.8) efg
	3	<2000	0.3 (0.0) cd	357 (72) c	6697 (1082) ab	40.0 (4.3) ab	23.0 (0.5) abcd	14.5 (0.6) ab
C3/M	3	<250	1.9 (0.3) cde	802 (94) b	10207 (978) b	68.2 (4.6) a	35.3 (1.7) cdef	24.6 (1.4) abcd
	3	<2000	0.5 (0.1) bcd	323 (28) c	4170 (813) bc	24.0 (4.2) bc	15.9 (1.9) fg	19.6 (5.7) abc
C4/M	3	<250	2.2 (0.2) bcd	1155 (486) ab	12690 (4438) a	87.8 (27.6) a	35.9 (7.2) abcd	32.2 (8.1) abc
	3	<2000	0.6 (0.1) abc	571 (95) ab	17263 (1205) a	107 (9) a	23.5 (2.9) abcd	77.1 (56.5) abcd
C5/D	3	<250	1.4 (0.3) ef	621 (128) bc	6870 (326) b	42.0 (2.9) bc	36.8 (8.6) abcd	19.6 (1.7) cdef
	3	<2000	0.3 (0.0) cd	380 (42) bc	3937 (417) bc	21.0 (2.1) c	24.6 (1.4) abc	13.3 (2.6) a
T1/R	3	<250	0.7 (0.1) f	134 (2) fg	2213 (86) e	14.3 (0.8) e	29.5 (7.7) cdef	12.7 (2.4) g
	3	<2000	0.2 (0.0) d	83.0 (17) ef	1165 (137) efg	4.67 (1.3) fg	21.7 (3.4) bcde	7.3 (0.9) f
T2/R	3	<250	2.4 (0.0) abc	777 (283) bc	5173 (507) bc	45.6 (5.1) b	30.3 (1.7) bcde	27.3 (2.3) abc
	3	<2000	1.4 (0.3) abc	1012 (572) ab	3163 (473) c	21.1 (4.1) c	20.4 (2.5) cdef	11.0 (1.4) bcde
T3/M	3	<250	2.5 (0.2) abc	2653 (650) a	16467 (3973) a	99.6 (21.1) a	66.8 (11.2) a	62.2 (4.4) a
	3	<2000	0.5 (0.1) ab	924 (197) a	18710 (10480) a	97.9 (56.8) a	28.5 (3.5) ab	27.5 (6.4) a
T4/D	3	<250	2.1 (0.1) bcde	1161 (125) ab	10147 (511) a	47.9 (3.4) b	42.9 (3.1) ab	40.2 (3.8) ab
	3	<2000	2.4 (0.2) a	664 (134) ab	8030 (724) a	38.6 (4.1) ab	24.5 (2.4) abc	22.3 (5.6) a

Table 12. Mean metals concentrations (±1 standard error) in sediment… continued.

Site/ type	n	Fraction (µm)	%TOC		Metals concentrations (µg/g) Lead		Zinc		Cadmium		Nickel		Copper	
S1/R	3	<250	1.9(0.2)	cde	181(24)	g	2410(170)	de	10.8(1.1)	ef	16.8(.6)	f	13.2(0.4)	g
	3	<2000	0.6(0.1)	abc	136(14)	d	1837(406)	de	7.01(0.7)	def	13.2(0.4)	g	8.6(0.8)	ef
S2/R	3	<250	1.6(0.2)	def	216(40)	e	2193(373)	de	8.22(0.8)	fg	22.7(3.7)	def	13.7(1.8)	g
	3	<2000	0.5(0.1)	abc	91.4(4)	efg	2737(1086)	cd	11.3(6.4)	def	13.1(0.9)	g	7.1(0.6)	f
S3/M	3	<250	2.4(0.3)	abc	421(150)	cd	5390(1807)	b	30.5(10.1)	cd	32.7(4.1)	abcd	23.6(3.8)	bcde
	3	<2000	0.3(0.0)	cd	274(47)	c	4250(1285)	bc	16.2(5.6)	cd	16.0(0.5)	fg	10.2(0.4)	bcde
S4/D	3	<250	2.2(0.3)	bcde	176(3)	ef	2213(189)	e	15.5(2.8)	ef	21.8(1.6)	ef	17.2(1.5)	efg
	3	<2000	0.5(0.1)	abc	131(12)	d	1530(206)	de	7.32(2.0)	ef	16.3(1.4)	efg	9.8(0.7)	cdef
S5/D	3	<250	3.3(0.2)	a	178(5)	ef	2340(21)	de	22.2(1)	d	28.3(3)	bcde	26.0(6)	bcde
	3	<2000	0.7(0.3)	abc	102(18)	de	1259(201)	ef	8.16(3)	def	19.6(1)	cdef	10.4(1)	bcde
S6/M	3	<250	2.4(0.3)	abc	232(10)	de	2623(54)	cd	22.2(2)	d	27.3(3)	cdef	19.1(1)	def
	3	<2000	0.6(0.1)	cd	137(17)	d	2210(290)	cd	12.6(0)	cde	17.9(1)	defg	8.98(1)	def
ANOVA														
$F_{(15,32)}$			5.91^{**1};2.94^{**2}		25.7^{**};29.3^{**}		31.7^{**};21.7^{**}		42.2^{**};19.9^{**}		4.08^{**};7.96^{**}		10.4^{**};6.33^{**}	
R^2			0.73;0.58		0.92;0.93		0.94;0.90		0.95;0.90		0.66;0.79		0.83;0.75	
PEC[3]			--		128		459		4.98		48.6		149	
PEC-10[4]			--		123		1702		9.1		--		--	
PEC-20[5]			--		179		2409		14.1		--		--	

Table 12. Mean metals concentrations (±1 standard error) in sediment… continued.

[1]<250-µm fraction
[2] <2000-µm fraction
[3]PEC = general probable-effect concentration (MacDonald et al. 2000)
[4]PEC_{10} = Tri-State Mining District-specific low-risk threshold associated with a 10% reduction in a measured endpoint (MacDonald et al. 2010)
[5]PEC_{20} = Tri-State Mining District-specific high-risk threshold associated with a 20% reduction in a measured endpoint (MacDonald et al. 2010)

Table 13. Mean metals concentrations (±1 standard error) in surface waters. Also shown are the results of one-way analysis-of-variation (ANOVA) as *F*-values, coefficients of determination (R^2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05). Censored values (< method detection limit; MDL) were replaced with 50% of the MDL for statistical computations.

Site/ type	n	Metals concentrations (µg/L) Lead		Zinc		Cadmium		Nickel		Copper	
J1/R	3	0.02 (0.01)	j	1.79 (0.45)	n	0.02 (0.01)	ij	0.26 (0.05)	g	0.47 (0.04)	fg
C1/R	5	0.01 (0.00)	j	1.05 (0.10)	n	0.01 (0.00)	j	0.31 (0.03)	g	0.33 (0.01)	h
C2/M	3	0.24 (0.02)	f	421 (5)	a	1.74 (0.03)	a	1.81 (0.04)	ab	0.49 (0.03)	fg
C3/M	5	0.74 (0.01)	c	255 (2)	d	0.50 (0.01)	c	1.65 (0.04)	bc	0.61 (0.02)	c
C4/M	3	0.46 (0.01)	e	261 (3)	c	1.06 (0.01)	b	1.54 (0.03)	cd	0.52 (0.01)	ef
C5/D	3	0.54 (0.00)	d	92.8 (2.3)	g	0.20 (0.01)	f	0.92 (0.02)	d	0.60 (0.01)	c
T1/R	3	0.43 (0.02)	e	156 (6)	f	0.31 (0.02)	e	0.99 (0.04)	d	0.47 (0.00)	fg
T2/R	3	0.30 (0.01)	f	67.0 (1.7)	h	0.42 (0.05)	d	0.65 (0.03)	e	0.62 (0.04)	c
T3/M	3	1.63 (0.05)	a	344 (17)	b	1.71 (0.06)	a	1.86 (0.09)	ab	0.74 (0.02)	ab
T4/D	3	0.81 (0.04)	b	230 (8)	e	1.33 (0.06)	b	2.13 (0.16)	a	1.88 (0.03)	a
S1/R	3	0.17 (0.00)	g	22.2 (0.5)	l	0.09 (0.00)	h	0.40 (0.06)	g	0.45 (0.03)	gh
S2/R	3	0.09 (0.00)	i	10.9 (2.1)	m	0.02 (0.01)	i	0.53 (0.02)	f	0.51 (0.02)	ef
S3/M	3	0.11 (0.00)	i	35.0 (0.1)	i	0.14 (0.00)	f	0.59 (0.05)	ef	0.54 (0.01)	de
S4/D	5	0.13 (0.00)	h	24.0 (0.3)	k	0.11 (0.00)	g	0.60 (0.03)	ef	0.65 (0.01)	bc
S5/D	3	0.10 (0.01)	i	29.2 (0.2)	j	0.07 (0.01)	h	0.58 (0.03)	ef	0.56 (0.00)	d
S6/M	3	0.17 (0.00)	g	91.4 (3.9)	g	0.29 (0.02)	e	0.63 (0.07)	ef	0.56 (0.01)	d
ANOVA											
$F_{(15,32)}$		268**		294**		217**		42.8**		37.4**	
R^2		0.99		0.99		0.99		0.94		0.94	
Missouri standard		5–7[1]		193–223[1]		0.4–0.5[1]		94–113[1]		13–16[1]	
Federal criteria		5–9[2]		93–145[2]		0.4–0.6[2]		215–337[2]		16–26[2];18[3]	

[1]Missouri Department of Natural Resources (2009), Chapter 7, http://www.sos.mo.gov/adrules/csr/current/10csr/10c20-7A-G.pdf warm-water fisheries
[2]U.S. Environmental Protection Agency hardness-based criteria (USEPA 2006)
[3]Estimated biotic-ligand model (BLM) value for copper; (USEPA 2007b, Appendix G)

Table 14. Mean densities, no-effect hazard concentrations (NEHC) of metals, and hazard quotients of *Orconectes neglectus neglectus* for receptor wildlife species. Values in bold exceed 1.0, indicating risk. Site-mean concentrations of metals are expressed as dry weight.

			Zinc			Cadmium			Lead		
Site/ type	Density (#/m^2)	Species	Mean conc. (µg/g)	NEHC[1]	Hazard quotient[2]	Mean conc. (µg/g)	NEHC	Hazard quotient	Mean conc. (µg/g)	NEHC	Hazard quotient
J1/R	28.5	Robin[3]	80.2	217	0.37	0.66	4.8	0.14	0.31	5.4	0.06
		Heron[4]	80.2	1836	0.04	0.66	40.8	0.02	0.31	45.3	0.01
		Shrew[5]	80.2	608	0.13	0.66	6.2	0.11	0.31	37.9	0.01
		Mink[6]	80.2	2693	0.03	0.66	27.5	0.02	0.31	168	0.00
C1/R	4.1	Robin	74.4	217	0.34	0.19	4.8	0.04	0.48	5.4	0.09
		Heron	74.4	1836	0.04	0.19	40.8	0	0.48	45.3	0.01
		Shrew	74.4	608	0.12	0.19	6.2	0.03	0.48	37.9	0.01
		Mink	74.4	2693	0.03	0.19	27.5	0.01	0.48	168	0.00
C2/M	5.9	Robin	356	217	**1.64**	8.67	4.8	**1.79**	6.79	5.4	**1.27**
		Heron	356	1836	0.19	8.67	40.8	0.21	6.79	45.3	0.15
		Shrew	356	608	0.59	8.67	6.2	**1.40**	6.79	37.9	0.18
		Mink	356	2693	0.13	8.67	27.5	0.32	6.79	168	0.04
C3/M	8.9	Robin	339	217	**1.56**	4.32	4.8	0.89	14.6	5.4	**2.72**
		Heron	339	1836	0.18	4.32	40.8	0.11	14.6	45.3	0.32
		Shrew	339	608	0.56	4.32	6.2	0.70	14.6	37.9	0.39
		Mink	339	2693	0.13	4.32	27.5	0.16	14.6	168	0.09
C4/M	9.4	Robin	399	217	**1.84**	7.29	4.8	**1.51**	9.33	5.4	**1.74**
		Heron	399	1836	0.22	7.29	40.8	0.18	9.33	45.3	0.21
		Shrew	399	608	0.66	7.29	6.2	**1.17**	9.33	37.9	0.25
		Mink	399	2693	0.15	7.29	27.5	0.27	9.33	168	0.06
C5/D	3.8	Robin	417	217	**1.92**	4.04	4.8	0.84	15.7	5.4	**2.93**
		Heron	417	1836	0.23	4.04	40.8	0.10	15.7	45.3	0.35
		Shrew	417	608	0.69	4.04	6.2	0.65	15.7	37.9	0.41
		Mink	417	2693	0.15	4.04	27.5	0.15	15.7	168	0.09

Table 14. Mean densities, no-effect concentrations, and hazard quotients (continued).

			Zinc			Cadmium			Lead		
Site/ type	Density ($\#/m^2$)	Species	Mean conc. (µg/g)	NEHC	Hazard quotient	Mean conc. (µg/g)	NEHC	Hazard quotient	Mean conc. (µg/g)	NEHC	Hazard quotient
T1/R	34.2	Robin	241	217	**1.11**	1.8	4.8	0.37	4.38	5.4	0.82
		Heron	241	1836	0.13	1.8	40.8	0.04	4.38	45.3	0.10
		Shrew	241	608	0.40	1.8	6.2	0.29	4.38	37.9	0.12
		Mink	241	2693	0.09	1.8	27.5	0.07	4.38	168	0.03
T2/R	29.0	Robin	220	217	**1.01**	2.97	4.8	0.61	7.74	5.4	**1.44**
		Heron	220	1836	0.12	2.97	40.8	0.07	7.74	45.3	0.17
		Shrew	220	608	0.36	2.97	6.2	0.48	7.74	37.9	0.20
		Mink	220	2693	0.08	2.97	27.5	0.11	7.74	168	0.05
T3/M	2.3	Robin	500	217	**2.30**	10.1	4.8	**2.09**	20.8	5.4	**3.88**
		Heron	500	1836	0.27	10.1	40.8	0.25	20.8	45.3	0.46
		Shrew	500	608	0.82	10.1	6.2	**1.63**	20.8	37.9	0.55
		Mink	500	2693	0.19	10.1	27.5	0.37	20.8	168	0.12
T4/D	0.7	Robin	346	217	**1.59**	8.58	4.8	**1.77**	19.7	5.4	**3.67**
		Heron	346	1836	0.19	8.58	40.8	0.21	19.7	45.3	0.44
		Shrew	346	608	0.57	8.58	6.2	**1.38**	19.7	37.9	0.52
		Mink	346	2693	0.13	8.58	27.5	0.31	19.7	168	0.12
S1/R	3.4	Robin	181	217	0.83	1.27	4.8	0.26	5.46	5.4	**1.02**
		Heron	181	1836	0.10	1.27	40.8	0.03	5.46	45.3	0.12
		Shrew	181	608	0.30	1.27	6.2	0.20	5.46	37.9	0.14
		Mink	181	2693	0.07	1.27	27.5	0.05	5.46	168	0.03
S2/R	2.2	Robin	157	217	0.72	0.4	4.8	0.08	3.54	5.4	0.66
		Heron	157	1836	0.09	0.4	40.8	0.01	3.54	45.3	0.08
		Shrew	157	608	0.26	0.4	6.2	0.06	3.54	37.9	0.09
		Mink	157	2693	0.06	0.4	27.5	0.01	3.54	168	0.02
S3/M	1.2	Robin	204	217	0.94	1.99	4.8	0.41	4.67	5.4	0.87
		Heron	204	1836	0.11	1.99	40.8	0.05	4.67	45.3	0.10
		Shrew	204	608	0.34	1.99	6.2	0.32	4.67	37.9	0.12
		Mink	204	2693	0.08	1.99	27.5	0.07	4.67	168	0.03
S4/D	1.3	Robin	160	217	0.74	1.49	4.8	0.31	2.72	5.4	0.51
		Heron	160	1836	0.09	1.49	40.8	0.04	2.72	45.3	0.06
		Shrew	160	608	0.26	1.49	6.2	0.24	2.72	37.9	0.07
		Mink	160	2693	0.06	1.49	27.5	0.05	2.72	168	0.02
S5/D	1.0	Robin	166	217	0.76	1.14	4.8	0.24	3.96	5.4	0.74
		Heron	166	1836	0.09	1.14	40.8	0.03	3.96	45.3	0.09
		Shrew	166	608	0.27	1.14	6.2	0.18	3.96	37.9	0.10
		Mink	166	2693	0.06	1.14	27.5	0.04	3.96	168	0.02
S6/M	1.1	Robin	235	217	**1.08**	2.16	4.8	0.45	4.46	5.4	0.83
		Heron	235	1836	0.13	2.16	40.8	0.05	4.46	45.3	0.10
		Shrew	235	608	0.39	2.16	6.2	0.35	4.46	37.9	0.12
		Mink	235	2693	0.09	2.16	27.5	0.08	4.46	168	0.03

Table 14. Mean densities, no-effect concentrations, and hazard quotients (continued).

[1]NEHC = No adverse-effect level-based toxicity reference value (TRV)/estimated daily food ingestion (DI); TRVs for birds, Cd =1.47, Pb =1.63, Zn =66.1; for mammals, Cd =0.77, Pb =4.7, Zn =75.4 (Table 5, Schmitt et al. 2008 and references therein)
[2]Hazard Quotient (HQ) = metal concentration in crayfish (dry weight)/NEHC; all assuming a diet of 100 percent crayfish
[3]American robin, *Turdus migratorius;* DI =1.52 kg/kg/d (USEPA 1993)
[4]Great blue heron, *Ardea herodias*; DI =0.18 kg/kg/d (USEPA 1993)
[5]Short-tailed shrew, *Blarina brevicauda*; DI =0.62 kg/kg/d (USEPA 1993)
[6]American mink, *Mustela vison*; DI =0.14 (USEPA 1993)

Table 15. Mean densities, no-effect hazard concentrations (NEHC) of metals, and hazard quotients of *Orconectes macrus* for receptor wildlife species. Values in bold exceed 1.0, indicating risk. Site-mean concentrations are expressed as dry weight.

			Zinc			Cadmium			Lead		
Site/ type	Density (#/m^2)	Species	Mean conc. (µg/g)	NEHC[1]	Hazard quotient[2]	Mean conc. (µg/g)	NEHC	Hazard quotient	Mean conc. (µg/g)	NEHC	Hazard quotient
J1/R	5.3	Robin[3]	81.1	217	0.37	1.11	4.8	0.23	0.38	5.4	0.07
		Heron[4]	81.1	1836	0.04	1.11	40.8	0.03	0.38	45.3	0.01
		Shrew[5]	81.1	608	0.13	1.11	6.2	0.18	0.38	37.9	0.01
		Mink[6]	81.1	2693	0.03	1.11	27.5	0.04	0.38	168	0.00
C1/R	13.0	Robin	104	217	0.48	0.34	4.8	0.07	0.72	5.4	0.13
		Heron	104	1836	0.06	0.34	40.8	0.01	0.72	45.3	0.02
		Shrew	104	608	0.17	0.34	6.2	0.05	0.72	37.9	0.02
		Mink	104	2693	0.04	0.34	27.5	0.01	0.72	168	0.00
S1/R	2.3	Robin	249	217	**1.15**	1.81	4.8	0.37	10.4	5.4	**1.94**
		Heron	249	1836	0.14	1.81	40.8	0.04	10.4	45.3	0.23
		Shrew	249	608	0.41	1.81	6.2	0.29	10.4	37.9	0.27
		Mink	249	2693	0.09	1.81	27.5	0.07	10.4	168	0.06
S2/R	0.8	Robin	181	217	0.83	0.39	4.8	0.08	6.08	5.4	**1.13**
		Heron	181	1836	0.10	0.39	40.8	0.01	6.08	45.3	0.13
		Shrew	181	608	0.30	0.39	6.2	0.06	6.08	37.9	0.16
		Mink	181	2693	0.07	0.39	27.5	0.01	6.08	168	0.04

[1]NEHC = No adverse-effect level-based toxicity reference value (TRV)/estimated daily food ingestion (DI); TRVs for birds, Cd =1.47, Pb =1.63, Zn =66.1; for mammals, Cd =0.77, Pb =4.7, Zn =75.4 (Table 5, Schmitt et al. 2008 and references therein)
[2]Hazard Quotient (HQ) = metal concentration in crayfish (dry weight)/NEHC; all assuming a diet of 100 percent crayfish
[3]American robin, *Turdus migratorius;* DI =1.52 kg/kg/d (USEPA 1993)
[4]Great blue heron, *Ardea herodias*; DI =0.18 kg/kg/d (USEPA 1993)
[5]Short-tailed shrew, *Blarina brevicauda*; DI =0.62 kg/kg/d (USEPA 1993)
[6]American mink, *Mustela vison*; DI =0.14 (USEPA 1993)

Table 16. No-effect hazard concentrations (NEHC) of metals and hazard quotient of *Orconectes neglectus neglectus* for receptor wildlife species using the minimum and maximum concentrations measured in O. n. neglectus collected from the study sites. Values in bold exceed 1.0, indicating risk.

Species	NEHC[1]	*Orconectes neglectus neglectus* Min. conc.	*Orconectes neglectus neglectus* Max. conc.	Hazard quotient[2] Min. conc.	Hazard quotient[2] Max. conc.
Zinc					
Robin[3]	217	74.4	500	0.34	**2.30**
Heron[4]	1836	74.4	500	0.04	0.27
Shrew[5]	608	74.4	500	0.12	0.82
Mink[6]	2693	74.4	500	0.03	0.19
Cadmium					
Robin	4.8	0.19	10.1	0.20	**2.09**
Heron	40.8	0.19	10.1	0.00	0.25
Shrew	6.2	0.19	10.1	0.03	**1.63**
Mink	27.5	0.19	10.1	0.01	0.37
Lead					
Robin	5.4	0.31	20.8	0.06	**3.88**
Heron	45.3	0.31	20.8	0.01	0.46
Shrew	37.9	0.31	20.8	0.01	0.55
Mink	168	0.31	20.8	0.00	0.12

[1]NEHC = No adverse-effect level-based toxicity reference value (TRV)/estimated daily food ingestion (DI); TRVs for birds, Cd =1.47, Pb =1.63, Zn =66.1; for mammals, Cd =0.77, Pb =4.7, Zn =75.4 (Table 5, Schmitt et al. 2008 and references therein)
[2]Hazard Quotient (HQ) = minimum and maximum concentration in crayfish (dry weight)/NEHC; all assuming a diet of 100 percent crayfish
[3]American robin, *Turdus migratorius;* DI =1.52 kg/kg/d (USEPA 1993)
[4]Great blue heron, *Ardea herodias*; DI =0.18 kg/kg/d (USEPA 1993)
[5]Short-tailed shrew, *Blarina brevicauda*; DI =0.62 kg/kg/d (USEPA 1993)
[6]American mink, *Mustela vison*; DI =0.14 (USEPA 1993)

Table 17. Hazard quotient for crayfish using maximum concentrations collected in the mainstem (2001–2002) and mainstem and tributaries of the Spring River in the Tri-State Mining District (TSMD); in the Old Lead Belt Mining District (OLBMD), and in the Virburnum Trend Mining District (VTMD) for receptor wildlife species. Values in boldface exceed 1.0 indicating risk.

	Hazard quotient[1]						
Species	***Orconectes neglectus neglectus* (TSMD, current study)**	***Orconectes macrus* (TSMD, current study)**	***Orconectes* spp. (TSMD, 2001-2002)[2]**	***Orconectes* spp. (TSMD, pre-2001)[2]**	***Orconectes* spp. (OLBMD, 1982)[2]**	***Orconectes luteus* (OLBMD, 2008)[3]**	***Orconectes hylas* (VTMD, 2004)[4]**
				Zinc			
Robin[5]	**2.30**	**1.13**	**6.60**	**12.5**	**3.80**	**1.51**	0.74
Heron[6]	0.27	0.14	0.80	**1.50**	0.40	0.18	0.09
Shrew[7]	0.82	0.41	0.20	0.50	0.10	0.54	0.26
Mink[8]	0.19	0.09	0.10	0.20	<0.10	0.12	0.06
				Cadmium			
Robin	**2.09**	0.37	0.40	**1.10**	0.90	**4.09**	0.41
Heron	0.25	0.04	<0.10	0.10	0.10	0.48	0.05
Shrew	**1.63**	0.29	0.30	0.90	0.30	**3.19**	0.32
Mink	0.37	0.07	0.10	0.30	0.10	0.72	0.07
				Lead			
Robin	**3.88**	**1.94**	0.90	**4.80**	**36.0**	**25.0**	**3.36**
Heron	0.46	0.23	0.10	0.60	**4.30**	**2.96**	0.40
Shrew	0.55	0.27	0.10	0.70	**5.10**	**3.54**	0.47
Mink	0.12	0.06	0.10	0.20	**1.80**	0.80	0.11

[1]Hazard Quotient; see Table 13 for definitions
[2]Schmitt et al. (2006), Table 8; converted to dw by using no adverse-effect level-based toxicity reference values (TRV) from Schmitt et al. (2008)
[3]Concentrations (µg/g dry weight) from Allert et al. (2010), Table 12 (Table 26 values corrected; correspondence to USFWS, July 2011)
[4]Concentrations (µg/g dry weight) from Allert et al. (2008), Table 6
[5]American robin, *Turdus migratorius;* DI =1.52 kg/kg/d (USEPA 1993)
[6]Great blue heron, *Ardea herodias*; DI =0.18 kg/kg/d (USEPA 1993)
[7]Short-tailed shrew, *Blarina brevicauda*; DI =0.62 kg/kg/d (USEPA 1993)
[8]American mink, *Mustela vison*; DI =0.14 (USEPA 1993)

Table 18. Mean (±1 standard error) probable-effects concentrations quotients (PEQs) for metals and sum of PEQs (∑PEQs) in <250-µm fraction of sediment for each sampling site. Also shown are the results of one-way analysis-of-variation (ANOVA) as *F*-values, coefficients of determination (R^2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05). Values in boldface exceed either low-risk (6.47) or high-risk (10.0) toxicity threshold $\sum PEQ_{Pb,Zn,Cd}$, indicating risk (MacDonald et al. 2010).

		Probable-effects concentrations quotients (PEQs)										
Site/ type	**n**	**Lead**		**Zinc**		**Cadmium**		**Nickel**		**Copper**		**∑PEQs**
J1/R	3	0.56 (0.22)	g	0.53 (0.11)	f	0.40 (0.08)	g	0.71 (0.04)	abcd	0.11 (0.01)	g	2.3 (0.4) f
C1/R	3	0.24 (0.00)	g	0.70 (0.03)	f	0.57 (0.02)	fg	0.52 (0.04)	cdef	0.09 (0.01)	g	2.1 (0.1) f
C2/M	3	3.25 (0.39)	cd	14.3 (0.4)	b	8.66 (0.64)	bc	0.52 (0.06)	cdef	0.12 (0.01)	efg	**27 (1)** b
C3/M	3	6.26 (0.74)	b	22.2 (2.1)	a	13.7 (0.9)	a	0.73 (0.03)	abc	0.16 (0.01)	abcd	**43 (4)** a
C4/M	3	9.02 (3.80)	ab	27.6 (9.7)	a	17.6 (5.6)	a	0.74 (0.15)	abcd	0.22 (0.05)	abc	**55 (19)** a
C5/D	3	4.85 (1.00)	bc	15.0 (0.7)	b	8.44 (0.58)	bc	0.76 (0.18)	abcd	0.13 (0.01)	cdef	**29 (2)** b
T1/R	3	1.05 (0.02)	fg	4.82 (0.19)	e	2.86 (0.16)	e	0.61 (0.16)	cdef	0.08 (0.02)	g	**9.4 (0.1)** e
T2/R	3	6.07 (2.21)	bc	11.3 (1.1)	bc	9.16 (1.02)	b	0.62 (0.04)	bcde	0.18 (0.01)	abc	**27 (4)** b
T3/M	3	20.7 (5.1)	a	35.9 (8.7)	a	20.0 (4.2)	a	1.37 (0.23)	a	0.42 (0.03)	a	**78 (12)** a
T4/D	3	9.07 (1.00)	ab	22.1 (1.1)	a	9.63 (0.67)	b	0.88 (0.06)	ab	0.27 (0.03)	ab	**42 (1)** a
S1/R	3	1.41 (0.19)	ef	5.25 (0.37)	de	2.17 (0.22)	ef	0.34 (0.01)	f	0.09 (0.00)	g	**9.3 (0.6)** e
S2/R	3	1.68 (0.31)	e	4.78 (0.81)	de	1.65 (0.15)	fg	0.47 (0.08)	def	0.09 (0.01)	g	**8.7 (1.3)** e
S3/M	3	3.29 (1.17)	cd	11.7 (3.9)	b	6.12 (2.03)	cd	0.67 (0.08)	abcd	0.16 (0.03)	bcde	**22 (7)** bc
S4/D	3	1.37 (0.02)	ef	4.82 (0.41)	e	3.11 (0.56)	e	0.45 (0.03)	ef	0.12 (0.01)	efg	**9.9 (0.9)** e
S5/D	3	1.39 (0.04)	ef	5.10 (0.05)	de	4.46 (0.15)	d	0.58 (0.06)	bcde	0.18 (0.04)	bcde	**12 (0)** d
S6/M	3	1.81 (0.08)	de	5.72 (0.12)	cd	4.46 (0.32)	d	0.56 (0.06)	cdef	0.13 (0.01)	def	**13 (0)** cd
ANOVA												
$F_{(15,32)}$		25.7*		31.8**		42.2**		4.08*		10.4**		45.3*
R^2		0.92		0.94		0.95		0.66		0.83		0.96

Table 19. Mean (±1 standard error) chronic toxic-unit scores and sum of scores (∑TUs) for surface waters; sites with scores <1.0 are predicted to non-toxic. Also shown are the results of one-way analysis-of-variance (ANOVA) as *F*-values, coefficients of determination (R^2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05). Chronic toxic-unit scores are based on USEPA (2006) hardness-based criteria.

Site/ type	**n**	**Chronic toxicity-unit scores**											
		Lead		**Zinc**		**Cadmium**		**Nickel**		**Copper**		**∑TUs**	
J1/R	3	0.000 (0.000)	j	0.01 (0.00)	l	0.000 (0.000)	i	0.002 (0.000)	ij	0.004 (0.000)	cde	0.02 (0.00)	m
C1/R	3	0.000 (0.000)	j	0.01 (0.00)	l	0.000 (0.000)	j	0.002 (0.000)	j	0.002 (0.000)	k	0.01 (0.00)	n
C2/M	3	0.002 (0.000)	fg	2.07 (0.02)	a	0.010 (0.000)	a	0.009 (0.000)	ab	0.002 (0.000)	jk	**2.10 (0.00)**	a
C3/M	3	0.005 (0.000)	bc	1.19 (0.01)	c	0.003 (0.000)	d	0.008 (0.000)	bc	0.003 (0.000)	ghi	**1.21 (0.01)**	c
C4/M	3	0.003 (0.000)	d	1.37 (0.01)	b	0.006 (0.000)	c	0.008 (0.000)	bc	0.003 (0.000)	hij	**1.38 (0.01)**	b
C5/D	3	0.004 (0.000)	c	0.52 (0.02)	e	0.001 (0.000)	f	0.005 (0.000)	de	0.003 (0.000)	def	0.54 (0.02)	f
T1/R	3	0.003 (0.000)	d	0.78 (0.04)	d	0.001 (0.000)	e	0.005 (0.000)	ef	0.002 (0.000)	jk	0.80 (0.04)	d
T2/R	3	0.002 (0.000)	e	0.34 (0.00)	f	0.003 (0.000)	d	0.003 (0.000)	hi	0.003 (0.000)	efg	0.35 (0.01)	g
T3/M	3	0.009 (0.000)	a	1.21 (0.04)	c	0.007 (0.000)	bc	0.006 (0.000)	cd	0.003 (0.000)	ijk	**1.23 (0.04)**	c
T4/D	3	0.007 (0.000)	ab	1.34 (0.04)	b	0.009 (0.000)	ab	0.012 (0.001)	a	0.011 (0.000)	a	**1.38 (0.04)**	b
S1/R	3	0.002 (0.000)	g	0.15 (0.00)	j	0.001 (0.000)	h	0.003 (0.000)	ij	0.003 (0.000)	fgh	0.16 (0.00)	k
S2/R	3	0.001 (0.000)	i	0.08 (0.01)	k	0.000 (0.000)	i	0.004 (0.000)	gh	0.004 (0.000)	cde	0.08 (0.01)	l
S3/M	3	0.001 (0.000)	i	0.25 (0.00)	g	0.001 (0.000)	f	0.004 (0.000)	fg	0.004 (0.000)	bc	0.26 (0.00)	h
S4/D	3	0.001 (0.000)	h	0.17 (0.00)	i	0.001 (0.000)	g	0.004 (0.000)	fg	0.005 (0.000)	a	0.18 (0.00)	j
S5/D	3	0.001 (0.000)	i	0.20 (0.00)	h	0.001 (0.000)	h	0.004 (0.000)	gh	0.004 (0.000)	bcd	0.20 (0.00)	i
S6/M	3	0.002 (0.000)	f	0.68 (0.03)	d	0.002 (0.000)	d	0.005 (0.000)	ef	0.004 (0.000)	ab	0.69 (0.03)	e
ANOVA													
$F_{(15,32)}$		168**		289**		152**		42.1**		27.4**		311**	
R^2		0.99		0.99		0.99		0.95		0.93		0.99	

Table 20. Mean (±1 standard error) wetted width, substrate coarseness (as measured visually), substrate homogeneity (standard deviation of substrate coarseness), water depth, and current velocity measured along lateral transects within riffle habitats. Also shown are the results of one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05).

Site/	Wetted width (m)			Substrate coarseness			Substrate homogeneity		Water depth (cm)			Mid-water current velocity (m/sec)			Substrate current velocity (m/sec)		
type	n			n					n			n			n		
J1/R	15	12.4 (1.4)	fgh	470	2.14 (0.06)	j	1.05 (0.04)	ab	94	13.4 (0.9)	g	64	0.13 (0.02)	e	81	0.11 (0.02)	g
C1/R	15	11.4 (0.5)	ghi	446	3.13 (0.03)	cde	0.71 (0.09)	cdef	89	23.3 (1.4)	ef	82	0.54 (0.03)	bcd	89	0.27 (0.02)	cde
C2/M	14	10.9 (1.5)	hi	406	2.75 (0.03)	hi	0.67 (0.07)	def	81	30.7 (2.6)	cde	81	0.48 (0.04)	cd	81	0.23 (0.02)	ef
C3/M	13	12.4 (0.5)	efgh	395	2.98 (0.04)	fg	0.75 (0.18)	cdef	79	32.9 (2.6)	bcd	74	0.50 (0.04)	cd	76	0.24 (0.02)	de
C4/M	11	13.6 (1.1)	efg	360	2.93 (0.03)	g	0.64 (0.03)	def	72	32.8 (2.3)	bc	69	0.58 (0.04)	bcd	72	0.25 (0.02)	cde
C5/D	15	15.3 (1.1)	def	510	2.71 (0.03)	i	0.57 (0.06)	f	103	27.6 (2.0)	de	92	0.53 (0.03)	bcd	96	0.26 (0.02)	de
T1/R	15	5.8 (0.6)	j	335	2.74 (0.04)	hi	0.75 (0.16)	bcde	67	11.9 (1.0)	g	41	0.21 (0.03)	e	61	0.13 (0.01)	g
T2/R	15	11.2 (0.8)	ghi	545	3.16 (0.03)	cd	0.64 (0.10)	ef	109	8.4 (0.7)	h	41	0.22 (0.03)	e	100	0.12 (0.01)	g
T3/M	15	9.4 (0.3)	i	606	2.90 (0.05)	fg	1.09 (0.19)	a	122	15.5 (0.9)	g	97	0.20 (0.02)	e	119	0.12 (0.01)	g
T4/D	15	16.1 (1).3	cde	430	2.77 (0.04)	hi	0.85 (0.06)	abcd	86	20.4 (1.4)	f	67	0.38 (0.02)	cd	86	0.23 (0.02)	f
S1/R	15	19.3 (0.7)	bc	520	2.98 (0.05)	def	1.06 (0.25)	ab	104	33.3 (1.8)	bc	93	0.63 (0.04)	bcd	104	0.27 (0.02)	bcd
S2/R	15	20.4 (2.6)	bcd	576	3.59 (0.04)	a	0.94 (0.16)	abc	115	38.9 (1.9)	a	109	0.56 (0.03)	cd	111	0.24 (0.02)	cde
S3/M	16	26.2 (0.8)	a	621	2.60 (0.05)	hi	1.26 (0.08)	a	106	27.3 (1.4)	a	98	0.66 (0.03)	b	104	0.29 (0.02)	bc
S4/D	15	16.9 (2.4)	def	530	3.02 (0.03)	efg	0.69 (0.17)	def	123	37.5 (1.5)	cde	121	0.64 (0.03)	bc	121	0.29 (0.02)	bc
S5/D	15	21.9 (1.3)	ab	555	3.28 (0.03)	b	0.59 (0.07)	ef	112	34.7 (1.6)	a	109	0.82 (0.03)	a	112	0.37 (0.02)	a
S6/M	15	19.0 (1.3)	bcd	430	3.16 (0.03)	c	0.63 (0.04)	ef	86	30.1 (2.3)	cde	75	0.71 (0.04)	b	85	0.30 (0.02)	ab
ANOVA																	
$F_{(15,32)}$		18.7**			59.0**		6.06**			42**			20.9**			35.0**	
R^2		0.56			0.10		0.74			0.29			0.14			0.29	

Table 21. Mean substrate coarseness (as measured visually; ±1 standard error), substrate homogeneity (e.g., standard deviation of substrate coarseness), water depth, and mid-water current velocity within kick-seine quadrats. Also shown are the results of one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05).

Site/ type	**Substrate coarseness**			**Substrate homogeneity**			**Water depth (cm)**			**Mid-water current velocity (cm/m)**		
	n			n			n			n		
J1/R	120	2.86 (0.07)	ghi	3	0.77 (0.09)	ab	120	17.3 (0.6)	hi	120	0.27 (0.01)	h
C1/R	120	3.17 (0.06)	cde	3	0.65 (0.06)	abcde	120	22.4 (0.6)	f	120	0.63 (0.02)	e
C2/M	120	3.05 (0.05)	efg	3	0.53 (0.14)	e	120	23.8 (1.1)	ef	120	0.80 (0.03)	c
C3/M	120	2.88 (0.05)	hi	3	0.59 (0.04)	de	120	27.8 (1.2)	d	120	0.64 (0.02)	e
C4/M	120	2.93 (0.05)	fghi	3	0.55 (0.07)	e	120	21.8 (1.0)	f	120	0.64 (0.03)	e
C5/D	120	2.67 (0.05)	j	3	0.56 (0.02)	e	120	21.0 (0.8)	fg	120	0.65 (0.03)	e
T1/R	120	2.79 (0.06)	ij	3	0.63 (0.07)	abcde	120	13.7 (0.5)	j	120	0.32 (0.01)	h
T2/R	120	2.99 (0.05)	efgh	3	0.60 (0.08)	bcde	120	10.5 (0.3)	k	120	0.30 (0.01)	h
T3/M	120	3.16 (0.08)	def	3	0.93 (0.12)	a	120	16.3 (0.5)	i	120	0.28 (0.02)	h
T4/D	120	2.89 (0.07)	ghi	3	0.78 (0.04)	a	120	19.1 (0.8)	gh	120	0.41 (0.02)	g
S1/R	120	3.37 (0.08)	bc	3	0.83 (0.29)	a	120	23.8 (1.0)	ef	120	0.52 (0.03)	f
S2/R	120	3.68 (0.08)	a	3	0.86 (0.14)	a	120	43.0 (0.6)	a	120	0.87 (0.02)	b
S3/M	120	3.41 (0.07)	b	3	0.75 (0.06)	abc	120	39.5 (1.1)	b	120	0.82 (0.02)	bc
S4/D	120	3.27 (0.05)	bcd	3	0.58 (0.07)	cde	120	25.4 (0.8)	de	120	0.83 (0.03)	bc
S5/D	115	3.43 (0.05)	ab	3	0.53 (0.03)	e	120	33.7 (0.8)	c	120	1.07 (0.02)	a
S6/M	120	3.04 (0.07)	efg	3	0.73 (0.11)	abcd	120	28.8 (1.4)	d	120	0.72 (0.03)	d
ANOVA												
$F_{(15,32)}$		18.0**			3.47*			101**			110**	
R^2		0.12			0.62			0.44			0.46	

Table 22. Mean substrate composition (by size class in mm; ±1 standard error) of sediment grab samples taken in riffle habitats at each site. Also shown are the results of one-way analysis-of-variation (ANOVA) as F-values, coefficients of determination (R2), and degrees of freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05). '--' = not available.

Site/	**Pecrent substrate composition (by size class in mm)**											
type	**n**	**>37.9**		**19–37.9**		**9–19**		**2–9**		**n**	**<2**	
J1/R	3	40.7 (9.5)	ab	29.2 (4.9)	abc	12.7 (2.9)	bcd	14.4 (2.7)	bcd	1	4.29 --	cd
C1/R	5	27.4 (5.7)	bc	30.2 (2.4)	abc	16.3 (1.1)	abc	18.9 (2.7)	abc	2	14.5 (0.4)	a
C2/M	3	39.5 (8.5)	ab	36.8 (5.4)	ab	11.7 (3.9)	bcd	8.3 (1.5)	cd	1	8.59 --	abc
C3/M	5	10.5 (4.1)	c	34.3 (5.5)	ab	19.7 (1.9)	ab	26.9 (2.7)	ab	5	8.70 (1.64)	abcd
C4/M	3	34.0 (10.8)	abc	26.4 (5.9)	abc	15.9 (2.6)	abc	17.7 (2.5)	abc	3	5.95 (0.32)	abcd
C5/D	3	10.8 (5.4)	c	41.4 (3.9)	a	25.8 (2.7)	a	17.6 (2.6)	abc	2	5.36 (1.49)	bcd
T1/R	3	32.7 (12.3)	bc	30.3 (3.8)	abc	18.7 (4)	abc	14.0 (3.6)	bcd	2	5.55 (1.17)	abcd
T2/R	3	26.5 (12.3)	bc	33.5 (7.6)	abc	14.6 (5)	abcd	20.8 (2.8)	ab	2	5.44 (0.11)	bcd
T3/M	3	43.6 (6.0)	ab	21.0 (4.5)	bc	12.3 (1)	bcd	17.8 (3.8)	abc	2	6.98 (1.55)	abcd
T4/D	3	19.9 (8.3)	bc	23.4 (4.4)	abc	15.4 (2)	abc	31.1 (1.4)	a	3	10.2 (2.47)	abc
S1/R	3	35.6 (25.5)	bc	32.4 (12.1)	abc	15.4 (6.2)	abc	12.7 (8.9)	bcd	1	11.1 --	ab
S2/R	3	39.9 (6.7)	ab	30.7 (12.1)	abc	11.2 (5.3)	bcd	12.9 (7.3)	bcd	1	14.1 --	ab
S3/M	3	88.5 (5.7)	a	9.1 (4.4)	c	1.9 (1.1)	d	0.6 (0.4)	d	0	-- --	
S4/D	5	41.2 (5.6)	ab	29.6 (3.4)	abc	13.0 (1.8)	bcd	14.3 (2.5)	bcd	2	3.06 (0.44)	d
S5/D	3	45.3 (13.2)	ab	23.0 (7.3)	abc	10.1 (1.4)	cd	17.8 (4.7)	abc	2	5.19 (1.31)	bcd
S6/M	3	15.2 (7.7)	bc	36.3 (5.8)	ab	25.1 (6.2)	ab	19.4 (4.6)	abc	1	10.2 --	abc
ANOVA												
$F_{(15,32)}$		2.93**		1.36ns		2.54ns		2.77**			1.83ns	
R^2		0.54		0.35		0.50		0.52			0.42	

Table 23. Mean values (±1 standard error) for in-situ surface-water quality. Also shown are the results of one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05). na = not available. Values for waste-water treatment plant (WWTP) not included in ANOVA. '--' = not available.

Site/ type	N	Temperature (°C)		pH		Specific conductance (µS/cm)		Dissolved oxygen (mg/L)		Turbidity (NTU)	
J1/R	3	21.3 (0.0)	jk	7.7 (0.1)	hi	316 (0)	n	8.3 (0.2)	abc	3.6 (0.7)	i
C1/R	3	22.5 (0.0)	i	8.1 (0.0)	abc	331 (0)	m	8.8 (0.0)	ab	11.8 (0.4)	fg
C2/M	3	21.6 (0.0)	j	7.8 (0.0)	ghi	430 (0)	e	7.3 (0.1)	gh	17.5 (0.2)	ab
C3/M	3	25.1 (0.0)	d	7.8 (0.0)	ghi	447 (0)	c	6.7 (0.1)	i	15.6 (0.7)	bc
C4/M	3	26.9 (0.0)	b	7.8 (0.0)	fg	411 (0)	f	7.8 (0.2)	bcd	15.3 (0.1)	bc
C5/D	3	25.3 (0.1)	c	8.2 (0.0)	ab	391 (0)	g	8.9 (0.1)	a	9.8 (1.3)	h
T1/R	3	24.5 (0.0)	e	7.7 (0.0)	i	438 (0)	d	6.4 (0.0)	i	8.6 (1.6)	h
T2/R	3	25.3 (0.0)	c	7.9 (0.0)	ef	430 (0)	e	7.4 (0.1)	efg	1.0 (0.4)	ii
T3/M	3	24.5 (0.1)	ef	7.9 (0.0)	de	558 (0)	a	7.7 (0.1)	def	0.8 (0.4)	i
T4/D	3	27.1 (0.0)	a	7.8 (0.0)	ghi	496 (0)	b	7.0 (0.0)	hi	0.4 (0.3)	i
S1/R	3	22.8 (0.0)	g	7.9 (0.0)	ef	365 (0)	h	7.5 (0.1)	fg	13.2 (0.1)	de
S2/R	3	22.6 (0.0)	hi	8.0 (0.0)	cd	338 (0)	l	7.3 (0.1)	gh	19.8 (0.6)	a
S3/M	3	22.6 (0.0)	h	8.0 (0.0)	bcd	331 (0)	m	7.7 (0.1)	cde	14.2 (0.4)	cd
WWTP	1	23.4 --		7.5 --		800 --		7.3 --		26.3 --	
S4/D	3	27.0 (0.0)	ab	8.2 (0.0)	a	361 (0)	i	9.4 (0.1)	a	9.5 (0.1)	h
S5/D	3	24.4 (0.0)	f	7.8 (0.0)	fgh	363 (0)	i	7.1 (0.4)	gh	11.1 (0.4)	gh
S6/M	3	19.5 (0.0)	k	8.0 (0.0)	cd	345 (0)	k	7.7 (0.0)	def	12.5 (0.1)	ef
ANOVA											
$F_{(15,32)}$		169*		25.0*		507*		21.0*		65.2**	
R^2		0.99		0.92		0.99		0.91		0.97	
Standard or criterion											
		32.0[1]		6.5–9[1]		na		5[1]		5.6[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*
[2]U.S. Environmental Protection Agency (2000), based on 25th percentile, range 0–18.8 NTU

Table 24. Mean alkalinity, hardness, and sulfate concentrations (±1 standard error) in surface water. Also shown are the results of one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05). Values for waste-water treatment plant (WWTP) not included in ANOVA.

Site/	Alkalinity ($mg\ CaCO_3/L$)			Hardness ($mg\ CaCO_3/L$)			Sulfate ($mg\ SO_4/L$)		
type	**n**			**n**			**n**		
J1/R	3	132 (2)	k	3	147 (1)	k	3	2.8 (0.3)	j
C1/R	5	146 (1)	e	5	163 (2)	g	3	0.7 (0.3)	j
C2/M	3	142 (1)	gh	3	207 (1)	bc	3	64 (1)	b
C3/M	5	142 (1)	gh	5	215 (1)	ab	3	65 (1)	b
C4/M	3	144 (0)	ef	3	197 (1)	de	3	48 (2)	cd
C5/D	3	153 (1)	bc	3	187 (2)	ef	3	27 (1)	e
T1/R	3	154 (1)	abc	3	204 (3)	cd	3	45 (1)	d
T2/R	3	161 (1)	ab	3	203 (1)	cd	3	25 (2)	e
T3/M	3	164 (1)	a	3	270 (4)	a	3	100 (6)	a
T4/D	3	135 (0)	jk	3	181 (1)	f	3	57 (2)	c
S1/R	3	149 (1)	cd	3	164 (0)	g	3	5.3 (0.7)	hi
S2/R	3	141 (1)	hi	3	157 (1)	hi	3	5.2 (0.3)	i
S3/M	3	138 (1)	ij	3	153 (2)	ij	3	7.4 (0.7)	g
WWTP	3	150 (2)		3	169 (1)		2	41 (4)	
S4/D	5	144 (0)	fg	5	158 (1)	h	3	5.0 (0.2)	i
S5/D	3	146 (1)	de	3	163 (3)	g	3	5.8 (0)	gh
S6/M	3	134 (0)	k	3	149 (1)	jk	3	9.1 (0.2)	f
ANOVA									
$F_{(15,32)}$		49.0**			85.3**			105.2**	
R^2		0.95			0.97			0.98	
Standard or criterion									
		20[1]			200[2]			1000[3]	

[1]U.S. Environmental Protection Agency (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]Sulfate plus chloride; Missouri Department of Natural Resources (2009), Code of Regulations, Chapter 7, *http://www.sos.mo.gov/adrules/csr/current/10csr/10c20-7A-G.pdf*

Table 25. Mean chlorophyll a, total suspended solids, particulate organic carbon, and dissolved organic carbon (±1 standard error) concentrations in surface waters. Also shown are the results of one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees-of-freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05). Values for waste-water treatment plant (WWTP) not included in ANOVA. '--' = not available.

Site/	Chlorophyll *a* (µg C/L)			Dissolved organic carbon (mg C/L)			Particulate organic carbon (mg C/L)			Total suspended solids (mg/L)		
type	**n**			**n**			**n**			**n**		
J1/R	3	1.48 (0.10)	ef	3	0.90 (0.08)	fgh	3	0.33 (0.28)	g	3	3.47 (0.71)	fgh
C1/R	5	1.19 (0.02)	h	3	0.93 (0.09)	efgh	5	0.67 (0.27)	ef	5	10.5 (1.3)	de
C2/M	3	1.44 (0.01)	efg	3	1.18 (0.07)	abcdef	3	0.77 (0.11)	abc	4	16.4 (0.2)	ab
C3/M	5	2.88 (0.07)	a	3	1.29 (0.16)	abcd	5	0.79 (0.11)	ab	7	18.3 (0.4)	a
C4/M	3	2.20 (0.03)	d	3	0.90 (0.04)	gh	3	0.78 (0.18)	abc	3	17.8 (0.9)	a
C5/D	3	2.27 (0.02)	cd	3	1.01 (0.04)	defgh	3	0.56 (0.10)	fg	3	8.70 (0.85)	efg
T1/R	3	1.45 (0.22)	fg	3	1.45 (0.06)	ab	3	0.55 (0.52)	fg	3	8.17 (2.60)	ef
T2/R	3	0.91 (0.03)	j	3	1.09 (0.11)	bcdefg	3	0.33 (1.66)	g	3	8.60 (4.76)	cde
T3/M	3	1.48 (0.16)	efg	3	1.24 (0.12)	abcde	3	0.19 (0.23)	g	3	1.93 (0.35)	gh
T4/D	3	1.02 (0.01)	ij	3	2.52 (0.06)	a	3	0.17 (0.15)	g	3	0.97 (0.09)	h
S1/R	3	1.06 (0.03)	hi	3	0.74 (0.10)	h	3	0.66 (0.73)	def	4	13.1 (0.8)	bc
S2/R	3	0.91 (0.02)	j	3	1.06 (0.11)	cdefgh	3	0.95 (0.70)	a	4	18.8 (0.6)	a
S3/M	3	1.29 (0.03)	g	3	1.11 (0.09)	bcdefg	3	0.72 (0.51)	cde	3	13.1 (0.8)	bc
WWTP	3	8.96 (1.59)		3	10.5 (0.6)		3	133 (5)		4	19.8 (2.1)	cde
S4/D	5	2.57 (0.04)	ab	3	1.13 (0.12)	bcdefg	5	0.74 (0.79)	bcde	5	10.4 (0.1)	
S5/D	3	2.45 (0.03)	bc	3	0.89 (0.11)	fgh	3	0.75 (0.20)	bc	4	11.9 (0.7)	bcd
S6/M	3	1.51 (0.02)	e	3	1.76 (0.48)	abc	3	0.74 (0.52)	bcd	5	10.9 (0.5)	cde
ANOVA												
$F_{(15,32)}$		67.3**			4.98**			15.6**			17.3**	
R^2		0.96			0.70			0.86			0.85	
Standard or criterion												
		81[1]			--			--			8.70[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*

Table 26. Mean ammonia (NH3), nitrite (NO3-N), total nitrogen (TN), total phosphorous (TP), concentrations (±1 standard error), and TN/TP ratio in surface waters. Also shown are the results of ranked one-way analysis-of-variance (ANOVA) as F-values, coefficients of determination (R2), and degrees of freedom for differences among sites (**P ≤0.01; *0.01≤ x ≤0.05; ns ≥0.05). Site means with the same lower case letter are not significantly different for each sample type (P >0.05). Values for waste-water treatment plant (WWTP) not included in ANOVA. '--' = not available.

Site/type	n	NH_3 (mg N/L)	n	NO_3-N (mg N/L)	n	TN (mg N/L)	n	TP (µg/L)	TN/TP
J1/R	3	0.218 (0.003) a	3	3.0 (0.1) a	3	3.0 (0.1) b	3	31 (1) i	96 (1) a
C1/R	3	0.001 (0.001) f	2	2.6 (0.1) b	3	2.6 (0.1) c	3	49 (3) h	53 (2) ab
C2/M	3	0.139 (0.002) ab	2	2.0 (0.0) e	3	2.1 (0.0) f	3	65 (5) gh	33 (2) bc
C3/M	3	0.197 (0.002) a	3	1.9 (0.2) de	3	2.3 (0.1) de	2	139 (5) de	17 (0) gh
C4/M	3	0.003 (0.000) ef	3	2.0 (0.0) e	3	2.1 (0.0) fg	3	71 (1) fg	29 (0) cd
C5/D	3	0.006 (0.000) bcd	3	2.0 (0.0) e	3	2.0 (0.0) gh	3	93 (5) ef	21 (1) ef
T1/R	3	0.008 (0.001) abc	3	0.7 (0.0) f	3	0.8 (0.0) hi	3	22 (4) jk	37 (7) c
T2/R	3	0.004 (0.001) cde	3	0.4 (0.0) fg	3	0.5 (0.0) ij	3	28 (4) ij	18 (3) fg
T3/M	3	0.001 (0.001) f	3	0.3 (0.0) g	3	0.4 (0.0) j	3	15 (2) k	25 (3) de
T4/D	3	0.003 (0.001) ef	3	3.6 (0.1) a	3	4.3 (0.6) a	3	741 (50) a	6 (1) l
S1/R	3	0.004 (0.000) de	3	2.7 (0.1) ab	3	3.1 (0.0) ab	3	173 (6) c	17 (0) fg
S2/R	3	0.193 (0.001) a	2	2.1 (0.1) cd	3	2.3 (0.0) e	3	158 (9) d	15 (1) hi
S3/M	3	0.003 (0.001) def	2	2.3 (0.1) c	3	2.4 (0.0) de	3	178 (3) c	13 (0) ij
WWTP	6	0.008 (0.000)	3	12 (1)	3	26 (4)	3	3942 (179)	6.5 (1)
S4/D	3	0.004 (0.002) de	3	2.3 (0.1) c	3	2.6 (0.0) c	3	227 (11) b	11 (1) k
S5/D	3	0.005 (0.001) cde	3	2.2 (0.1) c	3	2.4 (0.1) d	3	229 (7) b	11 (0) kl
S6/M	3	0.003 (0.001) ef	3	2.9 (0.1) b	3	3.0 (0.4) b	3	233 (11) b	12 (1) jk
ANOVA									
$F_{(15,32)}$		10.3**		46.8**		68.4 **		124**	64.5**
R^2		0.83		0.96		12.0		0.98	0.98
Standard									
		0.7–2.2[1]		10[1]		0.90[2]		75[2]	--

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

Table 27. Spearman coefficients for correlation among site-mean densities of Orconectes neglectus neglectus; Orconectes macrus; or combined densities of O. n. neglectus and O. macrus and concentrations of lead (Pb), zinc (Zn), cadmium (Cd), nickel (Ni), and cooper (Cu) in surface water, <250-µm fraction of sediment, detritus, and whole crayfish. Values listed in boldface are significant (P >0.05).

		Orconectes neglectus neglectus						***Orconectes macrus***	
Metal	**Matrix**	**Pb**	**Zn**	**Cd**	**Ni**	**Cu**	**Density**	**density**	**Combined density**
Pb	Surface water	**0.92**	**0.91**	**0.89**	**0.53**	0.05	0.09	-0.80	-0.08
	<250-µm sediment	**0.93**	**0.80**	**0.86**	**0.60**	0.30	-0.13	**-1.00**	-0.29
	Detritus	**0.86**	**0.86**	**0.91**	**0.59**	0.14	-0.09	-0.60	-0.26
	Crayfish		**0.90**	**0.89**	**0.63**	0.22	-0.04	-0.60	-0.21
Zn	Surface water	**0.80**	**0.93**	**0.94**	**0.62**	0.04	0.12	-0.80	-0.07
	<250-µm sediment	**0.94**	**0.90**	**0.92**	**0.65**	0.15	-0.08	-0.60	-0.25
	Detritus	**0.79**	**0.89**	**0.90**	**0.62**	-0.02	-0.02	-0.60	-0.19
	Crayfish	**0.90**		**0.93**	**0.74**	0.06	0.09	-0.60	-0.07
Cd	Surface water	**0.81**	**0.88**	**0.96**	**0.54**	0.14	0.11	-0.80	-0.07
	<250-µm sediment	**0.89**	**0.84**	**0.91**	**0.51**	0.18	-0.03	-0.60	-0.21
	Detritus	**0.79**	**0.84**	**0.89**	**0.51**	0.04	0.01	-0.60	-0.17
	Crayfish	**0.89**	**0.93**		**0.61**	0.16	0.03	-0.40	-0.15
Ni	Surface water	**0.82**	**0.89**	**0.93**	**0.56**	0.04	0.02	-0.80	-0.15
	<250-µm sediment	**0.66**	**0.64**	**0.61**	0.38		0.09	0.60	-0.03
	Detritus	**0.53**	**0.67**	**0.74**	**0.58**	0.26	-0.38	0.20	**-0.52**
	Crayfish	**0.63**	**0.74**	**0.61**		-0.01	-0.21	-0.60	-0.31
Cu	Surface water	**0.58**	0.45	**0.57**	0.20	0.24	-0.40	-0.80	**-0.54**
	<250-µm sediment	**0.70**	**0.58**	**0.69**	0.29	0.40	-0.25	0.60	-0.39
	Detritus	**0.68**	**0.68**	**0.81**	**0.59**	0.24	-0.28	0	-0.42
	Crayfish	0.22	0.06	0.16	-0.01		-0.37	-0.60	-0.15

Table 28. Metals concentrations in crayfish (Decapoda) opportunistically collected by kick seining by Wildhaber et al. (1997). Numbers in parenthesis are concentrations in *Orconectes neglectus neglectus* from this study.

	Metals concentrations in crayfish (μg/g dry weight)[1]				
Siteno	**Lead**	**Zinc**	**Cadmium**	**Nickel**	**Copper**
C5	18.4 (15.7)	421 (417)	3.85 (4.04)	1.87 (2.06)	258 (70.9)
T4	14.9 (19.7)	429 (346)	2.94 (8.58)	1.26 (1.50)	390 (85.4)
S2[2]	0.18 (3.54)[3]	73.4 (157)	0.18 (0.40)[3]	1.19 (1.56)[3]	251 (83.0)
S6	12.4 (4.46)	267 (235)	5.92 (2.16)	2.60 (1.46)	296 (91.8)

[1]Originally reported as wet weight; dry weight values computed using moisture content reported in Wildhaber et al. (1997); determined using inductively-coupled plasma-mass spectrometry
[2]Site located upstream of S2 at Missouri Department of Conservation's Tipton Ford Access point on Shoal Creek
[3]Below detection limit; half the reported value used in calculation

Table 29. Pore-water concentrations of lead (Pb), zinc (Zn), cadmium (Cd), and nickel (Ni) and toxic-units scores (∑TUs); concentrations of Pb, Zn, Cd, and Ni in sediment; surface-water specific conductance and sulfate concentrations; and percent survival and total reproduction of *Ceriodaphnia dubia* during 7-day toxicity test. Data from Allert et al. (1997).

	Pore-water Metals concentrations (µg/L)					Sediment metals concentrations (µg/g)				Surface water		*Ceriodaphnia dubia* 7-day test	
Site/ type	**Pb[1]**	**Zn[2]**	**Cd[1]**	**Ni[2]**	**∑TUs**	**Pb[2]**	**Zn[2]**	**Cd[2]**	**Ni[2]**	**Specific conductance (µS/cm)**	**Sulfate (mg SO_4/L)**	**Percent survival**	**Reproduction[3]**
C3/M	0.8	197	0.2	4.9	37.4	454	3720	19.5	15.5	490	32	22	0
C5/M	3.1	1681	2.6	5.7	1407	2120	13800	84.1	29.1	650	32	0	0
S3/M	1.3	77	1.0	7.2	33.3	116	1160	3.81	12.1	270	2	90	25.6
S6/M	0.8	87	1.4	5.6	11.3	113	901	4.21	18.7	295	8	90[4]	29.0[4]

[1] Determined using inductively-coupled plasma-mass spectrometry
[2] Determined using inductively-coupled argon plasma emission spectroscopy
[3] Total number of young per female; control (water) treatment reproduction =24.6
[4] 50% dilution of site water