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Ann L. Allert

*United States Geological Survey, aallert@usgs.gov*

Robert J. DiStefano

*Central Regional Office and Conservation Research Center*

Christopher J. Schmitt

*United States Geological Survey, cjschmitt@usgs.gov*

James F. Fairchild

*United States Geological Survey, jfairchild@usgs.gov*

William G. Brumbaugh

*United States Geological Survey, bbrumbaugh@usgs.gov*

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# Effects of Mining-Derived Metals on Riffle-Dwelling Crayfish in Southwestern Missouri and Southeastern Kansas, USA

Ann L. Allert · Robert J. DiStefano ·  
Christopher J. Schmitt · James F. Fairchild ·  
William G. Brumbaugh

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**Abstract** Riffle-dwelling crayfish populations were sampled at 16 sites in 4 tributaries of the Spring River located within the Tri-State Mining District in southwest Missouri. Crayfish density, physical habitat quality, and water quality were examined at each site to assess the ecological effects of mining-derived metals on crayfish. Metals (lead, zinc, and cadmium) were analyzed in samples of surface water, sediment, detritus, and whole crayfish. Sites were classified *a posteriori* into reference, mining, and downstream sites primarily based on metal concentrations in the materials analyzed. Three species of crayfish (*Orconectes neglectus neglectus*, *O. macrus*, and *O. virilis*) were collected during the study; however, only *O. n. neglectus* was collected at all sites. Mean crayfish densities were significantly lower at mining sites than at reference sites. Mean concentrations of metals were significantly correlated among the materials analyzed and were significantly greater at mining and downstream sites than at reference sites. Principal component analyses showed a separation of sites due to an inverse relationship among crayfish density, metals concentrations, and physical habitat quality variables. Sediment probable-

effects quotients and surface-water toxic unit scores were significantly correlated; both indicated risk of toxicity to aquatic biota at several sites. Metals concentrations in whole crayfish at several sites exceeded concentrations known to be toxic to carnivorous wildlife. Mining-derived metals have the potential to impair ecosystem function through decreased organic matter processing and nutrient cycling in streams due to decreased crayfish densities.

The Tri-State Mining District (TSMD) occupies an area of approximately 647,500 ha in southwestern Missouri, southeastern Kansas, and northeastern Oklahoma. The TSMD was mined for zinc (Zn) and lead (Pb) for >150 years, beginning in the mid-1800s and ending in the late 1960s, with peak production occurring during World War II (Stewart 1986). Many sites in the TSMD are contaminated by wastes from historical mining, ore processing, and smelting (Barks 1977; Czarnecki 1985; Davis and Schumacher 1992), which has resulted in effects on aquatic organisms and potential risk to humans and wildlife (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). The United States Environmental Protection Agency (USEPA) has established four Superfund National Priority List (NPL) sites within the TSMD.

Crayfish are an important structural component of many aquatic systems, including Ozark streams, where they have been found to be the predominant macroinvertebrate (Rabeni 1985). Crayfish also play an integral role in stream function and the cycling of nutrients and energy through stream food webs by shredding organic matter (Creed 1994; Momot et al. 1978; Momot 1995; Rabeni et al. 1995) and as an important prey item for fish and other aquatic and terrestrial vertebrates (DiStefano 2005 and references

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A. L. Allert (✉) · C. J. Schmitt · J. F. Fairchild ·  
W. G. Brumbaugh  
Columbia Environmental Research Center,  
United States Geological Survey, 4200 New Haven Road,  
Columbia, MO 65201, USA  
e-mail: aallert@usgs.gov

R. J. DiStefano  
Missouri Department of Conservation,  
Central Regional Office and Conservation Research Center,  
3500 East Gans Road, Columbia, MO 65201, USA

therein; Hobbs 1993; Rabeni et al. 1995). Recent research has showed that crayfish significantly affect aquatic microhabitats by way of ecosystem engineering (Zhang et al. 2004). Therefore, effects on crayfish may result in changes in the structure and function of Ozark stream ecosystems.

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

## Materials and Methods

Crayfish were sampled in riffle habitats of tributaries of the Spring River of southwestern Missouri and southeastern Kansas. The 16 sample sites were located in Jenkins Creek, Center Creek, Turkey Creek, and Shoal Creek (Table 1; Fig. 1). Sites were selected based on stream order (Strahler 1952) and proximity to areas with mine waste. Sites were classified based on distances downstream from known

sources of mining-related contaminants and metal concentrations in the materials analyzed from each site. Sites considered upstream of contaminated areas where metals concentrations were low (e.g., reference sites) included J1, C1, T1, T2, S1, and S2; sites directly downstream of highly contaminated areas where metals concentrations were high (e.g., mining sites) included C2, C3, C4, T3, S3, and S6; and sites considered to be further downstream of mining or less contaminated areas where metals concentrations were intermediate (e.g., downstream sites) included C5, T4, S4, and S5.

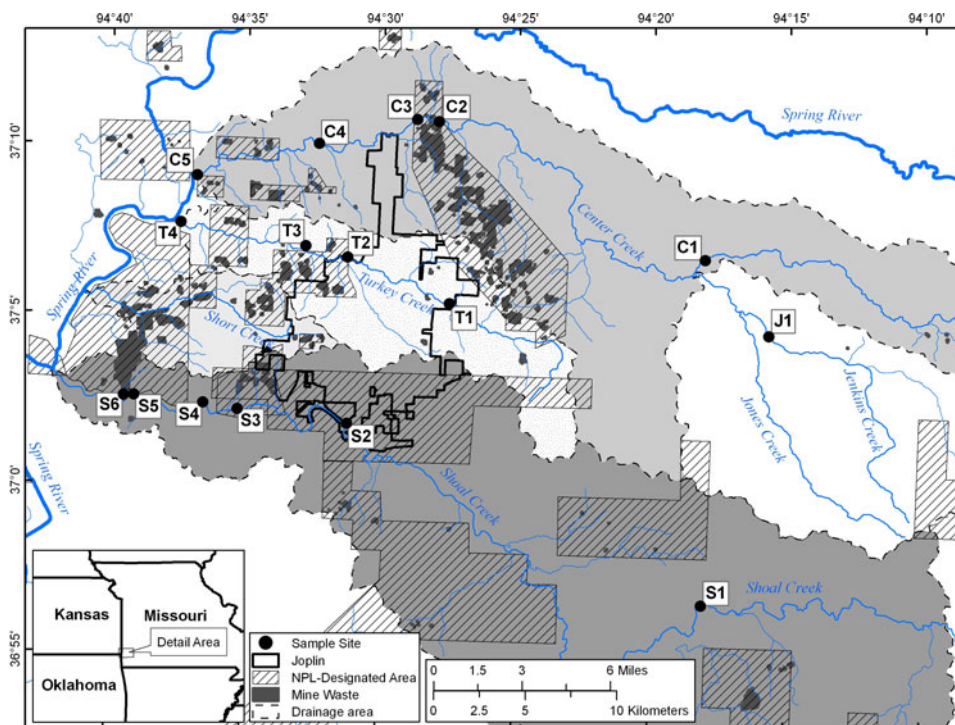
Crayfish were collected by disturbing the substrate inside a 1 m<sup>2</sup> weighted polyvinyl chloride (PVC) quadrat frame placed on the stream bottom directly upstream of a kick seine (1.5 m length × 1.5 m height) with a 3 mm diameter delta mesh on one date per site during the period of July 13 to 29, 2009 (15 sites) or 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  $n = 24/\text{site}$ ). Crayfish were identified as to species (Pfleiger 1996), and carapace length (CL) was 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 metal analyses were released alive to the stream.

**Table 1** Sampling locations in Jenkins, Center, Turkey, and Shoal creeks in southwestern Missouri and southeastern Kansas, USA

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 group
Jenkins creek									
J1	37°04'33.4"	94°15'40.1"	5.01	9,324	0.70	0.00008	5.1	3	R
Center creek									
C1	37°06'46.9"	94°18'02.9"	4.36	29,526	27.8	0.00094	15	5	R
C2	37°10'46.6"	94°27'56.2"	2.52	66,045	588	0.00891	3.4	5	M
C3	37°10'04.6"	94°32'21.3"	2.52	67,340	785	0.01166	0.6	5	M
C4	37°10'45.8"	94°28'44.7"	2.46	73,297	815	0.01112	3.9	5	M
C5	37°09'05.4"	94°36'50.7"	2.72	77,182	908	0.01176	1.0	5	D
Turkey creek									
T1	37°05'24.1"	94°27'27.9"	8.98	4,144	81.7	0.01971	2.7	2	R
T2	37°06'38.4"	94°31'17.2"	3.66	6,734	143	0.02128	4.1	3	R
T3	37°06'51.6"	94°32'44.9"	3.63	9,324	225	0.02417	1.1	4	M
T4	37°07'46.9"	94°37'35.2"	4.79	11,396	318	0.02790	4.5	4	D
Shoal creek									
S1	36°56'34.7"	94°18'03.9"	3.36	69,671	96.0	0.00138	6.8	5	R
S2	37°01'25.7"	94°31'11.0"	2.84	84,952	120	0.00142	5.1	5	R
S3	37°02'07.7"	94°35'16.5"	1.52	113,441	181	0.00160	2.6	5	M
S4	37°02'24.0"	94°36'30.1"	2.32	114,736	209	0.00182	2.4	5	D
S5	37°02'31.2"	94°39'08.0"	3.08	116,549	213	0.00183	7.1	5	D
S6	37°02'33.8"	94°39'23.6"	3.08	117,585	345	0.00294	0.5	5	M

R reference site, M mining site, D downstream site

**Fig. 1** Study sites, mine-related waste (i.e., chat piles), city of Joplin boundary, and designated areas within USEPA NPL (Superfund) sites, as well as drainages, which are distinguished by gray shades



Water depth and current velocity were measured at each 1 m<sup>2</sup> kick-seine quadrat just before crayfish sampling using a Marsh-McBirney 2000 portable flow meter (Hach Inc., Loveland, Colorado, USA) at 6/10 of measured depth (Bain and Stevenson 1999). Substrate particle size was visually estimated at five random points within a 0.5 × 0.5 m grid of each quadrat and assigned a value based on six coarseness categories (Bain et al. 1985).

A Hydrolab Quanta meter (Hach Inc., Loveland, Colorado, USA) 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 analyses of metals (Pb, Zn, and Cd), alkalinity, hardness, and sulfate concentration. Alkalinity and hardness were measured by titration (American Public Health Association 2005). Sulfate concentrations were determined by colorimetric detection using a Hach 2100 spectrophotometer (Hach Inc., Loveland, Colorado, USA). A subsample of each surface-water sample was filtered on-site into a precleaned polyethylene bottle using a polypropylene syringe and filter cartridge (0.45 μm pore size), placed on ice, refrigerated, and subsequently acidified to 1 % (v/v) with nitric acid within 4 days of collection. Water samples were analyzed for Pb, Zn, and Cd using semiquantitative multielement inductively-coupled plasma-mass spectrometry (ICP-MS).

Crayfish were subsampled from those collected during kick-seine sampling for metals analyses. Generally, five crayfish from each riffle at each site were composited for

analysis; however, samples from three sites (T4, S1, and S4) contained only three crayfish per riffle. Crayfish were rinsed with site water and placed in precleaned high-density polyethylene (HDPE) containers. Detritus (e.g., weathered leaves) was collected with a kick net and by hand on the day of crayfish sampling at the stream banks of each riffle sampled and placed in precleaned 125 mL HDPE bottles. Samples of crayfish and detritus were placed on ice, frozen (−20 °C) within 6 h of collection, and stored frozen until analyzed. Animal tissues and organic material were lyophilized (i.e., freeze-dried) and decreased 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 analyses. A dry mass of 0.25 g from each composite sample of crayfish or detritus sample was digested using concentrated nitric acid and microwave heating before being analyzed for Pb, Zn, and Cd using ICP-MS (Allert et al. 2008, 2009a; Besser et al. 2007). Surficial sediments (approximately the top 10 cm) were collected within the wetted stream channel of each riffle at each site using PVC scoops rinsed with site water before collection (Besser et al. 2009b). Sediment samples were composited and wet-sieved through a 2 mm stainless-steel mesh sieve in the field to remove coarse particles using a minimal quantity of site water (Besser et al. 2009b; Brumbaugh et al. 2007); placed in precleaned 125 mL glass bottles on ice; then refrigerated (−4 °C) until analyzed. In the laboratory, a subsample of the <2 mm sediment sample was sieved using a 250 μm stainless-steel

mesh sieve. Total recoverable (TR) metals (Pb, Zn, and Cd) in the <2 and <250  $\mu\text{m}$  sediment fraction were analyzed by ICP-MS (Brumbaugh et al. 2007; May et al. 1997).

Site-mean TR metals concentrations in the <250  $\mu\text{m}$  sediment fraction were converted to probable-effects quotients (PEQs) by dividing site-mean TR metals concentrations by TSMD-specific probable-effects concentrations (PECs; MacDonald et al. 2000, 2010) for each metal. Individual PEQs for the three metals were summed ( $\sum\text{PEQs}$ ) to estimate risks from the metal mixtures (Besser et al. 2009a; Ingersoll et al. 2001). In general,  $\sum\text{PEQs}$  greater than one are assumed to indicate a greater hazard of toxic effects (Besser et al. 2009a); however, MacDonald et al. (2010) calculated low-risk ( $\sum\text{PEQ} = 6.47$ ) and high-risk ( $\sum\text{PEQ} = 10.04$ ) toxicity thresholds for Pb, Zn, and Cd in TSMD sediments to better assess risks of toxicity in the TSMD.

The cumulative risk of toxic effects from metals in surface water was estimated using a toxic units (TU) approach (Wildhaber and Schmitt 1996). A TU is defined as the measured concentration of each dissolved metal in surface water divided by the chronic ambient water-quality criterion for the metal adjusted for hardness and the dissolved fraction of metal (USEPA 2005, 2006). TUs for metals were summed to produce a total toxicity estimate of the mixture (i.e., TU unit score =  $\sum\text{TU}$ ) for site means; values >1.0 generally indicate potential toxicity to aquatic biota.

Increased concentrations of metals in fish and crayfish within the TSMD represent a 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 to carnivorous wildlife. 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, 1999, 2007). This assessment used representative bird and mammal species based on body weight, such as the American robin (*Turdus migratorius*) and short-tailed shrew (*Blarina brevicauda*), 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 (NEHCs) to estimate daily contaminant intake rates; NEHCs are consensus-based no-adverse effect level-based toxic reference values normalized for estimated daily food ingestion rates (Schmitt et al. 2008). All assume a diet of 100 % crayfish. There is no analogous procedure to assess risk to predatory fish.

All statistical analyses were conducted using Statistical Analysis System (release 9.1; SAS, Chicago, IL) for

Windows. Before analyses, data were tested for normality and homogeneity of variance using Shapiro-Wilk statistic. Data were not normally distributed; therefore, all analyses were conducted using rank-transformed data (Conover and Iman 1981). Ranks of site means for crayfish density, water-quality variables, physical habitat quality variables, and metal concentrations were used in the statistical analyses. Censored values [less than method detection limit (MDL); Supplemental Table S1] were replaced with 50 % of the MDL for statistical computations, figures, and tables; only metal concentrations in surface water samples from reference sites were censored.

Differences among groups of sites (i.e., reference, mining, downstream) were tested using nested analysis of variance (ANOVA; riffles within sites) with site considered a fixed effect. Differences in measured variables were tested as planned nonorthogonal contrasts using single *df* *F*-tests. The within-site mean squares for ranked variables were used in all tests, which were conducted using PROC GLM. Associations between site means metal concentrations were examined using Spearman's rank correlation analyses (Supplemental Table S2) and indicated a high degree of correlation between metals. Separate linear regression of *O. n. neglectus* and *O. macrus* densities against physical and chemical habitat variables was performed using PROC REG with variable selection based on Akaike's information criterion (AIC; Burnham and Anderson 2002). In these analyses, models were evaluated relative to each other based on corrected AIC values (AICc). The AICc values are adjusted upward for sample size relative to the number of independent variables, which protects against over-fitting models due to small sample size (Burnham and Anderson 2002). The best-fit equally fit model (variables  $n = 30$ ) for *O. n. neglectus* density included five variables: current velocity; Pb and Cd in detritus; temperature and pH;  $R^2 = 0.89$ ; and AICc = 55.65. The best two equally fit models for *O. macrus* density included two variables: alkalinity and current velocity ( $R^2 = 0.99$ ; AICc = -6.68) or Pb in surface water and Zn in crayfish ( $R^2 = 0.99$ ; AICc = -5.99). The relationship among *O. n. neglectus* and *O. macrus* densities and the variables identified in the best-fit models were further examined with principal component analyses (PCA). A significance level of  $P < 0.05$  was used to judge all statistical tests. Supplemental material contains site mean summaries.

## Results

Crayfish were collected at all sites (Supplemental Table S3). Only one crayfish species, *Orconectes (Procericambarus) neglectus neglectus* Faxon (ringed crayfish), was

**Table 2** Mean densities of *O. n. neglectus*, water depth, and  $\sum$ PEQ and  $\sum$ TU for Pb, Zn, and Cd

Site group	<i>O. n. neglectus</i> density (n/m <sup>2</sup> )	$\sum$ PEQ	$\sum$ TU	Water depth (cm)
Reference <sup>a</sup>	16.9 (4.0)	9.2 (2.1)	0.23 (0.07)	21.8 (2.7)
Mining <sup>b</sup>	4.79 (1.33)	38.7 (6.3)	1.14 (0.14)	26.4 (2.4)
Downstream <sup>c</sup>	1.68 (0.41)	22.3 (4.0)	0.57 (0.15)	24.8 (1.9)
R versus M	$F_{(15,32)} = 15.0^{**}$	$F_{(15,32)} = 352^{**}$	$F_{(15,32)} = 2,191^{**}$	$F_{(15,32)} = 6.41^{**}$
R versus D	$F_{(15,32)} = 33.6^{**}$	$F_{(15,32)} = 111^{**}$	$F_{(15,32)} = 484^{**}$	$F_{(15,32)} = 4.88^*$
D versus M	$F_{(15,32)} = 5.44^*$	$F_{(15,32)} = 39.3^{**}$	$F_{(15,32)} = 395^{**}$	$F_{(15,32)} = 0.00$ ns

Data shown are arithmetic site groups means  $\pm$  SEs. Also shown are results of one-way ANOVA as  $F$  values and  $df$  for differences among site groups (\*\*  $p \leq 0.01$ ; \*  $0.01 \leq p \leq 0.05$ ; ns  $\geq 0.05$ ). Within-site mean squares for ranked variables were used in all tests

R reference site, M mining site, D downstream site

<sup>a</sup> Reference sites: J1, C1, T1, T2, S1, and S2

<sup>b</sup> Mining sites: C2, C3, C4, T3, S3, and S6

<sup>c</sup> Downstream sites: T4, C5, S4, and S5

collected at all sites. There were significant differences in mean densities of *O. n. neglectus* among site types; mean densities were significantly lower at mining and downstream sites than reference sites (Table 2). *Orconectes (Procericambarus) macrus* Williams (Neosho midget crayfish) was only collected at reference sites (J1, C1, S1, and S2). Mean densities of *O. macrus* were threefold greater than *O. n. neglectus* densities at C1; however, at the three other sites where both species were collected (J1, S1, and S2), mean densities of *O. n. neglectus* were 1.5- to 5-fold greater than *O. macrus* (Supplemental Table S3). A third species, *Orconectes (Gremicambarus) virilis* Hagen (virile crayfish), was collected only at J1 ( $n = 3$ ) and C1 ( $n = 2$ ).

Preliminary analyses showed there were no significant differences in mean CL between sexes for either *O. n. neglectus* ( $F_{(1,226)} = 2.29$ ;  $P = 0.13$ ) or *O. macrus* ( $F_{(1,54)} = 3.37$ ;  $P = 0.06$ ). However, there were significant differences in mean CL among sites ( $F_{(1,226)} = 2.29$ ;  $P = 0.13$ ) and site groups for *O. n. neglectus* collected for metal analyses: Crayfish from reference sites were significantly larger than crayfish collected at mining or downstream sites (reference vs. mining  $F_{(3,15)} = 159$ ;  $P < 0.001$ ; reference vs. downstream  $F_{(3,15)} = 123$ ;  $P < 0.001$ ; and mining vs. downstream  $F_{(3,15)} = 0$ ;  $P = 0.954$ ). *O. macrus* were only collected at reference sites; however, there were no significant differences in CLs of *O. macrus* collected for metals analyses among sites ( $F_{(3,52)} = 0.46$ ;  $P = 0.714$ ).

Mean concentrations of Pb, Zn, and Cd in *O. n. neglectus*, surface water, <250  $\mu$ m sediment fraction, and detritus were significantly correlated (Supplemental Table S2) and significantly greater at mining and downstream sites than at reference sites (Table 3; Supplemental Tables S4 through S7). Concentrations of metals in *O. macrus* were significantly greater than those in *O. n. neglectus* in

11 of the 12 comparisons (Supplemental Table S4). Mean concentrations of Pb, Zn, and Cd in the <250  $\mu$ m sediment fraction approached or exceeded TSMD-specific high-risk concentrations (PEC<sub>20</sub>; concentrations associated with a 20 % decrease in a measured end point at all site groups (Table 3; Supplemental Table S6; MacDonald et al. 2010). Mean concentrations of Pb in surface water did not exceed the chronic USEPA water-quality criterion at any site group; however, mean concentration of Zn and Cd did exceed these criteria at mining sites (Table 3).

Sediment  $\sum$ PEQs indicated risk of toxicity at mining and downstream sites (Table 2; Supplemental Table S8);  $\sum$ PEQs were greater than both the TSMD-specific low- ( $\sum$ PEQs = 6.47) and high-risk ( $\sum$ PEQs = 10.04) toxicity thresholds at mining and downstream sites (MacDonald et al. 2010). *O. n. neglectus* densities were decreased relative to reference sites where TSMD-specific  $\sum$ PEQs were exceeded (Fig. 2). Surface water  $\sum$ TUs were significantly greater at mining and downstream sites than at reference sites (Table 2; Supplemental Table S9). Surface water  $\sum$ TUs at all mining sites were <1, indicating potential risk to aquatic biota.

Criteria used to evaluate risks of Pb, Zn, and Cd concentrations in crayfish to wildlife indicated that metals concentrations at mining and downstream sites are potentially hazardous to carnivorous wildlife (Fig. 3). HQs were greater for birds than mammals in their respective size category (Supplemental Tables S10 and S11). HQs for mean concentrations of Pb and Zn in *O. n. neglectus* were >1.0 for robin-size birds at mining and downstream sites. The Cd hazard quotient was <1.0 for robin-sized birds and approached 1.0 for shrew-size mammals at mining sites (Fig. 3).

Mean water depth and current velocities were significantly greater at mining and downstream sites than at reference sites (Table 2; Supplemental Tables S12 and S13).

**Table 3** Metal concentrations in *O. n. neglectus*, detritus, <250 µm fraction of sediment, and surface water

Site group/criteria	Pb	Zn	Cd
	<i>O. n. neglectus</i> (µg/g dry weight)		
Reference <sup>a</sup>	3.65 (0.7)	159 (17)	1.22 (0.24)
Mining <sup>b</sup>	10.1 (1.7)	339 (26)	5.76 (0.77)
Downstream <sup>c</sup>	10.5 (2.6)	272 (35)	3.81 (0.94)
R versus M	$F_{(15,32)} = 47.3^{**}$	$F_{(15,32)} = 120^{**}$	$F_{(15,32)} = 531^{**}$
R versus D	$F_{(15,32)} = 22.3^{**}$	$F_{(15,32)} = 32.4^{**}$	$F_{(15,32)} = 155^{**}$
D versus M	$F_{(15,32)} = 2.05$ ns	$F_{(15,32)} = 17.0^{**}$	$F_{(15,32)} = 66.6^*$
	Detritus (µg/g dry weight)		
R	156 (36)	3,281 (663)	34 (11)
M	643 (70)	10,772 (1,438)	138 (20)
D	543 (124)	8,500 (1,261)	92 (20)
R versus M	$F_{(15,32)} = 167^{**}$	$F_{(15,32)} = 312^{**}$	$F_{(15,32)} = 129^{**}$
R versus D	$F_{(15,32)} = 71.9^{**}$	$F_{(15,32)} = 132^{**}$	$F_{(15,32)} = 41.6^{**}$
D versus M	$F_{(15,32)} = 9.59^{**}$	$F_{(15,32)} = 18.8^{**}$	$F_{(15,32)} = 13.6^{**}$
	<250 µm sediment fraction (µg/g dry weight)		
R	235 (73)	2,093 (409)	14.0 (3.7)
M	947 (231)	8,991 (1,435)	58.6 (8.7)
D	534 (128)	5,393 (1,011)	31.9 (4.2)
R versus M	$F_{(15,32)} = 155^{**}$	$F_{(15,32)} = 235^{**}$	$F_{(15,32)} = 341^{**}$
R versus D	$F_{(15,32)} = 51.2^{**}$	$F_{(15,32)} = 54.5^{**}$	$F_{(15,32)} = 93.4^{**}$
D versus M	$F_{(15,32)} = 15.9^{**}$	$F_{(15,32)} = 40.1^{**}$	$F_{(15,32)} = 47.1^{**}$
PEC <sub>20</sub> <sup>d</sup>	179	2,409	14.1
	Surface water (µg/L)		
R	0.17 (0.04)	43.2 (13.4)	0.14 (0.17)
M	0.56 (0.13)	235 (33)	0.91 (0.16)
D	0.40 (0.09)	93.9 (25.1)	0.43 (0.16)
R versus M	$F_{(15,32)} = 17.4^{**}$	$F_{(15,32)} = 8.68^{**}$	$F_{(15,32)} = 7.41^{**}$
R versus D	$F_{(15,32)} = 180^{**}$	$F_{(15,32)} = 373^{**}$	$F_{(15,32)} = 439^{**}$
D versus M	$F_{(15,32)} = 93.7^{**}$	$F_{(15,32)} = 482^{**}$	$F_{(15,32)} = 343^{**}$
WQC <sup>e</sup>	5–9	93–145	0.4–0.6

Data shown are arithmetic site groups means ± SEs. Also shown are results of one-way ANOVA as  $F$  values and  $df$  for differences among site group (\*\* $p \leq 0.001$ ; ns  $\geq 0.05$ ). Within-site mean squares for ranked variables were used in all tests

R reference site, M mining site, D downstream site

\* $0.01 \leq p \leq 0.05$

<sup>a</sup> Reference sites: J1, C1, T1, T2, S1, and S2

<sup>b</sup> Mining sites: C2, C3, C4, T3, S3, and S6

<sup>c</sup> Downstream sites: T4, C5, S4, and S5

<sup>d</sup> PEC<sub>20</sub> = TSMD probable-effects concentration high-risk threshold associated with a 20 % decrease in a measured end point (MacDonald et al. 2010)

<sup>e</sup> WQC = federal water-quality criteria adjusted for hardness and the dissolved fraction of the metal (USEPA 2006)

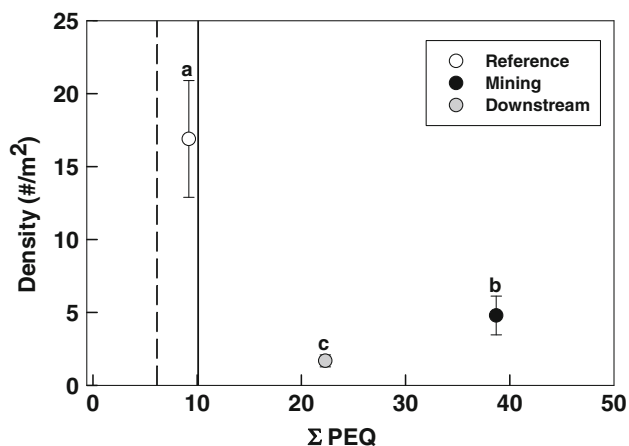
Substrate coarseness was not significantly difference among site groups (Supplemental Tables S12 and S13). In situ water-quality variables generally did not exceed state or federal criteria (Supplemental Tables S14 and S15). However, water-quality constituents associated with metal-mining, such as specific conductance and concentrations of hardness and sulfate, were significantly greater at mining than at reference sites (Supplemental Tables S16 and S17).

A series of interpretable ordinations were obtained by PCA for each species (Fig. 4). Each ordination resulted in the first two PCA axes explaining more of the variation (69–90 %) than expected by chance alone. In the ordination for the *O. n. neglectus*, variables with strong positive associations with PC1 included Pb (0.67) and Cd (0.64) in detritus. Current velocity (0.58) had a strong positive association, but density (−0.65) had a strong negative association with PC2. Reference sites were separated from all other sites with negative values for PC1, whereas

mining sites had positive values. In the ordination for *O. macrus*, density (−0.45) had a strong negative association with PC1, whereas, temperature (0.47) and alkalinity (0.41) had a strong positive association with PC1. Variables that had strong positive associations with PC2 included Pb in surface water (0.66) and Zn in the <250 µm fraction of sediment (0.47). Current velocity had a strong negative association (−0.46). These ordinations suggest that habitat variables and metals are important in explaining densities of *O. n. neglectus* and *O. macrus* among sites.

## Discussion

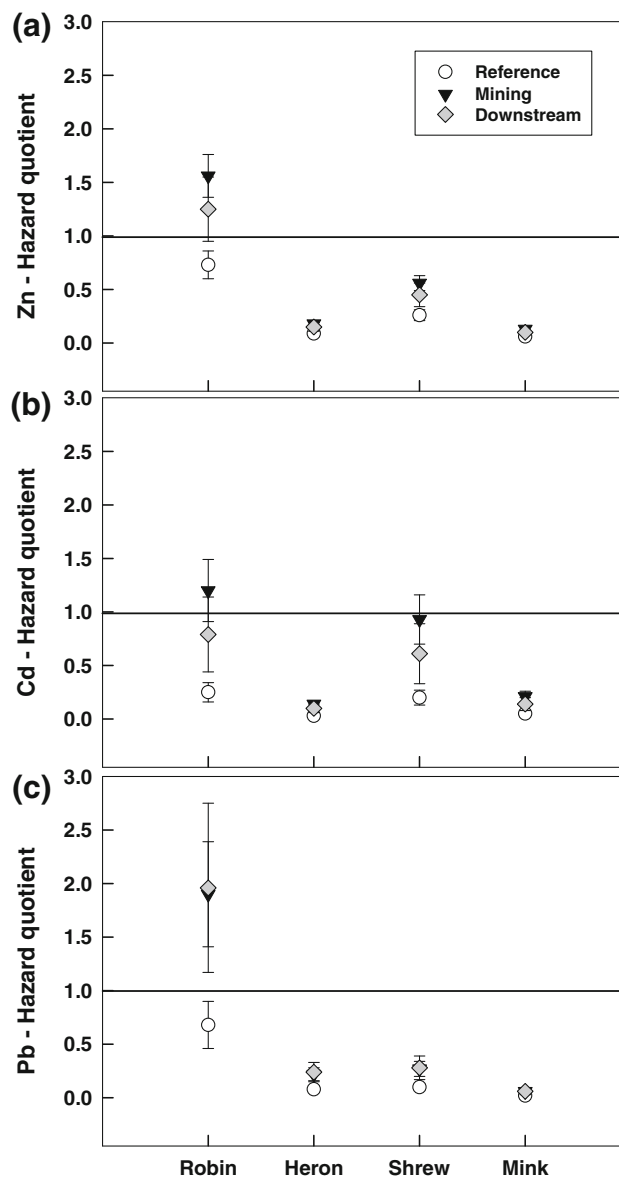
Our results indicate that environmental metals concentrations remain increased in the TSMD as indicated by high metal concentrations in surface water, sediment, detritus, and crayfish in the streams investigated. We found



**Fig. 2** Relationship between sediment  $\Sigma$ PEQs (MacDonald et al. 2010) and mean densities of *O. n. neglectus*. Lines represent TSMD low-risk (dashed line) and high-risk (solid line) toxicity thresholds (MacDonald et al. 2010)

decreased densities of *O. n. neglectus* and the absence of *O. macrus* at mining-affected sites as well as a negative association of crayfish densities with metal concentrations. Our results are consistent with previous investigations that have documented increased metal concentrations in macroinvertebrates, fish, detritus, and sediment in the TSMD (Angelo et al. 2007; Brumbaugh et al. 2005; MacDonald et al. 2010; Schmitt et al. 1993, 2006; Wildhaber et al. 1997, 2000) and that documented the absence or decreased densities of crayfish below mining-affected sites in the Viburnum Trend Mining District of southeast Missouri (Allert et al. 2008, 2009a). Allert et al. (2009a) also documented decreased survival of caged crayfish at sites directly downstream (0.4–3.7 km) of mining sites. Survival and biomass of caged crayfish were significantly lower at mining sites than reference or downstream sites, and survival was negatively correlated with metal concentrations in surface water, detritus, macroinvertebrates, stonerollers, and whole crayfish. In situ testing of crayfish was an important tool for showing that the absence of crayfish populations below mining sites was the result of metal exposure as opposed to habitat loss due to physical impairment by mine waste (e.g., sedimentation by mine tailings).

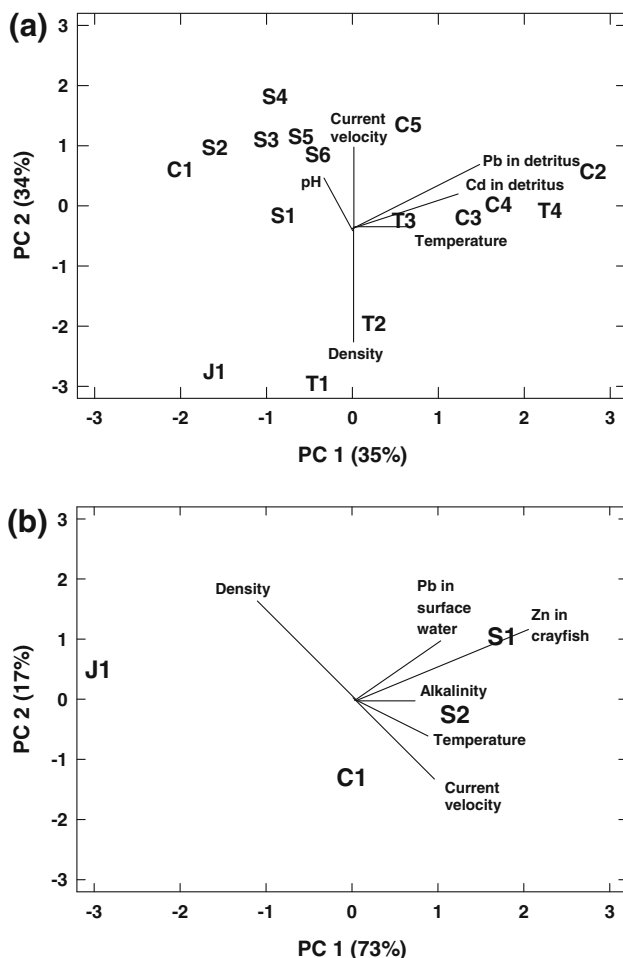
Although the diversity of crayfish in the Missouri Ozarks is high (>25 species; Pfeiffer 1996), many taxa are restricted to a single drainage and are further restricted within drainages due to macrohabitat partitioning between species (DiStefano et al. 2003; Flinders and Magoulick 2005; Rabeni 1985). *O. macrus* is endemic to the Ozark Highlands and is primarily found in the westward-flowing rivers north of the Illinois River in Oklahoma, including the Spring River. Within the Spring River drainage, *O. macrus* does not occur in the North Fork Spring River or in



**Fig. 3** HQs for dietary metal exposure for representative species for a Zn, b Cd, and c Pb in *O. n. neglectus*. Line represents risk threshold

portions of Shoal Creek (Dillman et al. 2010; Pfeiffer 1996). *O. 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 (Pfeiffer 1996). It is unclear why *O. macrus* is not found at sites in the lower reaches of Center Creek and Shoal Creek despite being present in the lower Neosho River and Spring River (Dillman et al. 2010). *O. macrus* may be more sensitive to metals than *O. n. neglectus*, or its sedentary nature within excavated tunnels may expose *O. macrus* to greater metal concentrations typical of porewater and sediment (Brumbaugh et al. 2007). Either or both mechanisms could have eliminated *O. macrus* from Center Creek and Shoal





**Fig. 4** PC ordination for selected physical habitat and water-quality variables and **a** mean densities of *O. n. neglectus* and **b** mean densities of *O. macrus*. Variables strongly associated with the axes are shown: the lengths of the lines leading from the centroid toward the labels are proportional to the strength of the correlation of that variable with the axes

Creek where metals concentrations are high (Allert et al. 1997, 2008, 2009a; Knowlton et al. 1983; Wigginton and Birge 2007).

Previous studies differed in their characterization of habitat selection by *O. neglectus*. Gore and Bryant (1990) found that *O. neglectus* partition their habitats, with young typically found in moderate-velocity (25–45 cm/s) cobble habitats and adults in low-velocity (0 cm/s) macrophyte beds or high-velocity (>65 cm/s) cobbled habitats. However, 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 in riffles measured in our study were comparable with previously reported values (Flinders and Magoulick 2005; Magoulick and DiStefano 2007; Rabalais and Magoulick

2006). Current velocities in riffles at sites in Center Creek and Shoal Creek (>0.60 cm/s) were greater than previously reported ranges for young *O. neglectus* (Gore and Bryant 1990), which may have limited densities in riffles of those creeks. Several studies (Flinders and Magoulick 2005; Rabalais and Magoulick 2006) have reported that *O. n. chaenodactylus* densities were negatively correlated with water depth in larger streams (stream order >3), possibly explaining the lower densities of *O. n. neglectus* in Shoal Creek, which was significantly deeper than the other streams sampled in our study. The inverse relation between crayfish density and stream size ( $\geq 5$  orders) or watershed size may be related to the proportional decrease in allochthonous materials in larger streams (Vannote et al. 1980), loss of habitat heterogeneity (Clark 2009; Mitchell and Smock 1991), smaller percentage of suitable habitat (Burkey and Simon 2010; DiStefano et al. 2008; Lodge and Hill 1994; Westhoff et al. 2006), or increased predation (Flinders and Magoulick 2003; Hill and Lodge 1995; Stein and Magnuson 1976).

Previous studies have used crayfish to assess the bioavailability of metals through the analysis of whole-body crayfish (Allert et al. 2008, 2009a, 2010; Besser et al. 2007; Schmitt et al. 2006, 2008). Crayfish, freshwater mussels, and benthic fishes have been identified as possible sentinel organisms in the investigation of heavy-metal pollution in the environment because of their important functional role in streams and limited home ranges (Allert et al. 2008, 2009a, 2009b, 2010; Angelo et al. 2007; Maret and MacCoy 2002). Dietary pathways have been identified as important routes of metal exposure (Besser et al. 2005; Farag et al. 1999). Therefore, the likelihood of food-chain transfer of metals from crayfish and other environmental media to wildlife in the TSMD is high and potentially hazardous.

## Conclusion

Crayfish densities were significantly decreased downstream of mine waste relative to upstream reference sites. Decreased crayfish densities imply a loss of ecosystem function because crayfish are a key structural and functional component of Ozark streams and their surrounding ecosystems. This study provides additional support for the use of crayfish in the assessment of the bioavailability and ecological effects of metals in aquatic ecosystems. However, 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 (e.g., mortality or other sublethal measurements). In situ and laboratory toxicity tests would provide additional information regarding metal exposure,

the bioaccumulation of metals, and the potential toxicity to known age classes of crayfish because it is impossible to observe temporal changes and bioaccumulation in the environment. Genomic research may provide information on the association of distribution and metal concentrations among crayfish in the TSMD.

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