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Effects of Lead-Zinc Mining on Crayfish (*Orconectes hylas*) in the Black River Watershed, Missouri, USA

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Abstract.— Mining has occurred in the New Lead Belt of southeast Missouri, one of the largest producers of lead ore in the world, since the 1960s. We studied populations of the crayfish *Orconectes hylas* at 13 sites to assess the ecological effects of mining-derived metals in the Black River watershed, which drains much of the New Lead Belt. Crayfish density, physical habitat, and water quality were examined at one reference site with no known upstream mining activities and at sites downstream of mining areas. Metals (Pb, Zn, Cd, Ni, Co) were analyzed in sediment pore water and whole crayfish. Mean crayfish densities were significantly greater ($P < 0.05$) at the reference site compared to mining and downstream sites. Crayfish densities were negatively correlated ($P < 0.05$) with sediment pore-water metal concentrations, Cd concentrations in whole crayfish, and temperature, but not with any measured physical habitat variable. These findings indicate that metals associated with current mining activities in the New Lead Belt have negative impacts on crayfish populations in Ozark streams. [*Keywords*.— crayfish; density; lead mining; metals; *Orconectes hylas*; toxicity]

INTRODUCTION

Lead (Pb) was discovered by early French explorers of the Mississippi River valley and has been mined in Missouri, USA, since the 1700s. Over the past three centuries, Pb and zinc (Zn) resources of Missouri have been heavily exploited. Previous studies (Schmitt and Finger 1982; Czarneski 1985) have documented elevated metal concentrations in water, sediment, and biota in aquatic ecosystems near mining areas in the "Old Lead Belt" located in eastern Missouri. Mining has occurred in the "New Lead Belt" since the 1960s. The mines, mills and smelters of the New Lead Belt exploit the Viburnum Trend, a geological formation containing significant Pb-Zn reserves. The mining district is located primarily in the Black River watershed of southeast Missouri, which drains portions of three Missouri counties (Crawford, Iron, and Reynolds), and is within the boundaries of the Mark Twain National Forest. Increased metal concentrations in water, sediment, detritus, invertebrates, and fish have also been documented in streams downstream of New Lead Belt mines (Gale et al. 1973; Duchrow 1983; Besser et al. 2006; Brumbaugh et al. 2007; Schmitt et al. 2007a, 2007b). More recently, exploration for Pb deposits has occurred in an area south of the New Lead Belt. The exploration area is located within the groundwater recharge area of the springs of the Ozark National Scenic Riverways, and the National Park Service has expressed concern about possible ecological effects of contamination of water resources from expanded mining in this area.

There are 26 species of crayfish in the Ozark Plateau Region of Missouri and Arkansas (Pflieger 1996). The woodland crayfish, *Orconectes hylas* (Faxon), is endemic to the Black, Meramec, and St. Francis River watersheds in Missouri (Pflieger 1996), and is the dominant crayfish species both in number and biomass in the Black River watershed. It is most commonly found in streams with silt-free gravelly substrates and low turbidity (Pflieger 1996). High densities of *O. hylas* are typical in riffle habitats, with reported densities of 25 – 37 m^{-2} (DiStefano et al. 2002).

Crayfish dominate the invertebrate biomass in Ozark streams (Rabeni et al. 1995). They are opportunistic omnivores that feed on varying proportions of fish, aquatic invertebrates, periphyton, and detritus throughout their life cycle (Hobbs 1993; Momot 1995; Whitledge and Rabeni 1997; Parkyn et al. 2001). Crayfish process a significant amount of organic matter, and facilitate particle size reduction and food availability for smaller invertebrates (Huryn and Wallace 1987; Usio 2000). Crayfish are the primary food resource of smallmouth bass *Micropterus dolomieu* Lacepède and other sport fishes in Ozark streams (Probst et al. 1984; DiStefano 2005), and smallmouth bass are a primary species sought by recreational anglers in the Ozarks (Weithman 1991; Mayers 2003). These characteristics make crayfish important to the ecology and economies of the Ozarks.

Crayfish serve as bioindicators of aquatic contamination by heavy metals (Anderson and Brower 1978; Roldan and Shivers 1987; Alikhan et al. 1990). Anderson and Brower (1978) showed that concentrations of metals in whole crayfish were inversely related to the distance from the metal source. However, the ratio of metals in whole crayfish may not reflect those in the environment because some metals (e.g., Zn) are physiologically regulated by

crayfish (Alikhan et al. 1990). Concentrations of metals such as cadmium (Cd) and Pb, which are not regulated (Dickson et al. 1979), are more likely to reflect environmental concentrations. Ecotoxicological studies of the effects of metals on crayfish are limited; however, Besser and Rabeni (1987) documented reduced survival and growth of crayfish in aquatic microcosms receiving inputs of metals from Pb-mine tailings from the Old Lead Belt.

Our study had the following objectives: 1) to characterize densities of *O. hylas* in streams of the Black River watershed; and 2) to evaluate relationships between *O. hylas* densities and (a) concentrations of mining-derived metals in sediment pore water and whole crayfish; (b) water quality; and (c) physical habitat characteristics of the study streams.

MATERIALS AND METHODS

Study Area

Crayfish samples and supporting data were collected at 13 sites in three tributaries of the Black River: Strother Creek, West Fork Black River (West Fork), and Bee Fork (Figure 1, Table 1) during August 2004. Sites were sampled once and were selected based on stream order (Strahler 1952); the absence of known upstream mining activities (reference site); the proximity to sites associated with mining activities (mining and downstream sites); and previously collected physical, chemical, and biological data (Besser et al. 2006; Brumbaugh et al. 2007). We classified one site as a reference site (WF1); three sites as mining sites (SC2, WF3, and BF4); and nine sites as downstream sites (SC3, MF2, MF3, WF2, WF4, WF5, WF6, BF5, and BF6). Site locations were documented by a hand-held global positioning system (GPS) receiver (±10 m); datum = Geodetic System (WGS) 84. We measured watershed area, tailings area, and distance to mines using ArcGIS 8.3 with the Arc Hydro extension (ESRI, Redland, CA, USA). Data layers were obtained from 30 m county Digital Elevation Models tiled from 1:24,000 U.S. Geological Survey (USGS) quadrangle sheets.

Crayfish Sampling

Densities of crayfish were determined by disturbing approximately one square meter of substrate directly upstream of a small kick seine (1 m length x 1.5 m height) with 3 mm delta mesh (Flinders and Magoulick 2005). Each site consisted of a reach containing three riffles large enough to accommodate the sampling. Five randomly located seine samples were collected within each of the three riffles (total n = 15 per site). We identified crayfish species and sex, and measured carapace length (CL, from the tip of rostrum to the posterior edge of the cephalothorax, to the nearest 0.1 mm). All crayfish, except those taken for metal analyses, were released alive to the streams.

Physical Habitat Measurements

Physical habitat variables were measured in each of the three riffles sampled for crayfish at all sites using methods of Platts et al. (1983) and Hamilton and Bergersen (1984). Depth (cm) and current velocity (m s^{-1}) were measured along transects across each riffle using a Marsh McBirney® (Frederick, MD, USA) Flow-mate 2000 current meter. Canopy cover was measured using a modified densitometer at three locations (e.g., right and left descending banks and center of stream) along the same transects (Bain and Stevenson 1999). Three 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 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 dried (60°C) for 24 h in the laboratory before weighing. Fine particulate organic matter (FPOM; < 2 mm) and coarse particulate organic matter (CPOM; > 2 mm) were determined by measuring weight loss after ignition (475°C; APHA 1992) for each substrate sample.

Water Quality Samples

We used a Hydrolab® (Loveland, CO, USA) Quanta meter to measure temperature, pH, conductivity, dissolved oxygen, and turbidity in each riffle. Surface water temperature was also measured hourly at each site from July to September using an Onset® (Pocassset, MA, USA) Temppro recorder. A surface water grab sample in each riffle was collected for additional water quality analyses (alkalinity, hardness, sulfate; APHA 1992). Recovery of reference standards for surface water quality parameters ranged from 95% to 108%, with the exception of two sulfate standards that were 77% and 84%.

Sediment Pore Water

We measured concentrations of metals in sediment pore water because of the close association of crayfish with sediment. Fine sediments were collected from stream gravel near the stream bank margins of the downstream end of riffles using a diaphragm pump equipped with an intake manifold covered with stainless steel screen (2 mm aperture) to exclude particles larger than coarse sand (Schmitt and Finger 1982; Brumbaugh et al. 2007). Sediment was transferred to the laboratory where it was homogenized; and pore water was extracted by centrifugation (USEPA 2005; Brumbaugh et al. 2007). Sediments were analyzed for both pore-water metal and total recoverable metals [Pb, Zn, Cd, nickel (Ni), and cobalt (Co); USEPA 2005; Brumbaugh et al. 2007]. Only pore-water metal concentrations are reported, because concentrations tracked closely with corresponding sediment concentrations (Brumbaugh et al. 2007) and pore-water metal concentrations can be compared with U.S. Environmental Protection Agency (USEPA) surface water quality criteria.

The risk of toxic effects from metals (Pb, Cd, Zn, Ni) in sediment pore water was estimated using the Toxic Units approach by Wildhaber and Schmitt (1996), which assumes that metal concentrations in pore water represent the bioavailable fraction of metals in sediments. A pore-water Toxic Unit (TU) is defined as the measured concentration of each dissolved metal in pore water divided by the chronic surface water quality criteria (WQC; USEPA 2002) for that metal (adjusted for hardness). There is currently no WQC for Co. Although these criteria were developed for surface water, they are reasonable estimates of the toxicity of pore waters to aquatic organisms. Toxic units for metals are summed to produce a total toxicity estimate (i.e., toxic unit score,

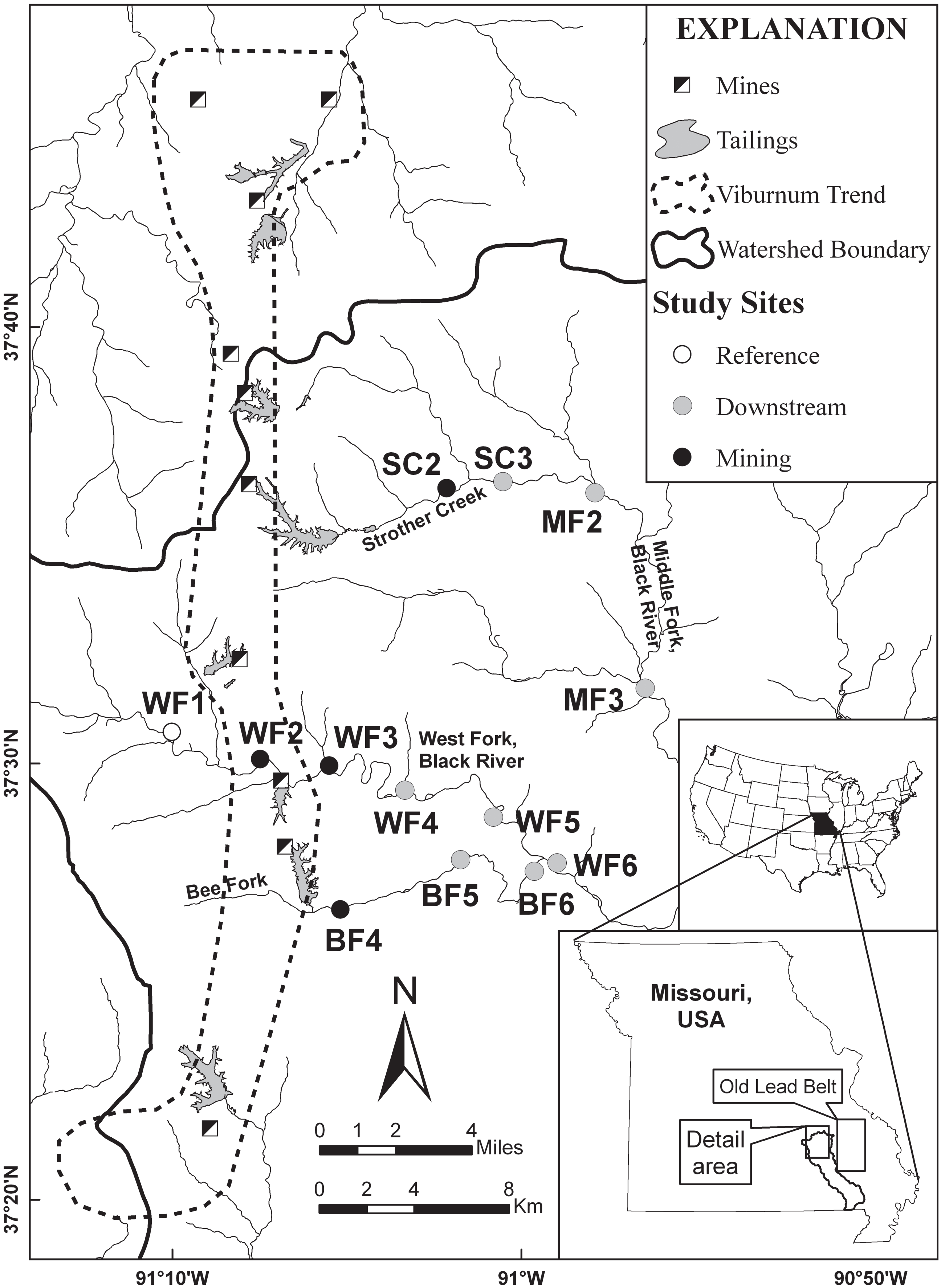

Figure 1. Map of study area in the Black River watershed of Missouri, USA.

TUs) for each sample, with values greater than 1.0 representing a risk of toxicity to sediment-dwelling biota.

Determination of Pore-water and Crayfish Metal Concentrations

Pore water was filtered using a polyethylene syringe and filter cartridge (0.45 μm pore size) into a pre-cleaned polyethylene bottle and acidified to pH < 2 with Ultrex® nitric acid (J.T. Baker Inc., Phillipsburg, NJ, USA). Pore-water samples were analyzed for metals by inductively coupled plasma-mass spectrometry (ICP-MS; May et al. 1997; USEPA 2005; Brumbaugh et al. 2007). A calibration blank and an independent calibration verification standard were analyzed every ten samples to confirm the calibration status of the ICP-MS during instrumental analyses of pore water in each of four batches. Method detection limits (MDLs) were: Pb 0.01 – 0.15 μg L^{-1}; Zn 0.82 – 2.06 μg L^{-1}; Cd 0.02 – 0.15 μg L^{-1}; Ni 0.07 – 3.00 μg L^{-1}; and Co 0.04 – 0.06 μg L^{-1}. Percent recovery of calibration verification standards ranged from 93% to 109%. Percent recovery of two reference solutions used as laboratory control samples ranged from 95% to 100%. Percent recovery of analytical spikes ranged from 92% to 100%. Relative percent differences between duplicate analyses of pore-water samples ranged from 0% to 4%.

At each site, several (n = 6 – 10) *O. hylas* collected during kick seining were placed in a pre-cleaned polyethylene bottle and frozen for metal analysis. Crayfish were analyzed for metals by ICP-MS (Brumbaugh et al. 2005; Besser et al. 2006). Whole specimens were lyophilized, followed by reduction to a coarse powder by mechanical crushing in a glass vial with a glass rod. Neither exoskeletons nor gut contents were removed before analyses. A dry mass of 0.25 g from each composited sample was digested. Quality control measures incorporated at the digestion stage of the crayfish analyses included digestion blanks, certified reference materials, replicates, and spikes. A calibration blank and an independent calibration verification standard were analyzed every ten samples to confirm the calibration status of the ICP-MS during instrumental analyses of digestates in each of eight batches. Method detection limits (MDLs) were: Pb 0.002 – 0.050 μg g^{-1}; Zn 1.41 – 3.25 μg g^{-1}; Cd 0.004 – 0.068 μg g^{-1}; Ni 0.16 μg g^{-1}, and Co 0.020 μg g^{-1}. Percent recovery of calibration blanks and verification standards ranged from 98% to 107%. Percent recovery of two reference solutions used as laboratory control samples ranged from 100% to 102%. Percent recoveries of metals in mussel and oyster tissue reference materials ranged from 94% to 107%. Instrumental precision determined by relative percent difference from the duplicate analysis of biota digestates was < 1%. Percent recovery of metals spiked in pre-digestion crayfish ranged from 92% to 108%. As a check for potential interferences, dilution percent differences (DPDs) based on 5x dilutions of the biota sample digestates were determined; DPDs were < 11% for all elements.

Statistical Analysis

Statistical analyses were conducted using Statistical Analysis System (SAS) for Windows (Release 9.1; SAS Institute, Cary, NC, USA). We did not test for significant differences in crayfish carapace length because crayfish were sampled in only one habitat type and crayfish are known to partition habitats based on size (DiStefano et al. 2003); however length data are reported, along with sex ratio of crayfish at each site. One-half of the MDL values for pore-water and crayfish metal concentrations were substituted for values less than the MDL for all computations. Crayfish density, water quality, physical habitat data, and metal concentrations were either log-transformed (measurement data) or angular-transformed (percent data) to improve normality. We also ranked-transformed all data and conducted all analyses using both log- and ranked-transformed data. The analyses produced similar results; log-transformed analyses are therefore reported. Differences in crayfish densities among sites and groups of sites were tested using nested analysis-of-variance (ANOVA; riffles nested within site), with site considered a fixed effect. Differences in crayfish densities among individual sites were evaluated with Duncan's multiple range test. Differences in crayfish densities among groups of sites were tested as planned non-orthogonal contrasts using single degree-of-freedom F-tests. The mean square for riffles within site was used in all tests, which were conducted using the PROC GLM module in SAS. Differences in water quality and physical habitat variables among individual sites and among groups of sites were also tested using the same procedures. Associations among crayfish densities, water quality, physical habitat variables, and metal concentrations were examined with Pearson correlation analysis. A significance level of $P < 0.05$ was used to judge all statistical tests. We examined the relationships among crayfish densities, water quality, physical habitat variables, and pore-water metal concentrations to detect similarities among sites using Principal Component Analyses (PCA; SIMCA-P Version 8.0; Umetrix Inc., Kinnelon, NJ, USA). We also used a generalized multiple regression model, Projection to Latent Structure (PLS; Abdi 2003), to predict crayfish density from pore-water metal concentrations, water quality, and physical habitat variables

RESULTS

Crayfish Density and Metal Concentrations

The predominant (> 99%) crayfish collected in the Black River watershed was *O. hylas*. Single *Cambarus hubbsi* Creaser females were collected at WF1 and BF6 and one *Orconectes punctimanus* (Creaser) female was collected at BF4. Female to male (F:M) ratios were 1:1 across sites with the exception of WF3 (where only one crayfish was collected), WF6, and BF4 (Table 2). Mean crayfish densities differed significantly among individual sites (Table 2). Mean crayfish densities ranged from 0 m^{-2} at SC2 to 20.8 m^{-2} at BF5. Mining sites had significantly lower densities of crayfish than the reference or downstream sites. When crayfish densities were analyzed among groups of sites, mean crayfish density at the reference site was significantly higher than mining and downstream sites. Mean crayfish densities at downstream sites were significantly higher than mining sites.

Metal concentrations in *O. hylas* from mining sites were higher than *O. hylas* from the reference or downstream sites (Table 2). Sex ratios (F:M) of *O. hylas* used in the metal analyses were close to 1:1 at each site except BF4, where it was 8:1. Metal concentrations in *O. hylas* from mining sites were eight- to ten-fold higher than at the reference site. Concentrations of Ni and Co in *O. hylas* from Strother Creek were two-fold higher than those collected in the West Fork or Bee Fork; however, concentrations

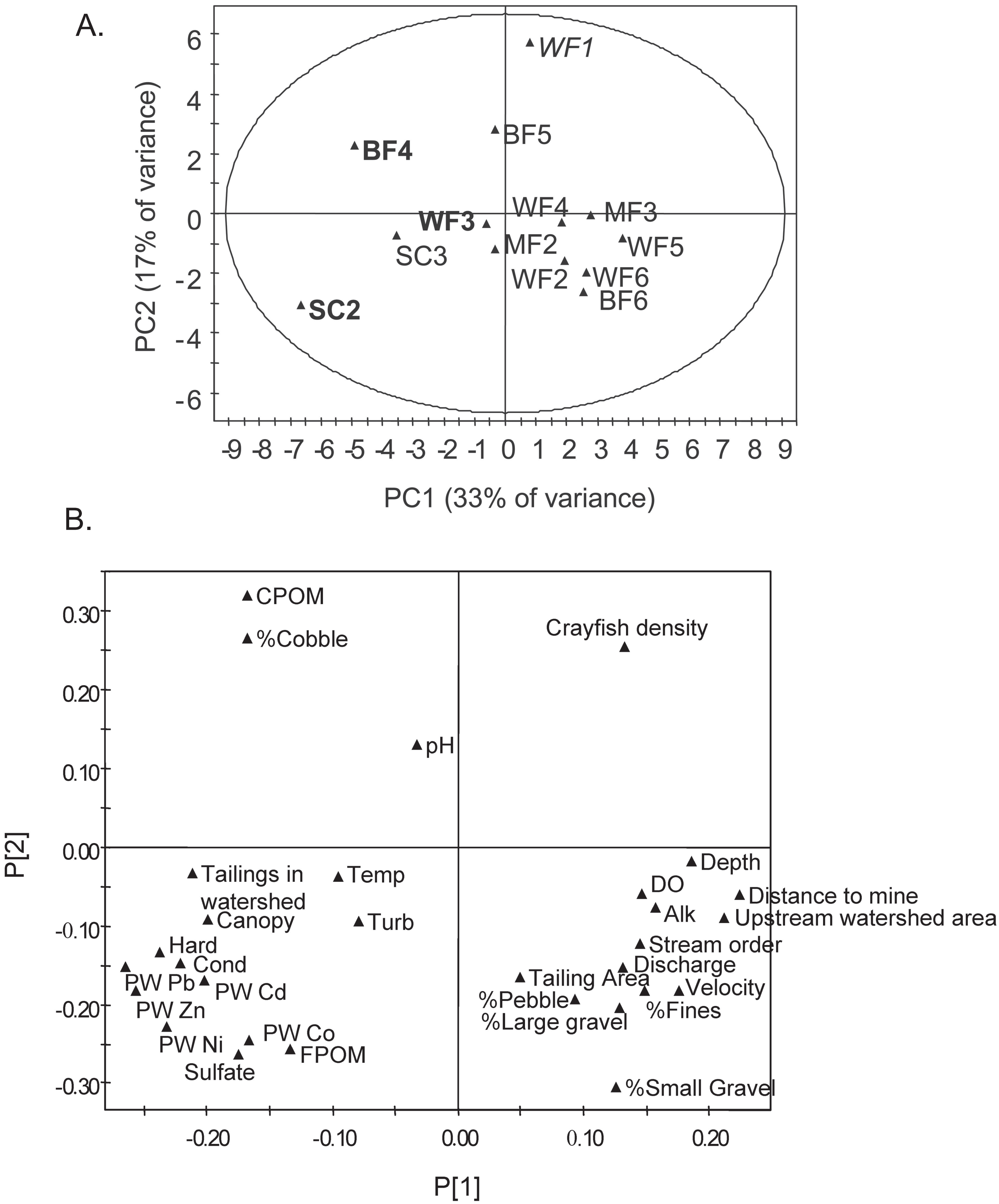

Figure 2. A, Principal Components Analysis (PCA) ordination showing the position of sampling sites along the primary and secondary axes. Mining sites are in bold. Reference site is italicized. B, Variables (loadings) that contribute to positions of sites.

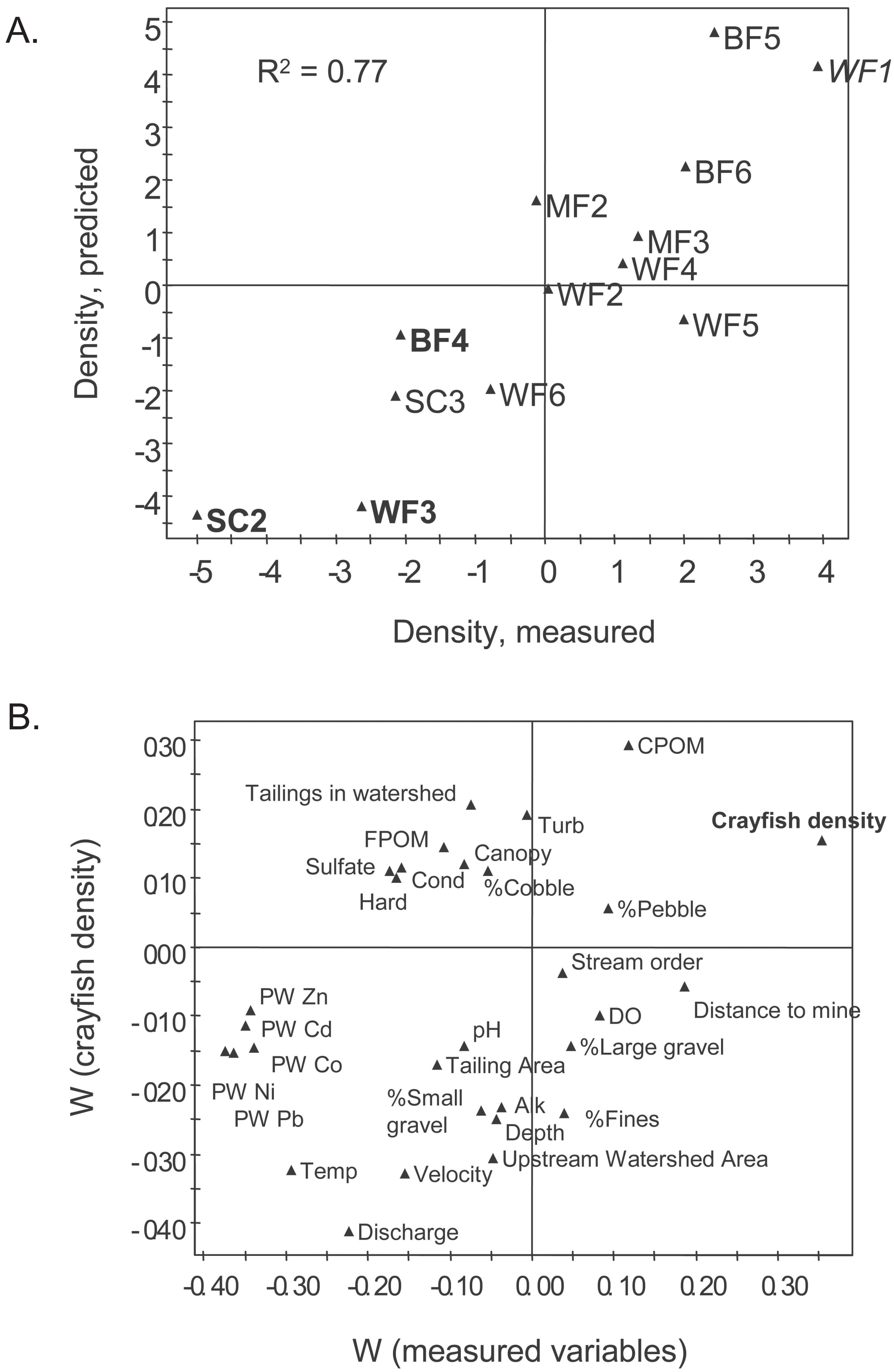

Figure 3. A, Projection to Latent Structure (PLS) or generalized multiple regression model describing predicted versus measured crayfish density. Mining sites are in bold. Reference site is italicized. B, Weights (W) of variable loadings that maximize correlation between the dependent variable (crayfish density) and pore-water metal concentrations, water quality, and physical habitat variables.

Table 1. Sampling locations in the Black River watershed. **M = mining site.** D = downstream site. *R = reference site.*

Site	Stream	Latitude, Longitude	Upstream watershed area (km²)	Stream order	Prominent mining feature in watershed	Stream distance from tailings or mine (km)	Tailings area/ watershed area (km²)	Discharge (m^3 sec^{-1})	Site type
Strother Creek									
SC2	**Strother Creek**	**37°36'07.2", 91°01'40.8"**	**39**	**2**	**Effluent pond; Buick tailings**	**3.7**	**0.017**	**7.3**	**M**
SC3	Strother Creek	37°36'14.4", 91°00'03.6"	92	2	Effluent pond; Buick tailings; Magmont tailings	6.3	0.012	10.2	D
MF2	Middle Fork Black R.	37°35'56.4", 90°57'25.2"	169	3	Effluent pond; Buick tailings; Magmont tailings	11.2	0.006	11.5	D
MF3	Middle Fork Black R.	37°31'26.4", 90°56'06.0"	377	3	Effluent pond; Buick tailings; Magmont tailings	22.4	0.003	28.2	D
West Fork									
WF1	*West Fork Black R.*	*37°30'39.6", 91°09'43.2"*	*95*	*2*	-	-	-	*5.4*	*R*
WF2	West Fork Black R.	37°30'00.0", 91°07'12.0"	153	2	Brushy Creek tailings	6.2	0.005	33.1	D
WF3	**West Fork Black R.**	**37°29'49.2", 91°05'13.2"**	**199**	**2**	**Brushy Creek tailings; West Fork tailings**	**2.2**	**0.006**	**53.4**	**M**
WF4	West Fork Black R.	37°29'13.2", 91°03'03.6"	217	2	Brushy Creek tailings; West Fork tailings	10.1	0.006	9.0	D
WF5	West Fork Black R.	37°28'33.6", 91°00'32.4"	236	2	Brushy Creek tailings; West Fork tailings	16.5	0.005	17.7	D
WF6	West Fork Black R.	37°27'28.8", 90°58'44.4"	343	3	Brushy Creek tailings; West Fork tailings; Fletcher clarification dam and tailings	14.6	0.008	63.0	D
Bee Fork									
BF4	**Bee Fork Black R.**	**37°26'31.2", 91°04'58.8"**	**49**	**1**	**Fletcher clarification dam and tailings**	**1.4**	**0.027**	**4.6**	**M**
BF5	Bee Fork Black R.	37°27'36.0", 91°01'30.0"	80	2	Fletcher clarification dam and tailings	7.4	0.017	5.0	D
BF6	Bee Fork Black R.	37°27'18.0", 90°59'24.0"	94	2	Fletcher clarification dam and tailings	13.7	0.014	5.7	D

Table 2. Number (n), sex ratio (F:M, female:male), density (mean ± 1 standard error), carapace length (mean ± 1 standard error) of *O. hylas* collected at sampling sites, and concentrations (µg g^{-1} dry weight) of metals in composite samples of *O. hylas*. Mining sites are in bold. Reference site is italicized. Sites with the same letter following mean density are not significantly different ($P < 0.05$).

Site	n	F:M ratio	Density (# m^{-2})		Carapace length (mm)	Composite metal sample							
						n	F:M ratio	Carapace length (mm)	Pb	Zn	Cd	Ni	Co
SC2	**0**	**-**	**0**	**g**	**-**	**0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
SC3	21	1:1	1.4 (0.4)	f	15.6 (0.7)	8	1:1	17.6 (1)	4	160	1.0	19	24
MF2	104	1:1	6.9 (1.2)	bc	14.8 (1.0)	9	1:1	23.0 (2)	1	97	0.7	5.0	5.0
MF3	79	1:1	5.2 (1.2)	bcd	17.7 (0.8)	8	3:1	20.7 (1)	1	96	0.6	2.0	1.0
WF1	*232*	*1:1*	*15.4 (1.8)*	*a*	*15.6 (0.6)*	*9*	*1:2*	*23.9 (1)*	*0.4*	*84*	*0.8*	*0.8*	*0.8*
WF2	68	1:1	4.5 (1.2)	cde	18.3 (0.7)	8	1:3	21.0 (1)	4	94	1.1	1.6	1.5
WF3	**1**	**0:1**	**0.1 (0.1)**	**g**	**28.0**	**6**	**1:1**	**16.1 (3)**	**14**	**97**	**1.4**	**4.7**	**3.7**
WF4	72	1:1	4.8 (1.0)	cde	14.7 (0.4)	9	1:1	16.7 (1)	1	85	0.7	1.1	0.9
WF5	54	1:1	3.6 (1.1)	def	15.8 (1.0)	10	1:1	20.0 (1)	2	95	0.8	1.6	1.2
WF6	24	1:3	1.6 (0.4)	f	17.9 (1.1)	9	1:1	18.6 (1)	1	92	1.7	1.0	0.7
BF4	**32**	**2:1**	**2.2 (0.3)**	**ef**	**18.0 (1.2)**	**9**	**8:1**	**23.4 (1)**	**18**	**100**	**2.0**	**6.6**	**3.5**
BF5	313	1:1	20.8 (4.0)	a	15.1 (0.7)	9	1:1	22.7 (1)	12	85	0.8	1.3	0.8
BF6	199	1:1	13.3 (3.9)	b	13.7 (0.6)	9	1:2	18.3 (1)	1	83	0.8	0.9	0.6

of Pb in *O. hylas* were two-fold higher in the West Fork and Bee Fork. Metal concentrations in *O. hylas* collected at downstream sites were lower than those from mining sites.

Physical Habitat Characterization

Mean depth and mean velocity in riffles differed significantly among individual sites (Table 3), generally increasing with distance downstream. Several sites (WF2, WF3, WF6) were sampled within 48 h of a significant (10 cm) rainfall, and these sites had the greatest mean depths, mean velocities, and discharge. Velocities were significantly different among groups of sites, with mining and downstream sites having significantly higher velocities than the reference site. Mean canopy cover also differed significantly among individual sites, with the reference and mining sites having significantly more canopy cover than downstream sites. There were significant differences among individual sites for all substrate sizes, except for the pebble size class. Fine particulate organic matter differed significantly among individual sites and groups of sites, but CPOM did not. Crayfish densities were not correlated with any measured physical habitat variable.

Water Quality Measurements

All water quality variables differed significantly among individual sites (Table 4). Water temperature was generally warmer in the West Fork and Strother Creek, and at upstream sites, except for WF1. The ranges of pH (7.5 – 8.0), turbidity (0 – 10 NTU), and dissolved oxygen (4.1 – 8.9 mg L^{-1}) were narrow, with the exception of low dissolved oxygen at BF4. The low reading at BF4 may have been due to measurement timing (i.e., early morning). Alkalinity was lower at sites in Strother Creek and Bee Fork than in the West Fork. Sites located in Strother Creek and Bee Fork had significantly higher conductivity, hardness, and sulfate concentrations compared to the reference site. Temperature, alkalinity, hardness, conductivity, and sulfate concentrations differed significantly among groups of sites, with mining sites having significantly higher values than either the reference site or downstream sites. Mean crayfish densities were negatively correlated with temperature (r = -0.64).

Metals in Sediment Pore Water

Pore-water metal concentrations differed significantly among individual sites for all metals (Table 5). Concentrations of all metals in sediment pore water differed significantly among groups of sites, with concentrations significantly greater at mining sites than the reference site. Concentrations of Ni and Co in pore water were ten-fold higher in Strother Creek than WF1. Generally, pore-water concentrations of all metals declined with distance downstream of mining; however, pore-water concentrations were higher at WF6 than WF4 and WF5. Pore waters from 10 of the 13 sites exceeded the chronic WQC for surface-water Pb, but no pore waters exceeded the chronic WQC for Zn or Cd. Pore waters from two of the mining sites (SC2 and WF3) exceeded the chronic WQC for Ni. Mining sites had the greatest toxic unit scores, which were 25 to 40 times greater than WF1. Hardness, conductivity, and sulfate concentrations had significant positive correlations with two or more metals in pore water (results not shown).

Crayfish density was negatively correlated with concentrations of all metals in pore water, TUs, and Cd concentrations in whole

Table 3. Physical habitat measurements (mean ± 1 standard error) at sampling sites. The range in the number of samples taken per site for depth and velocity = 73 – 169; for canopy = 10 – 26; for substrate composition = 3 – 4. FPOM = fine particulate organic matter. CPOM = coarse particulate organic matter. Mining sites are in bold. Reference site is italicized. Sites with the same letter are not significantly different ($P < 0.05$).

Site	Riffle depth (cm)	Riffle velocity (m sec^{-1})	Riffle % canopy	Substrate composition (by size class in mm)						
				% Cobble (> 38.1)	% Pebble (19 – 38.1)	% Large gravel (9 – 19)	% Small gravel (2 – 9)	% Fines (< 2)	FPOM (µg C g^{-1})	CPOM (µg C g^{-1})
SC2	**14.5 (1) efg**	**0.23 (0.01) de**	**89 (2) a**	**46 (8) bc**	**27 (4)**	**13 (3) bcd**	**13 (3) abcd**	**0 b**	**3 (1) c**	**5 (3)**
SC3	17.2 (1) def	0.26 (0.02) cde	38 (6) bcd	55 (7) ab	27 (3)	10 (2) bcd	7 (2) d	0 b	3 (2) c	3 (2)
MF2	22.6 (2) cd	0.27 (0.02) cd	33 (7) cd	50 (4) b	26 (6)	12 (3) bcd	12 (3) bcd	0 b	1 (0.2) c	5 (4)
MF3	24.4 (2) cd	0.58 (0.2) b	12 (3) f	35 (2) bcd	48 (2)	10 (3) bcd	7 (1) d	0 b	3 (1) c	1 (0.3)
WF1	*21.4 (2) cdef*	*0.15 (0.02) f*	*43 (6) bc*	*78 (7) a*	*13 (6)*	*6 (1) cd*	*3 (1) d*	*0 b*	*8 (2) a*	*1 (0.2)*
WF2	25.4 (1) bc	0.46 (0.03) b	16 (4) ef	32 (9) bcd	30 (6)	13 (2) bcd	20 (3) abc	4 (2) b	1 (0.1) c	2 (1)
WF3	**33.2 (2) a**	**0.55 (0.02) a**	**13 (3) f**	**78 (6) a**	**8 (2)**	**4 (3) d**	**8 (4) cd**	**2 (2) b**	**1 (0.1) c**	**1 (0.1)**
WF4	19.3 (2) def	0.33 (0.03) c	14 (4) f	33 (15) bcd	32 (12)	16 (4) bc	15 (1) abcd	3 (1) b	1 (0.1) c	1 (0.2)
WF5	20.4 (1) cde	0.31 (0.02) c	18 (3) ef	18 (11) cd	27 (10)	28 (6) a	23 (7) ab	4 (2) b	1 (0.1) c	1 (0.2)
WF6	29.0 (1) ab	0.60 (0.03) a	7 (2) f	49 (8) b	29 (4)	8 (3) cd	11 (2) bcd	1 (1) b	1 (0.1) c	1 (0.4)
BF4	**13.3 (1) g**	**0.20 (0.01) ef**	**29 (5) de**	**80 (10) a**	**14 (7)**	**3 (2) d**	**3 (1) d**	**0 b**	**7 (3) ab**	**2 (1)**
BF5	18.6 (1) cde	0.25 (0.02) cde	13 (4) f	56 (13) ab	29 (10)	9 (2) bcd	5 (1) d	0 b	4 (2) bc	1 (1)
BF6	17.7 (2) fg	0.33 (0.03) c	49 (7) b	10 (4) d	31 (9)	19 (3) ab	25 (7) a	14 (5) a	0.4 (0.1) c	3 (3)

crayfish (Table 6). Metal concentrations in pore water were highly inter-correlated, as were concentrations of Zn, Ni, and Co in whole crayfish. All pore-water metal concentrations except for Pb were also highly correlated with metal concentrations in whole crayfish.

An interpretable ordination of crayfish density, metal concentrations in pore water and physical habitat variables was obtained by PCA, with the first two axes containing substantially more information than expected by chance (Figure 2A). The plot shows that sites WF1 and BF5, which had the highest crayfish densities, and sites SC2, SC3, and BF4, which had the highest pore-water metal concentrations, were grouped separately from the remaining sites. Distance to mines, upstream watershed area, depth, and fine substrate were positively associated with axis 1, whereas pore-water metal concentrations, hardness, conductivity, and canopy were negatively associated with axis 1 (Figure 2B). Crayfish density, cobble substrate, and CPOM were positively associated with axis 2, whereas large and small gravel, FPOM, sulfates, and pore-water Co concentrations were negatively associated with axis 2 (Figure 2B).

When projecting crayfish density as the dependent variable using PLS, 77% of the variation in crayfish densities was explained by water quality, physical habitat variables, and pore-water metal concentrations (Figure 3A). Sites with the lowest mean crayfish density (SC2, WF3) were grouped in the lower left quadrant, whereas sites with the highest mean densities (WF1, BF5, BF6) were grouped in the upper right quadrant. Pore-water metal concentrations, temperature, and discharge were negatively correlated with crayfish density, as represented by the large distance between these variables (Figure 3B).

DISCUSSION

Metal Exposure and Crayfish Densities

Our data indicated that elevated exposure to toxic metals, as reflected by metal concentrations in sediment pore water and crayfish tissue, probably limits the densities of *O. hylas* in the Black River watershed. Sediment pore-water concentrations of Pb and Ni exceeded WQC in the three Black River tributaries investigated. We found significantly positive correlations between most pore-water metals and whole crayfish metal concentrations, including those that are physiologically regulated (e.g., Zn). Additionally, higher Cd concentrations in crayfish were associated with significantly lower crayfish densities. Temperature, physical habitat, and organic matter were similar at sites in the upper reaches of these tributaries where both the reference site and mining sites were located; however, crayfish densities were significantly higher at the reference site.

The general trend for lower densities at sites furthest downstream in all three tributaries may be due to differences in water quality and physical habitat characteristics, which differed significantly from the reference site. Temperature was negatively correlated with crayfish densities, which may reflect the influence of temperature on food consumption and growth. Whitledge and Rabeni (2002) examined the effect of four temperatures (18, 22, 26, and 30°C) on maximum consumption (C_{max}) in several Missouri *Orconectes* species, including *O. hylas*, and found that C_{max} and growth rates were greatest at 22°C. Survival of crayfish, and thus crayfish density, may have been reduced at higher temperatures due to increased molting frequency with higher growth rates. Trace metal uptake in freshwater crayfish likely occurs along with

Table 4. Surface water quality measurements (means ± 1 standard error) at sampling sites. Number of samples (n) taken for temperature = 1460. The range in number of samples for all other water quality = 3 – 5. Temp = temperature. Cond = conductivity. DO = dissolved oxygen. Turb = turbidity. Alk = alkalinity. Hard = hardness. Mining sites are in bold. Reference site is italicized. Sites with the same letter are not significantly different ($P < 0.05$).

Site	Temp (°C)	pH	Cond (µs cm^{-1})	DO (mg L^{-1})	Turb (NTU)	Alk (mg L^{-1} as $CaCO_3$)	Hard (mg L^{-1} as $CaCO_3$)	Sulfate (mg L^{-1})
SC2	**22.7 (0.04) a**	**7.7 (0.03) e**	**907 (1) a**	**7.9 (0.1) a**	**2 (1) abc**	**122 (1) e**	**444 (7) a**	**324 (9) a**
SC3	22.1 (0.04) ef	7.9 (0.02) ab	745 (1) b	8.5 (0.2) a	6 (1) a	138 (1) d	363 (6) b	233 (14) b
MF2	21.6 (0.04) g	7.8 (0.02) de	527 (10) c	8.1 (0.3) a	6 (5) abc	149 (1) c	264 (3) c	125 (5) c
MF3	21.7 (0.03) g	7.6 (0.03) f	418 (0) f	8.5 (0.03) a	0 (0) c	141 (1) d	202 (2) de	74 (0.3) e
WF1	*21.5 (0.05) h*	*7.9 (0.01) bc*	*340 (0.3) i*	*8.6 (0.1) a*	*0 (0) c*	*175 (1) a*	*183 (5) fg*	*1 (0.2) j*
WF2	22.3 (0.04) c	7.9 (0.01) bcd	338 (1) j	7.8 (0.1) a	0 (0) c	161 (2) b	171 (1) gh	16 (0.3) h
WF3	**22.2 (0.05) de**	**7.8 (0.01) cde**	**324 (1) k**	**7.6 (0.1) a**	**4 (1) ab**	**151 (1) c**	**165 (1) h**	**16 (0.2) h**
WF4	22.7 (0.05) ab	7.6 (0.01) f	382 (0) h	7.0 (0.1) a	2 (1) abc	171 (1) a	191 (1) ef	27 (0.5) g
WF5	22.6 (0.04) b	8.0 (0.01) a	354 (0) j	10.1 (0.2) a	0 (0) c	163 (1) b	180 (1) fg	19 (0.1) h
WF6	22.0 (0.04) f	7.9 (0.05) bcd	348 (0) i	8.1 (0.1) a	3 (2) abc	151 (1) c	173 (2) gh	25 (1) g
BF4	**22.3 (0.06) cd**	**7.8 (0.03) de**	**474 (1) d**	**4.1 (2) b**	**0.3 (0.3) bc**	**138 (4) d**	**209 (1) d**	**89 (2) d**
BF5	21.0 (0.05) i	7.8 (0.03) cde	444 (0) e	8.9 (0.1) a	10 (6) a	141 (1) d	200 (1) de	75 (1) e
BF6	19.9 (0.02) j	7.5 (0.01) g	401 (0.3) g	7.6 (0.01) a	3 (2) abc	147 (1) d	183 (10) fg	50 (1) f

the reabsorption of calcium from the old exoskeleton (Rainbow 1997) and may lead to increased mortality after ecdysis.

We did not find a significant relationship between discharge and crayfish densities; however, PCA analyses indicated that stream discharge was associated with crayfish density. Low crayfish densities at sites with high discharge (e.g., WF6) may reflect crayfish temporarily moving out of riffle habitats or deeper into the hyporheic zone after a rain event. However, pore-water concentrations of Ni and Co, which were negatively correlated with crayfish densities, were elevated at WF6, and may have contributed to reduced crayfish densities, even though this site was located 14 km downstream of mining areas.

Factors Influencing Crayfish Metal Exposure

Other factors that may influence whether tissue concentrations reflect environmental concentrations include internal regulation of metals by crayfish, bioavailability of metals, and seasonal and annual variation in metal availability (Thorp et al. 1979; Roldan and Shivers 1987; Mwangi and Alikhan 1993). Passive sampling probes used for monitoring selected samples during 2004 sediment toxicity tests indicated that large percentages of Co and Ni in laboratory-prepared sediment pore waters were labile (Brumbaugh et al. 2007). In contrast, most of the filterable Pb was comparatively non-labile, presumably because it was complexed by organic matter or was present as colloidal species (Brumbaugh et al. 2007). Pore-water concentrations of Ni and Co were highly correlated with Ni and Co concentrations in whole crayfish in our study.

Previous studies (Mirenda 1986a, 1986b; Vijayram and Geraldine 1996) demonstrated a significant relationship between metal concentrations in water and those in crayfish. Correlations between distance from a contaminant source and metal concentrations have also been reported (Bagatto and Alikhan 1987a, 1987b; Alikhan et al. 1990). Whole-body metal concentrations in our crayfish were higher at mining sites than the reference site, but we did not find a significant correlation between metal concentrations in crayfish and distance to mines. However, metal concentrations in whole crayfish were significantly correlated with pore-water metal concentrations, presumably a more direct indicator of metal bioavailability than distance from mining facilities.

Brumbaugh et al. (2007) summarized data collected during studies conducted from 2001 – 2005 in the Old and New Lead Belt mining districts, and showed that sediment, surface water, and pore-water metal concentrations differed between sampling years at some sites. Besser et al. (2006) reported elevated concentrations of Pb, Zn, and Cd in whole crayfish collected in 2001 and 2002 from sites in the Black River and surrounding watersheds associated with mining activity. Compared to our study, concentrations of Pb, Cd, and Zn in whole crayfish were similar at BF4 in 2001 ('BF' in Besser et al. 2006), but lower (one-fifth for Pb and one-fourth for Cd) at WF3 ('WF2' in Besser et al. 2006). Sediment metal concentrations were much lower at West Fork sites in 2004 than in 2002, presumably in part because operations at the West Fork mill were halted in late 2000 and severe flooding in 2002 (Brumbaugh et al. 2007). Temporal variation in metal concentrations may be due to seasonal or annual patterns in rainfall (Besser et al. 2006) and molting of crayfish (Knowlton et al. 1983).

There were also considerable differences between metal concentrations in pore water obtained from fine sediment collected along stream bank margins in 2004 and from pore water obtained from riffles by in-stream methods in 2003 and 2005 (Brumbaugh et al. 2007). Sampling of pore water was conducted in riffles in 2003

Table 5. Concentrations (µg L^{-1}; means ± 1 standard error) of metals measured in sediment pore water, and calculated chronic pore-water Toxic Units (TUs). TUs = pore-water concentration (PWC) / chronic water quality criteria (WQC). There is no water quality criterion for Co. TU score = sum of TUs for Pb + Zn + Cd + Ni. Mining sites are in bold. Reference site is italicized. Sites with the same letter are not significantly different ($P < 0.05$).

Site	n	Pb			Zn			Cd			Ni			Co	TU
		PWC	WQC	TU	PWC	WQC	TU	PWC	WQC	TU	PWC	WQC	TU	PWC	score
SC2	**3**	**102 (81) a**	**12**	**8**	**234 (81) a**	**418**	**0.56**	**0.3 (0.10) a**	**0.7**	**0.4**	**228 (53) a**	**184**	**1.2**	**769 (49) a**	**11**
SC3	2	17 (1) ab	10	2	58 (21) a	352	0.16	0.2 (0.10) ab	0.6	0.3	91(20) a	155	0.6	120 (410) ab	3
MF2	3	6 (3) cd	7	1	8 (7) de	269	0.03	0.1 (0.01) cdef	0.5	0.1	55 (15) abc	118	0.5	121 (57) ab	1
MF3	2	7 (2) cd	5	1	12 (7) de	214	0.06	0.1 (0.02) ef	0.4	0.1	17 (0.3) bcd	94	0.2	52 (2) bc	2
WF1	*2*	*1 (0.1) d*	*5*	*0.3*	*2 (1) e*	*197*	*0.01*	*0.04 (0.01) ef*	*0.4*	*0.1*	*4 (5) e*	*87*	*0.1*	*22 (13) d*	*0.4*
WF2	3	13 (2) b	4	3	22 (14) bcd	186	0.12	0.1 (0.01) bcde	0.4	0.2	14 (2) de	82	0.2	57 (18) bc	3
WF3	**4**	**59 (36) ab**	**4**	**14**	**67 (50) ab**	**181**	**0.37**	**0.2 (0.23) abcd**	**0.3**	**0.6**	**82 (27) a**	**79**	**1.0**	**63 (15) bc**	**16**
WF4	3	6 (3) cd	5	1	6 (3) de	204	0.03	0.1 (0.10) cdef	0.4	0.2	16 (4) d	90	0.2	28 (15) cd	2
WF5	2	5 (4) cd	5	1	4 (4) e	194	0.02	0.01 (0) f	0.4	0.03	9 (4) de	86	0.1	17 (7) d	1
WF6	2	13 (1) b	5	3	11 (2) de	188	0.06	0.1 (0.01) abcd	0.4	0.3	31 (34) d	83	0.4	131 (133) d	4
BF4	**3**	**83 (21) a**	**6**	**15**	**39 (7) abc**	**221**	**0.18**	**0.2 (0.04) abc**	**0.4**	**0.4**	**92 (95) ab**	**97**	**1.0**	**77 (60) bc**	**16**
BF5	3	7 (5) c	5	1	13 (0.3) cd	213	0.06	0.1 (0.03) def	0.4	0.1	5 (30) e	93	0.1	6 (2) d	2
BF6	3	6 (2) cd	5	1	8 (4) de	197	0.04	0.1 (0.04) bcde	0.4	0.2	17 (40) cd	87	0.2	29 (14) cd	2

and 2005 with diffusion samplers or "peepers" (Serbst et al. 2003; Brumbaugh et al. 2007) deployed in-stream for approximately two weeks. In 2003 and 2005, the average TUs for riffle pore water at WF1 was 0.09, while they ranged from 0.2 to 1.5 at mining sites. Although the TUs were comparably lower for in-stream samples obtained from riffles as compared to the laboratory-extracted pore-water samples obtained from fine (< 2 mm) sediments, these TUs also indicate an elevated risk of metal toxicity to aquatic organisms, particularly at the mining site in upper Strother Creek (SC2). Lower metal concentrations were expected for in-stream riffle samples as compared to laboratory-extracted pore waters because metals typically are greatly enriched in the fine particle fractions of sediments. In addition, homogenization and storage of 2004 sediment samples may have promoted the release of metals from sediment particle surfaces into the pore water as a result of reductive dissolution of iron and manganese oxyhydroxides. The positioning of in-stream samplers in coarse substrates of riffles also resulted in reduced measured metal concentrations because of the high exchange rate between pore waters and surface waters, which had low concentrations of metals (Brumbaugh et al. 2007). Each method of collecting pore water has advantages and disadvantages (USEPA 2005), but both sets of samples indicated risk to crayfish.

Diet may also influence bioaccumulation of metals in crayfish. Crayfish are omnivores and the primary shredders found in Ozark streams (Rabeni et al. 1995). Plant tissue may be a significant source of metal transfer through the trophic web. Besser et al. (2006) reported strong correlations between Pb, Zn, and Cd concentrations in crayfish, plant material, and benthic organisms, as well as a strong correlation between crayfish Pb and Cd tissue concentrations and those found in longear sunfish, *Lepomis megalotis* (Rafinesque). Crayfish are the predominant prey item of other centrarchid fishes in Ozark streams (DiStefano 2005) and also represent an important part (up to 20%) of the diet of longear sunfish (R.J. DiStefano, Missouri Department of Conservation, unpublished data). Therefore, high metal concentrations in longear sunfish and other centrarchids are likely related to high metal concentrations in crayfish. Stable carbon and nitrogen isotope analysis would be useful for determining pathways of metal accumulation in crayfish (Whitledge and Rabeni 1997; Parkyn et al. 2001).

Gherardi et al. (2002) and Wigginton and Birge (2007) have shown that metal accumulation and toxicity in crayfish is affected by both species and size of crayfish. Gherardi et al. (2002) found that the non-indigenous *Procambarus clarkii* (Girard) accumulated significantly more trace metals than the indigenous *Austropotamobius pallipes* (Lereboullet). Lethal concentrations of Cd varied across six species in two genera and two age classes of crayfish (Wigginton and Birge 2007). *Orconectes* species were more sensitive to Cd than *Procambarus* species, and juvenile crayfish were more sensitive than adult crayfish. Besser and Rabeni (1987) showed strong correlations of dissolved metal concentrations with metal concentrations in *Orconectes nais* (Faxon), survival, and growth; however, growth was not reduced in *O. nais* with increased tissue metal concentrations. Anderson and Brower (1978) and Mirenda (1986a, 1986b) found no relationship between the size of crayfish and the accumulation of metals (Pb, Zn, Cu, Mn, Cd); however, other studies (Knowlton et al. 1983; Bennet-Chambers and Knott 2002) suggested an inverse relationship between crayfish size and dissolved metal (Pb and Zn) concentrations. High surface area to volume ratio, metabolic rate, and permeability (due to more frequent molts) may result in higher metal concentrations in younger crayfish.

Table 6. Pearson product-moment coefficients (r) for correlations among crayfish density, metals concentrations in sediment pore water, TU scores, and metal concentrations in whole crayfish. Values in bold are statistically significant at ($P < 0.05$).

	Pore water						Whole crayfish				
Variables	**Pb**	**Cd**	**Zn**	**Ni**	**Co**	**TU scores**	**Pb**	**Cd**	**Zn**	**Ni**	**Co**
Crayfish density	**-0.79**	**-0.76**	**-0.74**	**-0.81**	**-0.74**	**-0.77**	-0.28	**-0.63**	-0.52	-0.50	-0.47
Pore water											
Pb		**0.89**	**0.91**	**0.83**	**0.65**	**0.89**	**0.79**	**0.79**	0.37	0.57	0.43
Cd			**0.93**	**0.88**	**0.74**	**0.93**		**0.70**	0.51	**0.67**	**0.62**
Zn				**0.83**	**0.71**	**0.92**			**0.62**	**0.72**	**0.66**
Ni					**0.87**	**0.78**				**0.81**	**0.74**
Co						**0.68**					**0.58**
Whole crayfish											
Pb						**0.72**		0.52	0.23	0.47	0.34
Cd						**0.60**			0.35	0.41	0.33
Zn						0.34				**0.89**	**0.94**
Ni						0.53					**0.97**
Co						0.46					

Crayfish Densities and Metal Exposure in Other Ozark Streams

We made additional crayfish collections outside the Black River watershed to gather baseline data for crayfish if comparable metal exposure were to occur in the exploration area, should mining commence. We sampled crayfish at sites in the Current River (Blair Creek; BC), Eleven Point River (Eleven Point; EP2), and Sinking Creek (SK) in the Black River watershed. We also sampled crayfish in the Meramec River watershed (Huzzah Creek; HZ) at a site located downstream of Pb-mining facilities. Sites in the Current River and Eleven Point River watersheds were located within the exploration area, where only prospecting has occurred.

The predominant crayfish collected at SK was *O. hylas*, and densities (13.6 m^{-2}) were not significantly different from those at WF1 (Table 7). The predominant crayfish collected at HZ was *Orconectes medius* (Faxon); numbers were two-fold higher than *Orconectes luteus* (Creaser). The majority (94%) of the crayfish collected at BC was *O. luteus*; however, *Orconectes ozarkae* Williams was also collected. The number of *Orconectes eupunctus* Williams collected at EP2 was five-fold higher than *O. ozarkae*. Mean crayfish densities at BC (20.1 m^{-2}) were not significantly different from those at WF1; however mean crayfish densities at HZ (8.3 m^{-2}) and EP2 (6.7 m^{-2}) were significantly lower than densities at WF1, but not significantly different than densities at downstream sites (e.g., WF4) in the Black River watershed. Female to male (F:M) ratios were 1:1 across sites and species with the exception of *O. ozarkae* at BC (2:1).

Whole crayfish from these additional sites were also analyzed for metals (Table 7). Sizes of crayfish used in metal analyses were similar to sizes of *O. hylas* from sites in the Black River watershed. Female to male (F:M) ratios of crayfish used in the metal analyses were 1:1 at all sites and for all species, except for *O. medius* (4:1). Metal concentrations in *O. hylas* at SK were comparable to *O. hylas* from WF1, as were metal concentrations in *O. luteus* at BC and in *O. eupunctus* at EP2. In contrast, Cd in *O. medius* at HZ was three-fold greater than those in crayfish collected at mining sites in the Black River watershed; however, concentrations of other metals were comparable to downstream sites in the Black River watershed. Relative densities of crayfish found in riffle habitats and the relative concentrations of metals in whole crayfish were similar across these watersheds and ecologically similar *Orconectes* species, suggesting that crayfish populations in the exploration area could be impacted by future Pb-Zn-mining.

Previous studies (Gale et al. 1973; Duchrow 1983; Besser et al. 2006; Schmitt et al. 2007a, 2007b) have shown both exposure and effects of mining-derived metals in aquatic biota in the New Lead Belt. Our study documents absence or reduced densities of crayfish, an important ecological component of Ozark streams, in streams contaminated with metals originating from Pb-Zn-mining. Additional laboratory and field toxicity tests are needed to determine the extent of the effects of mining on the functional ecology of streams draining the New Lead Belt, and to guide natural resource decision-making regarding exploration for and extraction of metals in the proposed mining area of the Mark Twain National Forest.

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Table 7. Number (n), sex ratio (F:M, female:male), site density (mean ± 1 standard error), carapace length (mean ± 1 standard error) of crayfish collected at sampling sites in other Ozark streams, and concentrations (μg g^{-1} dry weight) of metals in composite samples of crayfish. HY = *O. hylas*; MD = *O. medius*; LU = *O. luteus*; OZ = *O. ozarkae*; EU = *O. eupunctus*. Sites with an 'a' following mean density were not significantly different from WF1; sites with a 'b' following mean density were not significantly different than downstream sites ($P < 0.05$).

Site	Species	n	F:M ratio	Site density (# m^{-2})		Carapace length (mm)	Composite metal sample							
							n	F:M ratio	Carapace length (mm)	Pb	Zn	Cd	Ni	Co
SK	HY	204	1:1	13.6 (2)	a	15.7 (0.5)	9	1:1	21.9 (1)	0.2	77	0.4	0.8	1.4
HZ	MD	80	1:1	8.2 (2)	b	13.5 (0.9)	9	4:1	20.6 (1)	0.8	94	5.9	0.7	0.7
	LU	44	1:1					-	-	-	-	-	-	-
BC	OZ	20	2:1	20.1 (2)	a	13.7 (0.6)		-	-	-	-	-	-	-
	LU	282	1:1				9	1:1	20.0 (1)	0.3	79	0.6	0.6	1.0
EP2	EU	83	1:1	6.7 (1)	b	18.4 (0.5)	9	1:1	24.1 (1)	0.2	89	0.6	0.7	0.8
	OZ	18	1:1					-	-	-	-	-	-	-

as part of a Congressionally-funded investigation of the effects of mining in the Mark Twain National Forest of southern Missouri, USA. Crayfish were collected in accordance with a permit from the Missouri Department of Conservation. All procedures conformed to USGS and CERC guidelines for the humane treatment of test organisms during culture and experimentation. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The authors would like to thank the many private landowners who allowed access to the streams. CERC personnel who provided assistance were: J. Arms, E. Brunson, K. Echols, C. Ivey, R. Jacobson, L. Johnson, S. Koppi, J. Kunz, T. May, S. Olson, L. Sappington, D. Stoppler, M. Struckhoff, C. Vishy, C. Lawler, M. Walters, N. Wilhemi, D. Whites, C. Witte, R. Wright. Missouri Department of Conservation personnel who provided assistance were: T. Boersig, M. Combes, S. Geringer, S. Gao, S. Herleth-King, P. Horner, W. Mabee, and J. Westhoff. We thank C. Rabeni, J. Dwyer, J. Fetzner, J. Furse, M. McKee, and 2 anonymous reviewers for their comments which greatly improved the quality of this manuscript.

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