CERC Research Study Plan Title: Effects of lead-zinc mining on crayfish density in the Spring River watershed in southwest Missouri, Tri-State Mining District, USA

CERC Tracking # (provided when proposal submitted):

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USGS/BRD Center: Columbia Environmental Research Center

Facility Contact: Susan B. Jones

Project Contact: Ann L. Allert, James F. Fairchild, Robert J. DiStefano (Missouri Department of Conservation)

Proposed Date to be Initiated: May, 2009

I. **Rationale and Justification:** Lead was discovered in Missouri by early French explorers of the Mississippi River valley and has been mined since the 1700s. Lead and zinc resources of western Missouri, southwest Kansas, and northeastern Oklahoma (i.e., Tri-State Mining District) have been heavily exploited. Damage claims for injury to natural resources are being pursued by DOI trustees (U.S. Fish and Wildlife Service, Bureau of Indian Affairs, and several independent Native American tribes) in the Tri-State Mining District. Studies are designed to ascertain injury and guide remediation and restoration.

Previous studies by CERC have documented the release of metals from zinc-lead mining areas are linked to effects on aquatic organisms. Crayfish, in particular, have been shown to be sensitive to mining-derived metals. A series of studies (Besser et al. 2006; Brumbaugh et al. 2007; Allert et al. 2008a, 2008b in press) has demonstrated elevated concentrations of metals in crayfish in addition to lower densities or absence of crayfish at sites directly downstream of mining sites. Crayfish are an important prey item for fish and many other aquatic vertebrates (Probst et al. 1984; Rabeni et al. 1995; Whitledge and Rabeni 1997), waterfowl (DiStefano 2005 and references therein), and many terrestrial animals (Hobbs, 1993; DiStefano 2005). In addition, crayfish play a large role in the decomposition of organic matter in streams and the cycling of nutrients and energy through stream food webs (Momot 1995; Parkyn et al. 2001). Recent research has also demonstrated that crayfish significantly effect on aquatic microhabitats via ecosystem engineering (Zhang et al. 2004) which may have indirect implications for other species such as endangered mussels. Therefore, impacts of metals on crayfish can have significant direct and indirect effects on stream ecosystems.

II. Objectives

1) Determine densities of crayfish in riffle habitats and species composition at sites in selected streams in the Tri-State Mining Area of southwest Missouri;

2) Measure selected metal concentrations (Pb, Zn, Cd) in surface waters and crayfish tissues as estimates of potential metals exposures to Native Americans, migratory birds, and other trust resources;

3) Characterize physical habitat and water quality conditions of riffle habitats at selected sites in selected streams in the Tri-State Mining Area of southwest Missouri;

4) Evaluate relationships among concentrations of mining-derived metals in water, density of crayfish, and other water and physical habitat characteristics;

III. Listing of Studies: Effects of lead-zinc mining on crayfish density in the Spring River drainage in southwest Missouri, Tri-State Mining District, USA

A. Study 1: Measure riffle crayfish densities, crayfish species composition, metals, and habitat and water quality parameters at selected sites in the Spring River drainage in southwest Missouri, USA

1. **Principal Investigator(s):** Ann L. Allert, James F. Fairchild, Robert J. DiStefano (Missouri Department of Conservation)

2. **Specific Objectives:**

Crayfish density: A minimum of eight sites will be sampled, two of which will be reference sites. At each site, quantitative crayfish samples will be collected within three riffles. Crayfish will be sampled in riffles using a 1-m^2 quadrat sampler or1-m^2 kick seine according to established procedures (DiStefano et al. 1993; Flinders and Magoulick 2005; Larson et al. 2008). The method used will be dependent on habitat characteristics of the selected sites (i.e., water depth). Sampling will begin at downstream ends of riffles and proceed upstream. At each site, a total of 21 quadrat or kick-seine samples will be obtained by distributing 21 samples between the three or four riffles at that site. Crayfish collected will be identified to species (Pfleiger 1996), examined to determine sex, measured for carapace length (to nearest 0.1 mm), and released. Voucher specimens and unidentifiable crayfish will be placed on ice, returned to CERC for identification and archived in the walk-in freezer. All samples will be placed in pre-cleaned jars, stored on ice until they are returned to CERC and frozen until analyses.

Crayfish species composition: Crayfish will be collected via baited wire funnel trap at each of the crayfish density sampling sites to provide supplemental data (to crayfish collected in riffle quadrat or kick-seine samples) for crayfish species composition. Thirty traps baited with canned dog food (DiStefano et al. 2009) will be set in slower-flowing habitats (e.g., pools, backwaters, emergent vegetation patches); traps will be set no closer than 10 m apart. Traps will be deployed overnight and harvested the following morning. Crayfish collected will be processed as previously described. These data will be used in a qualitative manner, only to supplement crayfish species composition data collected during quadrat or kick-seine sampling.

Crayfish metals: At each site, crayfish will be selected from individuals collected during kick-seining or quadrat sampling. We will collect individuals that are within a similar size range (i.e., carapace length ±5 mm) for tissue metal analysis (Pb, Cd, Zn). Three replicate composites of 3 – 5 crayfish will be taken at each site. A replicate will be taken at each of the riffles. ***Individuals taken for metal analyses will be identified on datasheets.*** A single crayfish species will be collected for metals analyses, if possible. If more than one species is required per riffle or site because of availability, only one species should be placed in each sampling jar.

Surface water quality: Surface water quality (i.e., temperature, pH, conductivity, dissolved oxygen, turbidity) will be measured in situ at each site with a multiparameter water quality instrument (i.e., Hydrolab® Quanta) or equivalent instrument. A surface water grab sample from each site will also be collected for additional water quality analyses in the laboratory upon returning from the field (i.e., alkalinity, hardness, ammonia, total nitrogen, total phosphorous, dissolved organic carbon [DOC], sulfate) (APHA 2005). A surface grab sample for metal analyses will be filtered using a polyethylene syringe and 0.45-µm filter, and acidified to pH < 2 with Ultrex® nitric acid (Brumbaugh et al. 2007; May et al. 1997). Filter blanks will be taken at the time of sample collection. Reagent container blanks will be created at the time of sample acidification.

Riffle pore-water metals and water quality: If funding is available, pore water will be collected using sediment "peepers" (Brumbaugh et al. 2007) for metal analyses (Pb, Zn, Cd). Peepers will be constructed of HDPE 60-ml containers with 4-6 holes punched in the lid and a 0.45-µm polyethylene filter secured under the lid. Peepers will be filled with ultrapure water and transported to each site in Ziplock® bags filled with ultrapure water. Three peepers will be deployed at each site in riffle habitats. Peepers will be buried at a depth of 6-10 cm in the sediment for approximately 14 days. At the time of collection, the filter will be removed and the perforated lid replaced with a solid cap. Samples will be acidified to pH < 2 with Ultrex® nitric acid (Brumbaugh et al. 2007; May et al. 1997). "Peeper blanks" will be made and used at the time of deployment, sample collection, and sample acidification. In addition, water quality of pore water will be measured in the laboratory upon returning from the field (i.e., temperature, pH, conductivity, dissolved oxygen, alkalinity, hardness, ammonia, dissolved organic carbon [DOC] and sulfates).

Detritus: If funding is available, detrital material consisting of submerged, decomposing leaves will be collected from the site by using a kick net or seine at each site for metal analyses. Two samples will be collected from each site. Material will be rinsed within a 2-mm sieve and placed in pre-cleaned jars on ice, returned to CERC, where they will be frozen until analyses.

Habitat Measurements: Sites will be identified using a global positioning system receiver (GPS). Current velocity and depth will be measured at all riffle locations using Marsh McBirney flow meter and depth rod along transects set across each riffle. Substrate will be assessed using visual methods at each crayfish seining location (Bain et al. 1985; Bovee and Milhouse 1978). A substrate sample from each site will be taken for organic carbon analysis (APHA 2005). Stream discharge will be measured at each site. Selected landscape variables (i.e., watershed area, stream order, land-use area) will also be measured.

Sediment metals: If funding is available, a sediment sample will be taken from depositional areas for bulk metal analysis at each site. Samples will be analyzed using ICP-MS for Pb, Zn, and Cd using the simultaneously extracted metals (SEM) method (1-N HCL digestion) as described by Brumbaugh and Arms (1996) and applied by Besser et al. (2008).

Depositional sediment pore-water metals and water quality: If funding is available, a pore water sample will be collected in the laboratory by centrifugation from fine depositional sediments from each site (Besser et al. 2008). Water quality in the laboratory upon returning from the field (i.e., temperature, pH, conductivity, dissolved oxygen, alkalinity, hardness, ammonia, dissolved organic carbon [DOC]) will be measured. Pore water samples designated for metal analyses will be filtered, and acidified to pH <2 with Ultrex® nitric acid.

3. Experimental Design and Methodological Approaches: Potential sampling sites will be selected based on data collected in studies that evaluated metals concentrations in sediments and conducted laboratory sediment toxicity tests toxicity (Ingersoll et al. 2007 CERC Study Plan, personal communication). A subset of sites sampled in those studies will be selected for inclusion in the proposed study after additional consultation with the USFWS, USEPA, and state agencies. Studies will be conducted during base flow conditions July-September) from a minimum of eight sites.

4. Listing of SOP Numbers and Titles: Requirements for analyses, sample matrices, parameters, and standard operating procedures are listed in Tables 2 - 4.

5. Listing of Critical Data: Collection location (including latitude and longitude determined by GPS); date; time; physical site attributes (i.e., current velocity, depth, substrate characteristics); surface water quality; crayfish density; quantitative metal analysis of crayfish, and if funding is available, metal analysis of sediment, pore water, and detritus.

6. Statistical Analysis: Data will be analyzed using Release 9.1 of the Statistical Analysis System. Data will be analyzed using appropriated statistical methods to determine whether differences in measured endpoints exist among sites. Summary statistics for each endpoint will be computed and compared using parametric and non-parametric methods. Linear regression and correlation analyses will be conducted to ascertain the nature of relationships among endpoints.

7. Acceptance or Rejection Criteria for Results: Each endpoint will have its own quality assurance program that includes standards, reference materials, and blanks. Data outside the range of acceptable criteria will be clearly noted and discussed.

8. Special Safety Requirements: Department of Interior (DOI) Regulations state that all personnel should wear floatation devices when near water. Gloves are advised protection against infectious agents and parasites while handling fish. Red Cross-Certified First Aid/CPR personnel must be present during all field collections. A first aid kit should also be present in all field vehicles and boats.

9. Animal Care and Use Requirements: All personnel involved in research activities involving live organisms must adhere to the Columbia Environmental Research Center (CERC) Animal Welfare Plan, and implement the spirit and intent of the policies and regulations that assure humane and ethical treatment of research animals. The CERC Animal Welfare Plan outlines the Center's strategy for compliance with the AWA and associated amendments, principles and guidelines, and it is applicable to all laboratory and field research investigations using fish and other vertebrate species. We will comply with all CERC guidelines for the humane treatment of the test organisms during experimentation (e.g., collection).

10. Quality Assurance Requirements: Requirements for analyses, sample matrices, parameters, and standard operating procedures are listed in Tables 2 - 4.

11. Endpoint of Study: Completion of all chemical, biological, and statistical analysis; delivery of peer-reviewed project completion report. Prior to submission of a publication to a scientific journal or other outlet, the USGS will provide a copy for review to the Trustee Council. USGS will provide responses to the Trustee comments on the draft publication. The Trustees will also be provided copies of the journal review comments and proposed author responses for review and comment prior to submittal of the revised manuscript to the scientific journal.

12. Schedule of Study and Expected Outputs: Field collections will be conducted in the summer of 2009, if water levels allow. Laboratory analyses will be completed by December 2009, with a draft report in review by October 2010.

13. Place where Data will be Stored and Archived: CERC.

14. Relationship to Cooperator Needs: The USFWS, charged with protection of trust resources including migratory birds and endangered species including mussels and crayfish, seeks to demonstrate injury to a natural resource. Crayfish play a key role in Ozark streams because of their ecological dominance (Simberhoff 1998; Rabeni et al. 1995; Whitledge and Rabeni 1997); effects on microhabitats via ecosystem engineering (Zhang et 2004); importance as a food resource for migratory waterfowl (DiStefano 2005), and importance as prey for sport fishes such as smallmouth bass (*Micropterus dolomieui*), rock bass (*Ambloplites rupestris*), and longear sunfish (*Lepomis megalotis*) (Probst et al. 1984; DiStefano 2005).

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16. Signatures

Prepared by: Ann L. Allert Date: 11/12/08
Ann L. Allert

Approved by: Edward E. Little Date: 11/12/2008
Edward E. Little
Ecology Branch Chief

Approved by: Ryan Warbritton Date: Nov 12, 08
Ryan Warbritton
Animal Care and Use Committee Chair

Approved by: Paul R. Heine Date: 11/12/08
Paul R. Heine
Quality Assurance and Safety Officer

Approved by: Michael J. Mac Date: 11/12/08
Michael J. Mac
Center Director

Appendices: Lists of Tables, Proposed Budget, and In-Kind Support

Table 1: List of proposed study sites. Note: this is example of possible sites in Missouri. It is understood that more specific Missouri sites will be identified. Final site selection will be based on discussions with cooperators. A total of 12 sites is proposed, with a minimum of 8 sampled.

Stream	Site ID	Classification[1]	Comments
Center Creek	1	Reference	
Shoal Creek	2	Reference	
Mainsteam/ Upper Spring River	3	Reference	
Mainsteam/ Upper Spring River	4	Reference	
Center Creek	5	Low	
Shoal Creek	6	Low	
Shoal Creek	7	Low	
Center Creek	8	Moderate	
Shoal Creek	9	Moderate	
Shoal Creek	10	Moderate	
Mainsteam/ Upper Spring River	11	Moderate	
Mainsteam/ Upper Spring River	12	Moderate	
Turkey Creek	13	High	
Turkey Creek	14	High	
Center Creek	15	High	
Center Creek	16	High	
Shoal Creek	17	High	
Shoal Creek	18	High	
Mainsteam/ Upper Spring River	19	High	
Mainsteam/ Upper Spring River	20	High	

[1] Based on exceedance values developed by MacDonald et al. (2007) by lead concentrations in sediment collected by U.S. Fish and Wildlife Service in 2007.

Table 2: Requirements for accuracy, precision and detection limits.

Parameter	Estimated Accuracy for each matrix	Estimated Precision for each matrix	Precision Protocol for each matrix	Estimated Detection Limit
Chemical	Measure Values within 95% of CI or 10% of Mean	Replicate Values within ± 25%	Analyze duplicate at least once per run	Temperature (0.3°C)
				pH (0.1 unit)
				Turbidity (1 NTU)
				Conductivity (100 µmhos/cm)
				Dissolved oxygen (0.1 mg/L)
				Metals (varies)
				Nutrients (varies)
				DOC and POC (20 µg/L)
				Sulfates (1 mg/L)
				Alkalinity and hardness (2 mg/L)
				Total organic carbon (20 µg/L)
				GPS (10 m)

Table 3: Proposed quality assurance samples for various matrices.

Type	Matrix	Frequency	Analysis	Rationale
Field Duplicates	Water	1 per run	Hydrolab®, water quality	Measures precision of sample collection and degree of environmental variability
Blanks	DI water	1 per field samples	Metals	Monitors procedural contamination
Analytical duplicate	Crayfish, Water, Sediment, Detritus	1 per 20 analyses	Metals, water quality, PSA, carbon analyses	Monitors instrumental precision
Analytical Spike	Crayfish, Water, Sediment, Detritus	1 per analytical run per matrix	Metals	Monitors instrumental accuracy
Laboratory Control Sample	Crayfish, Water, Sediment, Detritus	2 per analytical run	Metals, water quality, carbon analyses	Monitors instrumental accuracy
Laboratory Control Sample	Crayfish	All Voucher specimens	Identification	Monitors technician accuracy
Calibration Standard	Crayfish, Water, Sediment, Detritus	1 per analytical run	Metals, Hydrolab® water quality, carbon analyses	Monitors accuracy

Table 4: Sample matrices, parameters and analytical methods or standard operating procedures (SOPs).

Matrix	Parameter	Analytical Methods
General Laboratory Practices		B4.01, B4.44, B5.03, B5.16, B5.40, B5.63, B5.106, APHA 2005
Water	Temperature	SOP B5.6, APHA 2005
Water	pH	SOPs B4.14; B4.56, B4.62, B5.239, APHA 2005
Water	Conductivity	SOP B5.31, APHA 2005
Water	Dissolved oxygen	Proposed, APHA 2005
Water	Turbidity	SOP B4.42, APHA 2005
Water	Alkalinity	SOP B4.16, APHA 2005
Water	Hardness	SOP B5.95, APHA 2005
Water	Sulfate	F5.31, B5.22
Water	Nutrients	APHA 2005
Water	DOC	SOP B5.21
Crayfish	Animal care	B5.72, B5148, B5.154, B5.160, B5.165
Sediment	Carbon	SOP B4.36, B5.253, APHA 2005
Sediment	Substrate characterization	Bain et al. 1985; Bovee and Milhouse 1978
Metals	Crayfish, water, leaves, detritus	SOPs C5.5, P.485, P.259, P.221, P.510, P.198, P.256, P.207
Leaves	Decomposition	In-prep
Habitat variables	Velocity, depth, in-situ substrate quality	MDC RAM or EPA IBI habitat protocols

Types of quality control for quantitative analysis by ICP-MS are indicated in SOPs C5.135, C5.212. Corrective actions are specified in SOP C5.209. Procedures for calculating QC statistics are as follows:

Percent Relative Standard Deviation (%RSD) = SD/Mean x 100
Relative Percent Difference or RPD = (D1-D2)/Mean x 100
% Spike Recovery = (Total Measured – Background)/Spike Amount x 100
Method Limit of Detection = 3 x $(SD_b^2 + SD_s^2)^{1/2}$ where

SD_b = standard deviation of a blank or low level standard and
Sd_s = standard deviation of a low level sample.

Table 5: Proposed water quality and biotic variables to be measured based on available funding.

Matrix	Variable	No. Reps / Site	Where measured
Surface/pore water	Temperature	3	In situ
Surface/pore water	pH	3	In situ
Surface/pore water	Conductivity	3	In situ
Surface/pore water	Dissolved Oxygen	3	In situ
Surface/pore water	Turbidity	3	In situ
Surface/pore water	Alkalinity	3	Lab
Surface/pore water	Hardness	3	Lab
Surface/pore water	DOC	3	Lab
Surface/pore water	Sulfate	3	Lab
Surface/pore water	Selected metals	3/3	Lab
Surface/pore water	Nutrients (NH3, TN, TP, SRP, NO2/NO3)	3	Lab
Crayfish	Density	21	In situ
Crayfish	Selected metals	3	Lab
Detritus	Selected metals	3	Lab
Depositional sediment	Selected metals	1	Lab

Table 6: Proposed habitat and sediment quality variables to be measured.

Matrix	Variable	No. Reps / Site	Where measured
Surface water	Current velocity	3 or 4 riffles	In situ
Surface water	Depth	3 or 4 riffles	In situ
Sediment	Sediment characterization	3 or 4 riffles	In situ
Surface water	Current velocity	21	In situ
Surface water	Depth	21	In situ
Sediment/seine or quadrat locations	Sediment characterization	21	In situ
Sediment	Sediment carbon	3	Lab
Surface water	Stream order	1	Lab
Site	GPS	3-4 (each riffle)	In situ
Site	Watershed area	1	Lab
Site	Land use	1	Lab
Site	Stream discharge	1	In situ

Table 7: Proposed project budget.

Category	Variable	Comments	Cost
Travel	Per diem	For 9 sites For 15 sites	9 x 100 x 2=1800 15 x 100 x 2=3000
Travel	Transportation	For 9 sites For 15 sites	6,500 8,000
Analytical	Water quality	$25/sample	9 x 3 x 25=675 15 x 3 x 25=1125
Analytical	Sediment carbon	$25/sample	9 x 3 x 25=675 15 x 3 x 25=1125
Analytical	Metals – crayfish	$125/sample 3 reps per site (Pb, Cd, Zn)	9 x 3 x 175=3375 15 x 3 x 175=5625
Analytical	Metals – surface water	$100/sample 3 reps per site (Pb, Cd, Zn)	9 x 3 x 150=2700 15 x 3 x 150=4500
Analytical	Metals – pore water	$125/sample 3 reps per site (Pb, Cd, Zn)	9 x 3 x 150=3375 15 x 3 x 150=5625
Analytical	Metals – detritus	$125/sample 3 reps per site (Pb, Cd, Zn)	9 x 3 x 175=3375 15 x 3 x 175=5625
Analytical	Metals – sediment	$125/sample (Pb, Cd, Zn)	9 x 125=1125 15 x 125=1875
Salary			20,000
Misc field and lab supplies			3,200
Total		For 9 sites For 15 sites	46,800 59,700
Total plus overhead, 7%		**For 9 sites** **For 15 sites**	**50,076** **63,879**
Total	Metals only crayfish; detritus	For 9 sites For 15 sites	42,300 (45,261) 52,200 (55,854)
Total	Metals only detritus (MDC pays for crayfish metals analyses	For 9 sites For 15 sites	36,225 (38,760.25) 42,075 (45020.25)

Appendices

Appendix 1: Surface Substrate Composition, Current Velocity, and Depth at Riffles

Objectives: To characterize microhabitats of riffles. Data will be used to determine whether surface substrate composition, current velocity, and depth help explain densities of crayfish, and whether the kick seine locations within riffles were representative of the riffle.

Data to be recorded: Site name; site number; lateral distance between measurements for each transect (e.g., measurements obtained at left and right wetted margin and at points along transects); distance of entire riffle (e.g., downstream to upstream distance or longitudinal length); GPS coordinates for each riffle (taken at downstream end of riffle); and surface substrate size, current velocity; and depth at points along transects in each riffle.

Methods: Transects will be set up across each riffle, and measurements will be taken along each transect (see below). Distance between transects and within transects will be determined by the riffle length and width. Start at the downstream end of Riffle 1 (the furthest downstream riffle at each site). Mark each transect with numbers, starting with "1" at the most downstream end of each riffle (i.e., renumber in each riffle).

Distance between stations on each transect:

Measure wetted width of stream.
If width is < 5 m, take velocity/depth measurements at 1-m intervals.
If width is 5 < x < 10 m, take velocity/depth measurement at 2-m intervals.
If width is 10 < x < 15 m, take velocity/depth measurements at 3-m intervals.
If width is 15 < x < 20 m, take velocity/depth measurements at 4-m intervals.

Distance to next transect:

If riffle length is <= 50 m; place next interval 10 m upstream.
If riffle length is 50 < x < 100 m; place next interval 20 m upstream.
If riffle length is > 100 m; place next interval 30 m upstream.

Velocity Measurements:

For water depths < 75 cm, measure velocity once at 0.6 of the depth from the water surface (e.g., if water is 50 cm deep, measure velocity at 30 cm from the water surface; 40 cm from the bottom surface).

For water depths > 75 cm, measure velocity twice at 0.2 d and 0.8 of the depth. Average these two readings to determine the velocity for that cross section.

Record velocity in m/sec; depth in cm.

Surface substrate composition measurements:

A grid (e.g., a piece of rebar welded into an 'X') will be used to characterize substrate at each point along each transect. The five-pointed grid will be haphazardly dropped down on the substrate at the point where depth and velocity readings were taken. Substrate will be classified at each of the four ends of the grid (or "X") as well as the center point (5 points in total), using the following categories (from a modified Wentworth scale; Bovee and Milhouse 1978):

Sand/silt (0.063 mm to 2 mm diameter), Gravel (2 mm to 16 mm diameter), Pebble (16 mm to 64 mm diameter), Cobble (65 mm to 250 mm diameter), Boulder (> 250 mm diameter) and Bedrock.

Each of those categories is assigned a numerical value:

Sand/silt = 1.0, gravel = 2.0, pebble = 3.0, cobble = 4.0, boulder = 5.0, bedrock = 6.0

The five numerical values (from each of the five grid contact points) are recorded and averaged to obtain a mean substrate value (to the tenths decimal place) for that particular riffle.

Appendix 2: Surface Substrate Composition, Current Velocity and Depth at Kick Seine Locations

Objectives: To characterize microhabitat at kick seine locations. Data will be used to determine whether surface substrate composition, current velocity, and depth help explain densities of crayfish, and whether the kick seine locations within riffles were representative of the riffle.

Data to be recorded: Site name; site number; riffle number; kick seine; substrate size class; current velocity; and depth at location of each kick seine sample.

Methods: Kick seines will be taken in 3-4 riffles. Placement will be determined randomly. Measurements will be taken at the center of each 1-m^2 square kick seine sample. Kick seines will be numbered by site number-riffle-number of kick seine with riffle (e.g., 1-1-5; 1-2-4; 1-3-3). Kick seines within riffles will be numbered in the order of which they are taken.

Velocity Measurements:

For water depths < 75 cm, measure velocity once at 0.6 of the depth from the water surface (e.g., if water is 50 cm deep, measure velocity at 30 cm from the water surface; 40 cm from the bottom surface). For water depths > 75 cm, measure velocity twice at 0.2 d and 0.8 of the depth. Average these two readings to determine the velocity for that cross section. Record velocity in m/sec; depth in cm.

Surface substrate composition measurements:

A grid (e.g., a piece of rebar welded into an 'X') will be used to characterize substrate at each kick seine. The five-pointed grid will be haphazardly dropped down on the substrate inside the square-meter sample. Substrate will be classified at each of the four ends of the grid (or "X") as well as the center point (5 points in total), using the following categories (from a modified Wentworth scale; Bovee and Milhouse 1978):

Sand/silt (0.063 mm to 2 mm diameter), Gravel (2 mm to 16 mm diameter), Pebble (16 mm to 64 mm diameter), Cobble (65 mm to 250 mm diameter), Boulder (> 250 mm diameter) and Bedrock.

Each of those categories is assigned a numerical value:

Sand/silt = 1.0, gravel = 2.0, pebble = 3.0, cobble = 4.0, boulder = 5.0, bedrock = 6.0

The five numerical values (from each of the five grid contact points) are recorded and averaged to obtain a mean substrate value (to the tenths decimal place) for that particular kick seine.

Appendix 3: Metal Samples

Crayfish: Crayfish samples will be taken and analyzed for metals to determine impacts of mining on biological community.

Methods: After crayfish are collected from kick seines, identified, sexed and measured, they should be placed in 4-oz. pre-cleaned polypropylene (PP) jars. Three to five crayfish of the dominant riffle species from each riffle at each site should be placed in separate 4-oz. PP pre-labeled jar (i.e., for a site, if two species are equal dominant and four riffles are sampled, there should be eight jars). Crayfish used for metal analysis **should be identified on the data sheet**. Jars should be placed on ice until they can be frozen at hotel or CERC. If only one riffle is sampled, three independent samples should be taken from that riffle.

Data to be taken: Total number of crayfish; carapace length (mm) of crayfish; metals (Pb, Cd, Zn) in crayfish tissue.

Detritus: Detritial samples will be taken and analyzed for metals as a measure of exposure of metals to crayfish.

Methods: Detritus will be collected from kick seines or d-nets and if necessary, other locations at the site. Only that detritus that is well weathered should be collected. Material will be rinsed with site water in a 2-mm stainless steel sieve or sieve bucket and placed in 4-oz. pre-cleaned polypropylene (PP) jars. Samples will be placed on ice until they can be frozen at hotel or CERC.

Data to be taken: Metals (Pb, Cd, Zn) in detritus.

Sediment: Depositional samples will be taken and analyzed for metals as a measure of exposure of metals to crayfish.

Methods: Sediment will be collected using a pre-cleaned scoop, 2-mm sieve bucket, and 19-L HDPE bucket. Material will be rinsed with site water through a 2-mm stainless steel sieve or sieve bucket and placed in the 19-L bucket. Samples will be placed on ice until they can be refrigerated at hotel or CERC.

Data to be taken: Metals (Pb Cd, Zn) in detritus.

Appendix 5: Water Quality

Objective: To characterized water quality in surface samples.

Methods: In-situ measurements will be taken in each riffle for temperature, pH, conductivity, turbidity and dissolved oxygen. A grab sample will also be taken in each riffle. Samples should be taken at the upstream end of the riffle.

A 4-L HDPE bottle will be used to collect a grab sample. Grab samples should be taken in at the upper end of each riffle, starting at the most downstream riffle. Bottles should be rinsed once with site water. Bottle should be placed completely under the water surface and filled. Cap bottle underwater, to insure the bottle is as full as possible. Place bottle in cooler with ice. Subsamples from the grab sample will be taken for water quality and metal analyses. See CERC SOPs for methods.

Data to be taken: temperature, pH, conductivity, dissolved oxygen, alkalinity, hardness, turbidity, sulfate, nutrients, metals, dissolved organic carbon (DOC), particulate organic carbon (POC), and total suspended solids (TSS).

Equipment needed: Coolers with blue ice; Hydrolab DataSonde 3 and Surveyor or Quanta or equivalent water quality instruments; calibration standards; meter log book; study log book; 4-L pre-labeled carboys; 125-ml pre-labeled bottles; 60-ml pre-labeled bottles.

Surface water grab at downstream end of each site:

1. Work downstream to upstream. Measurements will be taken in each riffle.
2. Take surface WQ with water quality instrument(s).
3. Take 4-L sub-surface grab sample.
4. From 4-L sample, collect one 20-mL filtered samples for the trace metals (e.g., Pb, Cd, Zn). Collect the metals sample using the syringe and straw and filter disc. Chemistry will provide syringe & straw, filter cartridges, and 20-mL bottles (all pre-cleaned) for each site (plus a few extras for dups). The syringe/straw and cartridges will be packaged in a single zip-lock. Be sure to place the straw only on a clean surface (e.g., in the zip-lock bag).
5. Place remaining 1-gal sample for all other WQ (e.g., alkalinity, hardness, turbidity, sulfate, ammonia, DOC). Preserve on ice until processing.
6. Samples will be filtered at CERC for sulfate (separate 125-ml bottle) and ammonia and DOC (60-ml bottle). Filter through 0.45-µm polycarbonate filter. Samples will be frozen until analyses.

7. Samples will be filtered at CERC for POC and TSS using glass fiber filters. Filters will be wrapped in aluminum foil, placed in Ziplock bags, and frozen until analyses.

8. Alkalinity, hardness and turbidity should be run ASAP.

9. TN/TP samples should be placed in 60-ml pre-label containers and frozen until analyses.

Filtration

Equipment needed: Vacuum pump; 0.45-µm polycarbonate filters; 60-ml pre-labeled bottles for ammonia/DOC; 125-ml pre-labeled bottles for sulfates; RO water; sulfuric acid; graduated cylinders (to measure ammonia/DOC sample prior to filtration); data sheets.

1. Take out surface water ammonia/DOC samples and place them in hotel laundry room refrigerator.

2. Be sure 1-L surface water samples have enough ice on them to remain at approximately 4 °C (or place in refrigerator, if there's enough room).

3. After transport to CERC, filtration should be completed before alk/hard/turb analysis is started.

4. Ammonia/DOC and sulfate samples will be filtered. Before each set of field samples, run RO water through both filtration systems. These will be Pre-Filter blanks. Two sets of filtration blanks should be run for sulfate and ammonia/DOC samples. Pre-filtration blanks for ammonia/DOC should be acidified with sulfuric acid.

5. Measure the **amount filtered** for ammonia/DOC, POC, TSS. Record on data sheet. Acidify samples with 2 drops of sulfuric acid. Between each sample, rinse well with RO water. Ammonia/DOC samples should be placed in a 60-ml bottle. Sulfate samples should be placed in 125-ml bottle.

6. After all field samples are taken, run RO water through both filtration systems. These will be post-filtration blanks. Again, two sets are needed for sulfates and ammonia/DOC. Post-filtration blanks for ammonia/DOC samples should be acidified.

7. COC forms can be filled out daily. COC should be kept separately for metal samples, ammonia/DOC, sulfate, alk/hard/turb.

Appendix 6: Field Sampling Filtration Procedure for Surface Water samples for Trace Metals

Wear powderless gloves and throughout the procedure, avoid handling the tip sections of the straws, filter discs, or syringes. After each new sample, the syringe and filter disc are discarded, but the straws are saved for cleaning and reuse. The procedure below is for collection of a 20-mL sample from a larger grab volume.

1. Attach a pre-cleaned sampling straw to the syringe and carefully insert into the grab water sample. Draw the syringe plunger to about two mL past the 20-mL mark. Invert syringe and draw plunger to the "stop" to remove all liquid from the straw.
2. Remove the straw and place in a plastic bag for return to the laboratory. Attach a cleaned filter disc and push the plunger first only to the 20 ml mark to expel a few mL of the filtered sample water to waste in order to rinse the filter cartridge with sample.
3. Displace the remaining 20 ml through the filter disc into a 30-mL sample bottle.
4. Discard the syringe and filter cartridge.
5. Cap bottle tightly and if possible, store on ice.

Appendix 7: Field procedures for in-situ peeper sampling of sediment pore water

Diffusion samplers (peepers) are buried 4-6 cm below the sediment surface for a period of 1 to 2 weeks (previous field tests of peepers indicated that equilibration was complete after burial in fine sediments for 4-5 days). The peepers are of a custom CERC design prepared from a 50-ml polypropylene snap-cap vial (Corning no. 1730) fitted with a 0.45-μm polyethersulfone filter membrane under the cap which had several 6-mm diameter holes punched into it to allow water entry. A nylon wire-tie is secured to the body of the vial so the tag end can remain above the sediment surface for retrieval purposes.

Upon retrieval, the peeper vials are rinsed thoroughly with site water and the membrane/perforated cap assembly is carefully removed and replaced with a pre-labeled non-perforated cap. During this process, it is important to avoid contamination of the liquid inside by fine sediment particles on the exterior of the peeper. If visible sediment particles are not readily removed by rinsing with site water, use DI water to rinse the exterior cap region before opening. All samples are placed in racks on ice in the field, and upon return to the laboratory they are acidified to 1% (v/v) HNO_3.