

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:

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. 2008, 2009) 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; Whitley 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 (lead, Pb; zinc, Zn; cadmium, Cd; nickel, Ni) in surface waters, sediments, detritus, 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 sites in selected streams in the Tri-State Mining Area of southwest Missouri;
- 4) Compare riffle crayfish densities and species composition to concentrations of Pb, Zn, Cd, and Ni in surface water, sediment, detritus, and crayfish.

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 nine sites will be sampled with at least one considered to be a reference site. At each site, quantitative crayfish samples will be collected within three riffles. Crayfish will be sampled in riffles using a 1-m² quadrat sampler or 1-m² 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, Ni). 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 and metals: 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. Three (approximately) 4-L surface-water grab sample from each site will be collected for additional water quality analyses in the laboratory upon returning from the field (i.e., alkalinity, hardness, ammonia [NH₃], total nitrogen [TN], total phosphorous [TP], nitrite/nitrate [NO₂/NO₃], soluble reactive phosphorous {SRP}, dissolved organic carbon, particulate organic carbon, chlorophyll *a*, total suspended solids, sulfate) (APHA 2005). A sub-sample (approximately 20 mls) of each grab sample will be filtered using a polyethylene syringe and 0.45- μ m filter and acidified to pH < 2 with Ultrex[®] nitric acid (Brumbaugh et al. 2007; May et al. 1997) for metals analyses (Pb, Cd, Zn, Ni). Filter blanks will be taken at the time of sample collection. Reagent container blanks will be created at the time of sample acidification.

Detritus: Organic material (henceforth detritus) primarily consisting of submerged, decomposing leaves will be collected using a kick net or seine at each site for metal analyses (Pb, Cd, Zn, Ni). Three samples will be collected from each site. Detritus 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.

Sediment metals: Three sediment samples will be taken from depositional areas along the edges of each riffle sampled for crayfish. Samples will be analyzed using ICP-MS for Pb, Cd, Zn, and Ni using the simultaneously extracted metals (SEM) method (1-N HCL digestion) as described by Brumbaugh and Arms (1996) and applied by Besser et al. (2009).

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.

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 nine 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, surface water, sediment, 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; Whitley and Rabeni 1997); effects on microhabitats via ecosystem engineering (Zhang et al. 2004); importance as a food resource for migratory waterfowl (DiStefano 2005), and importance as prey for sport fishes such as smallmouth bass (*Micropterus dolomieu*), rock bass (*Ambloplites rupestris*), and longear sunfish (*Lepomis megalotis*) (Probst et al. 1984; DiStefano 2005).

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16. Signatures

Prepared by: _____ Date: _____
Ann L. Allert

Approved by: _____ Date: _____
Facility Contact

Approved by: _____ Date: _____
Susan E. Finger
Program Coordinator

Approved by: _____ Date: _____
Edward E. Little
Ecology Branch Chief

Approved by: _____ Date: _____
Ryan Warburtin
Animal Care and Use Committee Chair

Approved by: _____ Date: _____
Paul R. Heine
Quality Assurance and Safety Officer

Approved by: _____ Date: _____
Michael J. Mac
Center Director

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

Table 1: List of proposed study sites. A total of 12 sites are proposed to be sampled (with a minimum of 9 sampled). Final site selection will be based on stream water levels and confirmation permission from landowners.

Stream	Site ID	Classification¹	Comments²
Center Creek	1	Moderate	CC-009
Center Creek	2	Moderate	CC-010
Center Creek	3	Moderate	CC-004/-005
Center Creek	4	Moderate	CC-002
Shoal Creek	5	Reference	SH-025
Shoal Creek	6	Low	SH-007 or Wildcat Conservation Area
Shoal Creek	7	Moderate	Site located downstream of Schermerhorn Park
Turkey Creek	8	Low	TC-014
Turkey Creek	9	Low	TC-009
Turkey Creek	10	Moderate	TC-008
Turkey Creek (mouth)	11	Moderate	TC-001
Spring River/North Fork	12	Reference	To be determined
Lost Creek		Low	LC-301; May be sampled if water levels remain high in other stream
Lost Creek		Moderate	LC-003; May be sampled if water levels remain high in other stream

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.

² We believe these are the site labels from MacDonald et al. (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 $\pm 25\%$	Analyze duplicate at least once per run	Temperature (0.3°C)
				pH (0.1 unit)
				Turbidity (1 NTU)
				Conductivity (100 μ mhos/cm)
				Dissolved oxygen (0.1 mg/L)
				Metals (varies)
				Nutrients (varies)
				DOC and POC (varies)
				Sulfates (1 mg/L)
				Alkalinity (20 mg/L); hardness (5 mg/L)
				Total organic carbon (varies)
				Total suspended solids (varies)
				Chlorophyll <i>a</i> (varies)
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	Total suspended solids	APHA 2005
Water	Nutrients	APHA 2005
Water	Particulate organic carbon; dissolved organic carbon, chlorophyll <i>a</i>	ASTM Method D4129-05, SOP B5.21, Standard Method 10200 H (ASTM 2005),
Crayfish	Animal care	B5.72, B5.148, 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, sediment, 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) = $SD/Mean \times 100$

Relative Percent Difference or RPD = $(D1-D2)/Mean \times 100$

% Spike Recovery = $(Total\ Measured - Background)/Spike\ Amount \times 100$

Method Limit of Detection = $3 \times (SD_b^2 + SD_s^2)^{1/2}$ 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
Water	Temperature	3	In situ
Water	pH	3	In situ
Water	Conductivity	3	In situ
Water	Dissolved Oxygen	3	In situ
Water	Turbidity	3	In situ
Water	Alkalinity	3	Lab
Water	Hardness	3	Lab
Water	Particulate organic carbon	3	Lab
Water	Dissolved organic carbon	3	Lab
Water	Chlorophyll <i>a</i>	3	Lab
Water	Sulfate	3	Lab
Water	Selected metals	3	Lab
Water	Total suspended solids	3	Lab
Water	Nutrients (NH ₃ , TN, TP, SRP, NO ₂ /NO ₃)	3	Lab
Crayfish	Density/species composition	21	In situ
Crayfish	Selected metals	3	Lab
Detritus	Selected metals	3	Lab
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

Table 7: Proposed project budget.

Category	Variable	Comments	Cost
Travel	Per diem	Proposed 12 sites (minimum of 9)	6,000
Travel	Transportation	Proposed 12 sites (minimum of 9)	15,704
Analytical	Water quality	\$30/sample	1,080
Analytical	Sediment carbon	\$30/sample	1,080
Analytical	Metals – crayfish	\$150/sample 3 reps per site (Pb, Cd, Zn, Ni)	5,400 ¹
Analytical	Metals – surface water	\$100/sample 3 reps per site (Pb, Cd, Zn, Ni)	3,600
Analytical	Metals – detritus	\$150/sample 3 reps per site (Pb, Cd, Zn, Ni)	5,400
Analytical	Metals – sediment	\$150/sample (Pb, Cd, Zn, Ni)	5,400
Salary			25,000
Misc field and lab supplies			1,000
Total		Proposed 12 sites (minimum of 9)	64,264
Total plus overhead, 7%		Proposed 12 sites (minimum of 9)	68,762.48 74,162.48 (with MDC contribution)

¹ Funds provided by Missouri Department of Conservation (MDC).

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-m² 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 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, Ni) in whole crayfish.

Detritus: Detrital 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 weathered detritus 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, Ni) 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, Ni) in sediment.

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; 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 each 4-L sample, one 20-mL filtered sample will be collected for the trace metals (e.g., Pb, Cd, Zn, Ni).
5. Place remaining 4-L sample for all other WQ (e.g., alkalinity, hardness, sulfate, ammonia, DOC, etc) on ice until processing/analyses.
6. Samples will be filtered at the hotel or CERC for sulfate (separate 125-ml bottle), NH₃, NO₂/NO₃, and DOC (60-ml bottle). Filter through 0.45- μ m polycarbonate filter. Samples will be frozen until analyses.
7. Samples will be filtered at CERC for POC and TSS using glass fiber filters. Filters for chlorophyll *a* and POC will be wrapped in aluminum foil, placed in Ziplock bags, and frozen until analyses. Filters for TSS will be dried and weighed immediately after filtration.

8. Alkalinity and hardness will be analyzed at CERC within seven days.
9. Samples for TN/TP analyses will 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 NH_3 /sulfate/DOC samples prior to filtration); data sheets.

1. Maintain 4-L surface-water samples at approximately 4 °C (or place in refrigerator, if there's enough room).
2. After transport to hotel or CERC, filtration for various water quality parameters (i.e., NH_3 /sulfate/DOC) should be completed before alk/hard/turb analysis is started.
3. With each set of field samples, run ultra-pure water through filtration systems. These will be filter blanks. Two sets of filtration blanks should be run for each analyses. Filtration blanks for NH_3 /DOC should be acidified with sulfuric acid.
4. Measure the **amount filtered** for each chlorophyll *a*, POC, and TSS. Record on data sheet. Between each sample, rinse well with RO water. Acidify NH_3 /DOC samples with 2 drops of ultra-pure sulfuric acid. Acidify POC samples to $\text{pH} < 2$ with ultra-pure sulfuric acid.
5. COC forms can be filled out daily. COC should be kept separately all analyses including metals, NH_3 /DOC, sulfate, alkalinity/hardness, SRP/ NO_2 / NO_3 , chlorophyll *a*, TSS, POC.

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

Wear powder-less 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.