CERC Research Study Plan Title: Effects of historic lead-zinc mining on crayfish density in the Big River in southeast Missouri

CERC Tracking # (provided when proposal submitted):

BRD BASIS+ Project / Task Number: C2G, Task 2

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USGS/BRD Center: Columbia Environmental Research Center

Facility Contact: Michael J. Mac

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

Date Initiated: June 2008

I. **Rationale and Justification** (Why): Lead was discovered in Missouri by early French explorers of the Mississippi River valley and has been mined since the 1700s. Since that time, lead and zinc resources of Missouri and adjoining areas of Kansas and Oklahoma have been heavily exploited. To date, lead and zinc mining has been focused in three primary areas: the "Old Lead Belt", in southeast Missouri, was active from about 1700 until the early 1970s; the "Tri-States District", in southwest Missouri, southeast Kansas, and northeast Oklahoma, was active from the late 1890s to the early 1970s; and the "New Lead Belt" in southeast Missouri, which became active in the 1960s, and where lead and other metals are still mined. In the Big River, damage claims for injury to natural resources are being pursued by DOI (U.S. Fish and Wildlife Service) and State trustees. Studies are designed to ascertain injury and guide restoration. Previous studies by CERC have documented the release of metals from the three mining districts that are linked to effects on aquatic organisms, particularly crayfish, where there were lower densities or absence of crayfish at sites directly downstream of mining sites (Allert et al. in press; Besser et al. 2006; Brumbaugh et al. 2007). Crayfish are an important prey item for fish and many other aquatic vertebrates, and provide significant food resources to many avian, waterfowl, and terrestrial species (Hobbs, 1993). 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). Therefore, impacts of metals on crayfish will likely have affects on additional components of stream and surrounding ecosystems.

II. Objectives (What):

1. Determine crayfish riffle densities and crayfish species composition in the Big River of southeast Missouri;

2. Measure selected metal concentrations in pore water, fish, invertebrates, detritus, and in tissues of crayfish as measures of metal (Pb, Zn, Cd, Co, Ni, Cu) exposure;

3. Characterize physical habitat and water quality conditions of the Big River used by crayfish;

4. Evaluate relationships among crayfish riffle densities, concentrations of mining-derived metals in water and crayfish, and other water and physical habitat characteristics.

5. Evaulate growth and survival of young crayfish using in-situ cages in relation to metals exposure.

III. Listing of Studies: Effects of historic lead-zinc mining on riffle crayfish density in the Big River in southeast Missouri (St. Francis, Washington, and Jefferson Counties)

A. **Study 1: Measure** riffle crayfish densities, crayfish species composition, metals, and water quality parameters in the Big River in southeast Missouri

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

2. Specific Objectives:

Crayfish density: A maximum 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 according to established procedures (DiStefano et al. 1993, Larson et al. in press). Sampling will begin at downstream ends of riffles and proceed upstream. At each site, a total of 21 quadrat samples will be obtained by distributing 21 samples between the three 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.

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 samples) for crayfish species composition. Thirty traps baited with canned dog food (DiStefano et al. in press) 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 identified to species (Pfleiger 1996),

examined to determine sex, measured for carapace length (to nearest 0.1 mm), and released. These data will be used in a qualitative manner, only to supplement crayfish species compostion data collected during quadrat sampling.

Crayfish metals: At each site, crayfish will be selected at random for tissue metal analysis. 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 should 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. 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, where they will be frozen until analyses.

Habitat measurements: Sites will be identified using a global positioning system (GPS) receiver. Current velocity and depth will be measured at each crayfish sampling location (i.e., quadrat), and along transects set across each riffle using Marsh McBirney flow meter and depth rod. Substrate composition will also be assessed using visual methods at each crayfish seining location (see attached protocols). Canopy cover will be measured along transects (see attached protocols). A substrate sample from each site will be taken for organic carbon analysis (APHA 2005). Stream discharge will be calculated at each site. Selected landscape variables (i.e., watershed area, stream order, land use area) will also be determined.

Surface water: general water quality: Surface water quality analyses (i.e., temperature, pH, conductivity, dissolved oxygen, turbidity) will be measured in situ at each riffle within a site with a multiparameter water quality instrument (i.e., Hydrolab® Quanta). A surface water grab sample from each riffle within a site will also be collected for additional water quality (i.e., alkalinity, hardness, ammonia, total nitrogen, total phosphorus, particulate organic carbon [POC], total suspended solids [TSS], sulfate) (APHA 2005).

Surface water: metals: From each surface water sample, an aliquot will be taken for metal analyses using a syringe and 0.45-μm polyethylene cartridge filter. Samples will be placed in polyethylene bottles, stored on ice, and acidified to pH <2 with Ultrex® nitric acid within 96-hr of collection (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.

Detrital metals: Detrital material consisting of fallen leaves will be collected from each site by a kick net or seine or by hand for analyzed using ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu (Allert et al. 2008). 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. Material will also be

placed in cages and serve as a food source for young-of-year *Orconectes sp.* (see below for cage study).

Sediment metals: At each site, a sediment sample will be taken for bulk metal analysis. Samples will be analyzed using ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu 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). Sediment samples will be collected by CERC Toxicology Section in association with a mussel/sediment toxicity study (separate work plan).

Pore-water water quality and metals: At each site, pore water will be collected at the laboratory by centrifugation from fine depositional sediments (Besser et al. 2008). Water quality (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. Sediment pore water samples will be collected by CERC Toxicology Section in association with a mussel/sediment toxicity study (separate workplan).

B. **Study 2: Measure** of crayfish survival and growth for in-situ exposures in the Big River in southeast Missouri

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

Specific Objectives:

Cage deployment: At four sites (two reference; two impact) we will deploy six cages [i.e., each containing 10 crayfish, detritus, supplemental food (Allert et al. 2008, Whitledge and Rabeni 1997) and rock refugia] to determine associations between survival and growth of crayfish and metals exposure. Crayfish (preferably *Orconectes luteus* or *O. harrisoni*; Pflieger 1996) will be used because it is a species with wide distribution in the river. All crayfish will be obtained from a field reference site, preferably from gravid females, and transported to CERC for hatching/grow-out. Juvenile crayfish (approximately 10-mm carapace length) will be deployed in cages for a period of approximately 56 days. Cages will be placed in habitats with adequate depth, most likely runs or pools in close proximity to riffles sampled for crayfish density or abundance. Proposed sampling dates for metal samples are days 0, 28, 56. Cages will be checked weekly for external biofouling and position in stream.

Cage design: Cages will be made of 3-mm mesh stainless steel cloth formed into a 16 x 36 x 7-cm box (Allert et al. 2008). A pre-determined amount of leaves (20-gm wet weight) and uniformly sized rocks (i.e., coarse gravel to small cobble collected from each site-specific location) will be added to each cage to provide food and

refugia. Rocks and leaves will be secured within a 6-mm mesh bag placed at the bottom of each cage. Cages will be deployed below riffles in run habitats along the streambank. Cages will be placed in the substrate to expose crayfish to pore water and allow access to benthic macroinvertebrates.

Crayfish metals: Three replicated samples consisting of 3 – 5 crayfish from the original pool of crayfish to be placed in cages will be taken prior to stocking of crayfish into cages. At day 28 and day 56, all crayfish within a cage will be placed into a pre-cleaned jar for metal analyses. Composite samples will contain 1 -10 crayfish per jar, depending on the survival of crayfish in each cage. The number of replicates per day per site = 3. All samples will be stored on ice until they are returned to CERC, where they will be frozen until analyses.

Detrital metals: At deployment, a subsample of detrital material taken for metal analyses (see above for protocol). Detritus will also sampled on days 28 and 56. Three samples will be taken from each site from materials remaining in each cage.

Fish metals: Fish (i.e., largescale stonerollers, *Campostoma oligolepis*, Pfleiger 1997) will be sampled from each site to supplement the diets of caged crayfish. Fish samples will be obtained at each site using seines, and if necessary, backpack electroshockers. Fish will be identified on site and total length (mm) of fish will be measured. Voucher specimens will be placed in 10% formalin and returned to CERC for identification. Fish will be transferred to 80% ETOH after two weeks. Approximately 10 fish from each site will be placed in pre-cleaned plastic bags or containers, and kept on ice during transport to CERC. Fish will be kept frozen at CERC until and after processing. Fish will be minced, homogenized and aliquots (n = 2) will be separated for residue analysis. Crayfish will be fed weekly at an approximate ration of 5% crayfish body weight.

Macroinvertebrate metals: Invertebrates make up a significant portion of the diet of young-of-year crayfish (Whitledge and Rabeni 1997). An invertebrate sample will be collected in coarse substrate (i.e., riffle) habitats at each site in the wadeable portion of the flowing stream using a kick net or seine or from detrital material collected for use in the cage study. Targeted organisms will include a range of macroinvertebrates. The proportion of the total number and weight of each type of macroinvertebrate will be recorded at the time of collection. Three samples will be taken at deployment at each site. Macroinvertebrates will also sampled on days 28 and 56. Three samples will be taken from each site from materials remaining in each cage.

Pore-water water quality and metals: We propose to collect pore water using sediment "peepers" in riffle habitats near locations where the cages are deployed. Peepers will be constructed of HDPE 60-ml containers with a 0.45-μm polyethersulfone filter at the top of the container (Brumbaugh et al. 2007). The filter will be protected by a HDPE lid with 4-6 holes punched in it. Peepers will be filled with deoxygenated ultrapure water and transported in 2-L polyethylene bottles filled

with deoxygenated ultrapure water. Three peepers will be deployed at each site for analysis of metals and two for measurement of pore-water quality parameters. Peepers will be buried 6-10 cm in the sediment for approximately 14 days, beginning on day 1 and again on day 14 or day 28. Pore water samples for metal analyses will be collected directly from the peeper. After retrieval, a pre-labeled cap will be placed on each peeper, and peepers transported on ice to CERC on ice. Samples will be acidified to pH < 2 with Ultrex® nitric acid within 96 hrs. Pore water quality (i.e., temperature, pH, conductivity, dissolved oxygen, alkalinity, hardness, ammonia, and sulfate will be measured (APHA 2005).

Surface water: general water quality: Surface water quality analyses (i.e., temperature, pH, conductivity, dissolved oxygen, turbidity) will be measured in situ at each riffle within a site with a multiparameter water quality instrument (i.e., Hydrolab® Quanta) on days 1, 14, 28, 42, and 56. A surface water grab sample from each riffle within a site will also be collected for additional water quality (i.e., alkalinity, hardness, ammonia, total nitrogen, total phosphorus, particulate organic carbon [POC], total suspended solids [TSS], sulfate) (APHA 2005) on days 28 and 56.

4. **Experimental Design or Methodological Approach:** Sampling sites will be selected based on data collected in previous studies that characterized lead concentrations in sediment (U.S. Fish and Wildlife 2007). Samples will be collected during base flow (July) from a maximum of eight sites.

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

6. **Listing of Critical Data:** Collection location (including latitude and longitude determined by GPS); date; time; physical variables (i.e., current velocity, depth, substrate particle size); water quality; quantitative metal analysis of water, crayfish and detritus, and crayfish density; and caged crayfish survival and growth.

7. **Statistical Treatment:** Data will be analyzed using Release 9.1 of the Statistical Analysis System. Data will be analyzed for normality, and appropriate statistical transformations will be made, if needed. Summary statistics for each endpoint will be computed and compared using parametric and non-parametric methods. Analysis of variance, linear regression, bivariate correlation, and multivariate techniques will be conducted to ascertain the nature of relationships among variables.

8. **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.

9. **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. A first aid kit should also be present in all field vehicles and boats. Ethanol is a Class-3 flammable liquid, and shipping regulations apply. Neutral-buffered formalin is considered non-hazardous at working concentrations (10%); however, it should be used outdoors or under a hood, with gloves and eye protection, and should be shipped by ground transport. Fish could potentially be collected by electrofishing; all electrofishing and watercraft safety regulations and guidelines apply. A DOI-Certified Electrofishing Team Leader must be present during all electrofishing operations. 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.

10. **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 (AWP), 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 AWP 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 culture and experimentation.

11. **Quality Assurance Requirements:** To the extent practicable, all analyses will comply with Good Laboratory Practices (GLPs). This includes descriptions of maintenance, inspections of instruments, and acceptance testing of instruments, equipment, and their components, as well as the calibration of such equipment and the maintenance of all records relating to these exercises. Documentation to be included with the final report(s) from each study will include field logs for the collection or generation of the samples, chain of custody records, and other QA/QC documentation as applicable. Requirements for analyses, sample matrices, parameters, and standard operating procedures are listed in Table 2 – Table 4.

12. **Endpoint of Study:** Completion of all chemical, biological, and statistical analysis; 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.

13. Schedule of Study and Expected Outputs: Field collections will be conducted in July 2008. Laboratory analyses will be completed by March 2009 with a draft report in review by May 2009.

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

15. Relationship to Cooperator Needs: The Department of Interior and the State of Missouri seek to demonstrate injury to biological resources. Crayfish play an important role in Ozark streams because of their ecological dominance (Rabeni et al. 1995; Whitledge and Rabeni 1997), and because they are a primary prey of sport fishes such as smallmouth bass (*Micropterus dolomieui*), rock bass (*Ambloplites rupestris*), and longear sunfish (*Lepomis megalotis*) (Probst et al. 1984; DiStefano 2005). The research conducted within this study plan has been specifically requested by the Missouri Department of Natural Resources and U.S Fish and Wildlife Service as a part of an NRDAR case. Data will be used in various regulatory and management programs related to the effects of mining on aquatic ecosystems.

16. Literature Cited:

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16. Signatures:

Prepared by: Ann L. Allert Date: 7/7/08
Ann L. Allert

Approved by: C Tyrrell for S Jones Date: 7/8/08
Susan B. Jones

Approved by: Edward Little Date: 7/8/08
Edward E. Little
Ecology Branch Chief

Approved by: Ryan Warburtin Warbritton Date: July 8, 08
Ryan ~~Warburtin~~ Warbritton
Animal Care and Use Committee Chair

Approved by: Paul R. Heine Date: 7/8/08
Paul R. Heine
Quality Assurance and Safety Officer

Approved by: C Tyrrell for M Mac Date: 7/8/8
Michael J. Mac
Center Director

Table 1: List of probable study sites.

Site ID		Classification[1]	River mile	Abundance Estimates	In-situ Cage Study	Comments
R-1	Above Irondale – downstream of Cedar Creek confluence	Low	-21	Yes	Yes	
R-2	Irondale (Hwy U)	Low	-19	Yes	Yes	
TH-1	Desloge (Hwy 67 bridge upstream of Flat River)	High	-5	Yes		
TH-2	Hwy K	High	6	Yes	Yes	
TM-1	Hwy 67 (north of Bonne Terre)	Medium	14	Yes	Yes	
TM-2	Mammoth Access	Medium	38	Yes		
TL-1	Washington State Park (upstream of Mineral Fork)	Low	33	Yes		
TL-2	Upstream of Cedar Hill Mill Dam	Low	49	Yes		Riffles have not been located at this site.

[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)
				Dissolved and particulate organic carbon (20 µg/L)
				Sulfates (1 mg/L)
				Particle size analysis (10% by class)
				Alkalinity and hardness (2 mg/L)
				Total organic carbon (sediment) (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	YSI or 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, Detritus	1 per 20 analyses	Metals, water quality, PSA, carbon analyses	Monitors instrumental precision
Analytical Spike	Crayfish, Water, Detritus	1 per analytical run per matrix	Metals	Monitors instrumental accuracy
Laboratory Control Sample	Crayfish, Water, Detritus, Sediment	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, Detritus, Sediment	1 per analytical run	Metals, YSI or 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	Dissolved organic carbon	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	Particle size	B5.179, APHA 2005
Metals	Crayfish, detritus, sediment, water	SOPs C5.5, P.485, P.259, P.221, P.510, P.198, P.256, P.207
Habitat variables	Velocity, depth, in-situ substrate quality	See attached 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 = $3 \times (SD_b^2 + SD_s^2)^{1/2}$ where

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

Table 4: Proposed water quality, sediment, and biotic variables to be measured.

Matrix	Variable	No. Reps / Site	Where measured
Surface	Temperature	1	In situ
Surface	pH	1	In situ
Surface/ pore water	Conductivity	1	In situ
Surface	Dissolved Oxygen	1	In situ
Surface	Turbidity	1	In situ
Surface/ pore water	Alkalinity	1	Lab
Surface/ pore water	Hardness	1	Lab
Surface/ pore water	DOC	1	Lab
Surface/ pore water	Sulfate	1	Lab
Pore water Pore water	Selected metals Selected metals	2 2 per date	Lab Field
Surface	Nutrients (NH_3, TN, TP, SRP, NO_2/NO_3)	1	Lab
Crayfish	Density	20 kicks per site	In situ
Crayfish	Mortality	6	In situ
Crayfish-density	Selected metals	3 composited samples per site	Lab
Crayfish-cage	Selected metals	3 composited samples per date	Lab
Fish, Invertebrates	Selected metals	2	Lab
Detritus	Selected metals	2	Lab

Table 5: Proposed habitat and sediment quality variables to be measured.

Matrix	Variable	No. Reps / Site	Where measured
Surface water	Current velocity	1- 4 riffles	In situ
Surface water	Depth	1-4 riffles	In situ
Sediment	Sediment particle size characterization	20	In situ
Sediment	Fine particle characterization	1	Lab
Sediment	Sediment carbon	2	Lab
Sediment	Selected metals	2	Lab
Surface water	Stream order	1	Lab
Site	GPS	1-4 (each riffle)	In situ
Site	Watershed area	1	Lab
Site	Land use	1	Lab
Site	Stream discharge	1	In situ

Table 6: Proposed project budget. NA = not applicable.

Category	Variable	Cost
Analytical	Water quality	1,500
Analytical	Metals – sediment, water and biota	23,825
Analytical	Landscape characterization	NA
Salary		20,000
Travel		7,000
Misc Field Supplies		3,200
Sub-Total		60,525
Overhead, 7%		4,236.75
Total		64,761.75

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. Velocity will also be measured at 2 cm above the substrate surface. Record velocity in m/sec; depth in cm.

Depth measurements:
Water depth will be measured using a standard depth gauge. Record depth in cm.

Surface substrate composition measurements:
A grid (e.g., a piece of rebar welded into an 'X' with each length measuring 0.5 m) 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 (<2 mm diameter), gravel (2 mm to 16 mm diameter), pebble (17 mm to 64 mm diameter), cobble (65 mm to 250 mm diameter), boulder (> 256 mm diameter), flat bedrock and irregular bedrock.

Each of those categories is assigned a numerical value (Bain et al. 1985):

Sand/silt = 1.0, bedrock = 1.0, gravel = 2.0, pebble = 3.0, cobble = 4.0, boulder = 5.0, irregular 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 location along each transect.

Appendix 2: Surface Substrate Composition, Current Velocity and Depth at Quadrat Sample Locations

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

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

Methods: Quadrat samples will be taken in three riffles. Placement will be determined randomly. Quadrat samples will be numbered by site number-riffle-number of quadrat with riffle (e.g., 1-1-5; 1-2-4; 1-3-3). Quadrat samples within riffles will be numbered in the order of which they are taken.

Velocity Measurements:
Velocity measurements will be taken immediately adjacent to the quadrat sampler because samples taken inside the quadrat would be affected by the actual sampler. Velocity will be measured 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), and at 2 cm above the substrate surface. Record velocity in m/sec.

Depth measurements:
Water depth will be measured in the middle of the 1-m^2 quadrat sample using a standard depth guage, and the spot where the velocity measurement is taken. Record 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 quadrat sample. 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 (<2 mm diameter), gravel (2 mm to 16 mm diameter), pebble (17 mm to 64 mm diameter), cobble (65 mm to 250 mm diameter), boulder (> 256 mm diameter), flat bedrock and irregular bedrock.

Each of those categories is assigned a numerical value (Bain et al. 1985):
Sand/silt = 1.0, bedrock = 1.0, gravel = 2.0, pebble = 3.0, cobble = 4.0, boulder = 5.0, irregular 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 quadrat sample.

Appendix 3: Canopy Cover Method

Have one person record and give visual estimate of bank vegetation shade and the other person read the densiometer.

Densiometer

1. Find transect that has been marked for depth/velocity measurements.
2. Stand on the transect with the densiometer 0.3 m from the left bank or nearest bank. (Left and right directions when facing downstream.).
3. Hold the densiometer 0.3 meters above the water surface.
4. Hold the densiometer so that it is level using the level bubble indicator and the top of your head just touches the point of the "V" as in Figure 1.

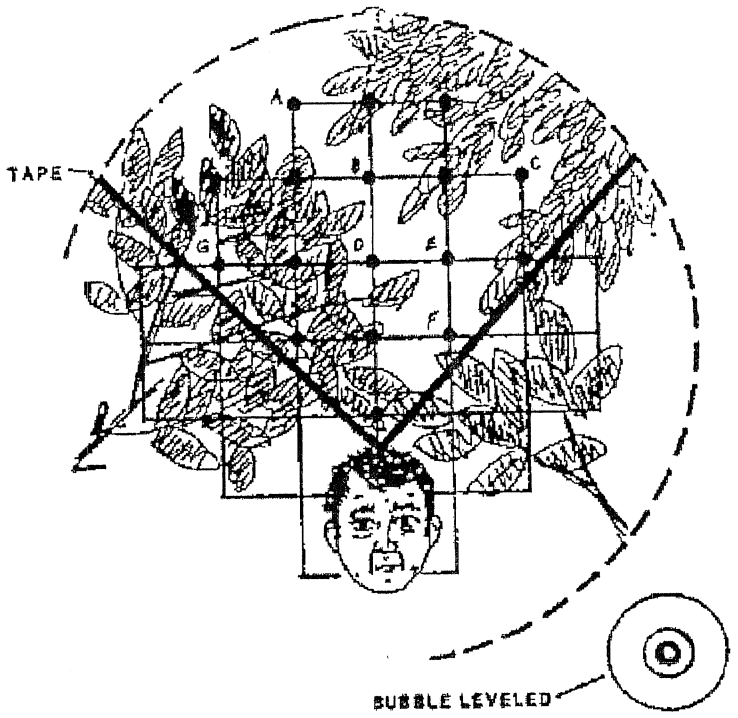

Figure 1. Schematic of modified convex spherical canopy densiometer. In this example, 10 of the 17 intersections show canopy cover, giving a densiometer reading of 10. Note proper positioning with the bubble leveled and the head reflected at the apex of the "V." (Mulvey et al. 1992).

5. Count the number of points covered by vegetation. Values will be between 0 for completely open and 17 for completely covered canopy.
6. Have the recorder record the value on the canopy cover form under "Left Bank".
7. Repeat steps 3 through 6 at the channel center facing upstream, the right bank, downstream and left bank. Record on the canopy cover form "Center-UP", "Center-Right", Center-Downstream", and "Center-Left". (Left and right directions when facing downstream.).

8. Repeat steps 2 through 6 for the right bank or farthest bank (Left and right directions when facing downstream.). Record on the canopy cover form "Right Bank". At this point you should have six measurements for the transect: four from the center and one at each bank.
9. Repeat steps 1 through 8 for each transect and record on a separate line of the canopy cover form.

Appendix 4: Crayfish, Fish, Detritus, Metal Samples

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

Crayfish: After crayfish are collected from quadrat samples, 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.

After crayfish are collected from cages, identified, sexed and measured, they should be placed in 4-oz. pre-cleaned polypropylene (PP) jars. All crayfish from a cage at each site should be placed in separate 4-oz. PP pre-labeled jar **(i.e., for a site, there should be three 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, Co, Cu) in whole crayfish.

Fish: After fish are collected from kick seines or electrofishing, identified, and measured, they should be placed in 4-oz. pre-cleaned polypropylene (PP) jars. Jars should be placed on ice until they can be frozen at hotel or CERC.

Data to be taken: Total number of fish; total length (mm); metals (Pb, Cd, Zn) in whole fish.

Detritus: After detritus is collected from kick seines or d-nets, it should be placed in 4-oz. pre-cleaned polypropylene (PP) jars. Jars should be placed on ice until they can be frozen at hotel or CERC.

Data to be taken: Metals (Pb, Cd, Zn, Ni, Co, Cu) 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 1-gal pre-cleaned 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, 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; 1-gal 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 1-gal sub-surface grab sample.

4. From 1-gal 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). See Appendix 6 for additional information.

5. Place remaining 1-gal sample for all other WQ (e.g., alkalinity, hardness, turbidity, sulfate, ammonia). Preserve on ice until processing.

6. Samples will be filtered at CERC or hotel for sulfate (separate 125-ml bottle) and ammonia (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 sample prior to filtration); data sheets.

1. Take out surface water ammonia 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 alkalinity, hardness, and turbidity analysis is started.

4. Ammonia 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 samples. Pre-filtration blanks for ammonia should be acidified with sulfuric acid.

5. Measure the **amount filtered** for ammonia, POC, TSS. Record on data sheet. Acidify samples with 2 drops of sulfuric acid. Between each sample, rinse well with RO water. Ammonia 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. Post-filtration blanks for ammonia samples should be acidified.

7. COC forms can be filled out daily. COC should be kept separately for metal samples, ammonia, 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 2 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.