

CERC Research Study Plan Title: Madison County Mines Site Resource Damage Assessment: Crayfish populations and in-situ toxicity testing of crayfish in the Little St. Francis River drainage

CERC Tracking # (provided when proposal submitted):

BASIS+ Project/Task/Subtask Number: SB00C2G Task 2

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USGS/BRD Center: Columbia Environmental Research Center

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I. Rational and Justification:

Southeast Missouri Lead Mining District (SEMOLMD) has been mined for lead-zinc ore for about 300 years. Metals, including lead, zinc, copper, cadmium, cobalt, and nickel were exploited to various degrees. Extraction and processing ore left a legacy of elevated concentrations of metals in stream sediment, water, and biota. The Madison County Mines (MCM) Superfund Site, located near Fredericktown, Missouri, is the southeastern end of the SEMOLMD. The principle drainage system for the MCM site is the Little St. Francis River and its tributaries. Contaminated areas in the MCM (e.g., chat, tailings, and contaminated soils) continue to be potentially primary sources of metals to the aquatic biota in the Little St. Francis River (USFWS 2015). Contaminated sediment provides exposure through incidental ingestion and by increasing metals concentrations in pore water of the sediment. Groundwater and surface water are also exposure paths for aquatic biota. Sediment metals concentrations in the Little St. Francis River have been found to be high enough to cause adverse effects to aquatic communities (Wooster-Brown 2006).

Crayfish are important to the ecology and economies of the Ozarks (DiStefano 2005). They eat/process live, dead and decaying plants as well as animal matter, and dominate invertebrate biomass in Ozark streams. They are prey for more than 200 animals, and are the primary food of popular sport fishes (e.g., smallmouth bass, rock bass, shadow bass; DiStefano 2005; Hobbs 1993). Crayfish have been identified as a critical ecosystem component of intermediate trophic position that may provide critical roles in facilitating both upward and downward flow of nutrients and energy (Momot 1995; Rabeni et al. 1995). It is likely that loss of crayfish populations in some mining-impacted areas leads to decreased rates of detrital decomposition thereby affecting functional processes in the

Ozark Plateau (Allert, unpublished data). Leachates from tailings piles and soils enter groundwater supplies for Ozark streams and have led to contaminated aquatic food chains and loss of biota including crayfish and sculpin (Allert et al. 2008, 2009a, 2009b, 2013, 2013; Besser et al. 2007). In-stream studies have revealed that crayfish are absent from locations immediately adjacent to lead mining operations and caged studies of crayfish have demonstrated a significant inverse relationship between survival of juvenile crayfish and dissolved trace metals in pore-water in the SEMOLMD and other mining districts in Missouri (Allert et al. 2008, 2009a, 2012, 2013).

II. Objectives (What):

We propose to conduct a two-tier investigation that includes a survey of crayfish populations and in-situ toxicity tests using endemic crayfish in the Little St. Francis River watershed in Madison County. Two candidate crayfish species for federal listing, *Orconectes quadruncus* and *Orconectes peruncus*, are found in the watershed and may be potentially impacted by metals contamination. Recent studies have demonstrated that the loss of endemic crayfish species alters macroinvertebrate structural and functional communities (Charlebois and Lamberti 1996; Freeland-Riggert 2014; Hanson et al. 1990). Data provided in these studies can be used to evaluate whether metals contamination in the Little St. Francis River cause injury to aquatic organisms and to wildlife, including migratory birds, which depend on those aquatic organisms. Analytical samples will be taken in support of biological studies. Trace metals concentrations in surface water, pore water, sediment, and biota (crayfish, fish, and other macroinvertebrates) will provide information to assess exposure of metals through food-web pathways.

III. Listing of Studies:

A. Study 1

1. **Principal Investigator(s):** Ann L. Allert, William G. Brumbaugh, Danielle Cleveland, Robert J. DiStefano (Missouri Department of Conservation)
2. **Specific Objectives:** a) Determine crayfish riffle/run densities and crayfish species composition in the Little St. Francis River drainage of southeast Missouri; b) measure selected metals (Pb, Zn, Cd, Co, Ni, Cu) concentrations in surface water, sediment, and crayfish as measures of metals exposure; c) characterize physical habitat in riffles/runs used by crayfish and water quality conditions of the Little St. Francis River drainage; and d) evaluate relationships among crayfish riffle/run densities, concentrations of mining-derived metals in water, sediment, and crayfish, and other water and physical habitat characteristics.
3. **Experimental Design or Methodological Approach:** Sites to be sampled will be selected based on data collected in previous studies that characterized metals concentrations in water, sediment, and animal tissue (MDNR 1986, 2004, 2005, 2010; USEPA 2006) and from pre-assessment X-ray fluorescence (XRF) meter data

collected for metals concentrations in sediment. We propose that eight sites be distributed within the Little St. Francis River drainage that are upstream, directly downstream, and remotely downstream of Operable Unit 2 (OU2; i.e., tailings or chat piles, mines).

Crayfish density: A maximum of eight sites will be sampled. If possible, two sites will be located upstream of the mining impact area and/or in a tributary(ies) of the Little St. Francis River without mining-sourced material. If possible, sites will be selected which have 1–6 riffle/run complexes. At each site, quantitative crayfish samples will be collected within 1–6 riffle/run complexes.

Crayfish will be sampled in riffle/runs by disturbing the substrate inside a 1-m² weighted polyvinyl chloride (PVC) quadrat frame placed on the stream bottom directly upstream of a kick seine (1.5 m length by 1.5 m height) with a 3-mm diameter delta mesh (Allert et al. 2012; DiStefano et al. 1993, 2003, 2009a; Williams et al. 2014). Sampling will begin at downstream ends of riffles and proceed upstream. At each site, a total of 21 kick samples will be obtained by distributing 21 samples between 1–6 riffle/runs at that site. Adult crayfish collected will be identified to species (Pfleiger 1996), examined to determine sex, measured for carapace length (from the tip of rostrum to the posterior edge of the cephalothorax to nearest 0.1 mm), and released. Voucher specimens and unidentifiable crayfish will be placed on ice, returned to Columbia Environmental Research Center (CERC) for identification and archived in the walk-in freezer.

Crayfish trace metals: At each site, we propose to take specimens of *O. quadruncus* collected during the population study at random for metals analysis. Three replicate composites of 3–5 crayfish will be taken at each site; one from each of the riffle/runs sampled. ***Individuals taken for metals analyses should be the same species for all riffles and sites and will be identified on datasheet (Appendix 1). In addition, crayfish should be similar in size across all riffles and sites.*** If more than one species is required per riffle or site for the metals samples because of the low number of individuals of the target species, only one species should be placed in each sampling jar. All samples for metals analyses will be placed in pre-cleaned jars, stored on ice until they are returned to CERC, where they will be frozen until preparation for analyses. Whole crayfish will be freeze-dried (SOP P.259) and cryogenically pulverized to a powder-like consistency (SOP P.213). Sub-samples (0.25 g dry) will be digested using a mixture of concentrated nitric and hydrochloric acids and with heating in a laboratory microwave oven (SOP P.636). The digestates will be analyzed using inductively-coupled plasma mass spectrometry (ICP-MS) for Pb, Zn, Cd, Ni, Co, and Cu (SOP P.241; Allert et al. 2008; Appendix 2).

Habitat measurements: Sites will be identified using a Thales® Mobile Mapper global positioning system (GPS) receiver. Current velocity and depth will be measured at each crayfish sampling location (i.e., kick seine quadrat), and along transects set across each riffle using Marsh McBirney® flow meter or a Hach® FH950.0 flow meter and depth rod. Substrate composition will also be assessed using

visual methods at each crayfish seining location (Appendices 3 and 4). Stream discharge will be calculated at each site. When possible, we will determine or calculate selected landscape variables such as watershed area, stream order, land use area, and area of mining-related materials using geographic information system (GIS) or other mapping tools.

Surface water: general water quality: Surface water quality analyses (i.e., temperature, pH, conductivity, dissolved oxygen, turbidity) will be measured in situ three times (in each riffle, if present) within a site with a multi-parameter water quality instrument (i.e., Hydrolab[®] Quanta). A sub-surface water grab sample three times (in each riffle, if present) 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] APHA 2005), major cations (Na, K, Mg, Ca, Fe, Mn, Sr) by ICP-MS (SOP P.241; anions (F, Cl, NO₂/NO₃, Br, SO₄, PO₄, by ion chromatography; SOP P.705; USEPA Method 300); and dissolved organic carbon by combustion and infrared detection; SOP P.722; USEPA Method 415.2). See Appendices 5 and 6.

Surface water: trace metals: From each surface water sample, an aliquot will be taken for metals analyses using a polypropylene syringe and will be filtered through a 0.45- μ m pore-size polyethersulfone membrane housed in a polypropylene cartridge (SOP P.566). Filtered samples will be transferred to 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; Appendix 5). Filtration blanks will be taken at the time of sample collection.

Sediment trace metals and carbon: At each site, a sediment sample will be taken for bulk metals analysis (Brumbaugh et al. 2007; Besser et al. 2009). Sediment samples will be sieved using a 2-mm sieve bucket in the field and a 250- μ m sieve in the laboratory. Samples of both fractions from each site will be analyzed using ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu following a total recoverable digestion method (SOP P.636; Brumbaugh et al. 2007). A subsample of both sediment fractions from each site will be analyzed for inorganic and total carbon (APHA 2005). The organic fraction will be determined by subtraction.

B. Study 2

- 1. Principal Investigator(s):** Ann L. Allert, William G. Brumbaugh, Danielle Cleveland, Robert J. DiStefano (Missouri Department of Conservation)
- 2. Specific Objectives:** Evaluate growth and survival of young crayfish using in-situ cages in relation to metals exposure.
- 3. Experimental Design or Methodological Approach:**

Cage deployment: We will deploy cages at four sites to investigate associations between survival and growth of crayfish with metals exposure. Crayfish (preferably *Orconectes luteus* or *O. quadruncus*; Pflieger 1996) will preferably be collected as eggs of gravid females from uncontaminated sites in either the Little St. Francis River or the St. Francis River watershed. Sites used to collect brood stock will not be the same sites used in the population study or in-situ toxicity study. Coordinates of site locations of brood stock will be recorded. Females with egg masses will be transported to CERC for egg hatching and larval grow-out. Juvenile crayfish (approximately 10-mm carapace length) will be deployed in cages for a period of approximately 56 days. Proposed sampling dates for metals samples are days 0, 28, 56. Cages will be checked weekly for external biofouling and position in stream.

Cage design: Environmental conditions (primarily depth) will determine the type of cage used. 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). Cages will contain 10 crayfish, leaves, rock refugia, and supplemental food (Allert et al. 2008, Whitley and Rabeni 1997). Rocks (i.e., coarse gravel to small cobble collected from each site-specific location) and leaves will be wrapped within 6-mm mesh bags secured at the bottom of each cage with stainless steel wire. 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. The bottom of cages will be placed on or below the substrate surface to expose crayfish to pore water and allow access to benthic macroinvertebrates.

Leafpacks will be a source of food and habitat for caged crayfish (Momot 1995). Leaves of five tree species [black willow (*Salix nigra*), sycamore (*Platanus occidentalis*), cottonwood (*Populus fremontii*), white oak (*Quercus alba*), and shagbark hickory (*Carya ovata*)] will be pre-leached, dried at 100–105 °C and weighed (Fairchild et al. 1987). A pre-determined amount of leaves (50 g dry weight for stainless cages) will be added to each cage.

Detrital trace metals: Some leaves will be taken at day 0, 28, and 56 for metals analyses. After sampled leaves are dried and weighed, they will be ground for metals analyses. Leaves will be digested using concentrated nitric acid and microwave heating and analyzed by ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu (Allert et al. 2008; Appendix 2).

Crayfish trace metals: Three replicated samples consisting of 5–10 crayfish from the stock of cultured young-of-year crayfish will be sampled prior to stocking of crayfish into cages near (<3 days) day 0 for metals concentrations. Crayfish will be measured for carapace length (from the tip of rostrum to the posterior edge of the cephalothorax to nearest 0.1 mm), and weighed prior to being placed in pre-cleaned containers. Voucher specimens will be archived in a walk-in freezer at CERC. At days 28 and day 56, all crayfish within cages (n = 3 per sampling date) will be measured for carapace length, and weighed then placed into a pre-cleaned jar for metals 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 for metals analyses will be placed in pre-cleaned jars, stored on ice until they are returned to CERC, where they will be frozen until preparation for analyses. Whole crayfish will be freeze-dried (SOP P.259) and cryogenically pulverized to a powder-like consistency (SOP P.213). Sub-samples (0.25 g dry) will be digested using a mixture of concentrated nitric and hydrochloric acids and with heating in a laboratory microwave oven (SOP P.636). The digestates will be analyzed using ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu (SOP P.241; Allert et al. 2008; Appendix 2).

Fish trace metals: If stainless steel cages are used, fish (i.e., largescale stonerollers, *Campostoma oligolepis*, Pflieger 1997) will be collected from each cage site using seines, and if necessary, backpack electroshockers. Fish will be identified on site and total length (mm) of fish will be measured. 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 =3) from each site will be separated for residue analysis. Fish tissues will be digested using concentrated nitric acid and microwave heating and analyzed by ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu (Allert et al. 2009a, 2012). Minced fish for crayfish food will be placed in pre-cleaned containers and kept frozen until placed into cages. Crayfish will be fed the minced fish weekly at a ration of at least 5% crayfish body weight (Appendix 2).

Voucher specimens of fish will be placed in 10% formalin and returned to CERC for identification. Fish will be transferred to 80% ETOH after two weeks.

Macroinvertebrate trace 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 from leaf material remaining in each cage (n =3 per sampling date per cage per site) or in habitats with coarse substrate at each site using a kick net or seine (n =3 per sampling date per site). Targeted organisms will include a range of macroinvertebrates. Samples will be collected on days 0, 28 and 56. Samples will be kept frozen until metals analyses. Macroinvertebrates will be digested using concentrated nitric acid and microwave heating and analyzed by ICP-MS for Pb, Zn, Cd, Ni, Co, and Cu (Allert et al. 2009a, 2012; Appendix 2).

Surface-water water quality and trace metals: Water quality monitoring and samples will be taken on days 0, 28 and 56 at each cage site. 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 multi-parameter 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], chlorophyll *a* [chl *a*], and major cations (Na, K, Mg, Ca, Fe, Mn, Sr) by ICP-MS and anions (F, Cl, NO₂/NO₃, Br, SO₄, PO₄) by ion chromatography (APHA 2005; Appendices 4 and 5) will be collected. In-stream water quality conditions will be monitored using YSI[®] multi-parameter units

(temperature, pH, conductivity, dissolved oxygen) and Onset[®] TempPro units (temperature).

Pore-water water quality and trace 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 LDPE 24-mL containers with a 0.45- μ m polyethersulfone filter at the top of the container (Brumbaugh et al. 2007; Appendix 7). 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 days 1, 14, 28, and 42. Pore-water samples for metals analyses will be collected directly from the peeper. After retrieval, it will be sealed tightly in a pre-labeled zip-seal plastic bag, and transported on ice to CERC. Samples will be acidified to pH <2 with Ultrex[®] nitric acid within 96 hrs.

At some locations where cobble habitats might make burial deployment of peepers impractical, a push-point (also known as a drive point) probe may be used. The probe is a narrow stainless steel sealed tube having six small slits cut into the bottom over a 2-cm length. The tube is lined with FEP tubing and will have a polypropylene mesh pre-filter sleeve fitted over the bottom end before inserting into the sediment. The probe will be driven into the substrate to a depth of about 4 cm, and a 20-mL sample of pore water will be drawn by using syringe that is attached to the FEP tubing housed inside the probe tube. Once drawn, the sample will be immediately filtered through a 45- μ m polyethersulfone filter.

Pore-water quality (i.e., temperature, pH, conductivity, dissolved oxygen, alkalinity, hardness, and ammonia) will be measured (APHA 2005).

4. Listing of SOP Numbers and Titles: Requirement for analyses, sample matrices, parameters, and standard operating procedures are listed in Tables 2–4, and SOP numbers and titles are listed in Appendix 8.

5. 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 metals analyses of water, crayfish and detritus, and crayfish density; and caged crayfish survival and growth (Tables 5–6).

6. Statistical Treatment: Data will be analyzed using Release 9.4 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 (Table 2) 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.

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. See Tables 2–3 for additional quality assurance information.

8. Special Safety Requirements: Gloves are advised protection against infectious agents and parasites while handling crayfish and fish. A first aid kit will be present in all field vehicles. Gloves, lab coats/plastic aprons, and protective eye-ware should be worn during the processing of water, sediment, and biological samples. Department of the Interior (DOI) regulations state that all personnel should wear floatation devices when near water. 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. All USGS personnel and/or contractors who potentially could drive a government vehicle must complete the National Safety Council Defensive Driver's Training Course.

9. Animal Care and Use Requirements: All personnel involved in research activities involving live organisms must adhere to the 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. Animal care SOPs are listed in Table 4 and Appendix 8.

Ice is not commonly approved to euthanize fish; however, body size and species-specific thermal tolerance should be considered when assessing the effectiveness and appropriateness of ice or an ice-slurry for the humane killing of fishes (Blessing et al. 2010; Wilson et al. 2009). Despite current guidance documents, Wilson et al. (2009) showed ice had a faster time to death and fewer signs of distress in small fish. The target species (either Largescale Stoneroller, *Camptostoma anomalum* or Central Stoneroller, *Camptostoma oligolepis*) are considered to be warm-water fish (e.g., avoids water temperatures <9 °C; Wismer and Christie 1987) and are small-bodied fish (average size <90 mm). Given this, we feel that the use of ice will be a humane, effective, and safe method of euthanasia.

We will have a contingency plan to use tricaine methanesulfonate (MS222) as our chemical anesthetic (dose >250 mg/L) on collected fish, if ice proves to be ineffective. Concentration solutions will be packaged securely and returned to CERC for disposal.

10. Biosecurity Requirements: We will comply with all CERC guidelines for the transfer of live organisms (LOTR). Transfer Request Forms will be completed prior

to crayfish being transferred (Appendix 9). We will use a modified version of CERC Fish Transfer Treatment Procedure (Appendix 10) to treat crayfish transferred to CERC's quarantine room.

We will comply with CERC Hazard Analysis Critical Control Point (HACCP) plans for the transfer of field-collected biological, water, and sediment samples and field-deployed passive samplers (Appendix 11). We will also comply with CERC HACCP plan for field work (Appendix 11; in review). Missouri Department of Conservation will comply with their cleaning and disinfecting protocols (Appendix 12).

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 and 4.

12. Endpoint of Study, Based on Accomplishments: Endpoint of study will be the completion of all chemical, biological, and statistical analyses and a 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 the Outputs Expected: Field collections will be conducted in June through August 2015, conditions permitting. Laboratory analyses will be completed by June 2016 with a draft report in review by December 2016. Annual progress reports will be provided by September 30th.

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

15. Relationship to Cooperator Needs: The Department of the Interior and the State of Missouri seek to determine injury to biological resources. Crayfish play an important role in Ozark streams because of their ecological dominance (Momot 1995; Rabeni et al. 1995; Whitley and Rabeni 1997), and because they are a primary prey of sport fishes such as smallmouth bass (*Micropterus dolomieu*), rock bass (*Ambloplites rupestris*), and longear sunfish (*Lepomis megalotis*), as well as prey for over 200 aquatic and terrestrial animals, including migratory waterfowl (Hobbs 1993; 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 a National Resource Damage Assessment

and Restoration. 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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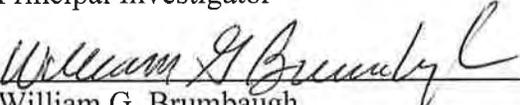
Wilson, J.M., Bunte, R.M., and A.J. Carty. 2009. Evaluation of rapid cooling and tricaine methansulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). *Journal of the American Association for Laboratory Animal Science* 48(6):785-789.

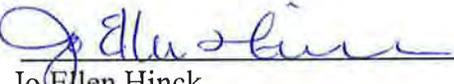
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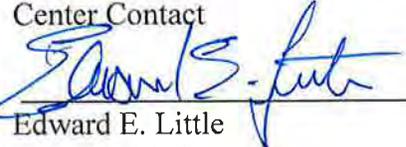
Wooster-Brown, C. 2006. Draft Report Madison County Mine Site Ecological Risk Assessment: Ecological Risk Assessment Madison County Mine Operable Unit 3 Superfund Site CERCLIS ID# MODO98633415-OU3. U.S. Environmental Protection Agency, Region 7, Kansas City, Kansas. 80pp.

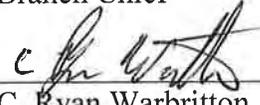
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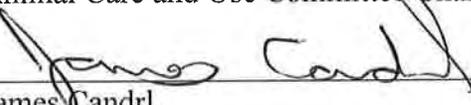
Prepared by:  Date: 4/21/15
Ann L. Allert
Principal Investigator

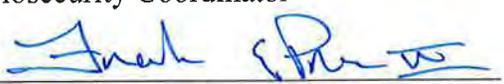
Prepared by:  Date: 4/21/15
William G. Brumbaugh
Research Chemist

Approved by:  Date: 4/21/15
Jo Ellen Hinck
Center Contact

Approved by:  Date: 05/20/15
Edward E. Little
Branch Chief

Approved by:  Date: May 06, 15
C. Ryan Warbritton
Animal Care and Use Committee Chair

Approved by:  Date: 5/7/15
James Candl
Biosecurity Coordinator

Approved by:  Date: 14 MAY 15
Frank Proa
Quality Assurance and Safety Officer

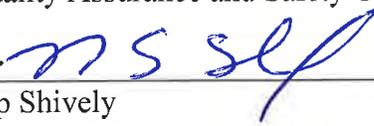
Approved by:  Date: 15-May-15
Rip Shively
Center Director

Table 1: List of proposed study sites for population study and in-situ toxicity test and metals concentrations in dried sediments as determined by X-ray fluorescence (XRF) meter data.

| Site ID | Description | Classification ^a | Abundance Estimates | In-situ Cage Study | Comments |
|---------|---|-----------------------------|---------------------|--------------------|----------|
| 1 | Upper Little St. Francis River off Tillman Road | Low | Yes | Yes | |
| 2 | Little St. Francis River downstream of Village Creek | | Yes | | |
| 3 | Saline Creek at South Chamber Street (City of Fredericktown Police Training Facility) | | Yes | Yes | |
| 4 | Little St. Francis River downstream of Saline Creek | | Yes | Yes | |
| 5 | Little St. Francis River downstream of Madison CR 504 (downstream of The Narrows) | | Yes | Yes | |
| 6 | Little St. Francis River at MDC Thompson Ford Access | | Yes | | |
| 7 | Chapel Creek off Madison CR507 | Low | Yes | | |
| 8 | Lower Little St. Francis River off CR527 | | Yes | | |

Table 1 (continued)...

| Sampling Location | Sediment size fraction | Mean (ug/g or mg/kg DW) | | | | | | | | |
|--|------------------------|-------------------------|-----------------------|-----------|------------------|----------|----------------|--|--|--|
| | | Pb | Cd ^a | Zn | Ni | Cu | Co | | | |
| Little St. Francis River at Tillman Road, St. Francis County, MO | <2mm | n=4 45.5 | n=4 <LOD ^b | n=4 47.3 | n=4 17.0 | n=4 20.5 | n=4 <LOD | | | |
| | >2mm | n=4 21.1 | n=1 6.0 | n=4 18.6 | n=4 8.3 | n=4 11.8 | n=4 <LOD | | | |
| Little St. Francis River dwnstrm Village Creek | <2mm | n=11 594.0 | n=3 17.0 | n=11 32.0 | n=11 24.0 | n=4 8.0 | n=8 <LOD | | | |
| | >2mm | n=4 405.3 | n=3 16.0 | n=4 38.8 | n=2 21.0 | n=4 <LOD | n=4 <LOD | | | |
| Saline Creek @ Police Training Facility (Off South Chamber) | <2mm | n=4 29.0 | n=2 11.0 | n=4 19.0 | n=4 68.0 | n=1 6.0 | n=3 44.0 | | | |
| | >2mm | n=4 15.0 | n=4 <LOD | n=4 18.2 | n=4 50.5 | n=4 <LOD | n=4 <LOD | | | |
| Little St. Francis dwnstr of Saline Creek | <2mm | n=4 811.0 | n=2 12.0 | n=4 24.0 | n=4 91.0 | n=4 23.0 | n=2 100 | | | |
| | >2mm | n=4 826.3 | n=2 11.0 | n=4 29.5 | n=4 105.5 | n=4 15.8 | n=1 51.0 | | | |
| Little St. Francis River dwnstr of The Narrows (off CR504) | <2mm | n=4 453.0 | n=3 9.0 | n=4 35.0 | n=4 89.0 | n=3 10.0 | n=2 55 | | | |
| | >2mm | n=4 352.8 | n=4 <LOD | n=4 24.9 | n=4 98.3 | n=3 11.3 | n=2 128 | | | |
| Little St. Francis River at Thompson Ford Access | <2mm | n=4 382.8 | n=4 <LOD | n=4 38.5 | n=4 78.5 | n=4 21.3 | n=4 <LOD | | | |
| | >2mm | n=4 76.7 | n=1 11.0 | n=4 46.5 | n=4 140.3 | n=4 57.3 | n=1 558 | | | |
| Chapel Creek off CR507, near Fredericktown, MO | <2mm | n=5 28.0 | n=5 <LOD | n=5 20.4 | n=5 16.2 | n=5 12.4 | n=5 <LOD | | | |
| | >2mm | n=5 20.1 | n=2 9.5 | n=5 19.0 | n=5 12.0 | n=5 14.5 | n=5 <LOD | | | |

| Sampling Location | Sediment size fraction | Mean (ug/g or mg/kg DW) | | | | | | | | | | | |
|--|------------------------|-------------------------|--------------|-----------------|-------------|------|--------------|------|-------------|------|--------------|------|-----------------------|
| | | Pb | | Cd ^a | | Zn | | Ni | | Cu | | Co | |
| Little St. Francis River off CR527, near Fredericktown, MO | <2mm | n=10 | 110.3 | n=10 | 8.7 | n=10 | 23.4 | n=10 | 43.2 | n=10 | 16.0 | n=1 | 33.0 |
| | >2mm | n=10 | 70.2 | | <LOD | n=10 | 44.8 | n=10 | 57.5 | n=10 | 12.8 | n=10 | <LOD |
| Probable Effects Concentrations ^e (above which harmful effects likely) | | | 128.0 | | 4.98 | | 459.0 | | 48.6 | | 149.0 | | ND^d |

^a Based pre-assessment XRF data and PEC values developed by MacDonald et al. (2000).

^b Missouri Department of Natural Resources XRF LOD is >20 ppm for Cd.

^c LOD = Level of Detection.

^d MacDonald et al. (2000).

^e Not determined by MacDonald et al. 2004. Canadian Maximum Concentration 110 µg/L or 4 µg/L 30-day average (Nagpal 2004; http://www.env.gov.bc.ca/wat/wq/BCguidelines/cobalt/cobalt_tech.pdf)

Table 2: Proposed 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 ($\pm 0.2^{\circ}\text{C}$) |
| | | | | pH (± 0.2 unit) |
| | | | | Turbidity ($\pm 5\%$ of reading ± 1 NTU @ temperature of calibration) |
| | | | | Conductivity (100 $\mu\text{mhos/cm}$) |
| | | | | Dissolved oxygen (± 0.2 mg/L ≤ 20 mg/L) |
| | | | | Metals (varies) |
| | | | | Nutrients (varies) |
| | | | | Dissolved and particulate organic carbon (20 $\mu\text{g/L}$) |
| | | | | Chlorophyll <i>a</i> (0.5 $\mu\text{g/L}$) |
| | | | | Cations/anions (varies) |
| | | | | Total suspended solids (1 mg/L) |
| | | | | Alkalinity and hardness (2 mg/L) |
| | | | | Total organic carbon (sediment) (20 $\mu\text{g/L}$) |
| Habitat | | | | GPS (10 m) |
| | | | | Stream order, watershed area, mining-related materials (varies) |

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 multi-parameter meter, trace metals | Measures precision of sample collection and degree of environmental variability |
| Filtration Blanks | DI water | 1 per field samples | Metals, water quality parameters | Monitors procedural contamination |
| Analytical duplicate or triplicate | Crayfish, Water, Detritus | 1 per 20 analyses | Metals, water quality parameters, 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 parameters, 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 parameters, carbon analyses | Monitors accuracy |

Table 4: Proposed 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, instrument manuals |
| Water | pH | SOPs B4.14; B4.56, B4.62, B5.239, APHA 2005, instrument manuals |
| Water | Conductivity | Proposed, APHA 2005, instrument manuals |
| Water | Dissolved oxygen | Proposed, APHA 2005, instrument manuals |
| Water | Turbidity | SOP B4.42, APHA 2005, instrument manuals |
| Water | Total suspended solids | SOP F6.2.15.022696 |
| Water | Alkalinity | SOP B4.16, APHA 2005 |
| Water | Hardness | SOP B5.95, APHA 2005 |
| Water | Anions | SOP P.705 |
| Water | Cations | SOP P.241 |
| Water | Nutrients | F6.2.22, APHA 2005, instrument manuals |
| Water | Dissolved organic carbon, chlorophyll <i>a</i> | SOP P.722, B5.37, APHA 2005, instrument manuals |
| Crayfish | Animal care | B5.72.091997, B5.13.052693, P.691 (B5.148.030789), P.683 (B5.154.091997), P.690 (B5.160.013189), B5.165.091997, B5.240.060392, F5-15-020488, P.691 |
| Sediment | Carbon | SOP B4.36, F.6.20.1.082396, APHA 2005, instrument manuals |
| Sediment | Particle size | B5.179, APHA 2005 |
| Metals | Crayfish, detritus, sediment, water | SOPs C5.5, P.485, P.259, P.221, P.510, P.636, P.198, P.241, P.213, P.184, P.239 |
| 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) = $SD/Mean \times 100$

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, sediment, and biotic variables to be measured.

| Matrix | Variable | No. Reps / Site | Where measured |
|---|---|--|-----------------------|
| Surface | Temperature | 3 | In situ |
| Surface | pH | 3 | In situ |
| Surface/ pore water | Conductivity | 3 | In situ |
| Surface | Dissolved Oxygen | 3 | In situ |
| Surface | Turbidity | 3 | In situ |
| Surface/ pore water | Alkalinity | 3 / 1 | Lab |
| Surface/ pore water | Hardness | 3 / 1 | Lab |
| Surface/ pore water | DOC | 3 / 1 | Lab |
| Surface | Anions, cations | 3 | Lab |
| Surface/ pore water | Selected metals | 3 / 1 | Lab |
| Surface | Nutrients (NH ₃ , TN, TP, TSS, POC, chl <i>a</i>) | 3 | Lab |
| Crayfish | Density | 21 kicks | In situ |
| Crayfish | Mortality | 3 cages (n =10) per date per species | In situ |
| Population study: crayfish | Selected metals | 3 composited samples per date | Lab |
| Cage study: crayfish | Selected metals | 3 composites (max. n =10) per date per species | Lab |
| Cage study: leaves, invertebrates, fish | Selected metals | 3 | Lab |

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

| Matrix | Variable | No. Reps / Site | Where measured |
|---------------|---|---------------------------------------|-----------------------|
| Surface water | Current velocity | minimum of 7 per riffle/ 21 per site | In situ |
| Surface water | Depth | minimum of 7 per riffle / 21 per site | In situ |
| Sediment | Sediment particle size characterization | minimum of 7 per riffle; 21 per site | In situ |
| Sediment | Sediment carbon | 3 | Lab |
| Sediment | Selected metals | 6 (3 per fraction) | Lab |
| Site | Stream order | 1 | Lab |
| Site | Coordinates | 1-4 (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 to conduct a survey of crayfish populations and in-situ toxicity study using endemic crayfish in the Little St. Francis River watershed in Madison County, Missouri, USA.

| Study | Cost | Comments |
|--------------------|------------------|--------------------|
| Population Survey | \$162,019 | |
| Analytical Support | \$28,800 | |
| Cage | \$57,000 | |
| Analytical Support | \$40,500 | |
| | \$288,319 | Total |
| Overhead (7%) | \$20,182 | Overhead |
| | \$308,501 | Grand Total |

| | |
|---|-----------|
| Level 1- Population survey only | \$190,819 |
| Level 2- Population survey + cage study | \$288,319 |

| Item | In-Kind Contributions | Comments |
|--------------------------------------|------------------------------|---------------------|
| <i>Survey, Cage & Analytical</i> | <i>\$468,550.00</i> | |
| <i>Laboratory Study</i> | <i>\$50,000.00</i> | |
| <i>MDC</i> | <i>\$37,500.00</i> | |
| | <i>\$556,050.00</i> | <i>Total</i> |

Appendices

Appendix 1: Datasheets



United States Department of the Interior
U.S.GEOLOGICAL SURVEY
Columbia Environmental Research Center
4200 New Haven Road
Columbia, Missouri 65201

II

**SAMPLE BATCH HISTORY INFORMATION
FOR SAMPLES RECEIVED AT ECRC**

PROJECT LEADER, STUDY DIRECTOR,
OR BIOMONITORING SPECIALIST: _____

FY: _____ PROJECT OR STUDY #: _____ WU#: _____

DATE: _____ OTHER INFORMATION: _____

BRIEF DESCRIPTION OF BATCH: _____

TOTAL NUMBER OF SAMPLES IN BATCH: _____

TYPE(S) OF SAMPLES IN BATCH: _____

COLLECTED BY: _____

DATE(S) COLLECTED: _____

COLLECTION SOP'S : _____, _____

GENERAL GEOGRAPHICAL AREA(S) OF COLLECTION: _____

HOW ARE SAMPLES PACKAGED? _____

WHO PACKAGED SAMPLES? _____

METHOD(S) OF PRESERVATION: _____

NUMBER OF COOLERS, BOXES, ETC. COMPRISING BATCH: _____

WERE SAMPLES STORED BEFORE TRANSMISSION? _____

IF SO, STORAGE TIME, METHOD, AND LOCATION: _____

SAMPLE BATCH HISTORY INFORMATION (continued)

TRANSMISSION DATE: ___/___/___

TRANSMITTED FROM: _____

TRANSMITTED TO: _____

MODE OF TRANSMISSION (HOW ARE YOU SENDING THE SAMPLES?):

ANALYSES REQUESTED: _____

DESCRIBE ANY DOCUMENTS ACCOMPANYING THE SAMPLES: _____

ADDITIONAL COMMENTS: _____

SIGNATURE OF INDIVIDUAL ULTIMATELY RESPONSIBLE FOR THE SAMPLES:

NAME: _____

ADDRESS: _____

PHONE: () _____ - _____

FAX: () _____ - _____

| Particulate Carbon Analysis | | | | | |
|---|-------------|--|--------------------------|----------|--|
| Study: MCM NRDA 2015; USGS BASIS+ (SB00C2G) | | USGS/CERC Ann Allert, Project Director | | | |
| Sample batch ID : | | | | | |
| Meter: | | | | | |
| Sample collection date(s) : | | | | | |
| Run date : | | Technician : | | | |
| Date proofed/Init. | | Date proofed/Init. | | | |
| Sample ID | Standard ID | Sample volume (L) | Coulometer output (ug C) | Comments | |
| 1 | | | | | |
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notes:

ProPlus Calibration

Date of Calibration: _____ Technician: _____
Instrument Serial Number: _____ Software Revision: _____ Cable Model Number: _____
Temperature Reading _____ Temperature Accurate: Y N
DO Sensor in use: Polarographic Galvanic Sensor notated in Sensor menu? Y N
DO membrane changed? Y N Color of Membrane _____ Color notated in Sensor menu? Y N

Record the following calibration values:

| | Pre Cal | After Cal | |
|--------------|---------|-----------|---|
| Conductivity | _____ | _____ | |
| ORP | _____ | _____ | |
| DO | _____ | _____ | True Barometric Pressure at time of calibration _____ |

| | Pre Cal | |
|-------|---------|---|
| pH 7 | _____ | pH mV value _____ Range 0 mV \pm 50 mV |
| pH 4 | _____ | pH mV value _____ Range +165 to +180 from 7 buffer mV value |
| pH 10 | _____ | pH mV value _____ Range -165 to -180 from 7 buffer mV value |

NOTE: See pH Cal tips section for additional information. Span between pH 4 and 7 and 7 and 10 mV values should be \approx 165 to 180 mV. 177 is the ideal distance or 59 mV per pH unit.

Record the following diagnostic numbers after calibration, by viewing the .glp file and reading the values for the day's calibration

Conductivity Cal Cell Constant _____ Range 5.0 +/- 1.0 acceptable
DO Sensor Value (uA) _____ (For yellow membrane cap (1.25 mil PE), Avg 6.15 uA (min. 4.31 uA, max. 8.00 uA)
pH Slope _____ (\approx 55 to 60 mV/pH, 59 ideal)
pH Slope% of ideal _____ (93.2% to 101.7%, 100% ideal)

Notes: _____



Qualitative Habitat Evaluation Index and Use Assessment Field Sheet

QHEI Score:

Stream & Location: _____ **RM:** _____ **Date:** ___/___/06

Scorers Full Name & Affiliation: _____

River Code: _____ **STORET #:** _____ **Lat./ Long.:** _____ / 18 _____ **Office verified location**

1) SUBSTRATE Check **ONLY** Two substrate **TYPE BOXES**; estimate % or note every type present

Check ONE (Or 2 & average)

| | | | | | | | | | |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| BEST TYPES | | POOL RIFFLE | OTHER TYPES | | POOL RIFFLE | ORIGIN | | QUALITY | |
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AJ SAMPLED REACH
Check ALL that apply

Comment RE: Reach consistency/ Is reach typical of stream?, Recreation/ Observed - Inferred, Other/ Sampling observations, Concerns, Access directions, etc.

METHOD

- BOAT
- WADE
- L. LINE
- OTHER

DISTANCE

- 0.5 Km
- 0.2 Km
- 0.15 Km
- 0.12 Km
- OTHER

CLARITY

- 1st --sample pass-- 2nd
- < 20 cm
- 20-<40 cm
- 40-70 cm
- > 70 cm/ CTB
- SECCHI DEPTH

CANOPY

- > 85% - OPEN
- 55% - <85%
- 30% - <55%
- 10% - <30%
- <10% - CLOSED

Cj RECREATION

- POOL: >100ft² >3ft

STAGE

- HIGH
- UP
- NORMAL
- LOW
- DRY

DJ MAINTENANCE

- PUBLIC / PRIVATE / BOTH / NA
- ACTIVE / HISTORIC / BOTH / NA
- YOUNG-SUCCESSION-OLD
- SPRAY / SNAG / REMOVED
- MODIFIED / DIPPED OUT / NA
- LEVEED / ONE SIDED
- RELOCATED / CUTOFFS
- MOVING-BEDLOAD-STABLE
- ARMoured / SLUMPS
- ISLANDS / SCURED
- IMPOUNDED / DESICCATED
- FLOOD CONTROL / DRAINAGE

EJ ISSUES

- WWTP / CSO / NPDES / INDUSTRY
- HARDENED / URBAN / DIRT&GRIME
- CONTAMINATED / LANDFILL
- BMPs-CONSTRUCTION-SEDIMENT
- LOGGING / IRRIGATION / COOLING
- BANK / EROSION / SURFACE
- FALSE BANK / MANURE / LAGOON
- WASH H₂O / TILE / H₂O TABLE
- ACID / MINE / QUARRY / FLOW
- NATURAL / WETLAND / STAGNANT
- PARK / GOLF / LAWN / HOME
- ATMOSPHERE / DATA PAUCITY

FJ MEASUREMENTS

- \bar{x} width
- \bar{x} depth
- max. depth
- \bar{x} bankfull width
- bankfull \bar{x} depth
- W/D ratio
- bankfull max. depth
- floodprone x^2 width
- entrench. ratio
- Legacy Tree:

BJ AESTHETICS

- NUISANCE ALGAE
- INVASIVE MACROPHYTES
- EXCESS TURBIDITY
- DISCOLORATION
- FOAM / SCUM
- OIL SHEEN
- TRASH / LITTER
- NUISANCE ODOR
- SLUDGE DEPOSITS
- CSOs/SSOs/OUTFALLS

Circle some & COMMENT

Stream Drawing:

Site Characterization Dataform

Study: MCM NRDA 2015; USGS BASIS+ (SB00C2G)
USGS/CERC Ann Allert, Project Director

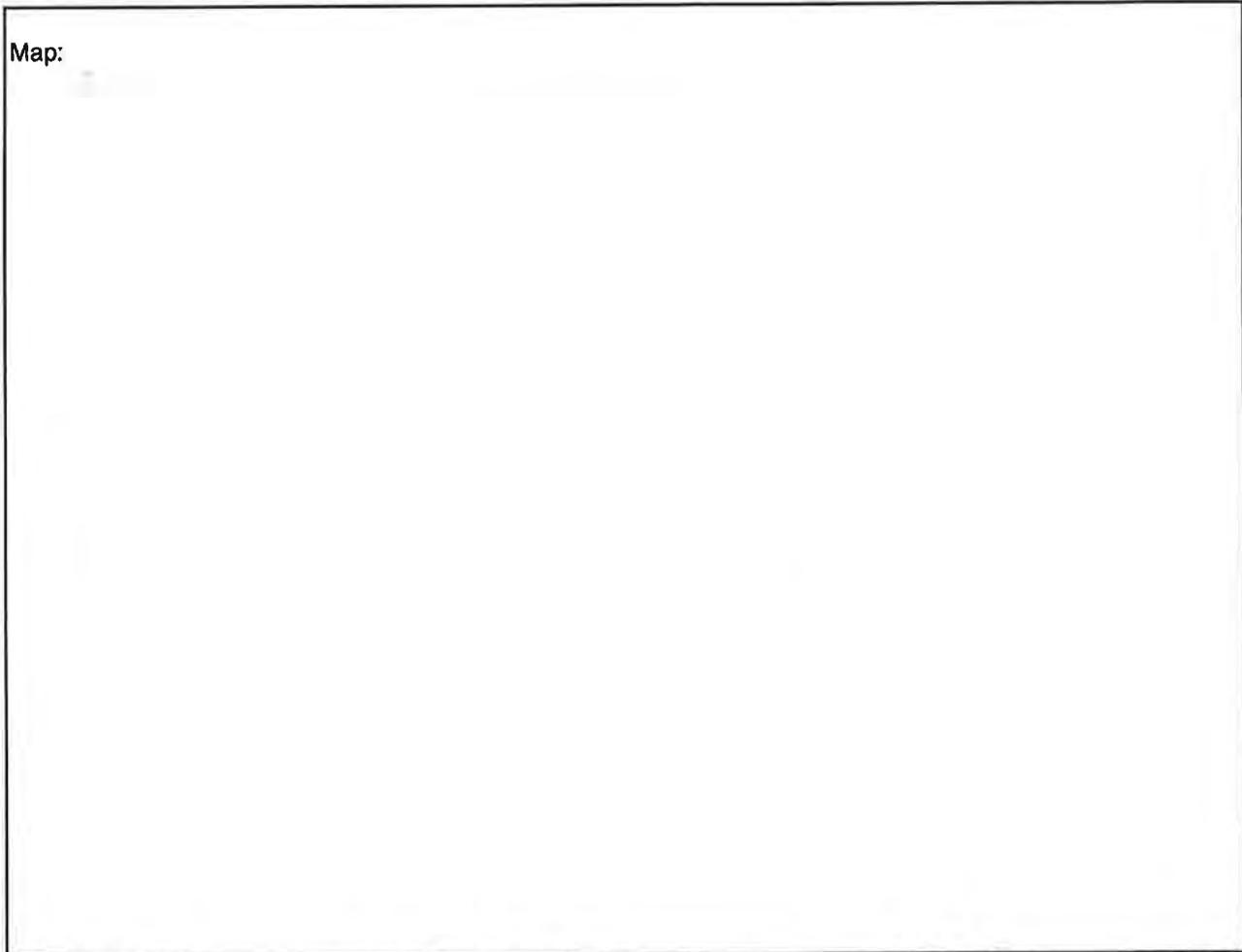
Technicians: _____

Site-Station: _____ **Date:** _____

Bank Condition: _____

Comments: _____

Map:



Start numbering at downstream riffle (most downstream riffle = 1; most upstream riffle = 3)

Appendix 2: Crayfish, Fish, and Detritus Metals Samples

Objective: Environmental samples will be taken and analyzed for metals to determine impacts of mining on crayfish populations and caged crayfish.

Crayfish: After crayfish are collected from quadrat samples, identified, sex determined and measured, they should be placed in 4-oz. pre-cleaned polypropylene (PP) jars. Three to five crayfish of the same species from each riffle at each site should be placed in separate 4-oz. PP pre-labeled jar. If only one riffle is sampled, three independent samples should be taken from that riffle. Crayfish used for metals analyses **will be identified on the data sheet**. Jars should be placed on ice until they can be placed in a freezer at hotel or CERC.

After crayfish are collected from cages, identified, sex determined, CL measured, and weighed, 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 metals analyses **will be identified on the data sheet**. Jars should be placed on ice until they can be placed in a freezer at hotel or CERC.

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 placed in a freezer at hotel or CERC.

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

Detritus: After leaves are collected from each cage, they should be placed in 4-oz. pre-cleaned polypropylene (PP) jars. Jars should be placed on ice until they can be placed in a freezer at hotel or CERC.

Data to be taken: Weight loss, metals (Pb, Cd, Zn, Ni, Co, Cu) in detritus.

Appendix 3: 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; length of transect (e.g., width of stream channel; 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: It is preferred that transect measurements start from the left descending bank. 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).

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 measurements:

Water depth will be measured using a standard depth gauge at 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' with each length measuring 0.5 m) will be used to characterize substrate at each point along each transect (Litvan et al. 2010). 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 or 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

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 4: 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; quadrat number; substrate size class; current velocity; and depth at location of each quadrat sample.

Methods: Quadrat samples will be taken in 1–6 riffles. Placement will be determined randomly. Quadrat samples will be numbered by site number-riffle-number of quadrat sampled at site (e.g., 1-1-1; 1-2-8; 1-3-21). 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). Record velocity in m/sec.

Depth measurements: Water depth will be measured in the middle of the 1-m² quadrat sample using a standard depth gauge at 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 (Litvan et al. 2010). 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 or 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

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 5: Processing for Water Quality Analyses

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 (3.785 L) pre-cleaned LDPE bottle will be used to collect three grab samples per site. Grab samples should be taken starting downstream and moving upstream. Bottles should be rinsed once with site water prior to the sampling being taken. 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.

Data to be taken: Temperature, pH, conductivity, dissolved oxygen, alkalinity, hardness, turbidity, total phosphorous (TP), total nitrogen (TN), ammonia, particulate organic carbon (POC), and total suspended solids (TSS), chlorophyll *a*.

Equipment needed: Coolers with blue ice; Hydrolab Quanta or equivalent water quality instruments; calibration standards; meter log book; study log book; 1-gal pre-labeled carboys; pre-labeled (20-, 60-, 500- 1000-ml) bottles; filtration equipment; filters; gloves; deionized water; forceps.

Surface water grab at each site:

1. Work downstream to upstream. Measurements will be taken in each riffle or three different locations at the site.
2. Using the water quality instrument(s), take a sub-surface reading of temperature, pH, conductivity, dissolved oxygen, and turbidity.
3. After in-situ readings are recorded, take 1-gal sub-surface grab sample.
4. Place 1-gal sample on ice or refrigerate (approximately 4 °C) until processing.

Processing of Sub-surface grab sample:

Equipment needed: Vacuum pump; 0.45- μ m polycarbonate filters; glass fiber filers; pre-labeled bottles; pre-labelled foil packs; RO water; sulfuric acid; graduated cylinders; data sheets.

1. Samples will be filtered at hotel (or on-site trailer) for ammonia (60-ml bottle; 0.45- μ m polycarbonate filter). Pre-, and post-filtration blanks should be taken.
2. Samples will be filtered at hotel (or on-site trailer) for chlorophyll *a* and POC using glass fiber filters. Filters will be wrapped in aluminum foil, placed in Ziplock bags, and frozen until analyses. Pre-, and post-filtration blanks should be taken.

3. An aliquot of the sample will be taken at hotel (or on-site trailer) for TN/TP analyses. A 60-ml sample should be placed in 60-ml pre-label containers and frozen until analyses.
4. An aliquot of the sample will be filtered at CERC TSS using nominal pre-weighed glass fiber filters. Filters should be dried immediately after filtration. The aliquot may be placed in a 1000-ml pre-cleaned bottle and preserved with ice or refrigeration prior to filtration. Pre-, and post-filtration blanks should be taken.
5. The remaining water in the sub-surface grab sample will be used for the alkalinity and hardness analyses. The container should be placed on ice or refrigerated until the analyses are conducted (which will be done within 96 hr).
6. Measure the **amount filtered** for ammonia, POC, TSS. Record on data sheet. Acidify ammonia samples with 2 drops of sulfuric acid, if analyses to be run after 24-hr.
7. Chain of Custody (COC) forms for each analyses (e.g., ammonia, TN/TP, TSS) can be filled out daily.

Appendix 6: Field Sampling Filtration Procedure for Surface Water samples for Trace Metals, Cations, Anions, and Dissolved Organic Carbon

Wear powderless gloves and throughout the procedure, avoid handling the tip sections of the straws, filter discs, or syringes. The same straw and syringe are reused to sub-sample sequentially for A: metals/cations, B: anions, and finally C: DOC (in that order), but with a new filter for each sample type. Note that the filter type for DOC is different (it has a glass mat prefilter (GMF) than the filter type used for metals/cations or anions (it has a polypropylene prefilter). After each new **stream grab sample**, a new syringe and straw are used. The procedure below is for collection and filtration of three 20-mL sub-samples from a larger grab volume.

1. Attach a pre-cleaned sampling straw to the syringe and carefully insert straw 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 clean plastic bag for reuse with next sub-sample (for anions step 6, then DOC step 7). Attach a **new** filter disc (be sure to use the proper cartridge type designated for either A: metals/cations/anions or B: DOC) 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 the appropriate sample container.
4. Cap bottle or vial containing the filtered sample tightly and place inside zip-seal plastic bag. Store on ice or in a refrigerator as soon as possible.
5. Discard the filter cartridge.
6. Using the same syringe & straw, repeat steps 1-5 for anions, but collect only 15 mL.
7. Using the same syringe & straw, repeat steps 1-5 for DOC. Collect 20 mL and dispense into amber DOC vial.
8. Discard the used syringe and filters, but save the straw(s) & return it to the laboratory for reuse after acid cleaning.
9. Preservative (nitric acid for metals & cations; sulfuric acid for DOC) will be added to each sample upon return to the laboratory, preferably within 96 h after sampling. Samples designated for anions will be kept refrigerated and in the dark (no preservative).

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 24mL polypropylene snap-cap vial fitted with a 0.45- μ m polyethersulfone filter membrane under the cap which has a large hole punched into it to allow water entry. A plastic 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 where they are acidified to 1% (v/v) HNO₃.

Appendix 8: List of SOP titles

- B4.01 Instrument design, maintenance, and calibration-general
- B4.14 Maintenance and Storage of Ross Combination pH Electrode
- B4.16 Alkalinity: Burette Method
- B4.36 Standard Operating Procedure for the Coulometrics Carbon Model 5020 Analyzer
- B4.42 Turbidity Sampling in Water using the Hach Model 2100A Turbidimeter
- B4.56 Combination pH Electrode, Ross Sure-flow Model 81-72 Preparation, Maintenance and Storage
- B4.62 Orion Model EA940 Expandable Ionalyzer
- B5.03 Preparation of Standard Solutions
- B5.13.052693 Procedure for Keeping Fish Culture Records
- B5.16 Glassware Washing Procedure for Analytical Biology Section
- B5.37 (Revised 2012) Calibration and Operation of the Turner Designs Model 10-AU-006 Fluorometer for the Analyses of Chlorophyll *a* and Pheophytin *a* from Water by In Vitro and In Vivo Methods
- B5.40 Invertebrate Glassware Cleaning Procedure
- B5.63 Storage, Handling, and Retrieval of Hand-written Material
- B5.72.091997 Anesthetization of Fishes for Sampling Purposes
- B5.95 Hardness
- P.691 Euthanasia and disposal of aquatic organisms (formerly B5.148.030789 Humane Disposal of Fish)
- P.683 Humane procedures of anesthetization and handling of research organisms for sampling purposes (formerly B5.154.091997 Humane Procedures for Anesthetization and Handling of Fish or Sampling Purposes)
- P.690 Reporting deficiencies in animal care and treatment (formerly B5.160.013189 Reporting Deficiencies in Animal Care and Treatment)
- B5.165.091997 Acclimation of Fish to Research Waters
- B5.179 Hydrometer Procedure for Particle Size Analysis of Sediment
- B5.239 Determining pH of Aqueous Samples with the Orion 290A pH Meter
- B5.240.060392 Assignment of Lot Numbers to Fish and Invertebrates
- F5.15.020488 Subduing Fish with Ice
- F6.2.22 Ammonia Determination using the Orion 290A pH/ISE Meter
- F6.2.15 Total Suspended, Fixed Suspended, and Volatile Solid Determination
- F6.20.1.082396 Particle Size Determination
- P.184 Type and Frequency of Quality Control Measurements Conducted For Elemental Analyses

- P.200 Sample Transmittal, Receipt, and Inventory
- P.213 Homogenization of Biological Tissue and Sediments By Brittle Fracture
- P.221 Mechanical Grinding Of Dried or Semi Dried Tissue Samples with The Bamix Mixer/Blender
- P.238 Homogenization of Samples by Manual Procedures
- P.239 Data Rejection Criteria and Corrective Action Procedures for Elemental Analyses
- P.241 Inductively Coupled Plasma-Mass Spectrometry for Environmental Sample Analysis
- P.254 Sample Storage and Disposal Protocol
- P.259 Lyophilization and Percent Moisture in Solid Samples
- P.510 Multiwave Microwave Acid Digestion of Environmental Samples
- P.566 Syringe and Disc Filtration of Water or Pore Water Samples for Trace Element Determinations
- P.570 Operation, Calibration, and Data Transfer of the YSI650 Multi-parameter Display System in Conjunction with the YSI6600 Multi-parameter Water Quality Monitor Unit
- P.578 Collection of Benthic Macroinvertebrate Samples For Measurement of Trace Elements
- P.579 Sampling Sediment for Elemental Analysis
- P.636 MULTIWAVE 3000 Microwave Acid Digestion of Environmental Samples
- P.705 Determination of Fluoride, Chloride, Nitrite, Nitrate, Bromide, and Sulfate in Water by Ion Chromatography with a Dionex ICS-1100
- P.722 Determination of Total Organic Carbon in Water with the Shimadzu TOC-L Analyzer

Appendix 9: CERC LIVE ORGANISM TRANSFER REQUEST (LOTR)

Revision Date: 9/4/14

DATE: **ESTIMATED DATE OF ORGANISM TRANSFER:**

COMPLETED BY: **CERC CONTACT:**

ORGANISMS TO BE TRANSFERRED:

REASON FOR ORGANISMS TO BE TRANSFERRED:

CERC STUDY PLAN # OR OTHER:

AGENCY OR COMPANY ASSISTING TRANSFER:

SIZE/AGE OF ORGANISMS:

QUANTITY:

WILD OR CAPTIVE:

ORIGIN:

DESTINATION (if not in the quarantine room, justify the reason):

CERC BIOSECURITY DESIGNATION (IF DETERMINED) AND JUSTIFICATION FOR EACH OF THE FOLLOWING CATEGORIES:

In the context of potential for release of non-native target organisms into the Missouri River watershed:

In the context of potential for release of non-native, non-target organisms to the CERC or into the Missouri River watershed:

In the context of potential for transfer or release of harmful pathogens to the CERC or into the Missouri River watershed:

KNOWN BIOSECURITY CONCERNS:

BIOSECURITY PLAN:

APPROVED BY:

DATE:

Appendix 10: CERC FISH TRANSFER STANDARD TREATMENT PROCEDURE

The following treatment procedure is recommended for all incoming fish transfers at CERC. This treatment should be used as a minimum procedure; additional processes may be applicable or required depending on the source of the fish and potential biosecurity risks associated with them. This treatment procedure has several purposes. First, it should allow the “hauling” water to be rinsed away from the fish and reduce the transfer of potential pathogens in the haul water. Second, the high concentration of salt will aid in killing or reducing external parasites from the fish’s body. Finally, it will allow potential non-target organisms to separate away from the fish and allow a visual period to observe the batch of fish for non-target organisms.

The treatment concentration may need to be lowered based on species and age of fish. It is recommended to test the treatment first with a single specimen and observe for signs of stress. Lower salt concentrations or shorter submersion times can still be effective at accomplishing the purpose of this treatment.

- 1) Separate dip nets should be used to transfer fish between hauling tank and treatment tank as well as between treatment tanks or nets should be placed in the salt bath with the fish.
- 2) Net crayfish out of the hauling tank and put into a 25 ppt [fish treatment =0 ppt (38g/gal or 10 g/L)] sodium chloride (NaCl) solution bath (well water) for 1 minute, then transfer crayfish to 50 ppt NaCl solution for four minutes. Following the first cycle of salt treatment, crayfish are placed into well water for five minutes. During this time, monitor the holding container for non-target organisms that may be at bottom of the tank or separated from the target organisms. Visible non-target organisms should be removed from the tank and euthanized immediately.
- 3) After the first cycle is completed, transfer the crayfish to another 25 ppt NaCl solution bath for an additional one minute; into 50 ppt NaCl for four minutes; into well water for five minutes. Again during this time, monitor the holding container for non-target organisms that may be at the bottom of the tank or separated from the target organisms. Non-target organisms should be removed from the tank and euthanized immediately.
- 4) After the second salt dip treatment, crayfish can be transferred to the appropriate rearing place at CERC.
- 5) At the completion of the salt water dip, the treatment tank solutions will be treated with a 200 mg/L chlorine solution for a minimum of one hour. The solution can then be neutralized with sodium thiosulfate (5.3g/gal) before being discharged if appropriate.

- 6) All water contained in the hauling tank or containers will be treated with 200 mg/L chlorine solution for a minimum of one hour before it is dumped into the sand trap behind the east boat barn.
- 7) The hauling tank or containers will then be surface sprayed with a disinfectant (200 mg/L bleach spray or 2% Virkon Aquatic®, recommended), rinsed and dried thoroughly for future use.
- 8) All equipment (waders, nets, buckets) associated with the fish transfer should be sprayed with an appropriate disinfectant (200 mg/L bleach spray or 2% Virkon Aquatic®, recommended), at the completion of the transfer.
- 9) All personnel involved in the transferring process will be instructed to follow this procedure.

Note: Salt treatment of crayfish was modified for second batch of ovigerous females (Johnson et al. 2003).

- 1) Separate dip nets should be used to transfer fish between hauling tank and treatment tank as well as between treatment tanks or nets should be placed in the salt bath with the fish.
- 2) Net crayfish out of the hauling tank and put into a 10 ppt sodium chloride (NaCl) solution bath (well water) for 10 minutes, then transfer crayfish to well water for five minutes. During this time, monitor the holding container for non-target organisms that may be at bottom of the tank or separated from the target organisms. Visible non-target organisms should be removed from the tank and euthanized immediately.
- 3) After the first cycle is completed, transfer the crayfish to another 10 ppt NaCl solution bath for an additional 10 minutes, then into well water for five minutes. Again during this time, monitor the holding container for non-target organisms that may be at the bottom of the tank or separated from the target organisms. Non-target organisms should be removed from the tank and euthanized immediately.

Appendix 11: CERC Hazard Analysis and Critical Control Point Plans (HACCPS)

CERC Biological Sample HACCP Final Plan

HACCP Step 1 – Activity Description

| Management Objective & Contact Information | |
|--|--------------------------------------|
| HACCP Plan Title: Importation of field-collected fresh or frozen, non-living biologic samples for toxic organic and inorganic chemical analysis | |
| Management Objective: Import field-collected fresh or frozen non-living biologic samples to the Columbia Environmental Research Center (CERC) for analysis of toxic organic and inorganic compounds. Provide this information to resource managers and regulatory agencies concerned about negative impacts on ecosystem health. | Contact Person: David Alvarez |
| | Phone: 573-441-2970 |
| | Email: dalvarez@usgs.gov |
| Activity Description i.e. Who; What; Where; When; How; Why | |
| <p>Who: Environmental Chemistry Branch What: Receiving fresh or frozen, non-living biologic samples for analysis of organic and inorganic compounds Where: CERC loading dock and quarantine room When: Throughout the year How: Biologic samples are received from collaborative partners via shipping carriers (Fedex , UPS, etc.) or collected by CERC personnel and transported to the lab and unloaded on the back loading dock. Cooler is taken to the quarantine room, opened up; an inventory of the samples is taken and if needed, sample containers and coolers are cleaned at this time. Samples are then typically stored in a walk-in freezer for later analysis. Why: To provide scientific data on the presence and/or concentrations of chemical constituents to collaborative partners such as state and federal agencies.</p> | |

HACCP Step 2 – Activity Flow Chart

Outline Sequential Tasks of Activity

| | |
|---------------|---|
| Task 1 | <p>Title: Coordination of sampling and shipping logistics.</p> <p>Description: Designated person coordinates with the partner agency to ensure that samples are collected and stored properly according to CERC Standard Operating Procedures (SOPs) and Chain of Custody (COC) forms if necessary. Informs staff members of approximate arrival time, intended purpose of the samples and designates appropriate staff to handle samples. Information related to potential biological non-targets at the sampling site and sample receiving are discussed.</p> |
| ↓ | |
| Task 2 | <p>Title: Shipping the samples and receiving of the shipment at CERC.</p> <p>Description: Samples are packaged in a cooler by field crews and shipped by a shipping carrier. Upon arrival at CERC, Samples are off-loaded by shipping carrier onto the back loading dock or occasionally at the front desk. Front desk notifies the recipient that the shipment has arrived.</p> |
| ↓ | |
| Task 3 | <p>Title: Processing and handling the shipment.</p> <p>Description: Cooler is opened up and an inventory of the samples is taken. If sample jars have broken, as much sample that can be recovered is transferred into a new container. Any residual water or sediment on the inside or outside of the cooler is removed. Both the sample jars and the cooler is cleaned thoroughly inside and out.</p> |
| ↓ | |
| Task 4 | <p>Title: Repacking and storage of the samples</p> <p>Description: Samples are either repacked in the cooler or placed in separate storage containers (boxes, tubs, freezer cages, etc.) and taken to the proper location for storage or direct processing of the samples.</p> |
| ↓ | |
| Task 5 | <p>Title: Processing and analyzing the samples.</p> <p>Description: Samples are processed in the lab according to the appropriate standard operating procedure. Excess sample will be disposed of according to CERC's hazardous substances and biosecurity guidelines.</p> |

HACCP Step 3 – Identify Potential Non-Targets

| |
|---|
| Non-Targets That May Potentially Be Moved/Introduced |
| Vertebrates: None |

Invertebrates: None

Plants: None

Other Organisms (pathogens, parasites, etc.) Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*), hepatitis, *Aeromonas/Pseudomonas*, *Batrachochytrium dendrobatidis* (chytrid fungus)

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|--|--|---|---|--|
| Task #1 Title: Coordination of sampling and shipping logistics. | Vertebrates: None | No | There is no risk of transferring non-target species because samples have not been shipped yet. | N/A | No | There are no significant non-targets during this task |
| | Invertebrates: None | No | | | | |
| | Plants: None | No | | | | |
| | Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms. | No | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|--|---|---|---|--|
| Task #2 Title: Receiving of the shipment at CERC. | Vertebrates: None | No | There is a very low risk of transferring non-target species because samples are contained in coolers and kept sealed. | N/A | No | There are no significant non-targets during this task |
| | Invertebrates: None | No | | | | |
| | Plants: None | No | | | | |
| | Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms. | No | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|--|--|--|--|--|---|--|
| Task #3 Title: Processing and handling the shipment. | Vertebrates: None | No | Once samples are on-station at CERC, the chance for non-target species to be transferred to an environment where they can spread or thrive is highly likely. | If samples are not processed immediately, the cooler and its entire contents should be placed in the walk-in freezer. Once removed from the freezer, the following procedures must be followed. Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with a 2% Virkon® solution and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, placed in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels. | Yes | This is a critical control point because this is the point where the transfer of a non-target species is most likely to occur. Surfaces that tissue samples are processed on has the highest risk of introducing a non-target species to CERC. |
| | Invertebrates: None | No | | | | |
| | Plants: None | No | | | | |
| | Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms. | Yes | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|--|--|---|---|--|
| Task #4 Title: Storage of the samples | Vertebrates: None | No | Risk is low for transferring non-target species because samples and storage cooler have been cleaned thoroughly before storage. Storage of samples in sealed containers in either a cooler or freezer minimizes any potential transfer of pathogens. | N/A | No | There are no significant non-targets during this task. |
| | Invertebrates: None | No | | N/A | | |
| | Plants: None | No | | N/A | | |
| | Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms. | No | | N/A | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|--|--|---|--|--|--|---|
| Task #5 Title: Processing and analyzing the samples. | Vertebrates: None | No | If work area is not thoroughly disinfected after processing the samples, the risk of transferring pathogens throughout the lab is present. | During the processing of samples, Best Lab Practices (BLP's) should be used at all times including wearing gloves while handling the samples. Upon completion of sample processing, all work and floor surfaces and equipment used during the processing must be sprayed down with a 2% Virkon® Aquatic or 2% chlorine solution and allowed to sit for ten minutes before surfaces are rinsed. Excess tissue samples can be placed in a bag and disposed of in the trash following a period of time after the final publication of data as agreed upon by CERC and its collaborator. For samples that cannot be disposed of (e.g., NRDAR projects), arrangements should be made for long-term storage at CERC or another facility during the planning stages of the project. | Yes | Samples will be exposed and handled on work surfaces potentially allowing pathogens to be transferred throughout the center and potentially infecting existing cultures or research projects. |
| | Invertebrates: None | No | | | | |
| | Plants: None | No | | | | |
| | Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms. | Yes | | | | |

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

| (Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet) One page for each Critical Control Point | | | | |
|---|---|---|----------------|---------------------------------------|
| Mangement Objective From Step 1 | Import field-collected non-living biologic samples to the Columbia Environmental Research Center (CERC) for analysis of toxic organic and inorganic compounds that may negatively influence ecosystems. Provide this information to resource managers and regulatory agencies. | | | |
| Critical Control Point: | Task # | 3 | Titl e: | Processing and handling the shipment. |
| Significant Non-Target(s) (Step 4, Column 3) | Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>), hepatitis, <i>Aeromonas/Pseudomonas</i> , <i>Batrachochytrium dendrobatidis</i> (chytrid fungus) | | | |
| Control Measure(s) (Step 4, Column 5) | Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, put in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels. | | | |
| Precribed ranges, limits, or criteria for control measure(s): (PRLC) | Sample bottles and cooler should be sprayed or wiped with 2% Virkon® for 10 minutes before rinsing or drying. Care must be taken to not damage sample labels. Coolers should be visually inspected after cleaning so there is no visible mud or sediment remaining outside or inside the cooler. | | | |
| Monitoring the Control Measure(s) | Who? | Chemistry technicians | | |
| | How? | Make sure all surfaces have been cleaned and sprayed with 2% Virkon and allowed to sit for ten minutes. | | |
| | Where? | Quarantine room | | |
| | How often? | When receiving a shipment of tissue samples. | | |
| Corrective Action(s) if Control | Repeat control measures from task 3. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon®. | | | |

| | |
|---|--|
| Measures Fail (or PRLC cannot be met) | |
| Supporting Documents (For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.) | |
| | |

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

| | | | |
|--|---|--|--|
| Management Objective From Step 1 | Import field-collected non-living biologic samples to the Columbia Environmental Research Center (CERC) for analysis of toxic organic and inorganic compounds that may negatively influence ecosystems. Provide this information to resource managers and regulatory agencies. | | |
| Critical Control Point: | Task # | 5 | Title: Processing and analyzing the samples. |
| Significant Non-Target(s) (Step 4, Column 3) | Bacteria and pathogens of concern to both human health and aquatic organisms. | | |
| Control Measure(s) (Step 4, Column 5) | During the processing of samples, Best Lab Practices (BLP's) should be used at all times including wearing gloves while handling the samples. Upon completion of sample processing, all work and floor surfaces and equipment used during the processing must be sprayed down with a 2% Virkon® Aquatic solution or a 2% chlorine solution and allowed to sit for ten minutes before surfaces are rinsed. | | |
| Precribed ranges, limits, or criteria for control measure(s): (PRLC) | All work surfaces and equipment should be sprayed or wiped with 2% Virkon® or 2% chlorine solution for 10 minutes before rinsing or drying. | | |
| Monitoring the Control Measure(s) | Who? | Chemistry technicians | |
| | How? | Make sure all surfaces have been cleaned and sprayed with 2% Virkon or 2% chlorine and allowed to sit for ten minutes. | |
| | Where? | Grinding room | |

| | | | |
|--|---|--|-----------------------------------|
| | How often? | When receiving a shipment of tissue samples. | |
| Corrective Action(s) if Control Measures Fail (or PRLC cannot be met) | Repeat control measures from task 5. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon® or 2% chlorine. | | |
| Supporting Documents <i>(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)</i> | | | |
| Development Team Members | | James Candrl and David Alvarez | |
| Date Developed: | 11/20/14 | Date(s) Reviewed: | 12/29/14 Biosecurity Committee |

*all fields in grey are required

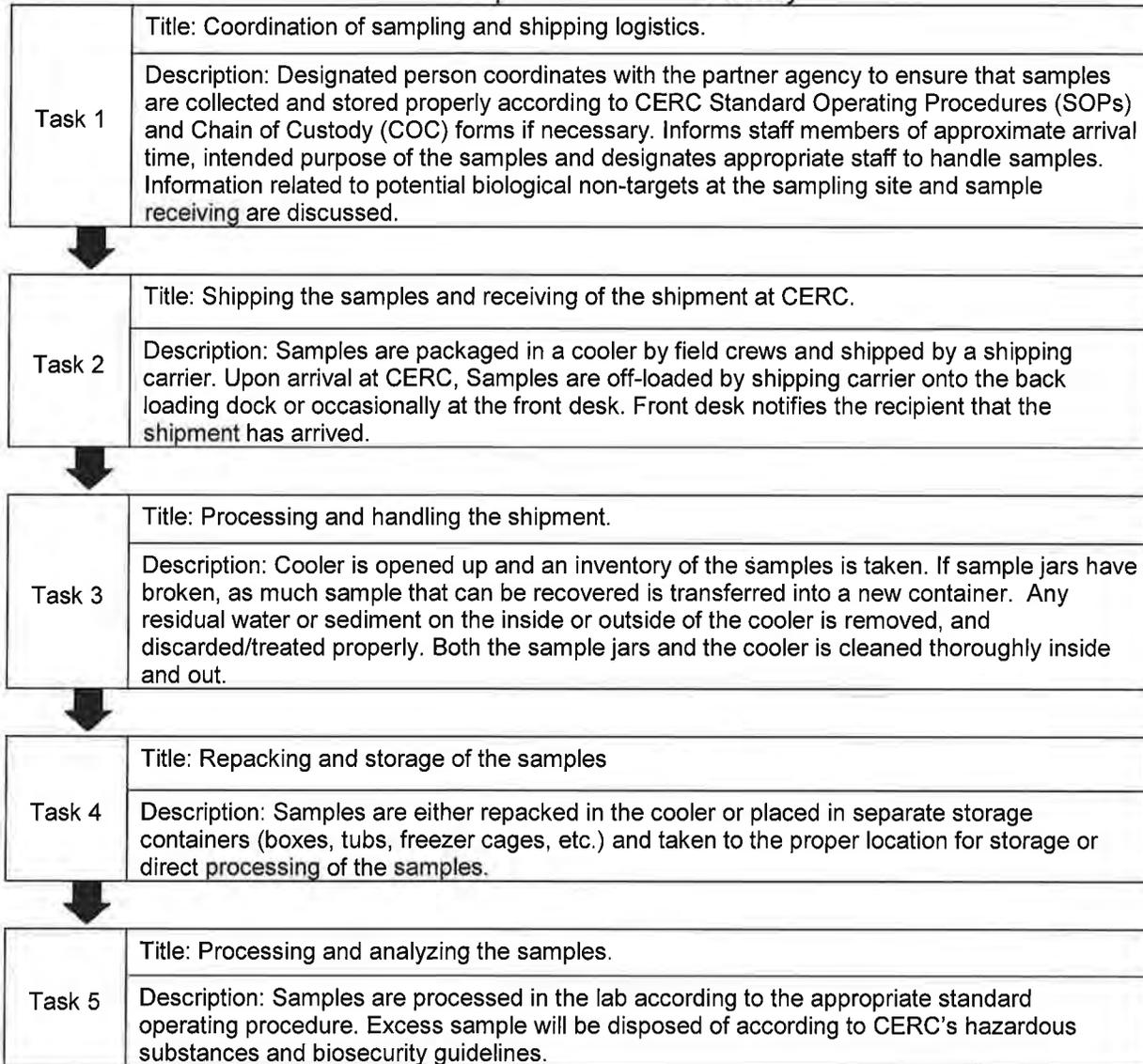
CERC Water Sample HACCP final Plan

HACCP Step 1 – Activity Description

| Management Objective & Contact Information | |
|--|--------------------------------------|
| HACCP Plan Title: Importation of water samples for organic and inorganic chemistry analysis | |
| Management Objective: Import field-collected water samples to the Columbia Environmental Research Center (CERC) for analysis of organic and inorganic compounds. Provide this information to resource managers and regulatory agencies concerned about negative impacts on ecosystem health. | Contact Person: David Alvarez |
| | Phone: 573-441-2970 |
| | Email: dalvarez@usgs.gov |
| Activity Description i.e. Who; What; Where; When; How; Why | |
| <p>Who: Environmental Chemistry Branch What: Receiving water samples for analysis of organic and inorganic compounds Where: CERC loading dock and quarantine room When: Throughout the year How: Water samples are received from collaborative partners via shipping carriers (Fedex , UPS, etc.) or collected by CERC personnel and transported to the lab and unloaded on the back loading dock. Cooler is taken to the quarantine room, opened up; an inventory of the samples is taken and if needed, sample containers and coolers are cleaned at this time. Samples are then typically stored in a walk-in freezer for later analysis. Why: To provide scientific data on the presence and/or concentrations of chemical constituents to collaborative partners such as state and federal agencies.</p> | |

HACCP Step 2 – Activity Flow Chart

Outline Sequential Tasks of Activity



HACCP Step 3 – Identify Potential Non-Targets

| |
|---|
| <h3 style="margin: 0;">Non-Targets That May Potentially Be Moved/Introduced</h3> |
| <p>Vertebrates: Small fish of various species</p> |
| <p>Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) could be potentially be transferred but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel</p> |

(Dreissena polymorpha) and quagga mussel (*Dreissena bugensis*) veligers.

Plants: Water meal and algae

Other Organisms (pathogens, parasites, etc.) Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|--|--|---|---|--|
| Task #1 Title: Coordination of sampling and shipping logistics. | Vertebrates: Small fish of various species | No | There is no risk of transferring non-target species because samples have not been shipped yet. | N/A | No | There are no significant non-targets during this task |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | | | |
| | Plants: water meal and algae | No | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|--|--|---|---|--|
| Task #2 Title: Receiving of the shipment at CERC. | Vertebrates: Small fish of various species | No | Shipping trucks, warehouses and CERC loading dock are dry, so environment for aquatic species to survive and spread is not likely. | N/A | No | There are no significant non-targets during this task |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | | | |
| | Plants: water meal and algae | No | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|--|--|--|--|---|---|--|
| Task #3 Title: Processing and handling the shipment. | Vertebrates: Small fish of various species | Yes | Once samples are on-station at CERC, the chance for non-target species to be transferred to an environment where they can spread or thrive is highly likely. | If samples are not processed immediately, the cooler and its entire contents should be placed in the walk-in freezer. Once removed from the freezer, the following procedures must be followed. If freezing is not advised due to risk sample containers breaking (a potential risk with very wet sediments in glass jars), then the outside of the cooler should be decontaminated in the quarantine room using a 2% Virkon® solution and then the cooler and its entire contents can be placed in the walk-in cooler. Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with a 2% Virkon® solution and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, placed in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels. | Yes | This is a critical control point because this is the point where the transfer of a non-target species is most likely to occur. Loose sediments or excess water in the cooler has the highest risk of introducing a non-target species to CERC. |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | Yes | | | | |
| | Plants: water meal and algae | Yes | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | Yes | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|---|--|--|--|--|
| Task #4 Title: Storage of the samples | Vertebrates: Small fish of various species | No | Risk is low for transferring non-target species because samples and storage cooler have been cleaned thoroughly before storage. Storage of samples in closed bottles in either a cooler or freezer does not allow for non-target species to remain viable. | N/A | No | There are no significant non-targets during this task. |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | N/A | | |
| | Plants: water meal and algae | No | | N/A | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | N/A | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|-----------------------------|---|---|---|--|--|---|
|-----------------------------|---|---|---|--|--|---|

| | | | | | | |
|--|--|----|--|---|----|--|
| Task #5 Title: Processing and analyzing the samples. | Vertebrates: Small fish of various species | No | The risk is low for transferring non-target species to CERC during this task. If samples have not previously been frozen or stored in an anaerobic environment for a period of time, they are processed using strict controls to minimize the risk for release of any non-target organisms | Excess water samples can be stored for a period suitable for the analytes of interest. Following this time, any un-used water should be disposed of by pouring down the quarantine room waste system for treatment. | No | There are no significant non-targets during this task. |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | | | |
| | Plants: water meal and algae | No | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | | | |

C. HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

| (Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet) One page for each Critical Control Point | | | | |
|---|---|---|--------|---------------------------------------|
| Management Objective From Step 1 | Import water samples to the Columbia Environmental Research Center (CERC) for analysis of organic and inorganic compounds that may negatively influence ecosystems. Provide this information to management and regulatory agencies. | | | |
| Critical Control Point: | Task # | 3 | Title: | Processing and handling the shipment. |
| Significant Non-Target(s) (Step 4, Column 3) | Small fish of various species, numerous small invertebrates (worms, crayfish, leeches) but specifically New Zealand Mud Snails, zebra and quagga mussel veligers, water meal, algae, numerous pathogens of concern to both human health (<i>E. coli</i> , <i>hepatitis</i>) and aquatic species. | | | |
| Control Measure(s) (Step 4, Column 5) | Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, put in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels. | | | |
| Prescribed ranges, limits, or criteria for control measure(s): (PRLC) | Sample bottles and cooler should be sprayed or wiped with 2% Virkon® for 10 minutes before rinsing or drying. Care must be taken to not damage sample labels. Coolers should be visually inspected after cleaning so there is no visible mud or sediment remaining outside or inside the cooler. | | | |
| Monitoring the Control Measure(s) | Who? | Chemistry technicians | | |
| | How? | Make sure all surfaces have been cleaned and sprayed with 2% Virkon and allowed to sit for ten minutes. | | |
| | Where? | Quarantine room | | |
| | How often? | When receiving a shipment of water samples. | | |
| Corrective Action(s) if Control Measures Fail | Repeat control measures from task 3. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon®. | | | |

| | | | |
|---|----------------|---------------------------------------|---|
| (or PRLC cannot be met) | | | |
| Supporting Documents | | | |
| <i>(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)</i> | | | |
| | | | |
| Development Team Members | | James Candri and David Alvarez | |
| Date Developed: | 11/7/14 | Date(s) Reviewed: | 12/29/14 Biosecurity Committee |

***all fields in grey are required**

CERC Sediment Sample HACCP final Plan

HACCP Step 1 – Activity Description

| Management Objective & Contact Information | |
|--|-------------------------------|
| HACCP Plan Title: Importation of field-collected soil and sediment samples for organic and inorganic chemistry analysis | |
| Management Objective: Import field-collected soil and sediment samples to the Columbia Environmental Research Center (CERC) for analysis of organic and inorganic compounds. Provide this information to resource managers and regulatory agencies concerned about negative impacts on ecosystem health. | Contact Person: David Alvarez |
| | Phone: 573-441-2970 |
| | Email: dalvarez@usgs.gov |
| Activity Description i.e. Who; What; Where; When; How; Why | |
| <p>Who: Environmental Chemistry Branch What: Receiving soil and sediment samples for analysis of organic and inorganic compounds Where: CERC loading dock and quarantine room When: Throughout the year How: Soil and sediment samples are received from collaborative partners via shipping carriers (Fedex , UPS, etc.) or collected by CERC personnel and transported to the lab and unloaded on the back loading dock. Cooler is taken to the quarantine room, opened up; an inventory of the samples is taken and if needed, sample containers and coolers are cleaned at this time. Samples are then typically stored in a walk-in freezer for later analysis. Why: To provide scientific data on the presence and/or concentrations of chemical constituents to collaborative partners such as state and federal agencies.</p> | |

HACCP Step 2 – Activity Flow Chart

Outline Sequential Tasks of Activity

| | |
|--------|---|
| Task 1 | <p>Title: Coordination of sampling and shipping logistics.</p> <p>Description: Designated person coordinates with the partner agency to ensure that samples are collected and stored properly according to CERC Standard Operating Procedures (SOPs) and Chain of Custody (COC) forms if necessary. Informs staff members of approximate arrival time, intended purpose of the samples and designates appropriate staff to handle samples. Information related to potential biological non-targets at the sampling site and sample receiving are discussed.</p> |
| ↓ | |
| Task 2 | <p>Title: Shipping the samples and receiving of the shipment at CERC.</p> <p>Description: Samples are packaged in a cooler by field crews and shipped by a shipping carrier. Upon arrival at CERC, Samples are off-loaded by shipping carrier onto the back loading dock or occasionally at the front desk. Front desk notifies the recipient that the shipment has arrived.</p> |
| ↓ | |
| Task 3 | <p>Title: Processing and handling the shipment.</p> <p>Description: Cooler is opened up and an inventory of the samples is taken. If sample jars have broken, as much sample that can be recovered is transferred into a new container. Any residual water or sediment on the inside or outside of the cooler is removed, and discarded/treated properly. Both the sample jars and the cooler is cleaned thoroughly inside and out.</p> |
| ↓ | |
| Task 4 | <p>Title: Repacking and storage of the samples</p> <p>Description: Samples are either repacked in the cooler or placed in separate storage containers (boxes, tubs, freezer cages, etc.) and taken to the proper location for storage or direct processing of the samples.</p> |
| ↓ | |
| Task 5 | <p>Title: Processing and analyzing the samples</p> <p>Description: Samples are processed in the lab according to the appropriate standard operating procedure. Excess sample will be disposed of according to CERC's hazardous substances and biosecurity guidelines.</p> |

HACCP Step 3 – Identify Potential Non-Targets

| |
|---|
| <h3 style="margin: 0;">Non-Targets That May Potentially Be Moved/Introduced</h3> |
| <p>Vertebrates: Small fish of various species, amphibians, fish and amphibian eggs depending on time of year</p> |

Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) could be potentially be transferred but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*) veligers. Various aquatic insects and/or eggs, fire ants (*Solenopsis* sp.)

Plants: Family Lemnaceae (Water meal *Wolffia* and *Wolffiella* spp. and Duckweed, *Lemna* spp.)

Other Organisms (pathogens, parasites, etc.) Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*), hepatitis, *Aeromonas/Pseudomonas*, *Batrachomyxium dendrobatidis* (chytrid fungus)

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|--|--|---|---|--|
| Task #1 Title: Coordination of sampling and shipping logistics. | Vertebrates: Small fish of various species | No | There is no risk of transferring non-target species because samples have not been shipped yet. | N/A | No | There are no significant non-targets during this task |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | | | |
| | Plants: water meal | No | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|--|--|---|---|--|
| Task #2 Title: Receiving of the shipment at CERC. | Vertebrates: Small fish of various species | No | Shipping trucks, warehouses and CERC loading dock are dry, so environment for aquatic species to survive and spread is not likely. | N/A | No | There are no significant non-targets during this task |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | | | |
| | Plants: water meal | No | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|--|--|--|--|---|---|--|
| Task #3 Title: Processing and handling the shipment. | Vertebrates: Small fish of various species | Yes | Once samples are on-station at CERC, the chance for non-target species to be transferred to an environment where they can spread or thrive is highly likely. | If samples are not processed immediately, the cooler and its entire contents should be placed in the walk-in freezer. Once removed from the freezer, the following procedures must be followed. If freezing is not advised due to risk sample containers breaking (a potential risk with very wet sediments in glass jars), then the outside of the cooler should be decontaminated in the quarantine room using a 2% Virkon® solution and then the cooler and its entire contents can be placed in the walk-in cooler. Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with a 2% Virkon® solution and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, placed in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels. | Yes | This is a critical control point because this is the point where the transfer of a non-target species is most likely to occur. Loose sediments or excess water in the cooler has the highest risk of introducing a non-target species to CERC. |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | Yes | | | | |
| | Plants: water meal | Yes | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | Yes | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|--|--|---|---|--|
| Task #4 Title: Storage of the samples | Vertebrates: Small fish of various species | No | Risk is low for transferring non-target species because samples and storage cooler have been cleaned thoroughly before storage. Storage of samples in closed bottles in either a cooler or freezer does not allow for non-target species to remain viable. | N/A | No | There are no significant non-targets during this task. |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | N/A | | |
| | Plants: water meal | No | | N/A | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | N/A | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|--|--|--|---|---|---|--|
| Task #5 Title: Processing and analyzing the samples. | Vertebrates: Small fish of various species | No | The risk is low for transferring non-target species to CERC during this task. If samples have not previously been frozen or stored in an anaerobic environment for a period of time, they are processed using strict controls to minimize the risk for release of any non-target organisms. | Sediment samples can be placed in a sealed ziplock bag and disposed of in the trash following a period of time after the final publication of data as agreed upon by CERC and its collaborator. For samples that cannot be disposed of (e.g., NRDAR projects), arrangements should be made for long-term storage at CERC or another facility during the planning stages of the project. | No | There are no significant non-targets during this task. |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | | | |
| | Plants: water meal | No | | | | |
| Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | | | | |

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

| | | | | |
|--|---|---|---------------|---------------------------------------|
| Mangement Objective From Step 1 | Import field-collected soil and sediment samples to the Columbia Environmental Research Center (CERC) for analysis of organic and inorganic compounds that may negatively influence ecosystems. Provide this information to management and regulatory agencies. | | | |
| Critical Control Point: | Task # | 3 | Title: | Processing and handling the shipment. |
| Significant Non-Target(s) (Step 4, Column 3) | Small fish of various species, numerous small invertebrates (worms, crayfish, leeches) but specifically New Zealand Mud Snails, zebra and quagga mussel veligers, water meal, bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>E. coli</i>), hepatitis, <i>Aeromonas/Pseudomonas</i> , <i>Batrachochytrium dendrobatidis</i> (chytrid fungus). | | | |
| Control Measure(s) (Step 4, Column 5) | Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample bottles should be removed from the cooler, dried off and sprayed down with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, put in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels. | | | |
| Precribed ranges, limits, or criteria for control measure(s): (PRLC) | Sample bottles and cooler should be sprayed or wiped with 2% Virkon® for 10 minutes before rinsing or drying. Care must be taken to not damage sample labels. Coolers should be visually inspected after cleaning so there is no visible mud or sediment remaining outside or inside the cooler. | | | |
| Monitoring the Control Measure(s) | Who? | Chemistry technicians | | |
| | How? | Make sure all surfaces have been cleaned and sprayed with 2% Virkon and allowed to sit for ten minutes. | | |
| | Where? | Quarantine room | | |
| | How often? | When receiving a shipment of soil or sediment samples. | | |

| | | | |
|--|--|---------------------------------------|--|
| Corrective Action(s) if Control Measures Fail (or PRLC cannot be met) | Repeat control measures from task 3. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon®. | | |
| Supporting Documents <i>(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)</i> | | | |
| Development Team Members | | James Candri and David Alvarez | |
| Date Developed: | 11/7/14 | Date(s) Reviewed: | 12/29/14 Biosecurity Committee |

***all fields in grey are required**

CERC Passive Samplers HACCP final Plan

HACCP Step 1 – Activity Description

| Management Objective & Contact Information | |
|--|--------------------------------------|
| HACCP Plan Title: Importation of passive samplers of organic and inorganic chemicals that were deployed in various aquatic environments. | |
| Management Objective: Import field-collected passive sample devices to the Columbia Environmental Research Center (CERC) for analysis of toxic organic and inorganic compounds. Provide this information to resource managers and regulatory agencies concerned about negative impacts on ecosystem health. | Contact Person: David Alvarez |
| | Phone: 573-441-2970 |
| | Email: dalvarez@usgs.gov |
| Activity Description i.e. Who; What; Where; When; How; Why | |
| <p>Who: Environmental Chemistry Branch What: Receiving passive sample devices for analysis of organic and inorganic compounds Where: CERC loading dock and quarantine room When: Throughout the year How: Passive sampling devices are received from collaborative partners via shipping carriers (Fedex , UPS, etc.) or collected by CERC personnel and transported to the lab and unloaded on the back loading dock. Cooler is taken to the quarantine room, opened up; an inventory of the sample containers is taken and if needed, samplers and coolers are cleaned at this time. Samplers are then typically stored in a walk-in freezer for later analysis. Why: To provide scientific data on the presence and/or concentrations of chemical constituents to collaborative partners such as state and federal agencies.</p> | |

HACCP Step 2 – Activity Flow Chart

Outline Sequential Tasks of Activity

| | |
|---|---|
| Task 1 | Title: Coordination of sampling and shipping logistics. |
| | Description: Designated person coordinates with the partner agency to ensure that samples are collected and stored properly according to CERC Standard Operating Procedures (SOPs) and Chain of Custody (COC) forms if necessary. Informs staff members of approximate arrival time, intended purpose of the samples and designates appropriate staff to handle samples. Information related to potential biological non-targets at the sampling site and sample receiving are discussed. |
|  | |
| Task 2 | Title: Shipping the samples and receiving of the shipment at CERC. |
| | Description: Samples are packaged in a cooler by field crews and shipped by a shipping carrier. Upon arrival at CERC, Samples are off-loaded by shipping carrier onto the back loading dock or occasionally at the front desk. Front desk notifies the recipient that the shipment has arrived. |
|  | |
| Task 3 | Title: Processing and handling the shipment. |
| | Description: Cooler is opened up and an inventory of the sample containers is taken. If any of the shipping cans containing the samplers are damaged or open, the passive samplers will be transferred to a new can. Any residual water or sediment on the inside or outside of the cooler is removed, and discarded/treated properly. Both the sample cans and the cooler is cleaned thoroughly inside and out. |
|  | |
| Task 4 | Title: Repacking and storage of the samples |
| | Description: Sample devices are either repacked in the cooler or placed in separate storage containers (boxes, tubs, freezer cages, etc.) and taken to the proper location for storage or direct processing of the samples. |
|  | |
| Task 5 | Title: Processing and analyzing the samples. |
| | Description: Samplers are processed in the lab according to the appropriate standard operating procedure. All passive samplers will be consumed during the processing. Sampler materials, such as the membranes, which remain following extraction, will be disposed of in the trash. Sampler hardware (metal and plastic components) will be placed in buckets prior to cleaning with acid, soapy water, and organic solvents. |

HACCP Step 3 – Identify Potential Non-Targets

Non-Targets That May Potentially Be Moved/Introduced

Vertebrates: Small fish of various species

Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) could be potentially be transferred but specifically New Zealand Mud Snails (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*) and quagga mussel (*Dreissena bugensis*) veligers.

Plants: Water meal and algae

Other Organisms (pathogens, parasites, etc.) Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*) and hepatitis.

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|---|--|---|---|---|--|
| Task #1 Title: Coordination of sampling and shipping logistics. | Vertebrates: Small fish of various species | No | There is no risk of transferring non-target species because the samplers have not been shipped yet. | N/A | No | There are no significant non-targets during this task |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | | | |
| | Plants: water meal and algae | No | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|---|--|--|---|---|--|
| Task #2 Title: Receiving of the shipment at CERC. | Vertebrates: Small fish of various species | No | Shipping trucks, warehouses and CERC loading dock are dry, so environment for aquatic species to survive and spread is not likely. | N/A | No | There are no significant non-targets during this task |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | | | |
| | Plants: water meal and algae | No | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|---|---|---|--|--|--|
| Task #3 Processing and handling the shipment. | Vertebrates: Small fish of various species | Yes | Once samplers are on-station at CERC, the chance for non-target species to be transferred to an environment where they can spread or thrive is highly likely. | If samplers are not processed immediately, the cooler and its entire contents should be placed in the walk-in freezer. Once removed from the freezer, the following procedures must be followed: Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Sample containers should be removed from the cooler, dried off and sprayed down with a 2% Virkon® solution and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, placed in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The samples can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels. | Yes | This is a critical control point because this is the point where the transfer of a non-target species is most likely to occur. Loose sediments or excess water in the cooler has the highest risk of introducing a non-target species to CERC. |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | Yes | | | | |
| | Plants: water meal and algae | Yes | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | Yes | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|---|--|---|---|---|--|
| Task #4 Title: Storage of the samples | Vertebrates: Small fish of various species | No | Risk is low for transferring non-target species because passive sampler containers and storage cooler have been cleaned thoroughly before storage. Storage of samples in closed containers in either a cooler or freezer does not allow for non-target species the potential to escape. | N/A | No | There are no significant non-targets during this task. |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | N/A | | |
| | Plants: water meal and algae | No | | N/A | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | N/A | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|--|---|--|--|--|---|--|
| Task #5 Title: Processing and analyzing the samples. | Vertebrates: Small fish of various species | No | The risk is low for transferring non-target species to CERC during this task. If samplers have not previously been frozen or stored in an anaerobic environment for a period of time, they are processed using strict controls to minimize the risk for release of any non-target organisms. | Any remaining materials (membranes) from the passive samplers following extraction are to be disposed of in the trash. Deployment hardware is placed in buckets or tubs to dry, after which they are cleaned using acids and solvents according to laboratory SOP P.585. | No | There are no significant non-targets during this task. |
| | Invertebrates: Numerous invertebrates (worms, crayfish, leeches, barnacles, crabs) but specifically New Zealand Mud Snails (<i>Potamopyrgus antipodarum</i>), zebra mussel (<i>Dreissena polymorpha</i>) and quagga mussel (<i>Dreissena bugensis</i>) veligers. | No | | | | |
| | Plants: water meal and algae | No | | | | |
| | Other Organisms Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>) and hepatitis. | No | | | | |

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

| | | | |
|--|---|---|---|
| Mangement Objective From Step 1 | Import passive sampler devices to the Columbia Environmental Research Center (CERC) for analysis of organic and inorganic compounds that may negatively influence ecosystems. Provide this information to management and regulatory agencies. | | |
| Critical Control Point: | Task # | 3 | Title: Processing and handling the shipment. |
| Significant Non-Target(s) (Step 4, Column 3) | Small fish of various species, numerous small invertebrates (worms, crayfish, leeches, crabs) but specifically New Zealand Mud Snails, zebra and quagga mussel veligers, water meal and algae, numerous pathogens of concern to both human health (<i>E. coli, hepatitis</i>) and aquatic species. | | |
| Control Measure(s) (Step 4, Column 5) | Start by preparing the sample processing table in the quarantine room. Clean off sufficient space and place bench paper on the work area. Carry the cooler directly from the loading dock into the quarantine room. Gloves should be worn while handling the shipment. Passive sampler containers should be removed from the cooler, dried off and sprayed down with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. Excess water in the cooler should be poured into a bucket dumping station. Excess sediment should be removed with gloved hands and paper towels, put in a sealed ziplock bag and placed in the trash can. The cooler should then be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. The sample containers can either be repacked in the same cooler or transferred to a clean container and then removed from the room. Bench paper should be rolled up and placed into the trash can. All work surfaces should be cleaned with Virkon® spray and paper towels. | | |
| Precribed ranges, limits, or criteria for control measure(s): (PRLC) | Sampler device containers and cooler should be sprayed or wiped with 2% Virkon® for 10 minutes before rinsing or drying. Care must be taken to not damage sample labels. Coolers should be visually inspected after cleaning so there is no visible mud or sediment remaining outside or inside the cooler. | | |
| Monitoring the Control Measure(s) | Who? | Chemistry technicians | |
| | How? | Make sure all surfaces have been cleaned and sprayed with 2% Virkon and allowed to sit for ten minutes. | |
| | Where? | Quarantine room | |
| | How often? | When receiving a shipment of passive sampler devices. | |
| Corrective Action(s) if Control Measures Fail (or PRLC cannot | Repeat control measures from task 3. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon®. | | |

| | | | |
|--|---------|---------------------------------------|-----------------------------------|
| be met) | | | |
| Supporting Documents <i>(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)</i> | | | |
| | | | |
| Development Team Members | | James Candri and David Alvarez | |
| Date Developed: | 11/7/14 | Date(s) Reviewed: | 12/29/14 Biosecurity Committee |

*all fields in grey are required

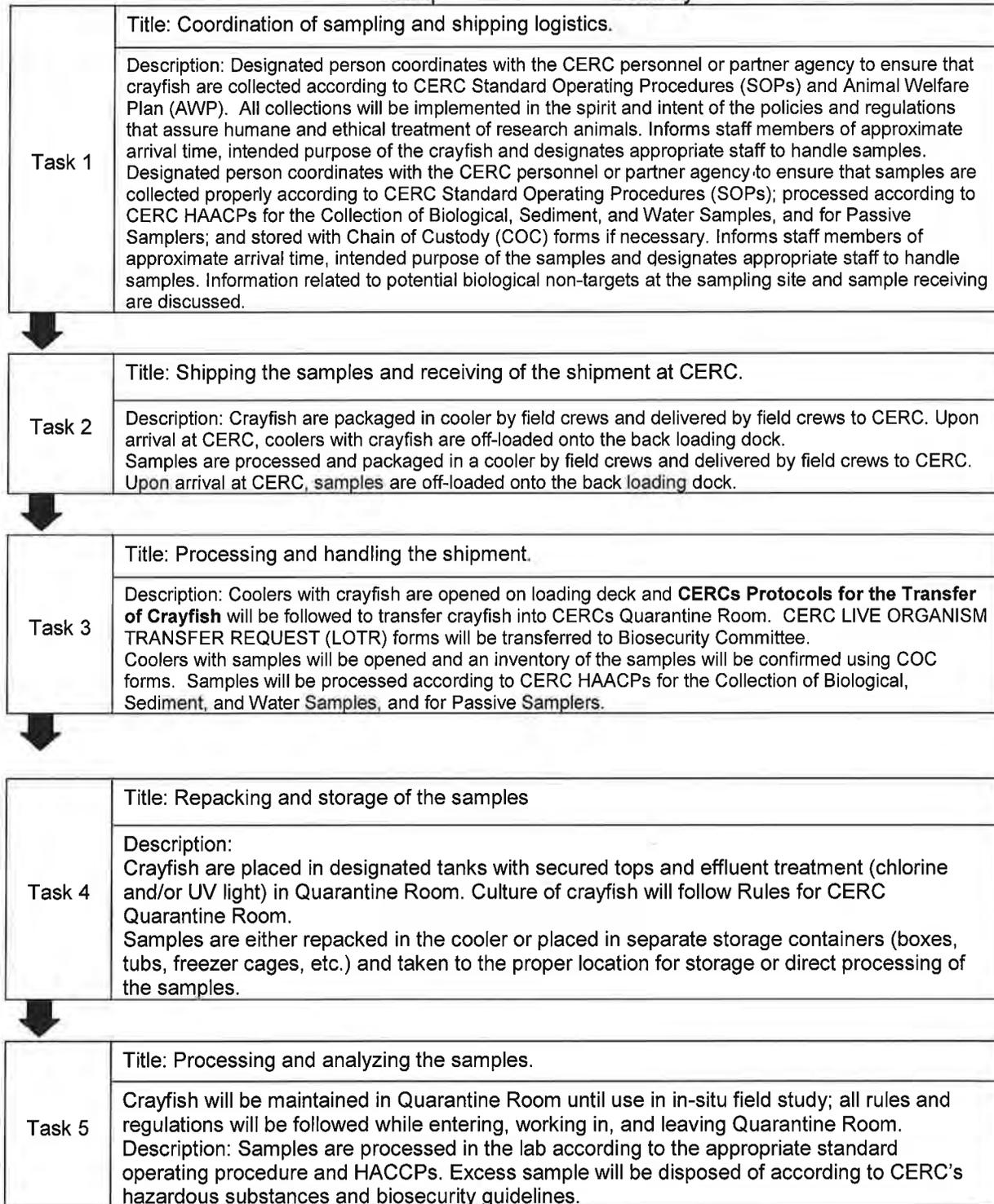
CERC Field Collection of Aquatic Organisms and Biological, Sediment, and Water Samples HACCP Plan (DRAFT)

HACCP Step 1 – Activity Description

| Management Objective & Contact Information | |
|---|-------------------------------|
| HACCP Plan Title: Field sampling for aquatic organisms (crayfish); Field collection of biological, sediment, and water samples for toxic inorganic chemical analysis | |
| Management Objective: Field sampling for aquatic organisms (crayfish); and field-collection of biological, sediment, and water samples for inorganic compounds. Provide this information to resource managers and regulatory agencies concerned about negative impacts on ecosystem health. | Contact Person: Ann L. Allert |
| | Phone: 573-876-1903 |
| | Email: aallert@usgs.gov |
| Activity Description i.e. Who; What; Where; When; How; Why | |
| <p>Who: Ecology Branch, Ecological Restoration Branch</p> <p>What: Field collection of crayfish for estimates of density and an in-situ toxicity test, and field collection of biologic samples biological, sediment, and water samples for toxic inorganic chemical analysis</p> <p>Where: Little St. Francis River and tributaries, St. Francis and Madison County, Missouri, USA; on-site processing of samples at Farmington, MO, USA</p> <p>When: March to August 2015 (conditions permitting)</p> <p>How: Field collection of crayfish for estimates of density will be done using a 1-m² quadrat or kick-seine sampler. Field collection of crayfish for in-situ toxicity test will be done using hand-nets or seines. Crayfish not used as voucher specimens or for inorganic analyses (metals) will be returned to the stream where they were collected. Crayfish not used in in-situ toxicity tests will be euthanized. Field collection of samples for inorganic analyses: A subsample of crayfish (n =3 per site; 3–5 crayfish per sample) will be placed in HDPE jars after they have been identified to species and carapace length measured. Sediment samples (n =3 per site) will be taken using a plastic scoop, 2-mm filtration bucket, and pre-cleaned jars. Subsurface grab samples of water (n =3 per site) will be taken using a pre-cleaned 1-gal container. Water will be filtered or placed in pre-cleaned sample containers depending on analysis. Equipment used to process samples will be rinsed with reverse-osmosis water and dried. Water used to rinse equipment will be put into the sanitary waste system. Pore water will be collected from sediments and placed into pre-cleaned jars. Macroinvertebrates, leaves, and crayfish will be taken from cages and each placed into pre-cleaned jars (n =3 per site) on two sampling dates.</p> <p>Why: To provide scientific data on the effects of trace metals on crayfish in the Little St. Francis River with cooperation from the Missouri Department of Conservation, in support of a National Damage Assessment (NRDAR) for US Fish and Wildlife Service.</p> | |

HACCP Step 2 – Activity Flow Chart

Outline Sequential Tasks of Activity



HACCP Step 3 – Identify Potential Non-Targets

Non-Targets That May Potentially Be Moved/Introduced

Vertebrates: USGS NAS database lists the following organisms non-native invasives in the St. Francis Drainage: Cyprinidae, *Cyprinus carpio*, Common carp; Centrarchidae (established Little St. Francis River in Madison County), *Ambloplites rupestris*, rock bass (established Little St. Francis River in Madison County), Cyprinidae, *Notropis buccatus*, silverjaw minnow (collected St. Francis River in Madison County).

Invertebrates: USGS NAS database lists the following organisms non-native invasives in the St. Francis Drainage: *Orconectes hylas* (Crustacean Decapoda; woodland crayfish; collected in St. Francis River and tributaries in St. Francis and Madison County); *Orconectes harrisonii* (Crustacean Decapoda; belted crayfish; collected in St. Francis River in St. Francis County); *Craspedacusta sowerbyi* (Coelenterates-Hydrozoan; freshwater jelly fish; collected in Cedar Lake and Lac Benet near Bonne Terre in St. Francis County); *Daphnia lumholtzi* (Crustaceans-Cladocerans; waterflea); Mollusks-Bivales, Corbiculidae, *Corbicula fluminea*; Asian clam (collected St. Francis River in Madison County).

Plants: Filamentous green algae (Chlorophyta/Charophyta) and potential other aquatic vegetation that is distributed Statewide in Missouri. None is listed for Little St. Francis River, but staff should be aware of the potential for their presence.

Other Organisms (pathogens, parasites, etc.) Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically *Escherichia coli* (*E. coli*), hepatitis, *Aeromonas/Pseudomonas*, *Batrachochytrium dendrobatidis* (chytrid fungus).

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|--|---|--|---|---|---|---|
| Task #1 Title: Coordination of sampling; and shipping logistics. | Vertebrates: Common carp; rock bass silverjaw minnow | No | Fish can be identified and euthanized prior to leaving site. | Bleach; MS-222; ice; Virkon® spray and paper towels | Yes | Preventative measures should be taken before leaving field sites. |
| | Invertebrates: several | No | When possible, invertebrates will be identified, removed, and/or euthanized prior to leaving sites. Gear will be cleaned between sites to prevent spread of organisms too small to visible identify. Gear will be cleaned according to Missouri Department of Conservation Cleaning and Disinfecting Gear and Equipment Protocols. Containers will be cleaned on-site according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers. | Bleach; MS-222; ice; Virkon® spray and paper towels | | |
| | Plants: several | No | Gear will be cleaned between sites to prevent spread of organisms too small to visible identify. Gear will be cleaned according to Missouri Department of Conservation Cleaning and Disinfecting Gear and Equipment Protocols. Containers will be cleaned according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers. | Bleach; MS-222; ice; Virkon® spray and paper towels | | |
| | Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms. | No | Gear will be cleaned between sites to prevent spread of organisms too small to visible identify. Gear will be cleaned according to Missouri Department of Conservation Cleaning and Disinfecting Gear and Equipment Protocols. CERC Protocols for the Receipt and Transfer of Crayfish will be followed. Containers will be cleaned according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers. | Salt; bleach; MS-222; ice; UV, Virkon® spray and paper towels | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|--|---|---|---|--|
| Task #2 Title: Receiving of crayfish and sample shipment at CERC. | Vertebrates: None | No | CERC Protocols for the Receipt and Transfer of Crayfish will be followed There is a very low risk of transferring non-target species because samples are contained in coolers and kept sealed and because containers will be cleaned on-site according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers. | N/A | No | There are no significant non-targets during this task. |
| | Invertebrates: several | Yes | | Salt, bleach, UV, temperature, Virkon® spray and paper towels | | |
| | Plants: None | No | | N/A | | |
| | Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms. | Yes | | Bleach; MS-222; ice; UV, Virkon® spray and paper towels | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures Can be applied during this task to reduce the risk of non-targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|--|--|--|--|---|---|---|
| Task #3 Title: Processing crayfish and handling the sample shipment. | Vertebrates: None | No | Once samples are on-station at CERC, the chance for non-target species to be transferred to an environment where they can spread or thrive is highly likely. | Crayfish should be processed immediately following CERC Protocols for the Receipt and Transfer of Crayfish. If samples are not processed immediately, the cooler and its entire contents should be placed in the walk-in freezer. Once removed from the freezer, the following procedures must be followed: CERC personal will follow CERC HAACPs for Biological, Sediment, and Water Samples, and for Passive Samplers upon return to CERC. Storage of samples in freezer should kill invertebrates. | Yes | This is a critical control point because this is the point where the transfer of a non-target species is most likely to occur. Surfaces that tissue samples are processed on have the highest risk of introducing a non-target species to CERC. |
| | Invertebrates: several | Yes | | | | |
| | Plants: None | No | | | | |
| | Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms. | Yes | | | | |

| 1 Tasks (From Step 2) | 2 Potential Non-Targets (From Step 3) | 3 Risk Assessment Are any non-targets significant? Yes or No | 4 Justification Justify your answer in Column 3 | 5 Control What Control Measures can be applied during this task to reduce the risk of non- targets? | 6 CCP Is this task a CCP? Yes or No | 7 Justification Justify your answer in column 6 |
|---|--|--|--|--|---|---|
| Task #4 Title: Culture of crayfish and storage of the samples | Vertebrates: None | No | Culture of crayfish will follow Rules for CERC Quarantine Room. Risk is low for transferring non-target species out of culture tank or through effluent of culture tank due to control measures to keep crayfish in tanks (tops) or treat effluent (UV/chlorine). All equipment and surfaces will be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. All wasted will be placed into the trash can then frozen before disposal. All materials including feces removed from tanks holding crayfish will be frozen before disposal. Risk is low for transferring non-target species because samples and storage cooler have been cleaned thoroughly before storage. Storage of samples in sealed containers in either a cooler or freezer minimizes any potential transfer of pathogens. | N/A | No | There are no significant non-targets during this task. |
| | Invertebrates: several | No | | | | |
| | Plants: None | No | | | | |
| | Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms. | No | | | | |

| | | | | | | |
|---|---|-----------|---|--------------|---|---|
| 1 | 2 | 3 Risk | 4 | 5 Control | 6 | 7 |
|---|---|-----------|---|--------------|---|---|

| Tasks (From Step 2) | Potential Non-Targets (From Step 3) | Assessment t Are any non- targets significant? Yes or No | Justification Justify your answer in Column 3 | What Control Measures Can be applied during this task to reduce the risk of non-targets? | CCP Is this task a CCP? Yes or No | Justification Justify your answer in column 6 |
|---|--|---|--|---|--|---|
| Task #5 Title: Processing and analyzing crayfish and the samples. | Vertebrates: None | No | If work area is not thoroughly disinfected after processing the samples, the risk of transferring pathogens throughout the lab is present. | All processing of crayfish will occur in the Quarantine Room and care should be given to not working over open drains. Constant checks around coolers should be made to ensure no crayfish have fallen to floor or escaped coolers. All equipment and surfaces will be sprayed with 2% Virkon® and allowed to sit for ten minutes before it is rinsed and wiped dry. All wasted will be placed into the trash can then be frozen before disposal. During the processing of samples, Best Lab Practices (BLP's) should be used at all times including wearing gloves while handling the samples. Upon completion of sample processing, all work and floor surfaces and equipment used during the processing must be sprayed down with a 2% Virkon® Aquatic or 2% chlorine solution and allowed to sit for ten minutes before surfaces are rinsed. Excess tissue samples can be placed in a bag and disposed of in the trash following a period of time after the final publication of data as agreed upon by CERC and its collaborator. For samples that cannot be disposed of (e.g., NRDAR projects), arrangements should be made for long-term storage at CERC or another facility during the planning stages of the project. | Yes | Samples will be exposed and handled on work surfaces potentially allowing pathogens to be transferred throughout the center and potentially infecting existing cultures or research projects. |
| | Invertebrates: several | No | | | | |
| | Plants: None | No | | | | |
| | Other Organisms Bacteria and pathogens of concern to both human health and aquatic organisms. | Yes | | | | |

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

| | | | |
|--|--|--|--|
| Mangement Objective From Step 1 | Import field-collected crayfish and field-collected non-living biologic, sediment and water samples and passive samplers to the Columbia Environmental Research Center (CERC) for analysis of toxic inorganic compounds that may negatively influence ecosystems and culture of crayfish for an in-situ toxicity test. Provide this information to resource managers and regulatory agencies. | | |
| Critical Control Point: | Task # | 3 | Title: Processing and handling the shipment. |
| Significant Non-Target(s) (Step 4, Column 3) | Bacteria, parasites and pathogens of concern to both human health and aquatic organisms; specifically <i>Escherichia coli</i> (<i>E. coli</i>), hepatitis, <i>Aeromonas/Pseudomonas</i> , <i>Batrachochytrium dendrobatidis</i> (chytrid fungus) | | |
| Control Measure(s) (Step 4, Column 5) | <p>Control Measures for the Field: Gear will be cleaned between sites to prevent spread of organisms too small to visible identify. Gear will be cleaned according to Missouri Department of Conservation Cleaning and Disinfecting Gear and Equipment Protocols.</p> <p>Disinfectant Sterilization: The disinfectant of choice is bleach (sodium hypochlorite). Disinfection is accomplished by using a 200-ppm bleach solution to immerse or rinse all field gear and equipment prior to going to another site. Gear and equipment (boots, waders, nets, traps, seines, wetsuits, etc.) will be completely sprayed or immersed in the disinfectant solution for 15 minutes or completely wetted with a bleach solution around 50% if immersing is not possible. Equipment needed include: a long handled brush 5–10 gallon bucket, sprayer. Brush should be used to remove mud, algae, and other debris from boots, waders, etc. It is best to allow the disinfectant solution to air dry on field gear and equipment if possible. If unable to air dry, rinse disinfectant solution with clean water on dry land not in the wetland. The remaining disinfectant solution should be poured on dry land not in water when finished.</p> <p>Desiccation Sterilization: Air drying of gear and equipment can also be used as a preventative for spreading and killing pathogens. Complete air drying, preferably in direct sunlight, at an air temperature of 86°F (30°C) or higher for 4 hours. Drying, as a strategy to sterilize gear and equipment, would have to be long enough to ensure that all water had evaporated. This method is the preferred method for scientific meters (i.e., water quality instruments, XRF).</p> <p>Control Measures for Receipt at CERC: CERC Protocols for the Receipt and Transfer of Crayfish will be followed. Containers will be cleaned according to CERC HAACPs for the Collection of Biological, Sediment, and Water Samples, and for Passive Samplers.</p> | | |
| Precribed ranges, limits, or criteria for control measure(s): (PRLC) | Sample bottles and cooler should be sprayed or wiped with 2% Virkon® for 10 minutes before rinsing or drying. Care must be taken to not damage sample labels. Coolers should be visually inspected after cleaning so there is no visible mud or sediment remaining outside or inside the cooler. | | |
| Monitoring the Control Measure(s) | Who? | Ecology and Chemistry technicians | |
| | How? | Make sure all surfaces and containers have been cleaned and sprayed with 2% Virkon or bleach and allowed to sit for ten minutes. | |
| | Where? | Quarantine room | |
| | How often? | When receiving a shipment of crayfish or biological, sediment, water samples or passive sampler . | |

| | |
|--|--|
| Corrective Action(s) if Control Measures Fail (or PRLC cannot be met) | Repeat control measures from task 3. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon® or bleach. |
| Supporting Documents <i>(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)</i> | |
| | |

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point

| | | | |
|---|---|--|--|
| Management Objective From Step 1 | Import field-collected crayfish and field-collected non-living biologic, sediment and water samples and passive samplers to the Columbia Environmental Research Center (CERC) for analysis of toxic inorganic compounds that may negatively influence ecosystems. Provide this information to resource managers and regulatory agencies. | | |
| Critical Control Point: | Task # | 5 | Title: Processing and analyzing the samples. |
| Significant Non-Target(s) (Step 4, Column 3) | Bacteria and pathogens of concern to both human health and aquatic organisms. | | |
| Control Measure(s) (Step 4, Column 5) | All processing of crayfish will occur in the Quarantine Room and care should be given to not working over open drains. Crayfish should be processed following CERC Protocols for the Receipt and Transfer of Crayfish. Constant checks around coolers should be made to ensure no crayfish have fallen to floor or escaped coolers. All equipment and surfaces will be sprayed with 2% Virkon® or bleach and allowed to sit for ten minutes before it is rinsed and wiped dry. All wasted will be placed into the trash can then be frozen before disposal. During the processing of samples, Best Lab Practices (BLP's) should be used at all times including wearing gloves while handling the samples. Upon completion of sample processing, all work and floor surfaces and equipment used during the processing must be sprayed down with a 2% Virkon® Aquatic solution or a 2% chlorine solution and allowed to sit for ten minutes before surfaces are rinsed. | | |
| Precribed ranges, limits, or criteria for control measure(s): (PRLC) | All work surfaces and equipment should be sprayed or wiped with 2% Virkon® or 2% chlorine solution for 10 minutes before rinsing or drying. | | |
| Monitoring the Control Measure(s) | Who? | Ecology and Chemistry technicians | |
| | How? | Make sure all surfaces have been cleaned and sprayed with 2% Virkon or 2% chlorine and allowed to sit for ten minutes. | |
| | Where? | Grinding room | |
| | How often? | When receiving a shipment of tissue samples. | |
| Corrective Action(s) if Control Measures Fail (or PRLC cannot be met) | Repeat control measures from task 5. Remove all visible dirt, sediments and water from cooler and spray with 2% Virkon® or 2% chlorine. | | |
| Supporting Documents (For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.) | | | |

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|---------------------------------|------------------------------------|--------------------------|------------------------------|
| Development Team Members | James Candri and Ann Allert | | |
| Date Developed: | 05/01/2015 | Date(s) Reviewed: | Biosecurity Committee |

***all fields in grey are required**

Appendix 12: Missouri Department of Conservation Cleaning and Disinfecting Gear and Equipment Protocols

Due to the potential of spreading pathogens, especially amphibian chytrid fungus (*Batrachochytrium dendrobatidas*) and Ranavirus (Iridoviridae), among survey sites, all field gear and equipment should be cleaned and disinfected.

Specific equipment should be cleaned between sampling sites. At minimum any gear and equipment (boots, waders, nets, traps, seines, wetsuits, etc.) that has been in contact with the soil or water should be cleaned and disinfected before, between, and after each wetland (river, pond, pool, etc.) either by use of disinfectant, desiccation, or heat sterilization.

1. **Disinfectant Sterilization:** The disinfectant of choice is bleach (sodium hypochlorite). Disinfection is easily accomplished by putting 4 ounces of bleach (half cup) in one gallon of clean water and using this solution (3% disinfectant solution) to immerse or rinse off all field gear and equipment prior to going to another wetland (river, pond, pool, etc.). Gear and equipment (boots, waders, nets, traps, seines, wetsuits, etc.) should completely be immersed in the disinfectant solution for 15 minutes or completely wetted with a bleach solution around 50% if immersing is not possible. For this disinfecting process, a long handled brush and 5–10 gallon bucket should be considered standard equipment for field investigators. Brush should be used to remove mud, algae, and other debris from boots, waders, etc. Bleach is the disinfectant of choice because it is the least hazardous chemical for disposal, and it is known to decompose rapidly in the environment. It is best to allow the disinfectant solution to air dry on field gear and equipment if possible. If unable to air dry, rinse disinfectant solution with clean water on dry land not in the wetland. The remaining disinfectant solution should be poured on dry land not in water when finished.
2. **Desiccation Sterilization:** Air drying of gear and equipment will also suffice as a preventative for spreading and killing pathogens. Complete air drying, preferably in direct sunlight, at an air temperature of 86°F (30°C) or higher for 4 hours will suffice. Drying, as a strategy to sterilize gear and equipment, would have to be long enough to ensure that all water had evaporated.
3. **Heat Sterilization:** Gear and equipment soaked in hot water will also eliminate pathogens. Heat sterilization at 160°F (71°C) for 20 minutes or 117°F (47°C) for 30 minutes will eliminate pathogens.

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