Effects of lead-zinc mining on fish density in riffle areas of the Big River (southeast Missouri)

MDC Workplan
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Statistical Advisors: Steven Sheriff, Ivan Vining

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Background and Study Justification:

The designated natural resource Trustees for Missouri in accordance with the Comprehensive Emergency Response, Compensation and Liability Act of 1980 (i.e. Superfund) are the U.S. Fish and Wildlife Service and Missouri Department of Natural Resources. The designated Trustees have decided that a Natural Resource Damage Assessment (NRDA) needs to be conducted for the Big River in Southeast Missouri. As part of the NRDA process, the Trustees have officially requested MDC’s assistance to assess the adverse impacts of mining activities on fish populations in the Big River.

Previous studies have documented that sediments in Big River from around Leadwood to the confluence with the Meramec River, and perhaps beyond, contain lead and/or zinc levels above the probable effects threshold (PEC) for aquatic invertebrates. Lead concentrations in fillet tissue of certain species are known to exceed the level considered by Missouri Department of Health and Senior Services (DHSS) to be safe for people to consume (i.e. 0.3 parts-per-million). This exceedance has resulted in DHSS issuing a fish consumption advisory for this reach of the Big River. Although data clearly show that fish in the Big River take up lead, biological impacts of lead exposure on fish are less well documented. Fish from the area are known to be affected by lead at the biochemical level as evidenced by inhibition of alpha-amino levulinic acid dehydrogenase in their blood. The purpose of this study is to determine if population level effects in fish populations are associated with the riffle areas of the Big River where sediments are contaminated with lead and/or zinc.

Study Objectives:

The research question for this study is as follows: “is the population size of benthic riffle fish in the Big River related to the amount of lead and/or zinc found in the sediment?” We have selected density of fishes per unit area as the metric to use to assess fish population size. We have selected riffle areas because previous data has shown that
benthic species of fish can be sensitive to mining wastes (Allert et al., personal communication).

The specific objective of the study is:

- to determine the density of riffle benthic fish species from select reaches of the Big River.
- to investigate the relationship between fish density of indicator species and the levels of lead and/or zinc in the sediment

**Experimental design:**

Density of riffle fishes will be determined in areas identified as containing background, low, moderate and high levels of lead in the sediment. Densities of each fish species will be regressed against the metal concentrations in the sediment to determine if there is a relationship. The Trustees will use this data to determine percent injury by measuring sediment metal concentrations and predicting level of injury based on the model developed in this study.

**Site selection:**

Sampling in 2007 by USFWS indicates that Big River sediment metal concentrations consistently exceed PECs from the area near Cedar Hills up to the Leadville area (Mosby personal communication). We characterized these sites as being background (<40 ppm lead), low (41-400 ppm lead), medium (401-800 ppm lead) or high (>800 ppm lead). These categories are not based on known or predicted biological impacts on fish since these data are not available. The categories were selected to discriminate between perceived differences in sediment contamination levels. The values used in this exercise were based on XRF readings which are suspected to be lower than traditional metal analysis using atomic absorption technology in the laboratory.

The sites selected for this study are shown in Table 1. Reference sites 1 and 2 are similar in physical habitat and biota as the Big River sites between these locations and the confluence with the Mineral Fork River. The Big River below the confluence with Mineral Fork River is a larger stream and may be considerably different from the upper Big River reference sites for the riffle fish sampled. For this reason, several reference sites of similar size in the nearby Bourbeuse River were identified and are indicated in Table 1.

**Site Characterization:**

Each riffle will be measured at one randomly selected lateral transect from the lateral transects established in the crayfish study conducted in July 2008 (Allert et al. 2008). If the transect is within 2 m of the measurements from the previous study, then this study will use the physical habitat descriptions from the crayfish study. If not, then the size and description of the habitat will be recharacterized using the methods in Allert et al. (2008).
The size of each of the pool and riffle areas sampled will be determined and recorded. The width of the riffle will be measured to the edge of where the water is moving. Backwater areas along the edge, such as water willow areas, will not be included in the width measurements. UTM coordinates should be recorded to delineate each pool and riffle. This information will be used to determine the amount of each habitat type in the sample site.

For each quadrat, the depth, water velocity and the substrate structure will be determined at the 4 corners and the middle. The temperature, pH and dissolved oxygen will be measured in a water sample from the middle of the quadrat. A picture of each quadrat will be taken from the downstream side viewing upstream,
Table 1. MDC study sites for 2008 Big River fish study

<table>
<thead>
<tr>
<th>Type of Site</th>
<th>County</th>
<th>Site ID</th>
<th>Site name</th>
<th>Order of Sampling</th>
<th>Miles from Desloge</th>
<th>Lead Conc.</th>
<th>Sediment Lead</th>
<th>Sediment Zinc</th>
<th>Y Proj</th>
<th>X Proj</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ref</td>
<td>Wash.</td>
<td>R-1</td>
<td>Above Irondale/Cedar Ck</td>
<td>5</td>
<td>-21</td>
<td>Bckgd</td>
<td>19</td>
<td>0</td>
<td>4188295</td>
<td>701719</td>
</tr>
<tr>
<td>Ref</td>
<td>Wash.</td>
<td>R-2</td>
<td>Irondale Access</td>
<td>4</td>
<td>-19</td>
<td>Bckgd</td>
<td>16</td>
<td>5</td>
<td>4189477</td>
<td>703293</td>
</tr>
<tr>
<td>Ref</td>
<td>Franklin</td>
<td>R-3</td>
<td>Bourbeuse River (4 miles below Union)</td>
<td>8</td>
<td>NA</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
</tr>
<tr>
<td>Ref</td>
<td>Franklin</td>
<td>R-4</td>
<td>Bourbeuse River (4 miles above Union)</td>
<td>10</td>
<td>NA</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
</tr>
<tr>
<td>Mined</td>
<td>St. Fran.</td>
<td>TH-1</td>
<td>Desloge (Hwy 67 bridge above Flat River)</td>
<td>9</td>
<td>3</td>
<td>High</td>
<td>813</td>
<td>957</td>
<td>4196518</td>
<td>718773</td>
</tr>
<tr>
<td>Mined</td>
<td>St. Fran.</td>
<td>TH-2</td>
<td>Hwy K</td>
<td>1</td>
<td>6</td>
<td>High</td>
<td>927</td>
<td>607</td>
<td>4200703</td>
<td>719663</td>
</tr>
<tr>
<td>Mined</td>
<td>St. Fran.</td>
<td>TM-1</td>
<td>Hwy 67 (north of Bonne Terre)</td>
<td>6</td>
<td>14</td>
<td>Mediu m</td>
<td>495</td>
<td>501</td>
<td>4203654</td>
<td>715024</td>
</tr>
<tr>
<td>Mined</td>
<td>Jefferson</td>
<td>TM-2</td>
<td>Mammoth Access</td>
<td>3</td>
<td>38</td>
<td>Mediu m</td>
<td>672</td>
<td>403</td>
<td>4221939</td>
<td>703711</td>
</tr>
<tr>
<td>Mined</td>
<td>Jefferson</td>
<td>TL-1</td>
<td>Washington State Park (Above Mineral Fork)</td>
<td>7</td>
<td>34</td>
<td>Low</td>
<td>229 (hwy CC)</td>
<td>110 (hwy CC)</td>
<td>4217325</td>
<td>703881</td>
</tr>
<tr>
<td>Mined</td>
<td>Jefferson</td>
<td>TL-2</td>
<td>Above Cedar Hill dam</td>
<td>2</td>
<td>74</td>
<td>Low</td>
<td>285</td>
<td>111</td>
<td>4247120</td>
<td>706082</td>
</tr>
</tbody>
</table>
The concentration of lead and zinc in the riffle area will be based on the data collected in the crayfish study conducted in July 2008 (Allert et al. 2008).

**Riffle fish sampling**

Riffles to be sampled will be the same as in the crayfish study (Allert et al. 2008). Each site in Table 1 will be made up of three riffles. The riffle fish will be collected at each site using block nets and backpack electroshocker.

**Sampling equipment**

The following needs to be available during the field sampling:
- 4 x 8 m enclosure net – 1/8” Ace mesh, 4’ deep, continuous lead line, anchored by 4’ or 6’ rebar at each corner and center of each panel
- Smith Root backpack electrofisher
- EF dip nets
- 8 m seine for lower enclosure
- tape measure
- Random numbers table
- Camera
- Field notebook

**Sampling unit**

Riffle fish will be sampled at a site by electroshocking fish in randomly placed 4 m x 8 m (32 sq. m) block nets. The location of sampling units among the 3 riffles at a particular site will be determined prior to going to the field. This will be done using the riffle dimensions determined in the Big River crayfish study conducted in July 2008. The size of the riffles will be estimated by plotting the riffle dimensions on graph paper for a particular site and then visually placing the maximum number of 4 x 8 m quadrats into each riffle. The total number of potential sampling units per site will be determined by summing the number of potential sites from each of the three riffles. A total of 3 quadrats will be randomly selected if there are <12 quadrats. If there are 12 to 15 potential locations 4 quadrats sites will be randomly selected. If there are greater than 15 potential locations then 5 (or 6 if possible) quadrats should be randomly selected.

Upon arriving at the sampling site in the field, the researchers will determine if the size of the riffles are similar to the dimensions used in the laboratory assignment of quadrat locations for that site. If no changes are noted, then the location of the downstream left corner of the quadrat should be positioned as indicated in the original laboratory drawings. If the riffle has changed slightly but no potential sampling locations need to be removed or added, then the location of the quadrat can be made by moving the lower left corner in an unbiased way to accommodate the changes in the riffle size. If the riffle has changed so much that potential quadrats need to be added or removed, then this should be done and the number of sampling units should be randomly selected from the new pool.
**Sampling method**

1. Drive 8 rebars into riffle for net supports. Enclosure will be 4 m wide (across riffle) by 8 m long (upstream to downstream). After sufficient time for the fish to settle down, the net will be carefully dropped into the water beginning at the downstream edge of the quadrat, then the sides and finally the top.
2. Trench lead line into substrate, by hand.
3. Place 4 m seine along downstream end of enclosure.
4. Each quadrat will be sampled using 3 sequential electrofishing runs; each run covering the entire quadrat. If no fish are captured in the first and second run for a particular quadrat, then no additional third run will be necessary to verify the absence of fish. However, if fish were captured in the first run but not in the second then a third run will be necessary to verify the absence of fish. Additional runs may be necessary beyond 3 runs in a sample if each subsequent run from the first has increasing number of fish. We need to have 3 consecutive runs with decreasing numbers of fish in each. This should be the norm given the above design. It is recommended that if the second run has more fish captured than the first and third then a fourth run be made.
5. Electrofishing operator and dip netters enter enclosure from upstream corners or other manner that minimizes potential impact on the fish.
6. Electrofish (EF) perpendicular to stream channel beginning at the top of the enclosure and sampling across the enclosure. The surface rubble should be lightly disturbed by kicking to ensure that stunned fish are not caught under rocks, etc. Large rocks may be moved, if necessary. EF operator maintains a straight line (across the enclosure) and slowly sweeps anode back and forth (roughly 2 m wide sections) until contacting opposite end of enclosure. Then, move downstream 2 m and repeat. A total of 4 passes should be made during each run to completely cover the enclosure.
7. All impacted riffle fishes should be retrieved by dip netters and placed into buckets.
8. After each run, the downstream seine should be pulled to retrieve fish that drifted past dip netters, then re-set.
9. All fish collected will be measured to the nearest 0.5 cm, identified to species and counted. If species cannot be identified in the field then it will be assigned a temporary name label and counted. A voucher specimen for all fish species will be preserved for subsequent identification. **Run data will be kept separate.**
10. Repeat run procedure from step #6.
11. The endpoint for the study will be the population estimate of benthic fish guild. This guild will be comprised of sculpins, darters, stonecats and madtoms. Because fish will be identified to species, the data can be used to explore other types of guild or taxonomic groupings *post-hoc*.

**Staff requirements:**
A total of 7 persons will be needed to sample a site:

- Backpack operator (1)
- Dipnetters (2)
- Seine holders (2)
- Miscellaneous helpers (2)

**Data analysis:**

The total number of benthic fishes will be estimated by examining the depletion curve for the total number of fish from the sculpins, darters, stonecats and madtoms captured during each run. Total density will be estimated using the general removal method (Willisam, et al., 2002; Amstrup et al., 2005). The percent injury will be derived by comparing the density in the “treated” sites to the density of fish at the “reference” sites.

**Timetable:**

- August 4- First draft of work plan
- August 8- Final work plan
- August 11 to September 15- Target dates for conducting studies
- October 31- Summary of findings
- January 15- Draft report
- February 1- Final report

**Literature Cited:**


ATTACHMENT #1
MDC Quality Assurance Plan

The DOI NRDA regulations require the trustees to develop a Quality Assurance Plan (QAP) that “satisfies the requirements listed in the NCP and applicable EPA guidance for quality control and quality assurance plans” (43 CFR §11.31(c)(2)). Such a plan is needed to ensure the validity of data collected as part of the NRDA and to provide a solid foundation for the Trustees’ subsequent decisions. Also relevant to this effort are the FWS guidelines developed under the Information Quality Act of 2001. All information developed in this NRDA will be in compliance with these guidelines.

A study plan must provide sufficient detail to demonstrate that:

- the project technical and quality objectives are identified and agreed upon;
- the intended measurements, data generation, or data acquisition methods are appropriate for achieving project objectives;
- assessment procedures are sufficient for confirming that data of the type and quality needed and expected are obtained; and
- any limitations on the use of the data can be identified and documented (EPA 2001).

Accordingly, specific study plans developed for this assessment will include the four elements called for by EPA:

- Project Management – documents that the project has a defined goal(s), that the participants understand the goal(s) and the approach to be used, and that the planning outputs have been documented;
- Data Generation and Acquisition – ensures that all aspects of project design and implementation including methods for sampling, measurement and analysis, data collection or generation, data compiling/handling, and quality control (QC) activities are documented and employed;
- Assessment and Oversight – assesses the effectiveness of the implementation of the project and associated QA and QC activities; and,
- Data Validation and Usability – addresses the QA activities that occur after the data collection or generation phase of the project is completed.

Project Management:

The project management organization will follow the guidance provided in Figure 1. The Principal Investigator for this project will be Mike McKee, MDC. The Principal Investigator will be responsible for drafting the project work plan and quality assurance plan. The PI will coordinate review of the documents and ensure that the information is communicated to all involved. A project master file will be
The Field Team Leaders for the study will be Jennifer Guyot for Washington County, Mike Reed (MDC) for St. Francois County and Kevin Meneau (MDC) for Jefferson County. They will be responsible for reviewing all support documents, procuring necessary equipment, training of support staff, field recording of data and ensuring worker safety.

The Assessment Manager (AM) will be Dave Mosby (USFWS). The AM’s role will be to review all project and QA plans to ensure that the USFWS-NRDA requirements are met. The PI will report to the AM any deviations from the work plan or from the quality assurance plan. These deviations will be documented in the Communications folder in the master file maintained by the PI. A separate Quality Assurance Coordinator will not be designated for this study. The role of the QAC will be fulfilled by the PI in cooperation with the AM.
Data Generation and Acquisition:

The project objectives, site selection rationale, experimental design and site description requirements are described in the main body of this plan. The data collection phase of this study will be principally related to procuring of fish by electroshocking and recording the number of fish captured in the sequential runs in each quadrat.

The method of procuring the fish from the water at each sampling site will depend on the species and location. The method of sampling will be electroshocking. Fish collection methods generally follow guidance provided in the second edition of *Fisheries Techniques* (Murphy and Wills, 1996) with emphasis on Chapters 7 and 8. Fish specimens will be identified to species in accordance with Pflieger (1997).

Copies of the field collection forms will be provided to the PI and the originals maintained in the Field Team Leader’s files. The PI will review all sheets for lack of clarity and review any discrepancies with the Field Team Leader. The corrected/approved data sheets with information notes will be retained in the Master File.

Assessment and Oversight:

The collection of fish and recording of field collection data are routine activities of the MDC field staff. The training and operation of electroshocking equipment will be subject to the standard procedures outlined in the training received by the MDC employees. The PI will make one field audit during the study. The PI will maintain oversight of the data recording methods, data quality control and data analysis. If there are any findings that affect the quality of the data collected, the PI will refer the issue to the Assessment Manager for resolution.

Data Validation and Usability:

The principal metric used in this study is the estimation of fish population density in the defined sampling sites. The study is self-validating in that it characterizes the detectability of each species by recording the progressive decline in capture rates in multiple passes with the electroshocker. The usability of the data will relate to the variability in estimates of the density metric estimated from the fish removal curves.

Literature Cited: