



**Monitoring Fish and Benthic Invertebrate Populations and Metal Tissue  
Concentrations in Lower Belt Creek - 2018**

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## **Background**

Decades of underground coal mining in the Great Falls Coal Field near Belt, Montana have resulted in acidic mine drainage (AMD) discharging into Belt Creek. The contaminants of concern (COC) in the AMD that exceed DEQ-7 criteria include dissolved aluminum (Al) and total recoverable (TR) concentrations of arsenic (As), beryllium (Be), cadmium (Cd), copper (Cu), iron (Fe), thallium (Tl), and zinc (Zn), while water quality exceedances downstream of the discharges in Belt Creek have generally been for dissolved Al and total recoverable Fe during baseflow conditions.

The Montana Department of Environmental Quality (MDEQ) is proposing to construct a water treatment plant in the area of Coke Oven Flats to treat several sources of AMD prior to it reaching Belt Creek and thereby improving water quality in Belt Creek. Treatment of the AMD water is expected to begin by late 2020 to early 2021. Montana Fish, Wildlife and Parks (MFWP), in cooperation with MDEQ, completed an initial study to document fish and benthic invertebrate populations, upstream and downstream of the AMD in 2015 (Mullen et al. 2018). This sampling event provided baseline fish and benthic invertebrate population data to allow for future comparisons following AMD water treatment. Additional sampling was conducted in 2018 to further assess population indices and document the baseline condition of metals in fish and benthic invertebrate tissue upstream and downstream of the AMD, prior to the start of treatment.

## **Methods**

### **Fish population indices**

Fish populations were sampled in the sections immediately upstream (control) and downstream (impact) from the AMD site (considered the Anaconda discharge for this report) in Belt Creek on November 2, 2015 (Figure 1 and Table 1). The control section is located approximately 100 m upstream from the AMD site and measured 229 m in length, while the impact section is located approximately 600 m downstream from the AMD site and measured 205 m in length.

Fish were temporarily stunned using a backpack electrofishing unit (Smith-Root LR-24) subsequently captured by two netters, and temporarily placed in a holding net. Two passes through the section were completed and fish captured from each pass were kept separate to allow for population estimates. All captured fish were identified to species, enumerated, measured, weighed, and released. Population estimates were later computed using the Zippin's depletion model (Zippin 1956). Model outputs were automatically computed per section length and were then standardized to 100 m.

### **Benthic macroinvertebrate population indices**

Benthic macroinvertebrate population sampling was conducted on July 13<sup>th</sup>, 2018 at a total of 5 sites (Figure 1). Sites were sampled on this date on the descending limb of the hydrograph to ensure water was flowing throughout the entire reach. Sampling had been conducted previously on September 30, 2015 and the current AML 4 site was dry at that time (Mullen et al. 2018). Five sites were sampled in 2018 on Belt

Creek to evaluate the downstream extent of impacts. The most upstream site would be upstream of the impacted area and serve as a reference site. Exact site locations were selected based on access and available habitat conditions (e.g., presence of suitable riffle habitat). Sampling protocols were consistent with the sampling efforts in the upper Belt Creek drainage and will allow for a more comprehensive evaluation of the health of Belt Creek.

One riffle within each site was selected for sampling of relative macroinvertebrate abundance and species composition. At each site, 3 Hess samples and 1 traveling kick were deployed for macroinvertebrate collection. The Hess sampler (600  $\mu\text{m}$  mesh) measures 16 inches in height, 13 inches in diameter, and covers a 133 sq. inch (0.086  $\text{m}^2$ ) bottom area. Hess samples were taken across the riffle as to sample left/right bank, and center of the stream. A traveling kick sample was taken in the riffle area for approximately 30 seconds with a D-frame kick net (900  $\mu\text{m}$  mesh; 12" width, 10"). All samples were placed in Nalgene bottles with 95% ethanol. Each sample was labeled with site, date, and sample type. The alcohol was replaced within 24 hours due to organic material in the jars.

All samples were sorted from debris using a magnifying light. The macroinvertebrates were preserved in vials with 95% ethanol and labeled according to site, date and sample type (hess or kick). Hess samples were counted entirely and analyzed for species composition and relative abundance. Rapid Bioassessment Protocol (RBP) III sorting methodology (Plafkin et al. 1989) were used to obtain a 300 organism ( $\pm 10\%$ ; 270-330 organisms) subsample for each kick-net collection and examined for additional species. Samples were identified to the lowest taxa possible (Stewart and Stark 1988; Merritt and Cummins 1996; Wiggins 1996).

### **Fish Tissue**

Two sites were sampled to collect fish tissues for metals analysis on September 10<sup>th</sup>, 2018, including a site upstream (control) and downstream (impacted) of the AMD near the Town of Belt Creek (Figure 2). The downstream site corresponded with the fish monitoring site sampled in 2015, near where the foot bridge was formerly located in the Town of Belt. The upstream site was sampled upstream of the AMD, near Armington Junction to reduce the chance of sampling a fish that had recently been downstream of the AMD. The downstream site was sampled first to guide in the selection of species and sizes at the upstream site, given the fish population was expected to be more limited at the downstream site. Fish sampled included 5 brown trout, 5 white suckers, and 5 rocky mountain sculpin from both the upstream and downstream sites. Brown trout (livers), white sucker (whole-bodies), and rocky mountain sculpin (whole-bodies) were analyzed for concentrations of Al, As, Cd, Fe, Pb, Ni, Se, and Zn in liver tissue. Brown trout muscle tissue was analyzed for Hg.

### **Benthic Invertebrate Tissue**

Benthic macroinvertebrates were collected on September 10<sup>th</sup>, 2018 for metals analyses from the same sites (upstream and downstream) as the fish tissue analysis (Figure 2). The downstream (impacted) site was sampled first in order to have a species list to target at the upstream (control) site. A kick-net was primarily used to dislodge benthic macroinvertebrates from rocks, then forceps were used to pick benthic

macroinvertebrates from the net or dissection tray. Larger rocks were also overturned in order to target uenoidae species that were attached to rocks. Sampling occurred until ~2 grams of tissue content were obtained from each of at least 3 taxa, or we sampled all available habitat within the sampling site. Each taxa was composited and analyzed for concentrations of Al, As, Cd, Fe, Pb, Ni, Se, and Zn. These methods resulted in a sample size of one for each taxa for qualitative comparison between upstream and downstream sites.

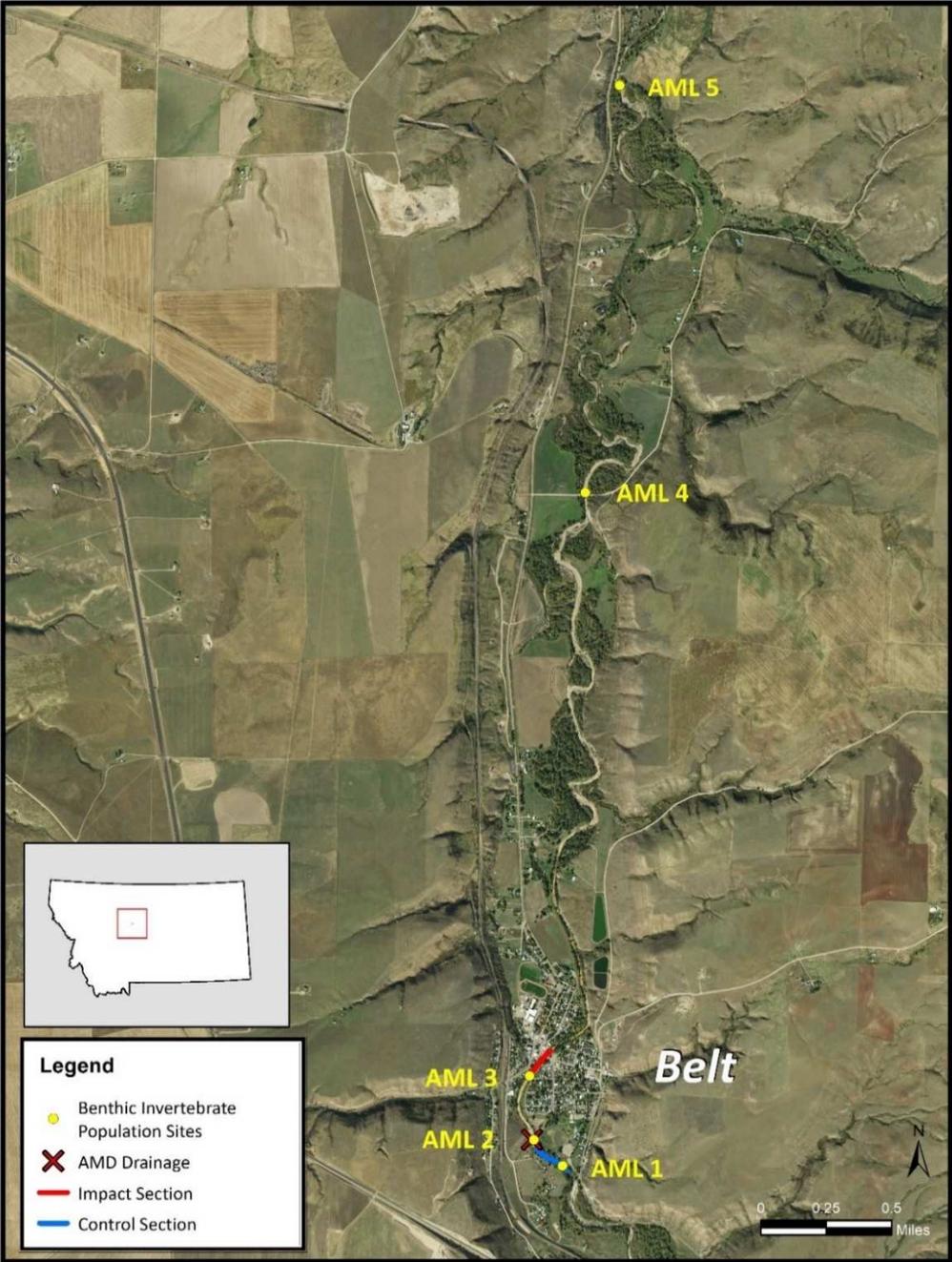


Figure 1. Sampling locations for benthic macroinvertebrate and fish population monitoring.

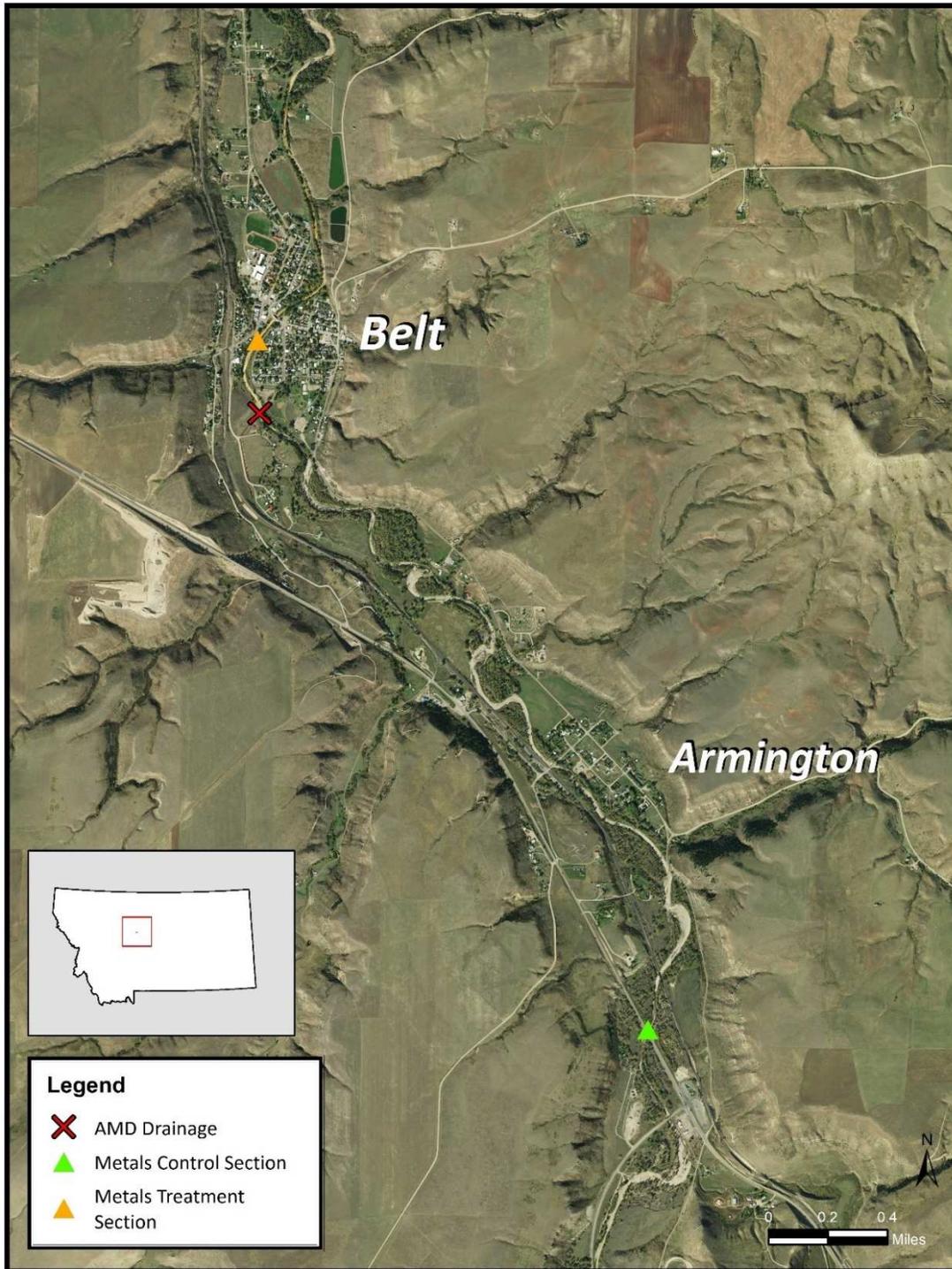


Figure 2. Sampling location for benthic macroinvertebrate and fish tissue monitoring.

## Results

### Fish Population Indices

Six species of fish were collected including brown trout, rainbow trout, longnose sucker, mountain sucker, sculpin, and longnose dace. Four of the six species were sampled in both sections, with mountain whitefish only collected in the control section, and white suckers only collected in the impact section. Population estimates were conducted on all species collected except for sculpin and longnose dace. Hundreds of sculpin and longnose dace of all size classes (young-of-year to adult) were observed in control and treatment sections, but their small size prohibited accurate population estimates.

Overall, we estimated approximately 2.4 times as many fish in the control section compared to the impact section (Figure 3). Population estimates were higher in the control than impact section for brown trout (1.8 times), rainbow trout (9.0 times), and mountain sucker (6.0 times). However, this trend was not true for longnose sucker, which were much more abundant (22 times) in the impact than the control section.

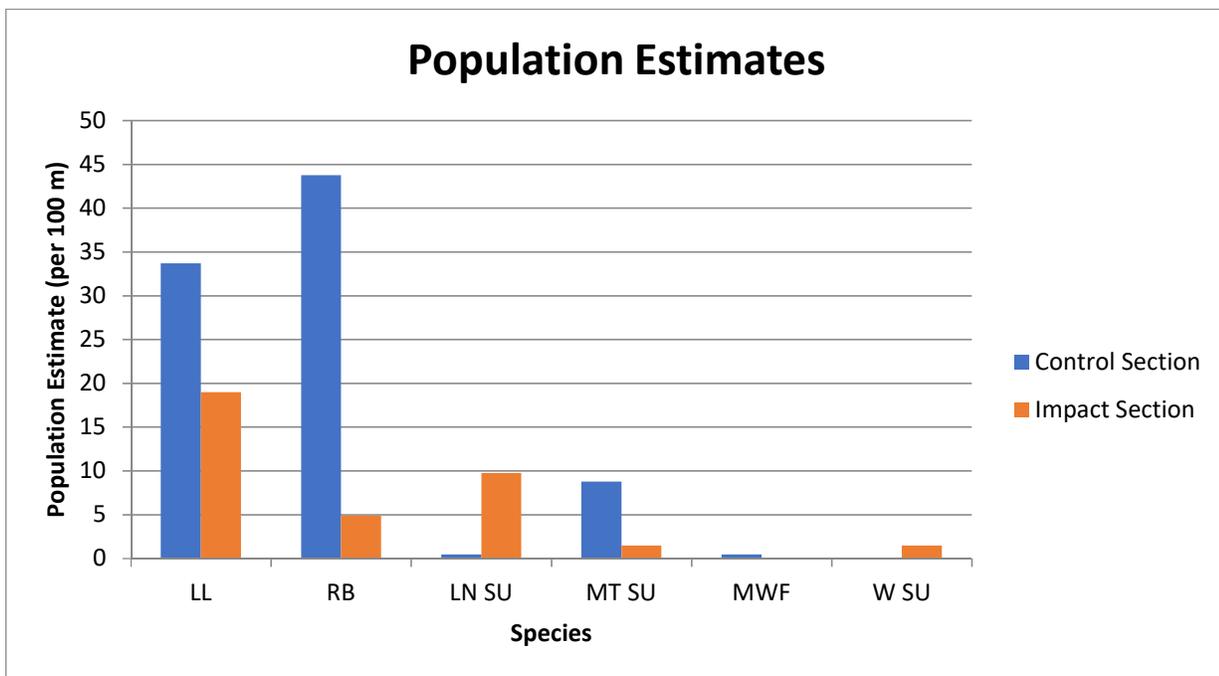


Figure 3. Population estimates for all species sampled in the control and impact sections of Belt Creek (excluding sculpin and longnose dace). No mountain whitefish were collected in the impact section and no white suckers were collected in the control section. Fish species abbreviations provided in Table 1.

Table 1. Abbreviations, tolerance values, and tolerance categories (intolerant, intermediate, or tolerant) of species sampled in the control and impact sections of Belt Creek. Possible tolerance values range from 0.0 – 10.0, with higher values indicating increased tolerance to anthropogenic disturbances.

<b>Species</b>	<b>Abbreviation</b>	<b>Tolerance Value</b> <i>(Whittier et al. 2007)</i>	<b>Tolerance Category</b> <i>(Barbour et al. 1999)</i>
Rainbow Trout	RB	2.1	Intolerant - Intermediate
Mountain Whitefish	MWF	2.5	Intolerant
Brown Trout	LL	2.7	Intolerant - Intermediate
Mountain Sucker	MT SU	4.4	Intermediate
Longnose Sucker	LN SU	4.6	Intolerant - Intermediate
White Sucker	W SU	7.6	Tolerant

Overall, trout sampled in the control section were larger than those sampled in the impact section (Figure 4). Medium-to-large size rainbow trout were absent in the impact section (no rainbow trout were sampled over 100 mm), whereas a fairly continuous range of rainbow trout sizes were sampled in the control section (approximately 21% were over 100 mm, with a maximum size of 315 mm). We also sampled an abundance of small young-of-year rainbow trout in the control section compared to only a few in the impact section. Furthermore, young-of-year brown trout were generally smaller in the impact than control section.

We also observed that fish in the impact section were noticeably lighter in color than fish in the control section. This observation may be related to the difference in water clarity between the two sections, as we observed much higher turbidity in the impact than control section.

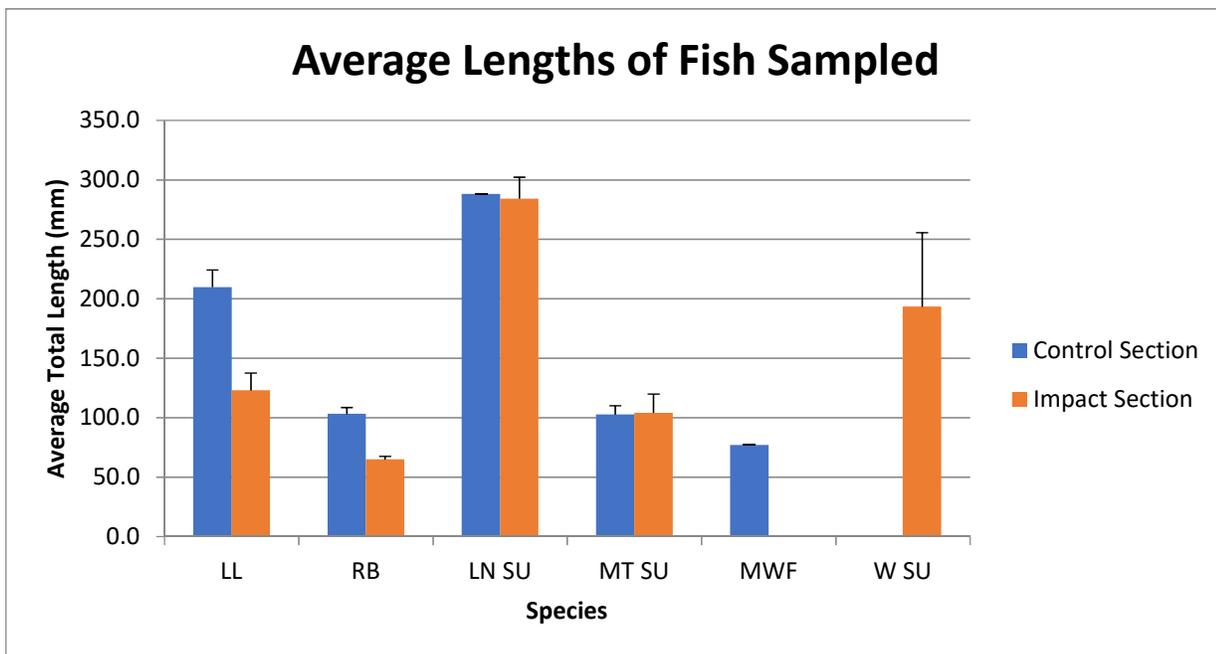


Figure 4. Average lengths of all species sampled (excluding sculpin and longnose dace). Bars represent standard error. No mountain whitefish were collected in the impact section and no white suckers were collected in the control section. Fish species abbreviations provided in Table 2.

### Benthic macroinvertebrate population indices

Benthic macroinvertebrates were sampled in both 2015 and 2018 for population indices. In 2015, sampling was conducted on September 30<sup>th</sup>. Site AML 4 was dry, but the remaining four sites were sampled. Mean total organisms (per m<sup>2</sup>) at the control site (AML 1) were 34 and 15 times greater than the impacted sites (AML 2 and AML 3, respectively), while the most downstream site (AML 5) had densities similar to the control (Figure 5). Sampling in 2018 was conducted on July 13<sup>th</sup>, when flow conditions were higher and allowed for sampling at AML 4. Mean total organisms at the control site (AML 1) in 2018 were higher than the next three downstream sites (2.9, 2.0, and 2.2 times higher), while the most downstream site (AML 5) had similar densities to the control (Figure 5).

Overall, benthic macroinvertebrates were impacted downstream of the AMD in both 2015 and 2018 sampling (Figure 5). Downstream of the control site (AML 1) impacts were more pronounced in 2015 than in 2018. The mean number of organisms sampled at the control site (AML 1) averaged 9,323/m<sup>2</sup> in 2015 and 2018 (Figure 5). Immediately downstream of the AMD at AML 2, the mean number of organisms were lowest, averaging 3,233/m<sup>2</sup> or 65% reduced abundance. Moving downstream to sites AML 3 and -4, mean numbers increased slightly to 4,741 and 4,180/m<sup>2</sup>, but were still 49 and 55% lower than the control site. The mean total number of organisms did not recover to control site abundance until AML 5 where mean numbers increased to 10,444/m<sup>2</sup>.

The total number of taxa showed a declining trend downstream of the control site (AML 1), being lowest at AML 3 in 2015, and at AML 4 when water was present in 2018 (Figure 5). In both years the most downstream site (AML 5) increased in taxa richness, but not to the same number as the control site.

The BMI results between 2015 and 2018 are difficult to compare since it would be hard to determine whether differences are due to annual variability, or simply seasonality difference due to higher flows and warmer water temperatures. Still the overall trends downstream of the AMD show impacts in both years. Overall, BMI densities appeared to recover by AML 5, however based on taxa richness, it appears there could still be influence at the most downstream site from the AMD. Though, this difference could also represent a natural change in the benthic invertebrate community due to the longitudinal difference in water quantity and quality (e.g., warmer temperatures related to less flow).

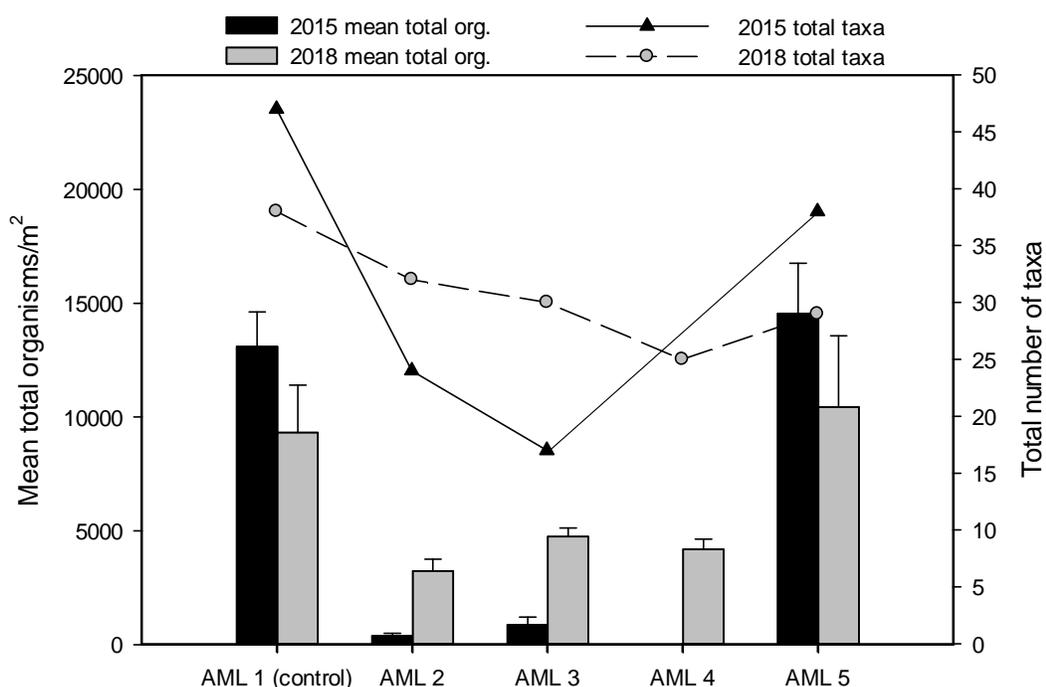


Figure 5. Benthic macroinvertebrate general population indices for sampling conducted in 2015 and 2018, including mean number of organisms/m<sup>2</sup> (left axis) and the total number of taxa (right axis) from the five sampling sites. AML 4 was dry in 2015 and not sampled. Error bars represent ± 95% CI.

### Benthic macroinvertebrate community composition

Benthic macroinvertebrate community composition results in 2018 were different to those observed in 2015. For example, a substantial decrease in number of EPT taxa and the percent EPT density was observed directly downstream of the AMD in 2015 (Figure 6). The two sites immediately downstream averaged 2.7 EPT taxa/11% EPT density (AML 2), and 2.0 EPT taxa/11% EPT density (AML 3), compared to 23.0 EPT taxa/55% EPT density observed at the Control site above the AMD in 2015 (Figure 6). In 2018, the two sites immediately downstream averaged 7.3 EPT taxa/62.8% EPT density (AML 2), and 10.7 EPT taxa/41.7% EPT density (AML 3), compared to 14.0 EPT taxa/51.3% EPT density observed at the Control site above the AMD. The decrease in EPT taxa and the percent EPT density at the two sites downstream of the control

coincided with a substantial increase in Coleoptera (beetles) at these sites in 2015. Higher EPT tax richness (16.7 EPT taxa) and percent EPT density (53%) was observed at the site located farthest downstream from the AMD (AMD 5) in 2015.

The timing of sampling likely influenced the % composition sampled between 2015 (September) and 2018 (July). During the July 2018 sampling, mayflies (Ephemeroptera) and true flies (Diptera) were more abundant, while the September sample from 2015 contained more beetles (Coleoptera), especially at the sites downstream of the AMD (Figure 6). Moreover, the same EPT taxa were found at the control site and downstream sites, so the similar composition were not simply due to less tolerant taxa making up the differences. In 2015, the substantial decrease downstream of the AMD was likely due to the contaminated flow from the AMD contributing to the majority of instream flow, while in July 2018, there was more upstream flow diluting the influence of the AMD, resulting in a similar composition of BMI present.

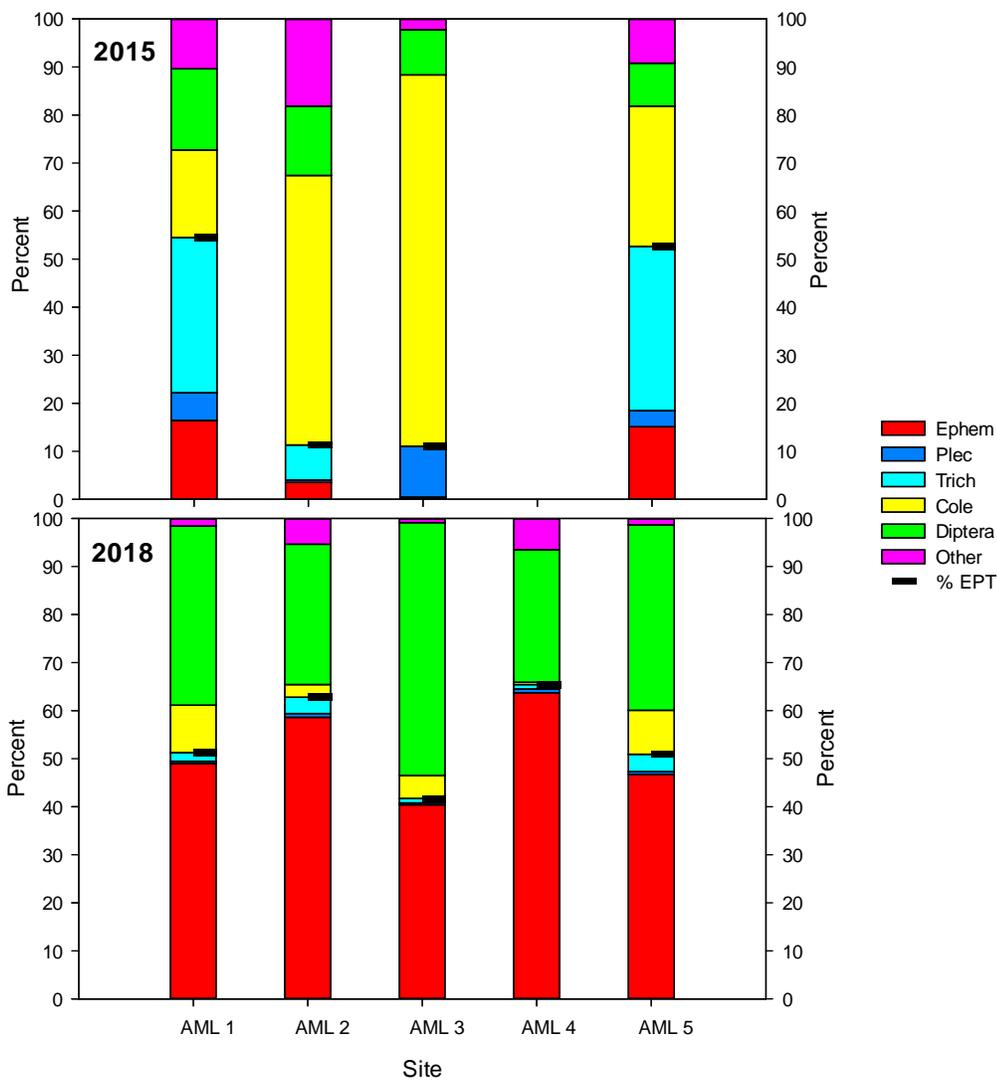


Figure 6. Composition of benthic macroinvertebrates sampled at each site sampled in 2015 (top graph) and 2018 (bottom graph). Percent EPT density is highlighted black. The AML 4 site was dry in 2015. Species

legend include Ephem (Ephemeroptera), Plec (Plecoptera), Trich (Trichoptera), Cole (Coleoptera), Diptera, and Other.

### Benthic Invertebrate Tissue

Benthic macroinvertebrates were collected for tissue metals analysis (Al, As, Cd, Fe, Pb, Ni, and Zn) downstream and upstream of the AMD. At the downstream impacted site, only 3 larger taxa were present (tipulidae, uenoidae, and pteronarcyidae) to allow for composite tissue comparisons. Of those, only a single (large) pteronarcyidae was found at the downstream site, so results for this comparison are shown, but were not obtained from a representative sample and should be viewed with that caveat in mind. At the upstream (control) site, five taxa were collected, including hydropsychidae and perlidae, taxa not found at the downstream site. For the three taxa collected at both sites, composite concentrations at the downstream impacted site were substantially higher for Al, Fe, and Ni (Figures 7, 10, and 12), and slighter higher for Zn (Figure 13). For the remaining metals (As, Cd, and Pb), there was no consistent pattern of metal concentrations between the upstream and downstream benthic macroinvertebrates (Figures 8, 9, and 11), with some metals higher at the upstream site, and some higher downstream.

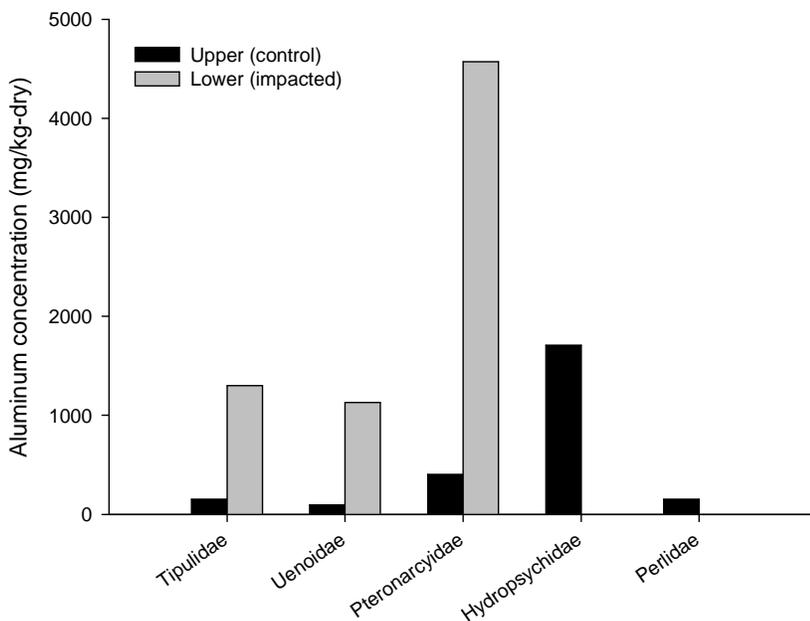


Figure 7. Benthic macroinvertebrate composite aluminum tissue concentrations found in five taxa from sites upstream (control) and downstream (impacted) of acid mine discharge into Belt Creek, Montana.

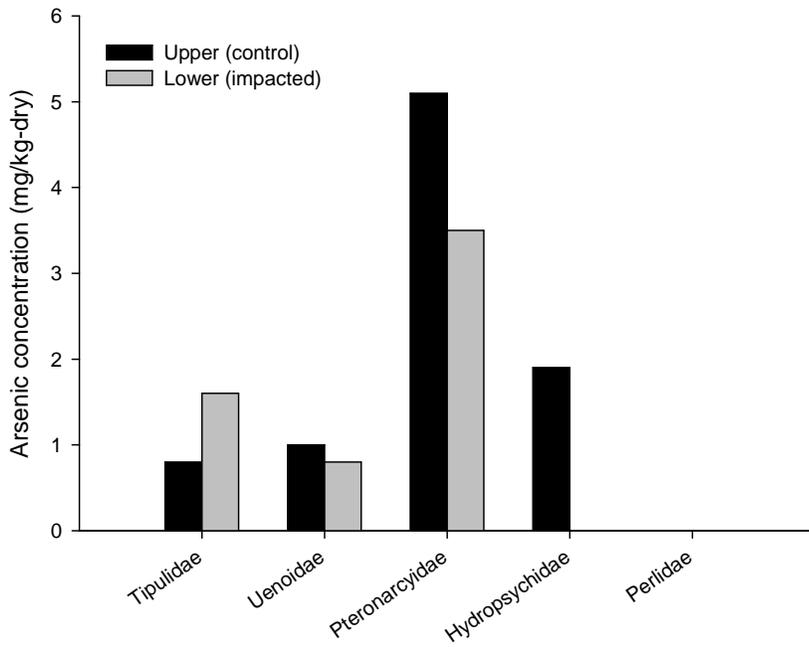


Figure 8. Benthic macroinvertebrate composite arsenic tissue concentrations found in five taxa from sites upstream (control) and downstream (impacted) of acid mine discharge into Belt Creek, Montana.

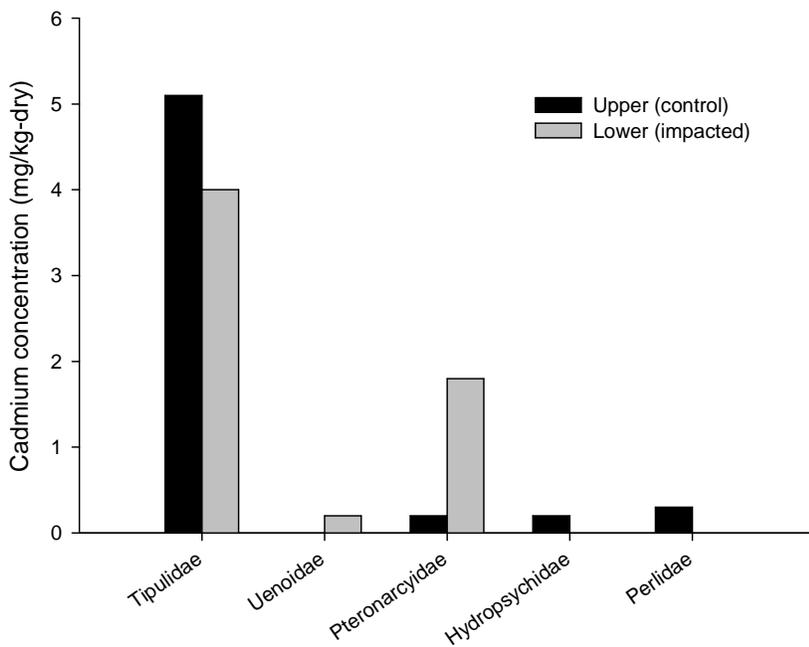


Figure 9. Benthic macroinvertebrate composite cadmium tissue concentrations found in five taxa from sites upstream (control) and downstream (impacted) of acid mine discharge into Belt Creek, Montana.

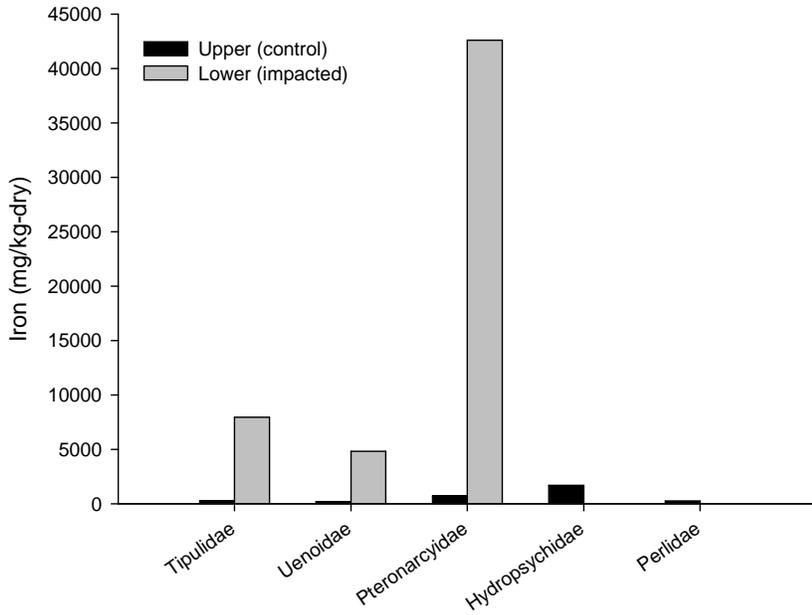


Figure 10. Benthic macroinvertebrate composite iron tissue concentrations found in five taxa from sites upstream (control) and downstream (impacted) of acid mine discharge into Belt Creek, Montana.

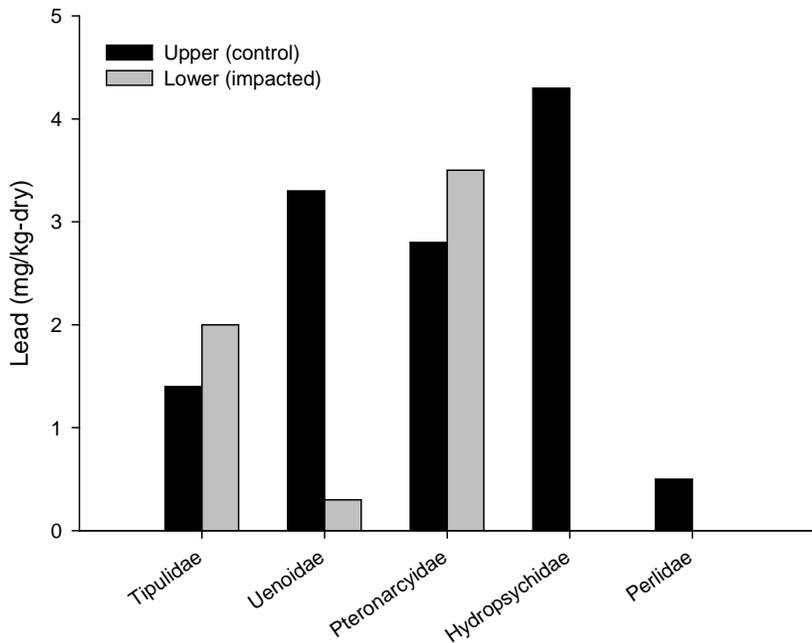


Figure 11. Benthic macroinvertebrate composite lead tissue concentrations found in five taxa from sites upstream (control) and downstream (impacted) of acid mine discharge into Belt Creek, Montana.

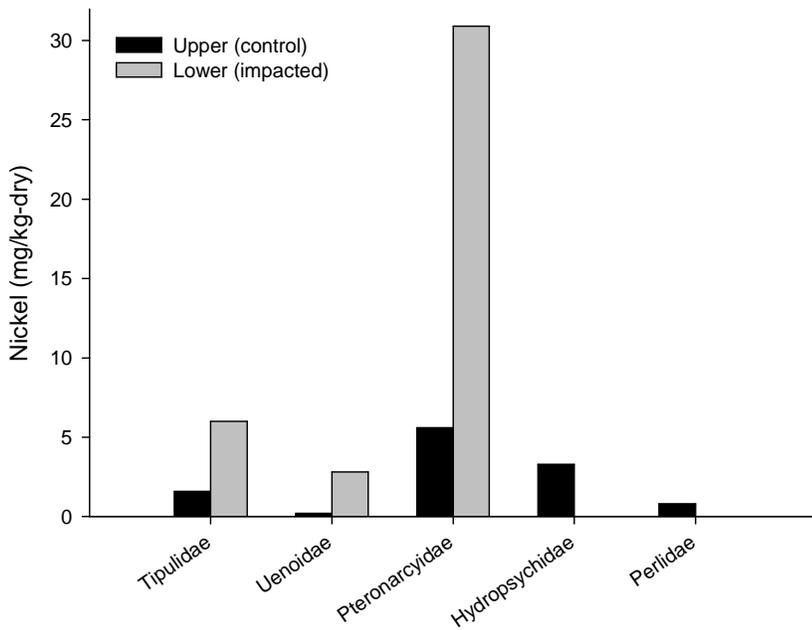


Figure 12. Benthic macroinvertebrate composite nickel tissue concentrations found in five taxa from sites upstream (control) and downstream (impacted) of acid mine discharge into Belt Creek, Montana.

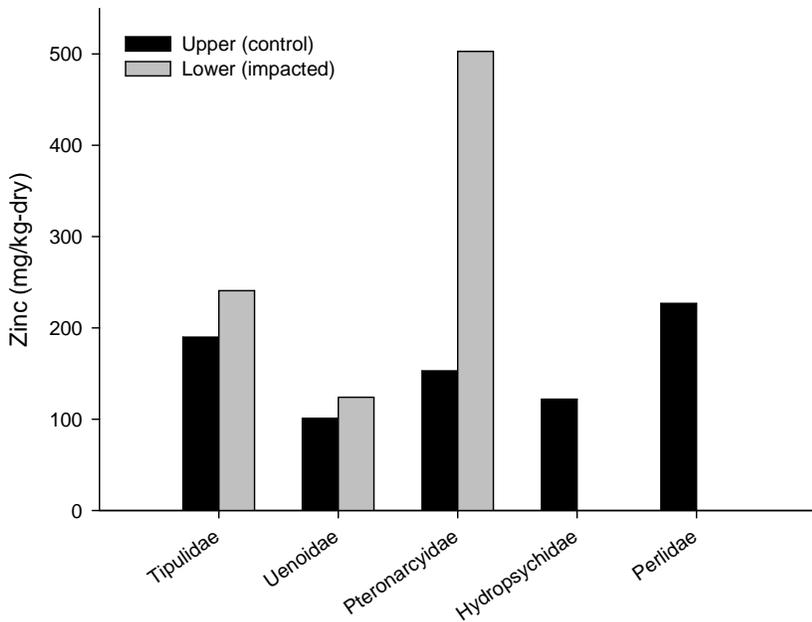


Figure 13. Benthic macroinvertebrate composite zinc tissue concentrations found in five taxa from sites upstream (control) and downstream (impacted) of acid mine discharge into Belt Creek, Montana.

## Fish Tissue

### Brown trout

Five adult brown trout were collected both upstream (305-376 mm TL) and downstream (271-330 mm TL) of the AMD and muscle tissue samples were analyzed individually for total Hg concentrations. The average Hg concentrations found were very low both upstream ( $0.028 \pm 0.005$  S.E. mg/kg wet weight) and downstream ( $0.026 \pm 0.001$  S.E. mg/kg wet weight) and based on fish consumption advisory guidance would have unrestricted consumption ( $<0.09$  mg/kg wet weight).

Brown trout livers were also removed and analyzed individually for Al, As, Cd, Fe, Pb, Ni, Se and Zn from five fish at each site, however due to a lab mistake, only three livers were analyzed from the upstream (control) site. For fish livers at both sites, concentrations for As, Pb and Ni were either non-detect or very low. Results for the remaining metals are shown in Figure 14. Aluminum concentrations in brown trout livers at the downstream site were 55% higher than upstream, although variability and low sample size (due in part to lab error) resulted in overlapping error bars and likely non-significant differences. The remaining metal concentrations were similar between sites and did not show any patterns of elevated concentration downstream of the AMD.

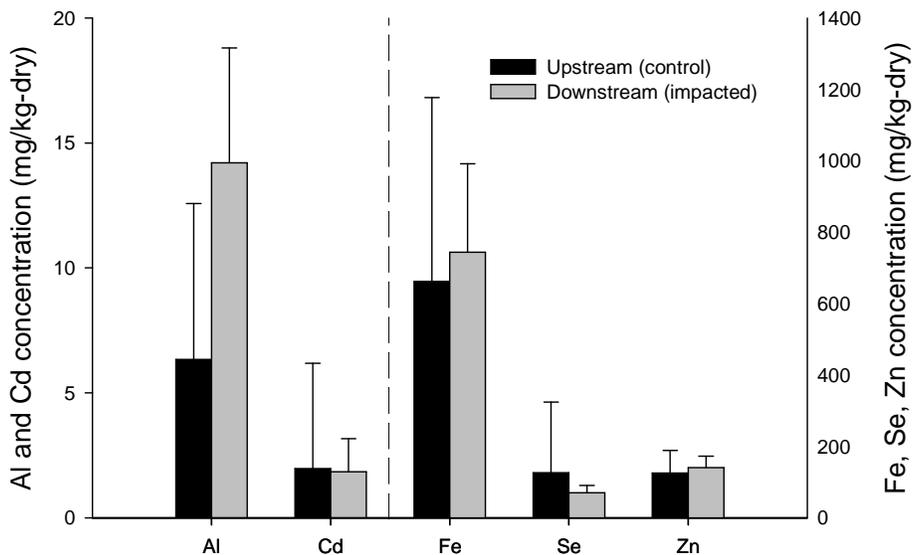


Figure 14. Mean metal concentrations found in brown trout livers, from sites upstream (control) and downstream (impacted) of acid mine drainage (AMD) into Belt Creek, Montana. Results for Al and Cd are referenced on the y-axis on the left; results for Fe, Se, and Zn are referenced on the y-axis on the right. Error bars represent 95% C.I.

### White suckers

White suckers were collected upstream (n=12; 134-187 mm TL) and downstream (n=9; 117-180 mm TL) of the AMD and three whole-body composites for each site (4 fish/composite upstream; 3 fish/composite downstream) were analyzed for metals, including Al, As, Cd, Fe, Pb, Ni, Se and Zn. Concentrations found in Al, Fe, and Ni were significantly higher at the downstream site (Figure 15). Whole-body concentrations of Zn and Cd were similar between sites, while Pb and Se concentrations were higher at the upstream site for unknown reasons. Arsenic concentrations were non-detect at both sites.

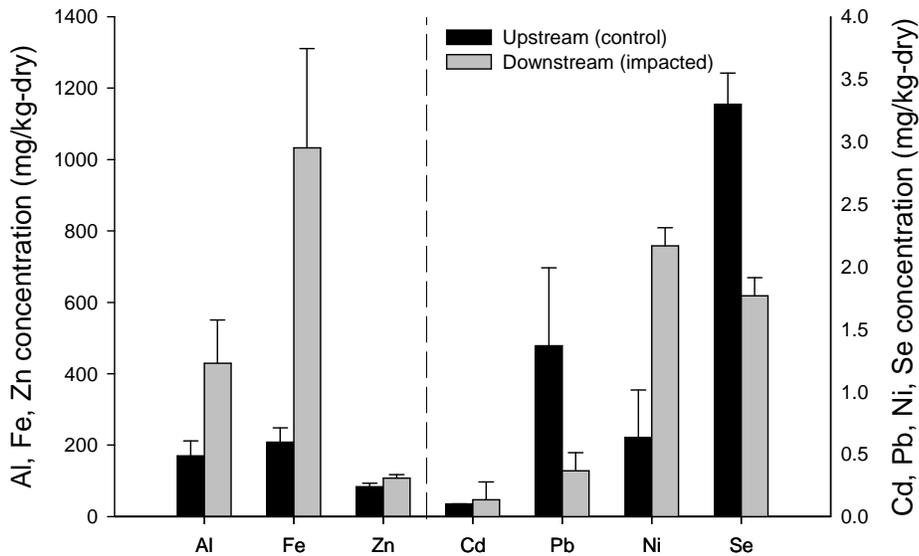


Figure 15. Mean metal concentrations found in white suckers analyzed whole-body, from sites upstream (control) and downstream (impacted) of acid mine drainage (AMD) into Belt Creek, Montana. Results for Al, Fe, and Zn are referenced on the y-axis on the left; results for Cd, Pb, Ni, and Se are referenced on the y-axis on the right. Error bars represent 95% C.I.

### Rocky mountain sculpins

Rocky mountain sculpins were collected upstream (n=10; 64-88 mm TL) and downstream (n=10; 65-87 mm TL) of the AMD and two, whole-body composites (5 fish/composite) for each site were analyzed for metals, including Al, As, Cd, Fe, Pb, Ni, Se and Zn. Concentrations found in Al and Fe were significantly higher at the downstream site (Figure 16). Whole-body concentrations of Cd and Se were similar between sites, while Zn, Pb and Ni concentrations were higher at the upstream site. Arsenic concentrations were non-detect at both sites.

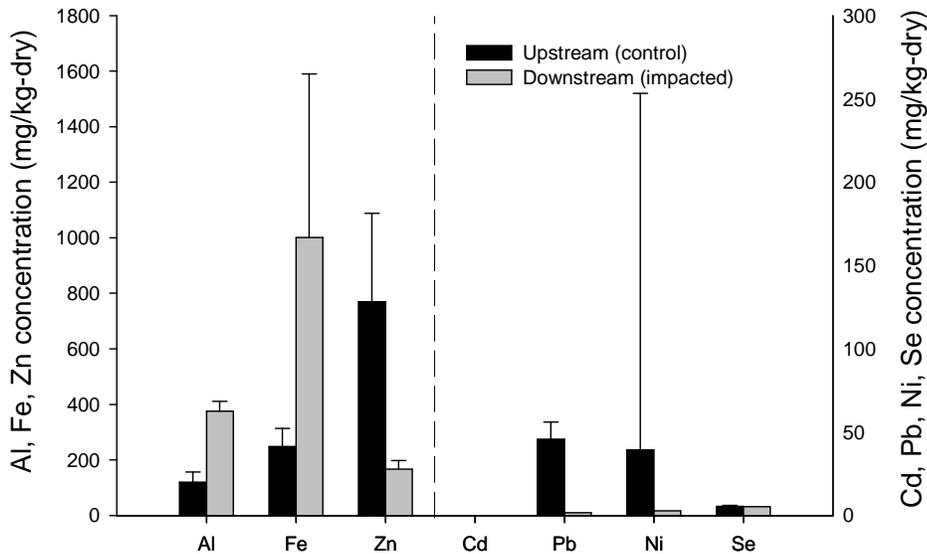


Figure 16. Mean metal concentrations found in rocky mountain sculpins analyzed whole-body, from sites upstream (control) and downstream (impacted) of acid mine drainage (AMD) into Belt Creek, Montana. Results for Al, Fe, and Zn are referenced on the y-axis on the left; results for Cd, Pb, Ni, and Se are referenced on the y-axis on the right. Error bars represent 95% C.I.

### Discussion & Recommendation

Fish sampling abundance results were related to tolerance levels to anthropogenic disturbances as quantified by Barbour et al. (1999) and Whittier et al. (2007). Relative tolerance values indicate that the more tolerant species (longnose suckers and white suckers) were captured in greater abundance in the impact section, while less tolerant species (trout, mountain whitefish, and mountain sucker) were captured in greater abundances in the control section (Table 1).

Overall, the benthic macroinvertebrate sites below the AMD are reduced in taxa richness and relative abundance, suggesting impacts from the metals released from the AMD site. Some recovery was observed at the downstream most site, downstream of Little Belt Creek, which exhibits more perennial flow than the reach just upstream. These data, collected prior to the implementation of the treatment facility, will serve as baseline conditions to compare to future monitoring efforts to track the recovery and improved health of the aquatic community.

The tissue data comprised of benthic macroinvertebrates, brown trout, white suckers, and rocky mountain sculpin, collected prior to the implementation of the treatment facility on Belt Creek, will serve as baseline conditions to compare to future monitoring efforts and track the recovery and improved health of the aquatic community. Overall, it appears that benthic macroinvertebrate tissue concentrations of Al, Fe, and Ni are the best indicators of AMD contamination. Aluminum and iron were also both higher in fish tissue at downstream sites than at upstream sites.

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