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Aquatic food webs and heavy metal contamination in the upper Blackfoot River, Montana

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AQUATIC FOOD WEBS AND HEAVY METAL CONTAMINATION IN THE UPPER BLACKFOOT RIVER, MONTANA

By

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ABSTRACT

Acid mine drainage (AMD), characterized by low pH and abundant heavy metals, is a widespread problem affecting water quality and fish habitat in Montana. Montana's upper Blackfoot River exhibits impaired water quality from historic mining that has significantly degraded aquatic habitat and reduced fish and invertebrate abundance in impacted streams. The goal of this study is to investigate the direct and indirect effects of mine-related heavy metals contamination on aquatic ecosystems by examining changes in aquatic community composition, bioaccumulation, and toxicity risk of heavy metals along a contamination gradient in the upper Blackfoot River. Three primary research questions were addressed in this study: 1) How are macro-invertebrate communities influenced by heavy metals contamination? 2) What are the implications of changes in food web structure for exposure pathways? 3) What levels of environmental contamination produce the greatest risk to upper trophic levels? Invertebrate and fish communities impacted by heavy metals in the upper Blackfoot River were sampled in 2009 and 2010 for community composition analyses and metals concentrations. The results of this study indicate that an increase in heavy metals contamination in the upper Blackfoot River results in important changes in exposure pathways of metals entering aquatic food webs through invertebrate food sources, as well as exposure pathways of metals to fish. The greatest exposure risk to upper trophic levels from the pool of bioavailable metals in invertebrates occurred at moderately contaminated sites where moderate invertebrate abundance and moderate sediment metals levels coincided. In addition, the highest metals concentrations in fish tissue were at sites with high exposure values in invertebrates, rather than sites with the highest sediment contamination levels. The results of this study indicate that biological mechanisms influencing the movement of heavy metals in aquatic food webs are important factors for assessing toxicity risk to upper trophic levels that may not be evident when considering environmental contamination alone.

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INTRODUCTION

Historic mining contamination is a widespread problem affecting water quality and fish habitat in Montana. Acid mine drainage (AMD), characterized by low pH and abundant heavy metals, can reduce aquatic invertebrate abundance and fish populations, even resulting in complete elimination of aquatic biota in extreme cases. The upper Blackfoot River exhibits impaired water quality from historic mining that has had significant impacts on aquatic habitat as well as on fish and invertebrate communities (Moore et al. 1991, Montana Fish Wildlife and Parks 1997). Changes in community composition can also have important implications for toxicity to upper trophic levels as species interactions and life history traits influence heavy metals movement in aquatic food webs (Hogsden and Harding 2012). The goal of this study is to investigate direct and indirect effects of mine-related heavy metals contamination on aquatic ecosystems by examining changes in aquatic community composition, bioaccumulation and toxicity risk of heavy metals along a contamination gradient in the upper Blackfoot River. Three primary research questions were addressed in this study: 1) How are macro-invertebrate communities directly influenced by heavy metals contamination? 2) What are the implications of changes in invertebrate community composition for fish exposure pathways? 3) What levels of environmental contamination produce the greatest risk to upper trophic levels?

Abandoned mines, acid mine drainage and heavy metals in aquatic environments

Water quality impairment from abandoned hardrock mines is a common occurrence across the western United States, posing water quality and human health risks from historic mining activities. The Bureau of Land Management's Abandoned Mine Lands (AML) program has identified approximately 46,000 sites as of 2014 with estimates of 6,000 abandoned mine sites existing in Montana alone (Pioneer Technical Services Inc. 1995, US BLM (Bureau of Land Management) 2014). The Boulder River, Clark Fork, and upper Blackfoot River in Montana are high-profile examples of rivers impacted by numerous abandoned mines in their headwaters. Several studies have documented impaired water quality and effects on aquatic biota from elevated heavy metal concentrations in these rivers (Moore et al. 1991, Martin 1992, USFWS (Fish and Wildlife Service) 1993, Farag et al. 2003). Despite cleanup efforts at many abandoned mine sites, heavy metal contamination will remain an important water quality issue in many streams across the western United States due to the large magnitude and broad scope of historic mining contamination.

Heavy metals can have profound impacts on aquatic communities with drastically reduced invertebrate abundance and species diversity in highly contaminated streams. The predictable response to metals contamination in aquatic invertebrate communities has been widely accepted as a useful bioindicator of water quality impairment (Karr et al. 1986, Hilsenhoff 1988, Cain et al. 1992). Numerous studies have expanded on these methods to investigate species specific and community level impacts from metals contamination (Poulton et al. 1995, Beltman et al. 1999, Clements et al. 2000, Hogsden and Harding 2012). Studies of food webs and knowledge of bioaccumulation and trophic transfer of contaminants have also been applied to investigate functional changes in aquatic ecosystems in response to mining contamination (Besser et al. 2001, Carlisle 2001, Campbell et al. 2003). Relatively few studies have applied these principles to evaluate how changes in community composition influence bioaccumulation and trophic transfer of contaminants to upper trophic levels and terrestrial consumers (Currie et al. 1997, Quinn et al. 2003, Kraus et al. 2014). Individual species are exposed to different levels of metals contamination due to feeding mode, behavior, and life history traits, which may potentially have important implications for the movement of heavy metals in aquatic food webs.

Aquatic species vary in their ability to cope with increased metals contamination. Mining impacted streams are typically devoid of metals sensitive taxa, forming the foundation for bioassessment methods for detecting water quality impairment (Cain et al. 1992). Species specific or genera abundance estimates of metal impacted streams can be compared to less impacted streams to evaluate metals bioavailability and toxicity in impaired streams (Chadwick et al. 1986, Hilsenhoff 1988, Cain et al. 1992). Despite the usefulness for identifying impaired streams, species or genera specific methods are limited in their ability to identify changes in community composition as individual species and functional groups respond differently to heavy metals contamination.

The movement of heavy metals in aquatic food webs via trophic transfer has been demonstrated with evidence of biomagnification of some metals in predators as well as bioaccumulation of metals in riparian food webs (Croteau et al. 2005, Ikemoto et al. 2008, Kraus et al. 2014). Bioaccumulation refers to the accumulation of metals in organisms from food sources and the environment. Biomagnification expands on this principle and occurs when metal concentrations increase with increasing trophic level due to additive contributions from prey, resulting in very high concentrations in upper trophic levels (Newman 2010). Metals accumulate in food sources such as invertebrates and are transferred to upper trophic levels through predation. In addition, differences in metals concentrations among invertebrate functional feeding groups has been attributed to differences in metals accumulation rates in food sources (Smock 1983a). As metals enter aquatic food webs, species interactions and community composition can have important implications for trophic transfer of contaminants and toxicity risk to upper trophic level consumers.

Bioaccumulation and trophic transfer of contaminants are complex, dynamic processes that depend on an organism's physiological characteristics and the physiochemical conditions of the environment (Kapustka et al. 2004). Metals concentrations in organisms vary by invertebrate size (Smock

1983b), trophic level (Quinn et al. 2003, Croteau et al. 2005), and functional feeding group (Smock 1983a, Farag et al. 1998, Besser et al. 2001). In addition, the influence of metals contamination in streams can have significant impacts on riparian food webs as prey abundance declines or metals toxicity impacts riparian consumers (Walters et al. 2008, Kraus et al. 2014). As community composition changes in response to metals contamination, it is important to consider the effects of metals bioaccumulation, trophic transfer, and ultimate toxicity risk to upper trophic levels as ecological factors confound the relationship between environmental contamination and organism level effects.

Effects of mining related pollution on aquatic communities

Direct physical and chemical impacts on environmental conditions and form of metals

Mining contamination can have a variety of effects on aquatic systems including physical and chemical modifications of streams. Oxidation of sulfides in mine discharge creates sulfuric acid, lowering pH levels in streams below lethal thresholds of aquatic organisms (Hogsden and Harding 2012). Contaminants associated with hard rock mining include arsenic, copper, lead, and mercury among others. As acidic water is diluted downstream of contamination sources, metals precipitate out of solution and coat the streambed in a cohesive layer, eliminating benthic habitats for periphyton and invertebrates (Hogsden and Harding 2012). Periphyton, bacteria, and fungi are also highly sensitive to changes in pH, and their absence in contaminated streams reduces food availability for grazing macroinvertebrates (Gray 1997). Acidity and metal toxicity influence the abundance and species diversity of macroinvertebrates, with only metal tolerant taxa such as chironomids and beetles present in heavily impacted streams (Gray 1997). As a result, streams impacted by AMD typically exhibit simplified food webs with drastically reduced species diversity and abundance compared to less impacted reference streams (Beltman et al. 1999, Clements et al. 2000).

Physical and chemical characteristics of streams influence the form, transport, and bioavailability of metals. In AMD impacted streams, low pH increases the solubility of metals and their downstream transport. As contaminated streams are diluted by groundwater and tributary sources, pH increases and aluminum, iron, and copper may precipitate and accumulate on the streambed and organisms. (Theobald et al. 1963, Chapman et al. 1983). A study of the effects of metals toxicity on fish in AMD streams found that acute toxicity was greatest where acidic streams mixed with uncontaminated tributaries and an increase in pH caused accumulation of iron and aluminum on the gills of fish (Henry et al. 1999). Despite high metal loading immediately downstream of discharge sources, heavy metals can be highly mobile and are affected by changing water chemistry and physical conditions throughout a stream network. In addition, increases in stream pH can create more hospitable conditions for aquatic organisms downstream of pollution sources, but also increase the acute toxicity of heavy metals. Dynamic physiochemical processes create a diverse pattern of metals toxicity along a contamination gradient in mining impacted streams.

Response of invertebrate community structure

Metals contamination can have significant effects on invertebrate community structure as the metal tolerances of different species are exceeded. Mining impacted streams often exhibit a predictable decline in invertebrate abundance and species richness with increasing metals contamination (Winner et al. 1980, Chadwick et al. 1986, Besser et al. 2001, Griffith et al. 2001, Quinn et al. 2003). Mayflies (*Ephemeroptera)* and stoneflies (*Plecoptera)* are among the most sensitive taxa to metals pollution, and their abundance is often used as indicators of water quality and mining contamination (Chadwick et al. 1986, Hilsenhoff 1988, Cain et al. 1992). The decline in invertebrate abundance in AMD streams can have important implications for community composition, trophic transfer of metals, and food availability for upper trophic levels.

Aside from the predictable loss of sensitive taxa in AMD streams, aquatic community response to metals contamination is complex, and total invertebrate abundance does not capture all of the changes in invertebrate communities. Metal tolerant taxa, such as some Chironomids, often do not exhibit a decline in abundance and can obscure the relationship between invertebrate density and metals contamination (Beltman et al. 1999). Several studies have not observed the typical decline in invertebrate abundance due to confounding community level effects of mining contamination (Leland et al. 1989, Kiffney and Clements 1994, Beltman et al. 1999). The relationship between metal tolerant taxa and invertebrate abundance was evident in an Idaho stream where Chironomids were the dominant taxa in both contaminated and reference sites. As a result, invertebrate densities were not significantly different among sites due to the metal tolerance of this taxon (Beltman et al. 1999). These observations emphasize the need for more detailed community composition analyses to fully understand invertebrate community response to mining contamination and implications for trophic transfer of metals.

Implications of changes in community composition for exposure pathways

Aquatic organisms are exposed to metals via water, sediment, and food. Organisms may accumulate metals from their environment as contaminants accumulate on gill filaments or adsorb onto the exoskeleton. Metals can also accumulate from food sources that include prey, sediment, and organic matter (Hare 1992). The partitioning of metals into various food sources and habitats found in streams has important implications for metals exposure of aquatic organisms.

Metals accumulation in invertebrates can be highly variable across taxa, body size, and functional feeding groups (Smock 1983b, Hare et al. 1991, Cain et al. 1992). Smaller invertebrates often accumulate higher concentrations of metals than larger invertebrates due to food selection or adsorption on the exoskeleton. Adsorption is an important accumulation source for some metals as surface to volume ratios increase from larger to smaller organisms (Smock 1983b, Hare 1992). Smaller invertebrates are also more limited in the type of material consumed, potentially influencing metals concentrations in small invertebrates compared to larger organisms (Smock 1983a). In addition, several studies have observed a decline in invertebrate biomass, but not abundance in contaminated streams as larger, metals sensitive invertebrates are eliminated (Leland et al. 1989, Kiffney and Clements 1993, Beltman et al. 1999). As a result, contaminated streams may be dominated by metal tolerant taxa with smaller body sizes that are more susceptible to metals accumulation.

Dietary preference and foraging behavior are also important factors for bioaccumulation as metals concentrations vary among different food sources. Biofilm and periphyton tend to accumulate higher metal concentrations than water or coarse sediment due to accumulation of fine particulate matter (Kiffney and Clements 1993, Besser et al. 2001). High concentrations of metals in periphyton can lead to metals concentrations in scraper and collector functional feeding groups that are higher than those in other FFG (Farag et al. 1998, Besser et al. 2001). Diet is also an important exposure pathway for upper trophic levels. A study of copper concentrations in rainbow trout found that dietary copper concentrations were a better predictor of copper body burden than exposure from waterborne copper concentrations (Miller et al. 1993). Differential concentrations of metals among food sources have important implications for accumulation of contaminants in aquatic food webs and transfer to upper trophic levels.

Trophic position is also an important factor dictating metals concentrations in aquatic organisms. Longer-lived, predatory organisms tend to accumulate higher concentrations of contaminants due to long exposure periods and biomagnification of some metals. Biomagnification refers to the accumulation of contaminants in higher concentrations as trophic level increases due to accumulation in food. Only a few metals have exhibited biomagnification, usually metals that do not function as important trace nutrients and that organisms are unable to regulate by excretion or sequestration (Newman 2010). Methyl-mercury and cadmium have been shown to biomagnify in some food webs whereas iron and copper typically show no relationship with trophic position or actually decrease with increasing trophic level (Quinn et al. 2003, Croteau et al. 2005). Differences in biological function and bioaccumulation ultimately dictate the tendency of metals to biomagnify in food webs. In addition, highly contaminated streams may exhibit simplified food webs with no upper trophic level consumers that are the necessary components for biomagnification (Newman 2010). Evidence of biomagnification of some mining associated metals, such as cadmium, in complex food webs emphasizes the need to assess community composition and food web complexity when evaluating toxicity risk to upper trophic levels.

The decline in invertebrate abundance and species richness with increasing levels of heavy metals may have important implications for exposure pathways of metals in contaminated streams (Winner et al. 1980, Quinn et al. 2003). Highly sensitive taxa such as mayflies and stoneflies are often reduced in mining contaminated streams and are replaced by more metal tolerant taxa such as caddisflies and chironomids (Farag et al. 1998, Clements et al. 2000). Metals sensitive taxa such as mayflies are typically classified in the scraper functional feeding group (FFG) whereas metals tolerant taxa such as midges or black fly larvae are classified in the filter-feeders FFG (Poff et al. 2006). A reduction in a key ecosystem function in response to decreased pH has been observed in an Appalachian stream as taxa in the shredder FFG were eliminated, leading to decreased leaf breakdown rates (Simon et al. 2009). This observation suggests that changes in water chemistry can result in significant changes in invertebrate functional groups and exposure pathways, but may not be a consistent response in all aquatic systems and invertebrate communities. As a result, the loss of metals sensitive taxa in mining impacted streams may result in a shift in the dominant dietary exposure pathway of metals entering aquatic food webs.

Macroinvertebrate life history traits may also have important implications for exposure pathways to upper trophic levels. Invertebrates become dislodged from substrate due to high water velocities, or enter the drift intentionally according to their life histories. Invertebrates suspended in the water column are more vulnerable to predation than benthic organisms, and trout tend to feed primarily on drifting invertebrates in mountain streams (Nakano et al. 1992). The propensity for aquatic macroinvertebrates to enter the drift varies by species and life stage and can change significantly between habitat types and seasons (Rader 1997). A study in Montana observed adult westslope cutthroat trout (*Oncorhynchus clarki lewisi*) feeding exclusively on drifting macroinvertebrates, emphasizing the importance of this food source for adult fish (Nakano et al. 1992). As community composition changes in contaminated streams, changes in the proportion of drifting invertebrates could have significant implications for food availability and metals exposure in fish populations.

Changes in invertebrate communities can also have significant impacts on resource subsidies and metals export to terrestrial food webs. Emergent taxa with a terrestrial life stage are important food sources for riparian organisms such as spiders and birds. Aquatic contaminants may also be transferred to riparian food webs as emergent taxa are consumed by terrestrial predators (Walters et al. 2008, Kraus et al. 2014). A study in Colorado documented a significant decline in the biomass of emergent insects and riparian spider communities with increasing metals contamination in streams. However, the greatest impact to terrestrial food webs in this study was from the decline of prey biomass, rather than metal toxicity to spiders (Kraus et al. 2014). The retention of metals during metamorphosis influences the metals concentration in winged adults of aquatic invertebrates. However, metals such as cadmium tend to be conserved through metamorphosis and have been shown to be transferred to riparian consumers

(Currie et al. 1997, Kraus et al. 2014). Aquatic contamination that reduces invertebrate abundance could have important implications for the export of contaminants from aquatic systems as well as food availability for riparian consumers.

Environmental contamination and toxicity risk for upper trophic levels

Environmental contamination levels in water and sediment are important indicators of potential metals exposure risk to upper trophic levels. However, complex species interactions and biological mechanisms can also be important factors for determining ultimate toxicity and bioaccumulation of metals in aquatic food webs (Currie et al. 1997, Quinn et al. 2003, Kraus et al. 2014). Due to the complex and dynamic interplay of physiochemical conditions, biological interactions, and community structure influencing metals movements in aquatic food webs, areas with the highest environmental metals concentrations may not always represent the greatest risk to upper trophic levels. Metals exposure risk in mining impacted streams may be better evaluated by a comprehensive analysis of changes in community composition and exposure pathways in aquatic food webs.

Invertebrate biomass represents the pool of metals that are bioavailable to upper trophic levels and riparian food webs via predation. As invertebrate abundance declines in contaminated streams, the total pool of metals in biota declines as well due to decreased biomass. The flux of metals transferred through predation is especially important in upper trophic levels as diet is the primary route of metals exposure in organisms such as fish (Miller et al. 1993). As a result, the relationship between environmental contamination levels and aquatic invertebrate abundance has important implications for the biological flux of contaminants and ultimate toxicity risk to upper trophic levels.

Moderately contaminated sites may represent the greatest risk of metals toxicity to upper trophic levels because such sites support higher densities of invertebrates and more complex food webs that influence bioaccumulation and trophic transfer of metals. In addition, upper trophic level predators such as fish are more likely to be present, providing the link for biomagnification. The interaction between more hospitable environmental conditions, increased flux of contaminants from higher invertebrate biomass, and complex food webs may produce the greatest risk to upper trophic levels in moderately contaminated sites.

Despite the increased potential for bioaccumulation and trophic transfer of contaminants in moderately contaminated sites, the ultimate exposure risk to upper trophic levels in these food webs will be dictated by species interactions and exposure pathways. Changes in community composition in response to heavy metals contamination can have important effects on the dominant functional feeding group, densities of drifting invertebrates or emergent taxa, and food web complexity. As a result, evaluating how community composition changes across a metal contamination gradient and its effect on exposure pathways of metals in aquatic food webs will help elucidate the greatest exposure risk to upper trophic levels in mining impacted streams.

Upper Blackfoot Mining Complex

Mike Horse Dam on the headwaters of the Blackfoot River, Montana, burst during spring runoff in 1975, contributing nearly 200,000 cubic yards of toxic mine tailings and sediment to the river. Immediately following the failure, a visible sediment plume extended more than 15 miles downstream (Montana Fish Wildlife and Parks 1997). Despite a quick response to fix the breach in the dam, contaminated ground water continued to seep from the compromised reservoir for nearly three months before full repairs were finished. Initial investigations by Montana Fish, Wildlife, and Parks documented a drastic decline in fish and macroinvertebrate abundance in impacted sections of the river (Montana Fish Wildlife and Parks 1997). Fortunately, the most highly impacted habitats were confined to the headwaters due to wetlands four miles below the dam, which captured much of the material that was flushed from the reservoir. However, a significant amount of toxic sediment was deposited throughout the floodplain and river channel, creating a source of future water quality impairments that continue to be detected downstream of the wetlands (Moore et al. 1991, Vandeberg et al. 2011).

The Mike Horse Mine area is a federal superfund site known as the Upper Blackfoot Mining Complex (UBMC) (TetraTech 2013). Following a settlement between the State of Montana and American Smelting and Refining Company (ASARCO) in 1991, several mitigation actions have been implemented, including installation of a water treatment facility to treat AMD flowing from old mine adits on the upper Blackfoot River. Removal of contaminated mine tailings impounded behind the Mike Horse dam began in 2014 and is projected to be completed by 2018 (Pioneer Technical Services 2015). The superfund site includes approximately six square miles surrounding the historic mine sites to the wetland complex downstream on the Blackfoot River (Pioneer Technical Services 2015). This area includes the main channel of the Blackfoot River and portions of the floodplain downstream to Hogum Creek.

Since the 1975 Mike Horse Dam failure, several studies have documented persistent water quality and habitat impairments in the Blackfoot River and impacted tributaries (Moore et al. 1991, Schmitz et al. 2010, Vandeberg et al. 2011). Despite restoration efforts of the tailings dam and contaminated sediments, numerous mine adits continue to discharge contaminated water with low pH levels and severely degraded water quality and fish habitat throughout the headwaters of the Blackfoot River. Several tributaries contain no fish populations and have severely reduced invertebrate abundance (Moore et al. 1991). A study in 1991 detected elevated cadmium concentrations in fish and invertebrates 75km downstream from the UBMC, indicating that contaminants can be highly mobile and can bioaccumulate in this system (Moore et al. 1991).

Even with the removal of the tailings dam and floodplain sediments, contaminated ground water and smaller mine adits may continue to impact aquatic food webs in the future. The persistent impacts from AMD in the upper Blackfoot River underscores the necessity of evaluating aquatic community response to contamination levels. In addition, the contamination gradient extending from the Mike Horse dam downstream provides an excellent opportunity to evaluate changes in community composition at varying metals levels. Evaluating changes in community composition and implications for bioaccumulation and trophic transfer of heavy metals will be an important component of remediating historic mine sites such as the Upper Blackfoot Mining Complex.

METHODS

Invertebrate, fish, sediment and water samples were collected during the summers of 2009 and 2010 to assess metals contamination and aquatic community impacts from AMD in the upper Blackfoot River. Sampling locations were selected throughout the headwaters of the Blackfoot River to provide a range of contamination levels. Sites included six on the mainstem Blackfoot River downstream of the Mike Horse dam, and nine tributary sites representing seven mining impacted and eight reference sites (Figure 1). The term "reference site" in this context was used to describe sites that have minimal impact from historic mining. Naturally occurring sources of heavy metals as well as other land use practices may influence aquatic communities in all streams in this study area. The use of the term "reference" in this study describes sites that provide a comparison for streams that are more heavily impacted by historic mining activities.

The mainstem sites include one location above the water treatment facility, two sites between the treatment facility and natural wetlands, one site between two large wetlands, and two sites downstream of the wetland complex that trapped the majority of sediment from the 1975 dam breach (**Error! Reference source not found.**). Sampling reaches were 80-200m long with biological sampling occurring in August of 2009 and 2010. The data used in this study were collected in support of a monitoring study to investigate physical and chemical processes of metals transport in the upper Blackfoot River prior to remediation (Wilcox et al. 2014).

Sediment and water sampling

Water and sediment samples were collected in 2009 and 2010 to evaluate potential metal exposure to aquatic organisms. Thirteen Metals/metalloids were analyzed in sediment and water samples including: As, Ba, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, and Zn. Water samples were collected eight times throughout the summer sampling season (May through October) and were collected in 1-liter acid-washed bottles and stored on ice for transport to the laboratory within 24hrs. Samples were analyzed for metals by Inductively Coupled Plasma Optical Emission or Mass Spectrometry (ICP-OES or ICP-MS) following standard US Environmental Protection Agency (USEPA) methodology. Quality control was maintained by using field and procedural blanks and external and internal standards according to USEPA methods (USEPA (Environmental Protection Agency) 1991).

In addition to trace metals concentrations, pH, alkalinity, total suspended solids, conductivity and temperature water quality metrics were collected coinciding with each water sample collection. Conductivity was measured with a Mettler Toledo Model MC126 portable conductivity meter/IP67 conductivity cell. Water temperature was measured with the same meter, as well as the water level pressure transducers, and pH was measured with an ORION Model 265A portable pH meter and gel pH probe.

Sediment samples were composed of sieved fine grained (<63 μm) bed sediments collected from the upper 1-3cm of stream substrate. Sediment samples were collected 2-4 times in 2009 and 3-4 times in 2010 throughout the summer sampling season and analyzed for the 13 metals listed earlier.

Stream habitat sampling

Habitat measurements included pool number (per 100m), depth, large woody debris, longitudinal profiles and channel cross sections. Five channel cross sections were surveyed with one cross section location coinciding with stream discharge measurements. Stream discharge was continuously measured using pressure transducers installed at each site, with semi-monthly physical streamflow measurements using a SonTek FlowTracker acoustic Doppler velocimeter to link stage and discharge. Three Wolmann pebble counts were conducted at each reach in order to quantify substrate size.

Macroinvertebrate sampling

Macroinvertebrate samples for community composition were collected from several locations within three distinct reaches at each site. A 0.093 m^2 Surber sampler was used to collect invertebrates from riffle habitat. Substrate within the Surber's frame was scraped free of organisms and detritus and the remaining sample was decanted of excess water and preserved with a 95% ethyl alcohol solution.

Macroinvertebrate community composition samples were sorted and identified in a North American Benthological Society certified taxonomic laboratory (Rhithron Associates, Inc) following field collections. Samples were sub-sampled to a minimum of 500 randomly selected organisms using a 30 grid Caton tray and random number generator. Percent substrate sub-sampled was recorded in order to estimate invertebrate density. All organisms were identified to at least genus and to species when possible. Ten percent of the community composition samples were re-identified by a second taxonomist for taxonomic quality control.

A separate invertebrate sample was collected at each site for whole body metals analysis. Arsenic, copper, cadmium, and zinc were the metals of primary concern based on previous studies and concentrations of these four metals were analyzed in biota. Samples for metals analysis were collected using a d-frame kick net and a sampling period of one minute. Invertebrates selected for metals analyses in 2009 were chosen in order to match previous studies and provide continuity among projects (Moore et al. 1991). The six target taxa included *Hydropsyche sp., Arctopsyche grandis*, *Limnephilus sp.*, *Brachycentrus sp. (americanus/ occidentalis), Classenia sabulosa*, and *Hesperoperla pacifica.* However, not all target species were present at all sampling stations and limited comparison among sites. The invertebrate taxa selected for metals analysis in 2010 were present at each site and included *Rhyacophila sp., Drunella coloradensis, Arctopsyche grandis, Brachycentrus sp., and Claassenia sabulosa*. A total of 150 individuals collected in 2009 and 2010 were analyzed for metals concentrations.

A minimum of ten individuals or 50mg dry mass per taxon were collected in order to meet laboratory metals detection limits. Samples were transferred from the field to a laboratory in mercury-free scintillation vials for metals analysis. All organisms were identified and enumerated to determine metal concentrations per individual. A sub-sample of each taxon was re-identified by a taxonomist in order to maintain taxonomic quality control. A 50mg or greater dry weight composite of similarly sized individuals was selected for metals analysis. Each composite was digested following USEPA standard methods and analyzed by ICP-OES (USEPA (Environmental Protection Agency) 1991).

Fish sampling

Fish populations were sampled using a backpack electrofisher in 200m block netted reaches at each site. Fish were present at eight sites (MC, SG, AC, B1, B2, B3, B4, B5), and population size was estimated using a two-pass electrofishing depletion estimate. Seventy-seven fish total were sacrificed for metals analysis of livers. The target fish species for metals analysis was non-native brook trout (*Salvelinus fontinalis*) which are relatively abundant in this system. Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) were collected where brook trout were absent. Ten analytical samples were collected from each site, composed of either 10 large fish or a composite of smaller individuals. Fish were euthanized with an overdose (2 g/L) of MS-222 (tricaine methanesulfonate). Livers were removed immediately and placed on ice for transport to the laboratory. Liver samples were digested by USEPA standard methods and analyzed by ICP-OES for metals concentrations (USEPA (Environmental Protection Agency) 1991).

Data Analysis

Metals concentrations in sediment rather than water were used to characterize contamination levels at each site because metals concentrations in water were below detection thresholds for many samples and varied significantly over the sampling period compared to sediment. Cumulative Criterion Units (CCU) were used to account for additive toxicity effects of multiple metals in sediment to aquatic biota. CCU is the ratio of observed sediment concentrations to the consensus-based Probable Effect Concentration (PEC) reported by Macdonald et al. (2000). The consensus-based PEC’s were developed from 17 field studies conducted throughout the United States, including the Clark Fork River, MT (USFWS (Fish and Wildlife Service) 1993, MacDonald et al. 2000). Sediment metals concentrations greater than the PEC are likely to cause toxicity effects to aquatic biota. The sum of the ratio of observed metals concentrations to PEC’s for individual metals was used to calculate the CCU.

$$\mathrm{CCU} = \sum \frac{Metal_i}{PEC_i}$$

CCU = cumulative criterion concentrations Metal_i = observed metal concentration for the *i*th metal

PEC_i = probable effect concentration for the i^{th} metal

As a result, CCU values greater than one indicate potential toxic effects to aquatic biota from additive contributions of multiple metals. Arsenic, cadmium, chromium, copper, lead, and zinc were used to calculate the CCU for each site. The median CCU for each year at individual sites was used to account for seasonal variability in sediment metals concentrations over the summer sampling period. Metals concentrations in sediment were considerably higher in the spring compared to the rest of the sampling periods, therefore the median was used to provide a more representative characterization of each site without the effect of the spring outlier concentration.

Invertebrate density, species diversity, and species richness were used to assess community composition using the standardized Surber samples and Shannon H diversity index (Bersier et al. 2002). Shannon’s H species diversity index accounts for both species richness and evenness, providing a more complete measure of species diversity. Shannon’s H (diversity index) was calculated as follows:

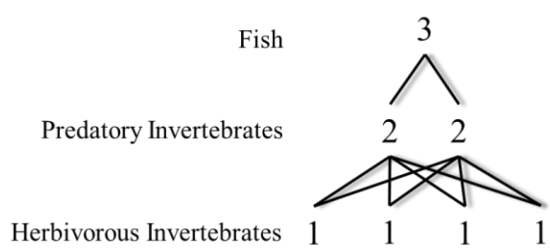
$$H = -\sum p_i \ln p_i$$

Where (p_i) refers to the proportion of individuals in species (*i)* relative to the total number of individuals. Invertebrate species richness was calculated as the total number of invertebrate species at each site.

Invertebrate diet provides information about the pathway of metals entering the food web as well as trophic position. Functional feeding groups (FFG) were assigned using specific feeding traits for lotic aquatic invertebrates in North America (Poff et al. 2006). Functional feeding groups evaluated include collector-filterer, collector-gatherer, predator, scraper/grazer, and shredder. The proportions of functional feeding groups for each invertebrate community was calculated by dividing the density of each FFG by the total density of invertebrates at each sampling reach. Trophic levels were also assigned for each taxon according to their FFG, which included 1) herbivorous invertebrates, 2) predatory invertebrates, and 3) fish.

Food web complexity was used to evaluate potential changes in species interactions and trophic relationships. Food web complexity was represented by the number of trophic links at each site exclusive of the plant-herbivore link. The number of trophic links were calculated by multiplying the number of species in one trophic level by the number of species in the next highest trophic level, summed for all trophic groups present. Only three trophic levels were used in calculating complexity in this study including 1) herbivorous invertebrates, 2) predatory invertebrates, and 3) fish.

Food web complexity example:



$$\text{Food web complexity} = (T^1 \times T^2) + (T^2 \times T^3)$$

T^i = number of species in trophic level *i*

In order to assess changes in drifting macroinvertebrates, invertebrate propensity to drift data were used to assess changes in food availability for fish (Rader 1997). Rader (1997) ranked invertebrate taxa propensity to drift by three categories: 1) weak propensity to drift, 2) medium propensity to drift, 3) strong propensity to drift. The data for these categories were retrieved from primary literature (Ward and Kondratieff 1992). The propensity to drift rankings reflect a species' relative availability to fish and were used to quantify food availability at each site and trophic transfer of metals from invertebrates to fish.

Species present in this study that are not represented in the Rader 1997 paper were calculated following the same methods from the data available in Ward and Kondratieff, 1992 or Poff et al, 2006.

Exposure values for As, Cd, Cu, Zn were used to evaluate the potential transport of these metals up the food chain to fish by the invertebrate biomass. Exposure values were calculated for each invertebrate analyzed for metals concentrations. These values were calculated by multiplying the metals concentrations by the observed densities of individual species at each site. As a result, the exposure values provide a quantitative estimate of the flux of contaminants potentially transported by each species.

Multiple linear regression models were fit for several community composition metrics using sediment metals contamination and habitat characteristics as explanatory variables. All data used in multiple regression models were scaled by dividing a variable by the mean of that variable to center the data and dividing the centered data by the standard deviation of that variable. Akaike Information Criterion (AIC) model selection was used to identify the model with the best fit (Akaike 1973). The model that minimized the AIC was selected as the final model.

Community composition data collected in 2009 and 2010 were analyzed separately to evaluate annual variations and sampling error. Data from metals analyses in fish and invertebrates were combined for both years due to the consistency in habitat and community composition metrics among years as well as the small amount of metals data available. Simple linear regression models were used to evaluate relationships between sediment contamination and exposure pathways. Model assumptions were checked using residual plots, variable inflation factors, and normal quantile plots to avoid collinearity among explanatory variables and ensure an appropriate model was selected. The natural log transformation was used on the median CCU data in the simple linear regression models to provide a linear relationship between community composition metrics and sediment contamination levels and satisfy model assumptions. All statistical analyses were computed using R statistical software (R Core Team 2015).

RESULTS

Impacts to aquatic communities from heavy metal contamination

Cumulative Criterion Units (CCU) were used to characterize potential toxicity from heavy metals contamination in sediment in the upper Blackfoot River. CCU among all sites ranged from 0.9 to 132.0 with an overall average CCU of 24.3 (Figure 2). The least contaminated site was Cadotte Creek (CD) and the most contaminated site was the Blackfoot River below the water treatment plant (B1). The average CCU value of 2.6 at the reference sites suggests that naturally occurring sources of heavy metals may be an important factor in this study area or that CCU tends to overestimate toxicity of metals in sediments. However, all contaminated sites had considerably higher CCU values with an average CCU of 41.9, confirming that historic mining and the Mike Horse Mine tailings dam breach have had significant impacts on heavy metals contamination of aquatic sediments in this region.

The CCU was calculated as the ratio of observed metals concentrations to the probable effect concentration, summed for Arsenic and five metals (As, Cd, Cr, Cu, Pb, and Zn). The CCU accounts for additive toxicity effects of multiple metals at each site, but may vary in the relative contributions from each metal. The percent contributions of each metal to the overall CCU at each site was calculated in order to evaluate the most abundant metals at each sampling location. When all of the study sites are considered, lead, copper, and zinc were the most influential metals contributing to the CCU values. An average of 25% of the CCU among all sites was attributed to lead, while zinc represented an average of 24% of the CCU and copper represented an average of 22% of the CCU among all sites. Arsenic represented an average of 19% of the CCU and cadmium contributed an average of 8% of the CCU.

When only reference sites are considered, arsenic, copper, and zinc contributed the most to site CCU. Arsenic represented an average of 31% of the CCU, copper contributed 28% of the CCU and zinc was the third highest at 18% of the CCU. Among impact sites only, lead, zinc, and copper were the most important metals, representing an average of 33%, 29%, and 16% respectively to the CCU. As a result, it is evident that zinc and copper are ubiquitous metals in this study area and potentially have important toxicity effects for aquatic biota.

A stepwise multiple regression model was used to evaluate physiochemical variables that influence sediment metals concentrations in the upper Blackfoot River mainstem sites only (B0, B1, B2, B3, B4) including distance from source, alkalinity, temperature, and stream discharge (Figure 3). Distance from source was defined as the distance in river kilometers from the Mike Horse dam. Distance from source and alkalinity were the only two predictor variables retained in the model after AIC model selection (Table 1). Distance from source was the most significant predictor variable with a p-value of 1.85×10^{-8}. Alkalinity provided only suggestive evidence as a predictor of sediment contamination with a p-value of 0.12. This model explained 70% of the variation observed in median CCU values and indicates that the best predictor of sediment contamination in the mainstem Blackfoot River sites is the distance from the Mike Horse dam, which is the largest source of contamination.

A stepwise multiple regression model was fit for four invertebrate community metrics, including invertebrate density (Figure 4), Shannon H diversity index (Figure 5), species richness (Figure 6), and food web complexity (Figure 7). For the 2009 sampling period, median CCU was retained in all four models in addition to several other physiochemical explanatory variables (Table 2, Table 4, Table 6, Table 8). For the 2010 data, median CCU and sediment characteristics were retained in each of the four regression models (Table 3, Table 5, Table 7, Table 9). Median CCU was the most significant term in each regression model, indicating that metals contamination is an important factor influencing each of these community composition metrics, in addition to habitat characteristics.

The density model for 2009 data showed a strong negative relationship with median CCU values and the highest adjusted R^2 value with 66% of the variation explained by this model (Figure 4, Table 2). Median CCU was the strongest explanatory variable with a p-value of 2.83 x 10^{-5}. Temperature and pH explanatory variables were also retained in this model with p-values of 0.049 and 0.0012 respectively.

For the 2010 data, only median CCU and percent fines were retained as explanatory variables after AIC model selection (Table 3). Median CCU was the most significant term with a p-value of 0.0061. Percent fines produced suggestive evidence as a predictor of invertebrate density with a p-value of 0.044. This model explained 55% of the variation observed with a negative relationship between invertebrate density and log median CCU. Both 2009 and 2010 regression models indicate a strong negative relationship between invertebrate density and sediment contamination levels.

The relationship between invertebrate diversity (Shannon H) and sediment contamination showed much more variation compared to invertebrate density, but also exhibited a negative relationship with sediment contamination levels (Figure 5). Only 33% of the variation was explained by the 2009 model, due to the large variability in diversity across all levels of sediment contamination (Table 4). Median CCU, pH, and percent fines explanatory variables were retained in this model with pH being the most significant term (p-value = 0.0268) and percent fines producing a p-value of 0.5481. Median CCU produced a p-value of 0.0438, indicating that sediment contamination levels influenced species diversity in addition to the presence of pH and percent fines in the model.

For the 2010 invertebrate diversity data (Shannon H), median CCU, D50 grain size, and percent fines were retained as explanatory variables after AIC model selection (Table 5). Median CCU produced a p-value of 0.0122, D50 returned a p-value of 0.0047, and percent fines returned a p-value of 0.0005. The 2010 Diversity model explained 76% of the observed variation. These results indicate that sediment contamination levels are important predictors of invertebrate diversity in addition to sediment habitat variables used in this study. However, it appears that habitat characteristics play a stronger role than sediment contamination in structuring invertebrate diversity in this study area.

Species richness also exhibited a strong negative relationship with median CCU with considerable variation across low to moderate levels of sediment contamination (Figure 6). 72% of the variation was explained by the 2009 model with median CCU being the most significant term (p-value = 1.34×10^{-5}) (Table 6). Temperature, pH, and percent fines were also retained in the model with p-values of 0.1168, 0.0090, and 0.0620 respectively. The 2010 model retained median CCU, D50 grain size, and percent fines as explanatory variables with median CCU being the most significant term (p-value 0.0026)

(Table 7). 72% of the variation was explained by the 2010 richness model. As a result, sediment contamination appears to be the most important predictor of invertebrate species richness in this study.

Food web complexity, represented by the number of trophic links, showed a strong negative relationship with median CCU values (Figure 7). 72% of the variation was explained by the 2009 model with median CCU being the most significant term (p-value = 1.07×10^{-5}) (Table 8). Drainage area, pH, and percent fines were also retained in this model with p-values of 0.0351, 0.0140, and 0.1065 respectively. In the 2010 food web complexity model, 66% of the variation was explained by the explanatory variables median CCU, D50 grain size, and percent fines (Table 9). Median CCU was highly significant with a p-value of 0.0069, while D50 grain size and percent fines produced a p-value of 0.0801 and 0.0095 respectively.

Changes in community composition and exposure pathways

The proportions of invertebrates representing each functional feeding group (FFG) were used to evaluate changes in exposure pathways among invertebrate communities at different contamination levels (Figure 8). In 2009, the proportion of Scrapers showed a negative relationship with median CCU, while proportion of collector-filterers showed a positive relationship with CCU. Proportions of predators and shredders did not exhibit any pattern across contamination levels in 2009. Proportion of omnivores showed a moderate negative relationship with median CCU. In 2010, the proportion of scrapers also declined with increasing sediment contamination. In contrast to the 2009 data, the proportion of collector-filterers did not show an obvious relationship with sediment contamination levels while the proportion of shredders showed a strong positive relationship with sediment contamination levels. The proportion of predators also showed a positive relationship with log median CCU but with considerable variation across all levels of contamination. These results indicate that invertebrate food web exposure to metals from periphyton declines with increasing sediment contamination, represented by the decline in the proportion of scrapers in both years.

Simple linear regression models were fit for proportions of scrapers, collector-filterers, and omnivores in 2009 and proportions of scrapers, shredders, and predators in 2010 versus log median CCU. All three models produced for the 2009 data returned a relatively small adjusted R^2 value with only 18% of the variation explained in the scrapers model, 11% explained in the collector-filterers model, and 32% of the variation explained in the omnivores model (Table 10). Log median CCU was significant in all three models (p-value < 0.05). All three models for the 2010 data also returned a relatively small R^2 with 35% of the variation explained in the scrapers model, 23% of the variation explained in the shredders model, and 13% of the variation explained in the predators model (Table 11). Log median CCU was significant in all three models (p-value < 0.05). As invertebrate density declined with increasing sediment contamination levels in both years, the responses of individual functional feeding groups varied with communities in highly contaminated sites being dominated by collector-filterers or shredders. Only proportion of scrapers consistently showed a strong negative relationship with log median CCU across both years.

Invertebrate metals concentrations by FFG were plotted in order to evaluate the implications for metals bioaccumulation from changes in proportions of FFG's (Figure 9). Arsenic concentrations in invertebrates were mostly below detection thresholds. For sites with sufficient data on Cd, Cu, and Zn, it appears that collector-filterers generally exhibited higher metals concentrations than predators and scrapers. However, this relationship was not apparent at all sites due in part to small sample sizes. For copper and zinc at site B1, the most highly contaminated site in this study, collector-filterers had significantly higher concentrations of these metals than predators and scrapers. In addition, site B4 shows higher concentrations of cadmium, copper, and zinc in collector-filterers than predators or scrapers. As a result, there is suggestive evidence that collector-filterers tend to have higher metals concentrations than either predators or scrapers. In addition, no sites show evidence of metals concentrations that are significantly higher in predators or scrapers compared to collector-filterers. As a result, it appears that food sources targeted by collector-filterers may result in increased metals bioaccumulation in these taxa.

Invertebrate propensity to drift may be an important factor determining food availability and metals exposure for fish. Both 2009 and 2010 data showed similar relationships for proportions of each drift category versus sediment contamination (Figure 10). The proportion of weak drifters showed a negative relationship with sediment contamination levels while the proportion of strong drifters showed a positive relationship with sediment metals concentrations. Medium drifters showed no obvious relationship with sediment contamination levels with a high degree of variability across all CCU levels.

Simple linear regression models were fit for both strong and weak drifters using log median CCU as the explanatory variable. For the 2009 data, 45% of the variation was explained in the weak drifter model with a p-value of 3.14×10^{-5} for the median CCU explanatory variable (Table 12). 26% of the variation was explained in the strong drifter model with a p-value of 0.00214 for the median CCU explanatory variable. For the 2010 data, 25% of the variation was explained in the weak drifter model with a p-value of 0.00458 for the median CCU explanatory variable (Table 13). 15% of the variation was explained in the strong drifter model with a p-value of 0.0257 for the median CCU explanatory variable. The high variation and low adjusted R^2 values for these regression models indicate that sediment contamination is not a strong predictor of the proportions of invertebrates representing each drift category and that habitat variables may play a stronger role in influencing these life history characteristics. However, the proportion of invertebrates with a strong propensity to drift increased with increasing sediment contamination in both 2009 and 2010, possibly due to indirect effects of mining contamination and habitat degradation.

Analyses of invertebrate metals concentrations by propensity to drift categories were also limited by data availability and detection thresholds. For cadmium, sites B1, B2, and B4 showed slightly higher concentrations in invertebrates with a strong propensity to drift compared to weak propensity to drift (Figure 11). However, invertebrates with a weak propensity to drift at site B0 showed significantly higher cadmium concentrations than invertebrates with a strong propensity to drift. No obvious pattern was apparent across all sites for copper concentrations. Site B0 showed higher concentrations in invertebrates with a weak propensity to drift compared to a strong propensity to drift. The other five sites showed similar metals concentrations across all three drift categories. Zinc was also highly variable across the mainstem sites for the three drift categories. Site B0 showed significantly higher zinc concentrations in invertebrates with a weak propensity to drift compared to a strong propensity to drift, while sites B1, B2, and B4 showed the opposite relationship with higher concentrations in strong propensity to drift compared to weak propensity to drift.

Analyses of changes in density and proportions of invertebrates with an emergent adult life stage were used to evaluate changes in resource subsidies and metals export to riparian food webs with increasing metals contamination. The density of emergent taxa declined with increasing metals contamination, coinciding with the observed relationship between total invertebrate density and sediment contamination. However, the proportion of invertebrates with an emergent life stage remained high at all levels of sediment contamination with a slight increase in the proportion of emergent taxa with increasing sediment contamination (Figure 12). Two significant outliers exist at site WC2 in 2010 with significantly lower proportions of emergent taxa compared to the other sites. A simple linear regression model was fit for the proportion of emergent taxa and log sediment CCU. Log sediment CCU returned a p-value of 0.00148 in the 2009 model (Table 12) and a p-value of 0.0889 in the 2010 model (Table 13). Both models produced positive slopes, suggesting that the proportion of emergent taxa increased with sediment contamination levels. However, both models produced low adjusted R^2 values with only 28% of the variation explained in the 2009 model and 7% explained in the 2010 model. The lack of a strong relationship between the proportion of emergent taxa and sediment CCU indicates that habitat conditions are stronger predictors of emergent taxa abundance or that the tendency for these types of streams to be dominated by emergent taxa obscures any relationship between the proportion of emergent invertebrates and sediment contamination. However, the decrease in emergent taxa density corresponding to the overall decrease in invertebrate density could have important implications for resource subsidies to riparian food webs in highly contaminated streams.

Organism metals concentrations at three trophic levels were plotted to evaluate any pattern of biomagnification or biodilution (Figure 13). The strongest evidence in previous studies for biomagnification has been observed for methyl-mercury, with weaker evidence for cadmium and selenium and little evidence for other metals (Sanchez-Bayo et al. 2012). In contrast, biodilution or biominification may occur if organisms are able to efficiently excrete metals and concentrations decrease with increasing trophic position. Comparisons among all contaminated sites shows evidence for biodilution of copper and zinc. These elements are essential trace nutrients for organisms, and biodilution is the expected relationship since organisms have evolved to cope with excess amounts of these metals. Sites B1, B3, and B4 in particular showed strong evidence of decreasing copper and zinc concentrations with increasing trophic level. Cadmium did not exhibit an obvious pattern among the impacted sites but did show suggestive evidence of biomagnification at site B2. However, there is not a statistically significant difference between trophic levels and this pattern is confounded by apparent biodilution at other sites. Interpretation of arsenic concentrations were limited by organism concentrations that were below detection thresholds, however site B2 did show considerably higher concentrations in fish compared to invertebrates. The suggestive evidence of biomagnification of arsenic and cadmium at site B2 was largely influenced by significant outliers, and it appears that the overall pattern for all metals is biodilution or no relationship among trophic position and metals concentrations.

Environmental contamination levels and toxicity risk to upper trophic levels

Exposure values (invertebrate density x invertebrate metal concentrations for each taxon) represent the potential flux of contaminants potentially transported by the invertebrate community to upper trophic levels through predation. Low invertebrate densities and high invertebrate metals concentrations may result in lower levels of contaminants bioavailable to fish compared to moderate densities with moderate invertebrate metals concentrations. The plots of exposure values vs sediment metals concentrations exhibit a similar pattern across all four metals (Figure 14). With two notable exceptions, the highest exposure values were observed at moderately contaminated sites with sediment CCU values of 64.8. Two outliers exist for arsenic and cadmium with very high exposure values coinciding with low sediment contamination levels.

Exposure value plots by drift categories indicate that invertebrates with a strong propensity to drift have higher exposure values at moderately contaminated sites compared to invertebrates with a weak propensity to drift (Figure 15). The weak drift category did not show a clear pattern and had similar exposure values across all contamination levels. However, the weak drift category also had fewer data points available. The high exposure values for invertebrates with a strong propensity to drift at moderately contaminated sites could have important implications for fish that target drifting invertebrates as a food source.

Fish populations are the top trophic level predators in this study. As a result, it is important to compare fish population sizes and metals concentrations coinciding with the observed exposure values in order to evaluate potential toxicity risk. Fish populations declined rapidly with increasing sediment contamination levels (Figure 16). Fish populations were significantly reduced above sediment CCU levels of 10. In contrast, the highest invertebrate exposure values were generally observed at CCU values of 64.8. The highest fish density was observed at Anaconda Creek (N = 100 per 100m) and the lowest fish density occurred at sites B1 and B2 on the mainstem of the Blackfoot River (N= 2 per 100m and 4 per 100m respectively). No fish were observed at the contaminated sites B0 in both 2009 and 2010, UB and B1 in 2009, and B3 in 2010.

Despite the drastically reduced fish populations in contaminated sites, the highest metals concentrations in fish that were sampled occurred at moderately contaminated sites with a CCU value of 64.8 (Figure 17). Arsenic, cadmium, and copper showed significantly higher concentrations in fish tissue at these sites with zinc showing considerable variation across all sites. The highest metals concentrations in fish tissue coincided with the highest invertebrate exposure levels at site B2 with CCU values of 64.8. As a result, it appears that there is a strong relationship between metals concentrations in fish and the pool of metals bioavailable to fish from invertebrates represented by invertebrate exposure values. However, fish sampled at each location may have arrived just prior to capture and may not provide an accurate representation of long term metals exposure to fish at each site.

DISCUSSION

The results of this study indicate that aquatic environments in the upper Blackfoot River impacted by historic mining and the Mike Horse dam breach are highly contaminated with heavy metals with significant implications for aquatic organisms and community structure. Despite the strong evidence of an impact to aquatic communities from historic mining contamination, naturally occurring metals remain an important component structuring aquatic communities in this region. Historic mines were located in regions with obvious mineralization and valuable ore deposits that were readily available to primitive technology. As a result, aquatic communities in the upper Blackfoot River have likely coexisted and evolved with naturally occurring metals levels that are higher than those found outside of this mineralized region and influence the species and invertebrate densities supported in these habitats. However, in sites that are heavily impacted by historic mining, sediment metals concentrations far exceed reference environmental contamination levels and exhibit significantly reduced invertebrate abundance and diversity.

Aquatic habitats impacted by heavy metals in the upper Blackfoot River exhibit significantly reduced invertebrate abundance and species diversity. These changes in community composition in response to heavy metal contamination also have important indirect effects on fish by reducing available food and by changing the exposure pathways of metals entering the aquatic food web. The proportion of scrapers declined with increasing sediment contamination and this may have important implications for metals exposure to aquatic invertebrate communities. In addition, the proportion of invertebrates with a strong propensity to drift increased with increasing sediment contamination potentially facilitating the transfer of metals to upper trophic levels through predation. However, the most significant observation in this study is the relationship between invertebrate abundance, metals concentrations in invertebrates, and metals concentrations in fish. These results indicate that species interactions and the biological movements of heavy metals in aquatic food webs play an important role in predicting heavy metals exposure in upper trophic levels in the upper Blackfoot River.

Impacts to aquatic communities from heavy metal contamination

Persistent acid mine drainage from historic mines and the failure of the Mike Horse dam in 1975 have severely impacted water quality and aquatic habitats in the upper Blackfoot River. Accordingly, the mainstem Blackfoot River sites (B0, B1, B2, B3, B4) have higher sediment metals concentrations compared to reference sites with the highest CCU values observed at sites B0 and B1 immediately downstream of the Mike Horse dam (Figure 2). In addition, the mainstem sites have significantly higher metals concentrations compared to one impact site (SG) located on a tributary not directly impacted by the Mike Horse dam failure. Distance from the Mike Horse dam was also the most significant predictor of sediment contamination at these sites, confirming that the Mike Horse dam was the primary source of heavy metals in the upper Blackfoot River.

A decline in invertebrate density was the strongest response to sediment contamination in the upper Blackfoot River invertebrate communities (Figure 4). Species diversity and food web complexity showed much more variation across all sites but also exhibited a decline with increasing contamination levels (Figure 5, Figure 7). These relationships are consistent with other studies of invertebrate community response to mining contamination (Kiffney and Clements 1994, Beltman et al. 1999, Clements et al. 2000). Invertebrate abundance is often reduced as conditions become more inhospitable, but metals tolerant taxa typically maintain some species diversity and functional groups in impaired streams. Habitat conditions are also important predictors of invertebrate density, however median sediment CCU was the most significant predictor variable in the multiple regression models, providing strong evidence that sediment contamination can dramatically decrease invertebrate abundance in the upper Blackfoot River.

The community composition data from 15 sites and 45 sampling locations over the period of two years provides a detailed picture of the aquatic community response to metals contamination in the upper Blackfoot River. The goal of this study was to investigate the implications of changes in community composition for the bioaccumulation and trophic transfer of contaminants. Accordingly, it is necessary to link the community composition data with metals analysis in aquatic biota and the environment. The metals data collected for invertebrate and fish populations was limited by sample size and detection limits, however all functional feeding groups and trophic levels were represented by this data set. In addition, several potentially important patterns have emerged from the community composition data that could help guide future studies investigating the processes of bioaccumulation and trophic transfer of heavy metals.

Changes in community composition and exposure pathways

Exposure pathways represent the primary route of metals entering food webs or individual trophic levels. A primary exposure pathway for metals entering aquatic food webs is represented by food sources exploited by aquatic invertebrates. As metals bioaccumulate and are transferred through the food web, metals concentrations and life history traits of prey become important factors for assessing exposure pathways to upper trophic levels and riparian food webs. Comparisons of the invertebrate FFG proportions among sites show potentially important changes in community composition that may affect exposure pathways of metals. The 2009 data exhibit a decline in the proportion of invertebrate scrapers with increasing sediment contamination and a respective increase in the proportion of collector-filterers (Figure 8). The 2010 data also exhibit a decrease in the proportion of scrapers but with an increase in the proportion of shredders instead of collector-filterers. Despite the variation among years, it appears that metals exposure from periphyton and biofilm decreases with increasing sediment metals contamination in this study due to the decline in the proportion of scrapers in both years. In highly contaminated sites, the relatively high proportion of collector-filterers or shredders may result in allochthonous material comprising a larger portion of resources supporting the aquatic food web. As a result, aquatic derived contamination entering the aquatic food web through food sources may be comparatively lower in highly contaminated sites than sites with low sediment metal levels in this study because of the higher proportion of invertebrates targeting food sources originating from terrestrial sources.

Several studies have observed higher metals concentrations in periphyton compared to sediment or other food sources (Smock 1983a, Farag et al. 1998, Besser et al. 2001). As a result, invertebrates in the scraper FFG are expected to have higher metals concentrations compared to other functional feeding groups. However, the metals data in this study do not exhibit a clear pattern in metals concentrations among FFG (Figure 9). This may indicate that water borne metals are the primary route of metals exposure for aquatic invertebrate communities in the upper Blackfoot River. In addition, limited data availability and detection limits may obscure any relationship observed in this study. A more detailed analysis of metals concentrations in aquatic invertebrates and the addition of metals concentrations in periphyton and biofilm may help elucidate any patterns in metals accumulation varying by functional feeding groups or food sources.

Environmental contamination levels and toxicity risk to upper trophic levels

Changes in community composition may also have important implications for metals exposure in fish and riparian food webs. The results of this study indicate that the proportion of invertebrates with a strong propensity to drift increases with sediment contamination levels (Figure 10). This may increase food availability for fish that prey primarily on this food source. However, no fish populations were observed at highly contaminated sites, which eliminates the possibility for metals transfer to upper trophic levels. Fish were observed at moderately contaminated sites that may have a higher proportion of drifting invertebrates to transfer contaminants to upper trophic levels. Food availability, in addition to habitat conditions, dictate fish abundance that are supported by streams. A higher proportion of drifting invertebrates coinciding with higher sediment metal levels, could create more hospitable conditions for fish populations than would otherwise be evident from habitat analyses alone. Ultimately, moderately contaminated sites that support fish populations and have a higher proportion of drifting invertebrates provides the link necessary for increased trophic transfer of contaminants.

The relationship between sediment contamination levels and drifting invertebrates would be especially important if invertebrates with a high propensity to drift exhibited higher metals concentrations than invertebrates with a low propensity to drift. However, the results of this study do not indicate a significant difference in metals concentrations between drift categories (Figure 11). This is likely due to the importance of water borne metals for exposure in invertebrates, as well as the similarity in food sources exploited by taxa in each drift category. As a result, fish that feed primarily on drifting invertebrates are not exposed to higher metals concentrations than fish feeding primarily on more sedentary organisms in the upper Blackfoot River.

The density of emergent taxa declined significantly with increasing sediment contamination levels, corresponding to the overall decrease in invertebrate abundance (Figure 12). However, the lack of a strong relationship between the proportion of emergent taxa and sediment CCU suggests that the largest effect of aquatic metals contamination on riparian food webs is the reduction in aquatic derived resources in contaminated streams. This is in agreement with previous studies that concluded that the greatest impact to riparian consumers was the reduction of invertebrate biomass, rather than metals toxicity from consuming emergent invertebrates (Walters et al. 2008, Kraus et al. 2014). All of the invertebrates analyzed for metals concentrations in this study were emergent taxa, limiting the ability to compare metals concentrations between emergent and non-emergent taxa. In addition, metals retention during metamorphosis to a winged adult life stage can be highly variable and would require additional comparisons of metals concentrations at all life stages of aquatic invertebrates (Currie et al. 1997, Kraus et al. 2014). However, the decline in invertebrate abundance with increasing metals contamination will reduce food available for riparian consumers and result in potentially significant indirect impacts to riparian food webs from aquatic contamination.

Exposure values for invertebrates analyzed in this study are intended to represent the flux of contaminants transported to upper trophic levels or riparian food webs. Highly contaminated streams may have very high metals concentrations in sediment and water, but also have drastically reduced invertebrate and fish biomass, reducing the trophic transfer of contaminants. In contrast, moderately contaminated sites support a relatively large biomass of invertebrates that accumulate metals and are prey sources for fish and riparian consumers. The highest exposure values observed in this study occurred at moderately contaminated sites with a CCU of 64.8 (Figure 14). A notable exception was an outlier at the lowest CCU level that was the highest exposure value observed. In addition, low sediment contamination levels produced relatively high exposure values as invertebrate density was highest at these locations. Sediment CCU values declined significantly in the mainstem Blackfoot River sites with minimal variation as the distance from the Mike Horse dam increased. In contrast, invertebrate exposure values exhibit considerably more variation with high exposure values at moderately contaminated sites. If environmental contamination were the strongest predictor of toxicity risk to upper trophic levels, exposure values would be expected to decline as sediment contamination declines. However, these results indicate that biological mechanisms are important factors determining metals concentrations in aquatic biota and ultimate toxicity risk to upper trophic levels. In addition, it appears that moderately contaminated sites in this study produced the greatest risk to upper trophic level consumers due to the presence of fish, relatively high invertebrate densities and moderately elevated metals contamination.

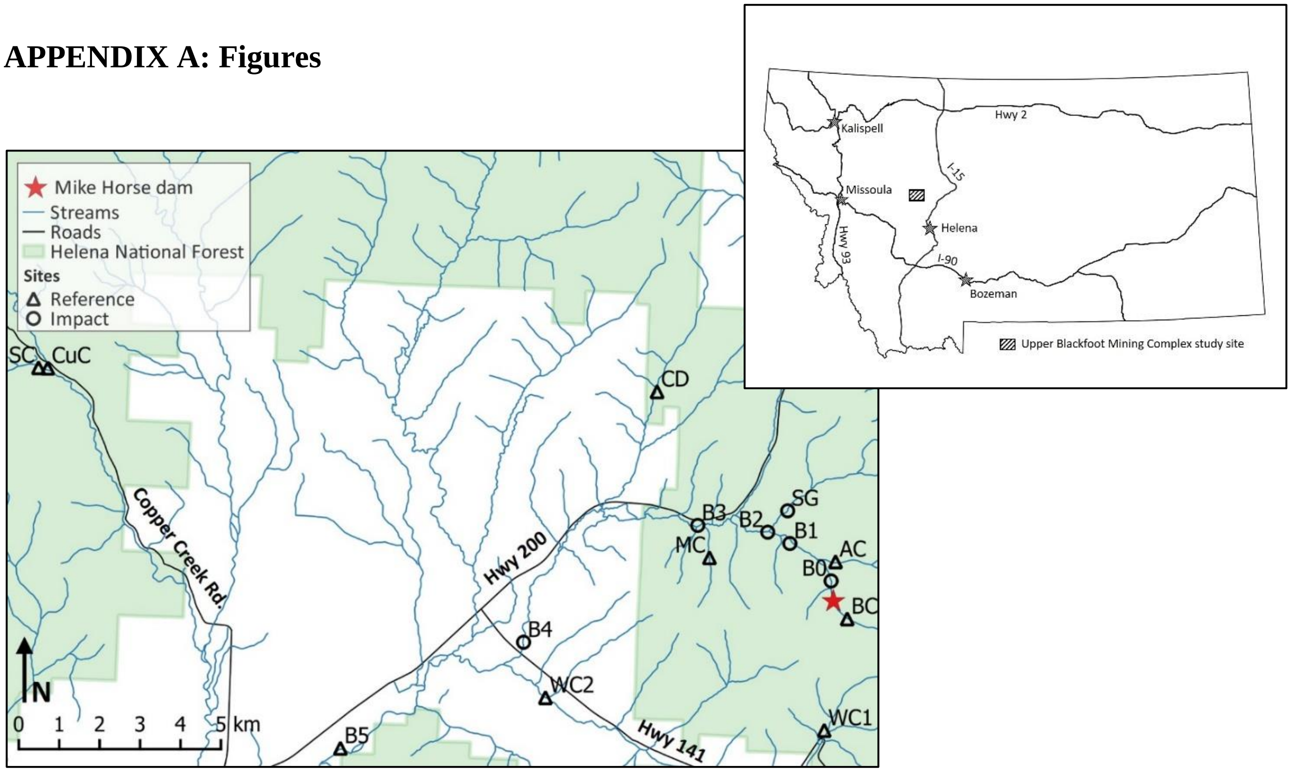
High invertebrate exposure values could also simply be the result of high invertebrate densities as exposure values are calculated by multiplying invertebrate density by the invertebrate metals concentration. A species with very high densities may produce a high exposure value even if the metals concentrations in this species were low, producing misleading results about the potential metals transport to upper trophic levels. However, Figure 14 shows that the highest invertebrate exposure value for arsenic was observed in a species with moderate densities compared to other species in this study. In addition, numerous low exposure values were observed in species with high densities. Heavy metals exposure to invertebrates likely reduces the abundance of that species due to metals toxicity. As a result, it appears that invertebrate density does not solely drive the observed exposure values which are a product of both high biotic metal levels as well as invertebrate abundance.

It is important to link invertebrate exposure values with the fish abundance and metals contamination in order to elucidate evidence of trophic transfer of contaminants from invertebrates to fish. Fish populations were drastically reduced at the most contaminated sites compared to reference sites in this study (Figure 16). This is likely due to direct metals toxicity and degraded habitats that do not support high densities of fish. However, fish that were sampled at moderately contaminated sites exhibited significantly higher metals concentrations than either reference or highly contaminated sites (Figure 17). Sites that contained fish with the highest metals concentrations coincided with sites that had the highest invertebrate exposure values. In contrast, highly contaminated sites did not exhibit elevated metals concentrations in fish tissue despite very high metals exposure in sediment and water. Fish livers were used for metals analysis in this study and provide a good approximation of metals exposure in fish as metals tend to accumulate rapidly in livers and remain elevated after exposure (Jezierska and Witeska 2006). Food sources are also important routes of metals exposure in fish, and these results indicate that moderately contaminated sites with relatively high invertebrate densities produce significantly higher metals concentrations in fish tissue.

CONCLUSION

The results of this study and similar efforts to investigate the biological movement of heavy metals in aquatic food webs will have important implications for assessing toxicity risk in AMD impacted streams. Species interactions, food web complexity, and community composition can have important effects on the biological movements of heavy metals in addition to the toxicity effects from direct exposure to contaminants in water and sediments. The results of this study identify several changes in community composition with important implications for bioaccumulation and trophic transfer of heavy metals including a shift in exposure pathways, decline in invertebrate abundance and species diversity, and ultimate metals accumulation in upper trophic levels. The most important observation is the link between invertebrate abundance, the pool of bioavailable metals in invertebrate communities, and metals concentrations in upper trophic levels observed in the upper Blackfoot River. In addition, the decline in the proportion of invertebrate scrapers and increase in invertebrates with a high propensity to drift with increasing sediment contamination levels could have important effects on metals exposure to invertebrate communities and fish in mining impacted streams. Fish were excluded from highly contaminated sites in this study likely due to direct toxicity effects of heavy metals. Moderately contaminated sites supported fish populations due to reduced direct toxicity effects, and the primary exposure source of heavy metals to upper trophic levels at these sites may be through trophic transfer. The results of this study emphasize the importance of biological factors that influence bioaccumulation and trophic transfer of metals in AMD streams and highlights the necessity for evaluating community composition response, in addition to environmental contamination levels, when evaluating ultimate toxicity risk to fish and other species in upper trophic levels.

APPENDIX A: Figures



Site	Code	Type	Latitude (N)	Longitude (W)	Elevation (m)
Beartrap Creek below tailings dam	B0	Impact	47.03030	112.35454	1650
Blackfoot River below water treatment plant	B1	Impact	47.03829	112.36839	1603
Blackfoot River below Shave	B2	Impact	47.04063	112.37572	1540
Blackfoot River below 1st wetland	B3	Impact	47.04156	112.39856	1576
Blackfoot River below 2nd wetland	B4	Impact	47.01413	112.45378	1520
Blackfoot River at Hogum Creek	B5	Impact	46.98900	112.51200	1463
Shave Gulch	SG	Impact	47.04554	112.36950	1629
Anaconda Creek	AC	Reference	47.03461	112.35333	1643
Upper Beartrap Creek (above tailings dam)	BC	Reference	47.02200	112.34885	1711
Cadotte Creek	CD	Reference	47.07096	112.41341	1610
Copper Creek (above Snowbank Creek confluence)	CuC	Reference	47.07100	112.61200	1615
Meadow Creek	MC	Reference	47.03447	112.39437	1612
Snowbank Creek	SC	Reference	47.07100	112.61500	1615
Willow Creek above Flesher Pass Road	WC1	Reference	46.99700	112.35500	1707
Willow Creek below Sandbar Creek	WC2	Reference	47.00200	112.44600	1550

Figure 1: Map and descriptions of sites sampled in the upper Blackfoot River during the summers of 2009 and 2010 for metals contamination in sediment, water, and aquatic organisms.

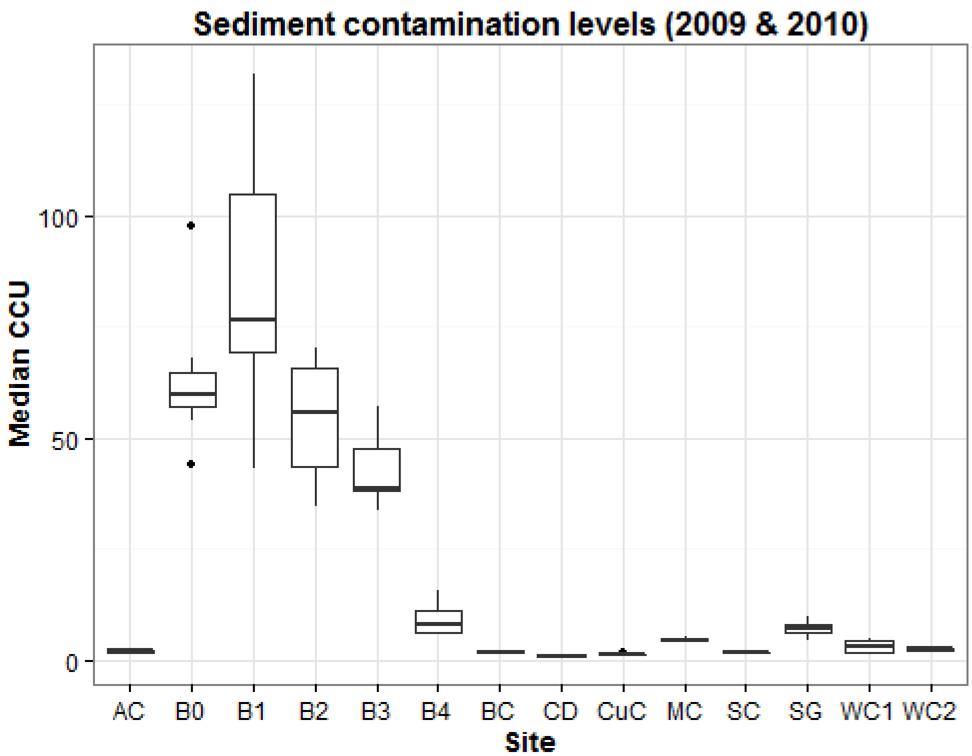


Figure 2 : Boxplot of cumulative criterion units (CCU) at each site in the upper Blackfoot River sampled in 2009 and 2010. Metals concentrations in sediment were not collected from site B5. See Figure 1 for site codes

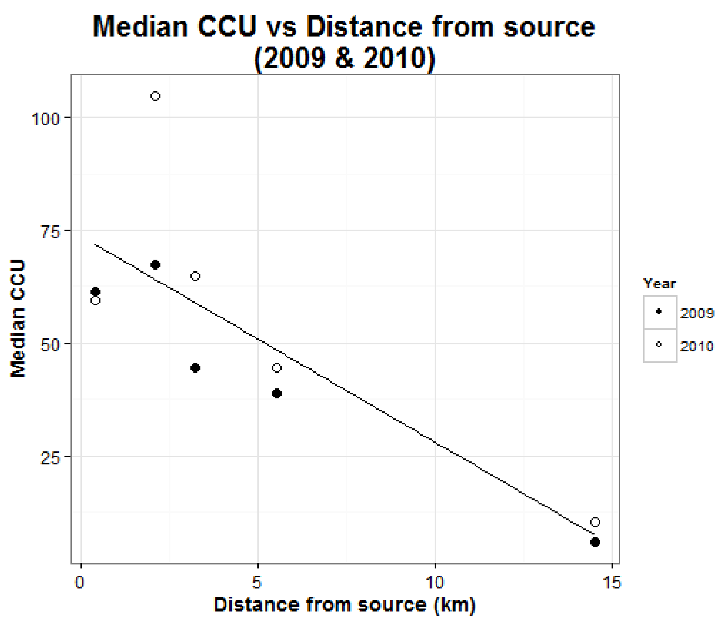


Figure 3 : Median CCU (cumulative criterion units) versus distance from Mike Horse dam (km) for mainstem sites only (B0, B1, B2, B3, B4) sampled in 2009 and 2010 in the upper Blackfoot River.

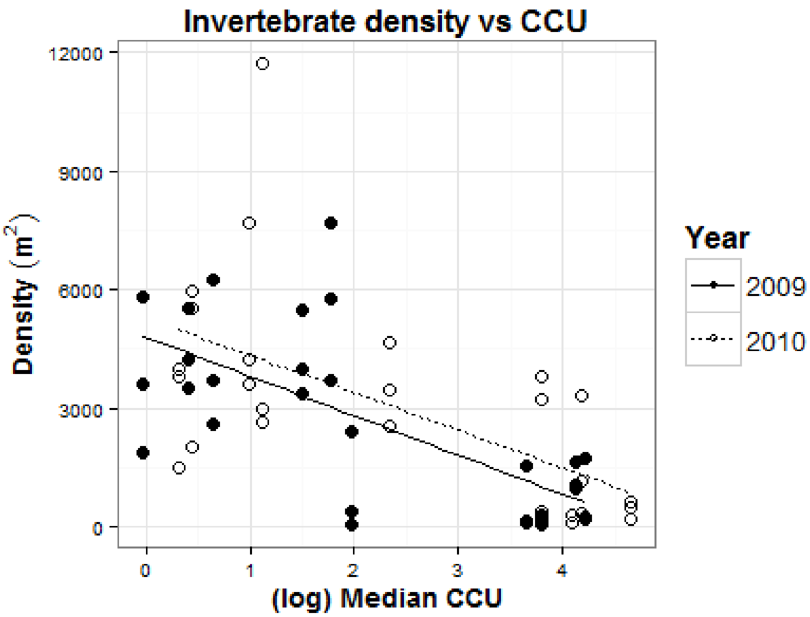


Figure 4: Invertebrate density vs sediment contamination (CCU = cumulative criterion units) for all upper Blackfoot River sites sampled in 2009 and 2010. Solid line is the regression line for 2009, dashed line is the regression line for 2010 (2009 adj. $R^2 = 0.61$, 2010 adj. $R^2 = 0.53$). Each point represents the average invertebrate density from three sampling reaches at individual sites.

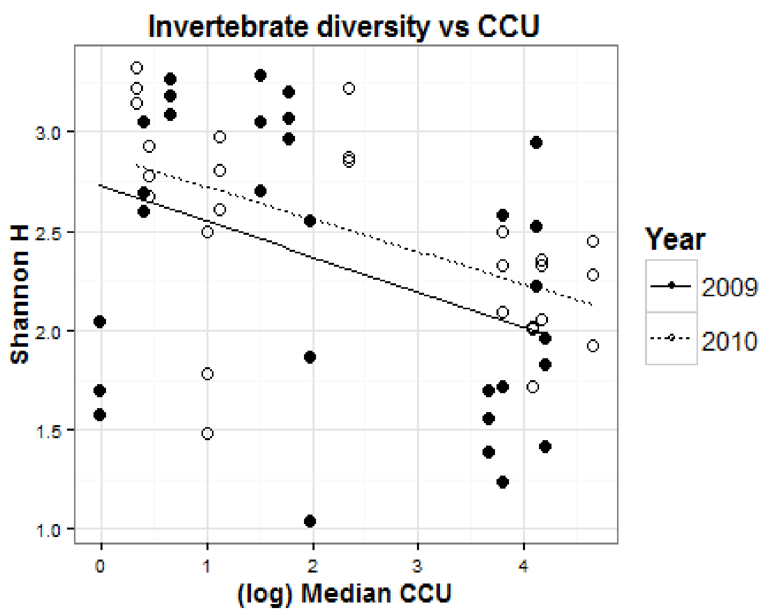


Figure 5: Invertebrate diversity (Shannon H) vs sediment contamination (CCU = cumulative criterion units) for all upper Blackfoot River sites sampled in 2009 and 2010. Solid line is the regression line for 2009, dashed line is the regression line for 2010(2009 adj. $R^2 = 0.3$, 2010 adj. $R^2 = 0.75$). Each point represents the average invertebrate diversity from three sampling reaches at individual sites.

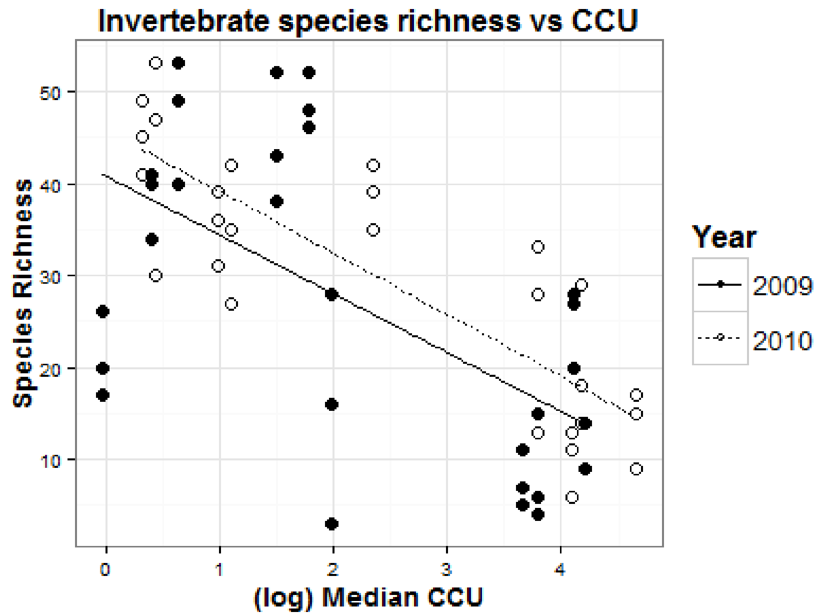


Figure 6: Invertebrate species richness vs sediment contamination (CCU = cumulative criterion units) for all sites upper Blackfoot River sampled in 2009 and 2010. Solid line is the regression line for 2009, dashed line is the regression line for 2010 (2009 adj. R^2 = 0.54, 2010 adj. R^2 = 0.34). Each point represents the average species richness from three sampling reaches at individual sites.

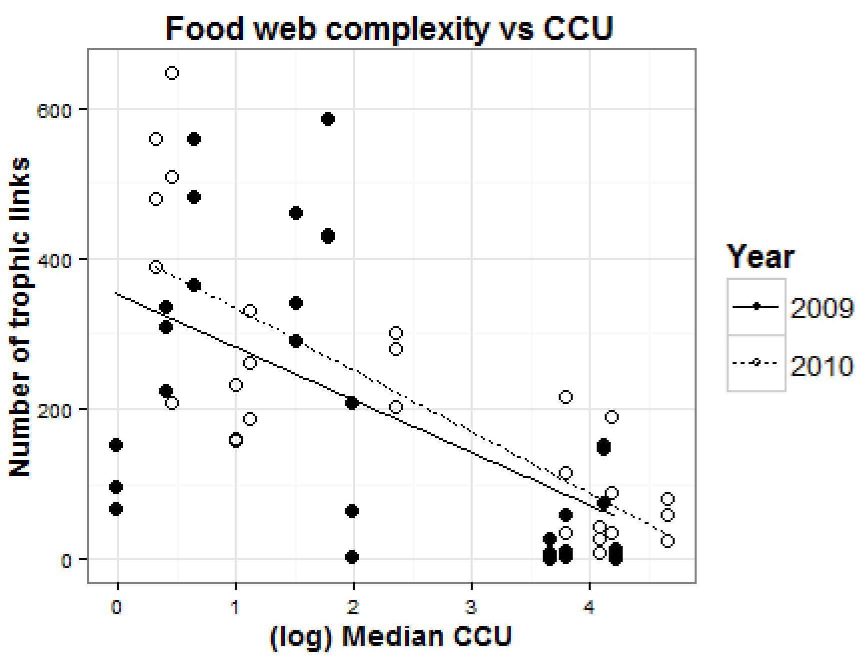


Figure 7: Invertebrate food web complexity vs sediment contamination (CCU = cumulative criterion units) for all upper Blackfoot River sites sampled in 2009 and 2010. Solid line is the regression line for 2009, dashed line is the regression line for 2010 (2009 adj. R^2 = 0.58, 2010 adj. R^2 = 0.29). Each point represents the average number of trophic links from three sampling reaches at individual sites.

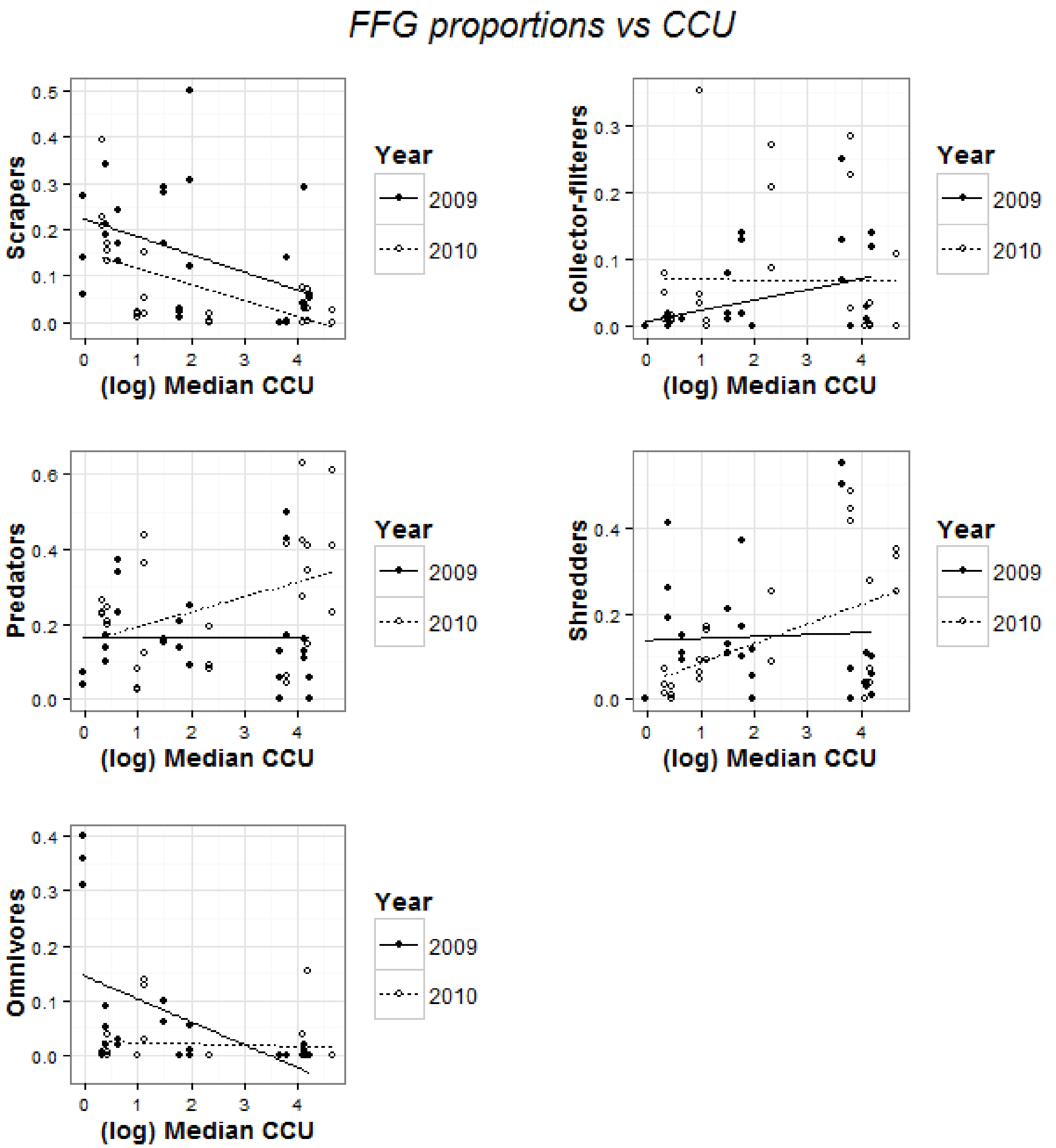


Figure 8: Proportions of functional feeding group vs sediment contamination (CCU = cumulative criterion units) for all upper Blackfoot River sites sampled in 2009 and 2010. Solid line is the regression line for 2009, dashed line is the regression line for 2010. Each point represents the average proportions from three sampling reaches at individual sites.

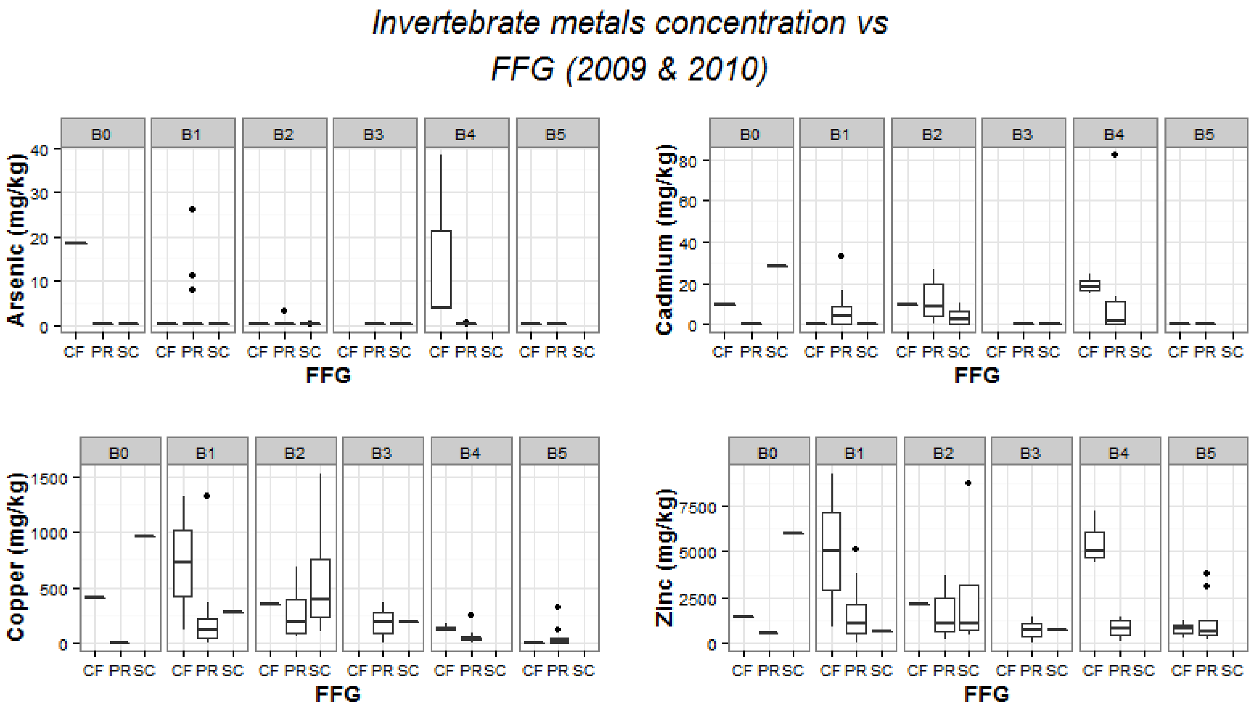


Figure 9: Boxplot of invertebrate metals concentrations by invertebrate functional feeding group for mainstem sites in the upper Blackfoot River sampled in 2009 and 2010 (CF = collector-filterers, PR = predator, SC = scraper, FFG = Functional feeding group).

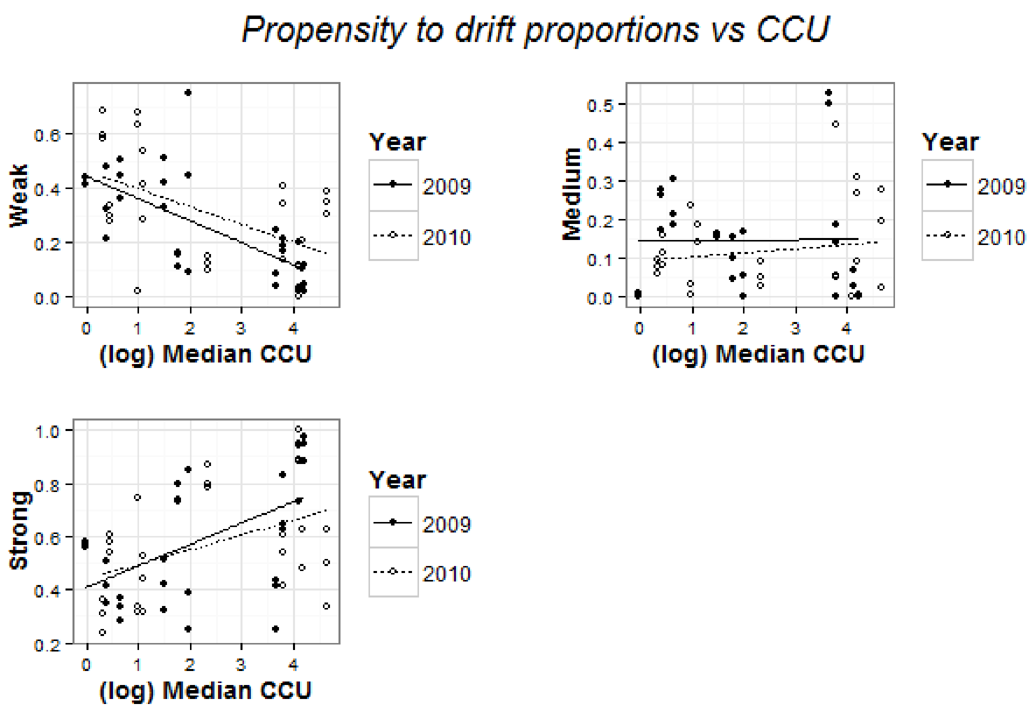


Figure 10: Proportion of invertebrates in three propensity to drift categories vs. sediment contamination (CCU = cumulative criterion units) for all upper Blackfoot River sites sampled in 2009 and 2010. Each point represents the average proportions from three sampling reaches at individual sites.

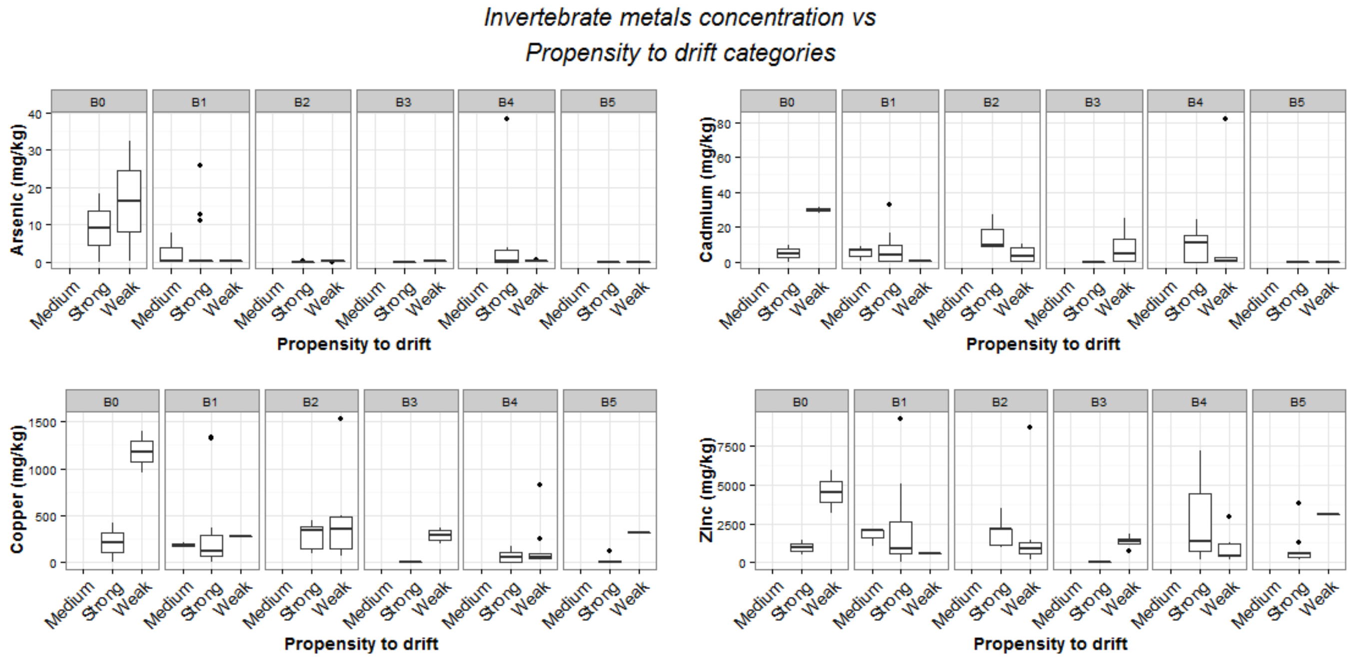


Figure 11: Invertebrate metals concentrations for three propensity to drift categories for mainstem sites in the upper Blackfoot River sampled in 2009 and 2010. See Figure 1 for site codes.

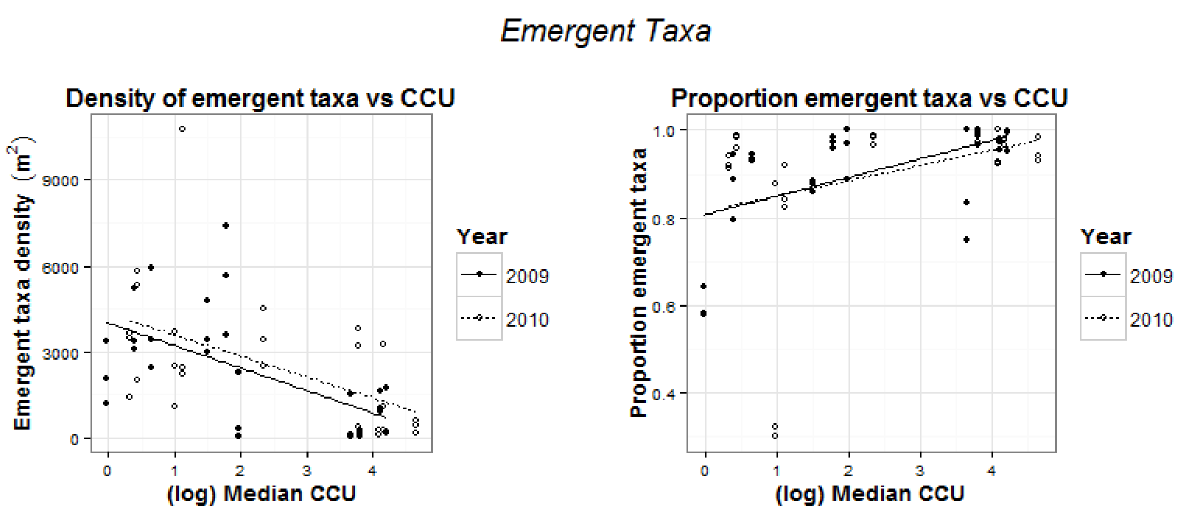


Figure 12: Density and proportions of emergent invertebrate taxa vs. sediment contamination (CCU = cumulative criterion units) for all upper Blackfoot River sites sampled in 2009 and 2010. Each point represents the average invertebrate density from three sampling reaches at individual sites.

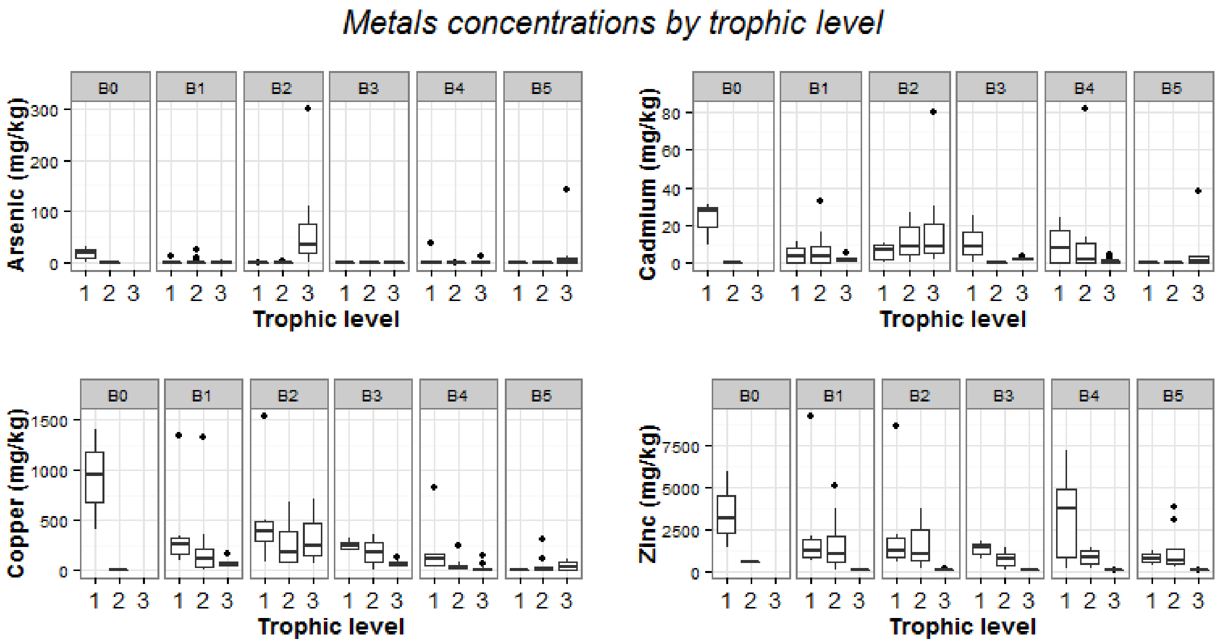


Figure 13: Boxplot of invertebrate and fish metals concentrations by trophic level for all mainstem sites in the upper Blackfoot River sampled in 2009 and 2010. Trophic levels: 1 = herbivorous invertebrates, 2 = predatory invertebrates, 3 = fish.

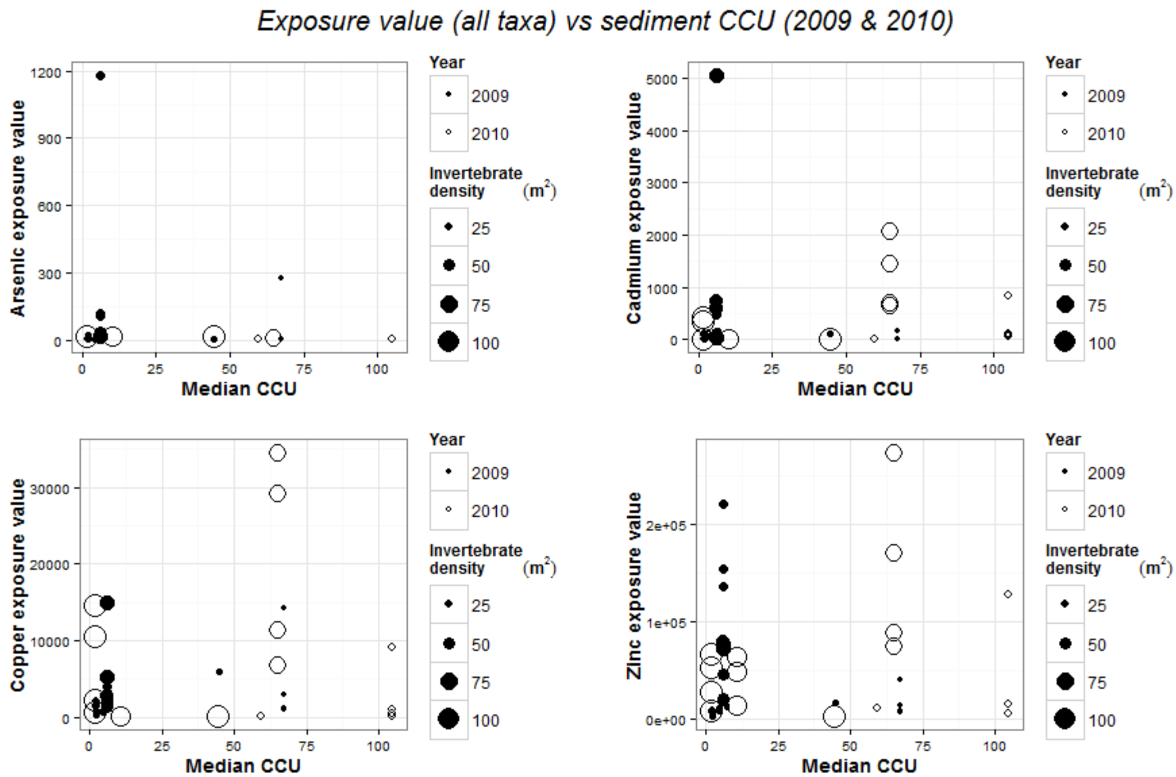


Figure 14: Exposure values for all taxa and four metals vs. sediment contamination (CCU = cumulative criterion units) for all upper Blackfoot River sites sampled in 2009 and 2010. Point size reflects the invertebrate species' density with larger points corresponding to higher densities. Solid circles identify 2009 data and open circles identify 2010 data. Each point represents exposure values for an individual species.

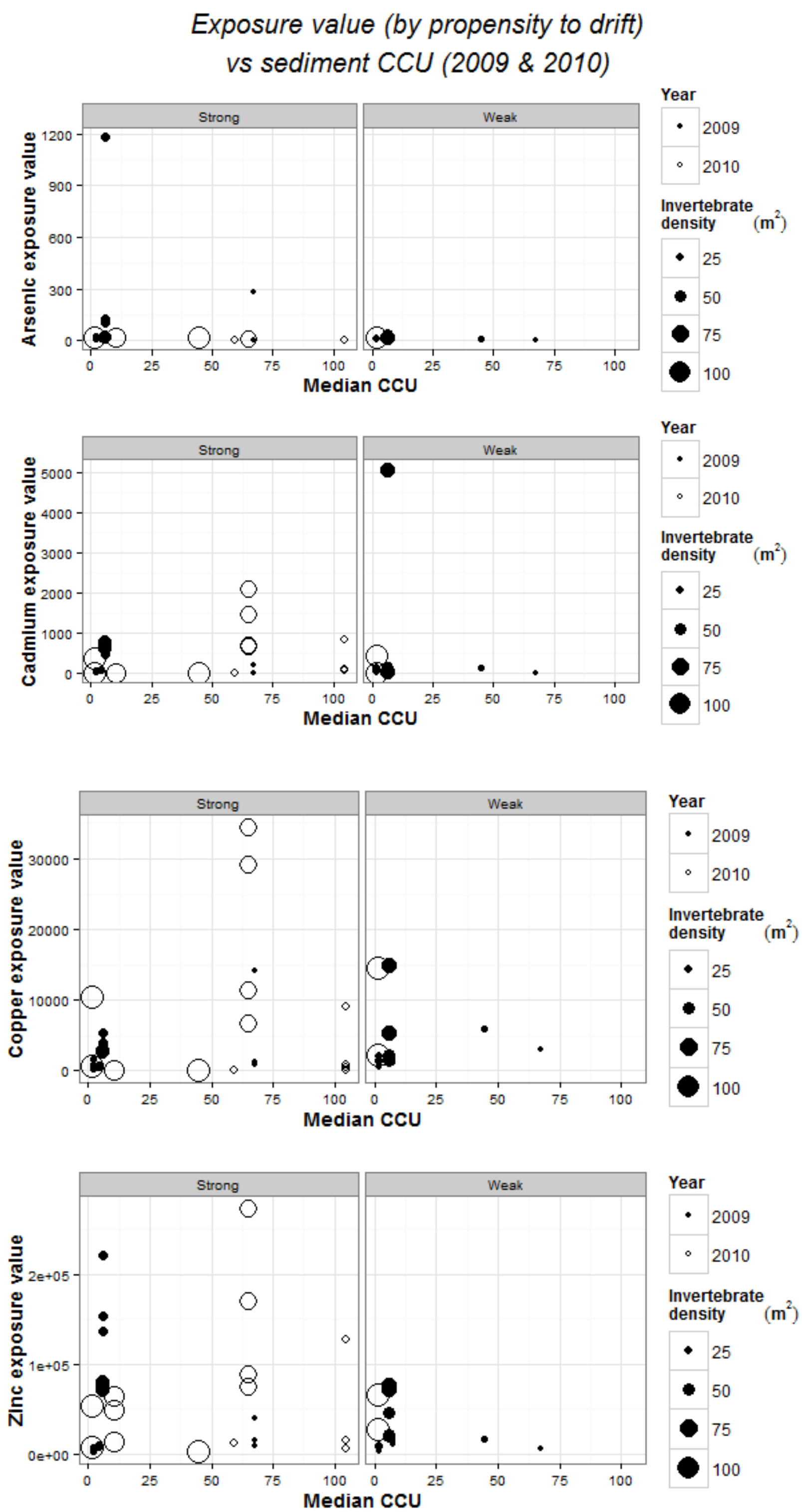


Figure 15: Exposure values for invertebrates with strong and weak propensity to drift vs. sediment contamination (CCU = cumulative criterion units) for all upper Blackfoot River sites sampled in 2009 and 2010. Point size reflects the invertebrate species' density with larger points corresponding to higher densities. Solid circles identify 2009 data and open circles identify 2010 data. Each point represents exposure values for an individual species.

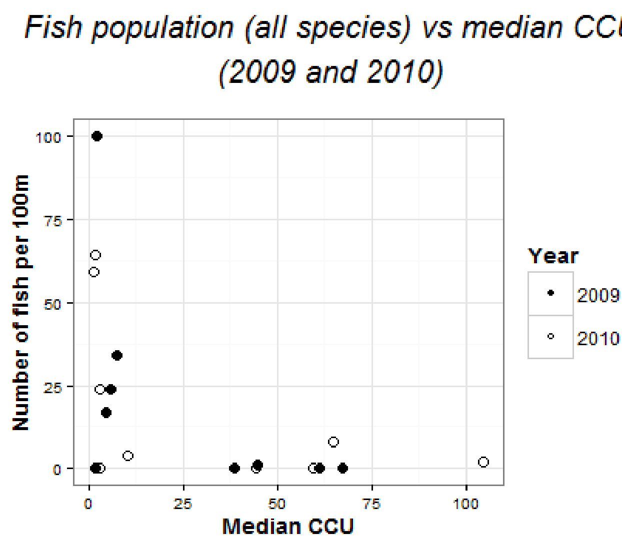


Figure 16: Fish population size vs. sediment contamination (CCU = cumulative criterion units) for all upper Blackfoot sites sampled in 2009 and 2010. Solid circles identify 2009 data and open circles identify 2010 data. Each point represents fish population estimates from individual streams.

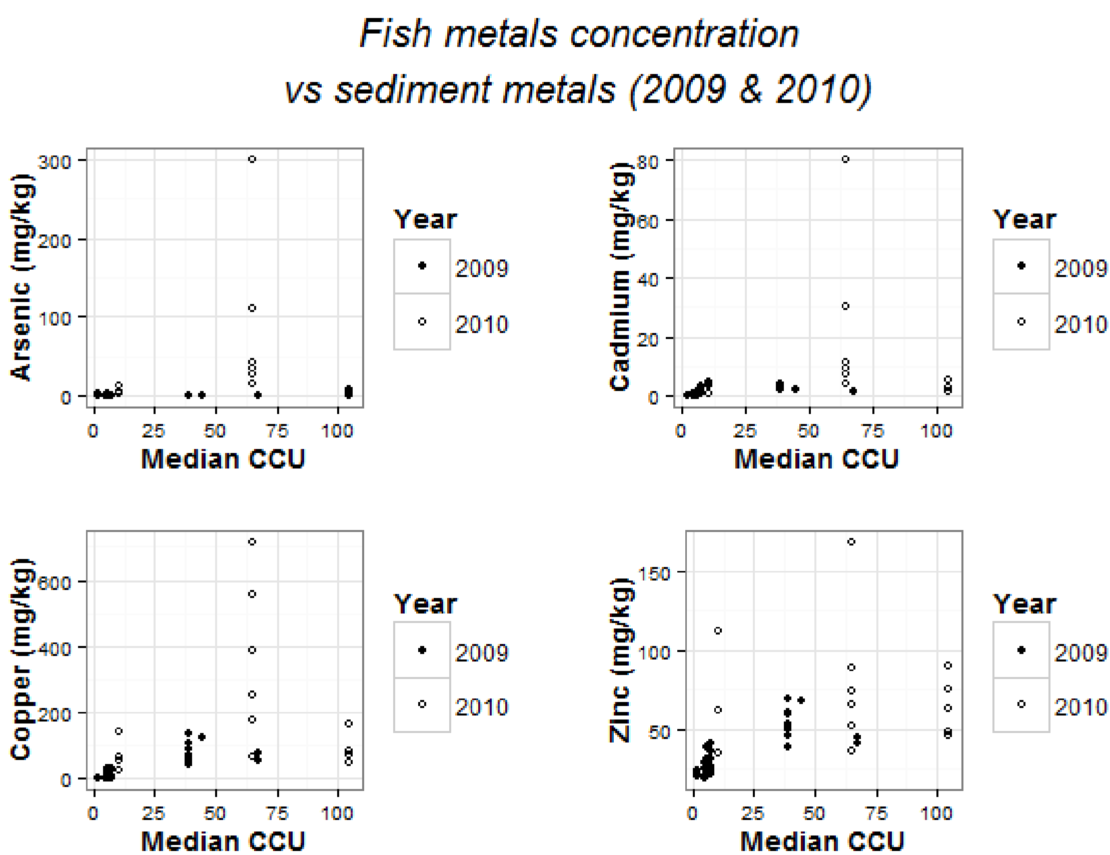


Figure 17: Metals concentrations in fish vs. sediment contamination (CCU = cumulative criterion units) for all upper Blackfoot River sites sampled in 2009 and 2010. Solid circles identify 2009 data and open circles identify 2010 data. Each point represents observed metals concentrations in livers of individual fish.

APPENDIX B: Regression model output

Table 1: 2009 and 2010 sediment contamination model

Stepwise AIC model selection results:

Initial Model:

medianCCU ~ River_km + temp + alkalinity + discharge

Final Model:

medianCCU ~ River_km + alkalinity

Step		variable	Df	Deviance	Resid. Df	Resid. Dev	AIC
1					25	7.724768	-30.703
2	-	discharge	1	0.208913	26	7.933681	-31.9024
3	-	temp	1	0.230616	27	8.164297	-33.0428

Model results:

formula = medianCCU ~ River_km + alkalinity

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	5.48E-17	1.00E-01	0	1
River_km	-8.10E-01	1.03E-01	-7.868	1.85E-08
alkalinity	1.65E-01	1.03E-01	1.607	0.12

Residual standard error: 0.5499 on 27 degrees of freedom

Multiple R-squared: 0.7185, Adjusted R-squared: 0.6976

F-statistic: 34.45 on 2 and 27 DF, p-value: 3.703e-08

Table 2: 2009 Invertebrate density model

Stepwise AIC model selection results:

Initial Model:
density ~ (medianCCU) + pH + temp + drain_area + D50 + fines

Final Model:
density ~ medianCCU + pH + temp

Step			Df	Deviance	Resid. Df	Resid. Dev	AIC
1					20	6.716703	-23.5635
2	-	drain_area	1	0.181027	21	6.89773	-24.8454
3	-	fines	1	0.434614	22	7.332344	-25.1956
4	-	D50	1	0.515002	23	7.847346	-25.3629

Model results:

formula = density ~ medianCCU + pH + temp

Residuals:

Min	1Q	Median	3Q	Max
-2429.02	-1026.21	43.28	799.72	2484.49

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-19508	5937.63	-3.285	0.00324
medianCCU	-52.88	10.17	-5.202	2.83E-05
pH	2779.98	752.36	3.695	0.0012
temp	193.82	93.12	2.081	0.04872

Residual standard error: 1330 on 23 degrees of freedom
Multiple R-squared: 0.6982, Adjusted R-squared: 0.6588
F-statistic: 17.73 on 3 and 23 DF, p-value: 3.491e-06

Table 3: 2010 Invertebrate density model

Stepwise AIC model selection results:

Initial Model:

density ~ (medianCCU) + temp + D50 + fines + drain_area

Final Model:

density ~ medianCCU + fines

Step			Df	Deviance	Resid. Df	Resid. Dev	AIC
1					10	5.151312	-6.03198
2	-	drain_area	0	0	10	5.151312	-6.03198
3	-	temp	1	0.171841	11	5.323153	-7.53977
4	-	D50	1	0.074022	12	5.397176	-9.33262

Model results:

formula = density ~ medianCCU + fines

Residuals:

Min	1Q	Median	3Q	Max
-1.18520	-0.31328	-0.01209	0.39420	0.90569

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-1.38E-16	1.73E-01	0	1
medianCCU	-6.04E-01	1.82E-01	-3.321	0.0061
fines	-4.09E-01	1.82E-01	-2.25	0.044

Residual standard error: 0.6706 on 12 degrees of freedom
Multiple R-squared: 0.6145, Adjusted R-squared: 0.5502
F-statistic: 9.564 on 2 and 12 DF, p-value: 0.003283

Table 4: 2009 Invertebrate diversity model

Stepwise AIC model selection results:

Initial Model:

shannonH ~ (medianCCU) + pH + temp + drain_area + D50 + fines

Final Model:

shannonH ~ medianCCU + pH + fines

Step			Df	Deviance	Resid. Df	Resid. Dev	AIC
1					20	10.36728	-11.8439
2	-	temp	1	0.002745	21	10.37002	-13.8368
3	-	drain_area	1	0.304797	22	10.67482	-15.0546
4	-	D50	1	0.444624	23	11.11944	-15.9528

Model results:

formula = shannonH ~ medianCCU + pH + fines

Residuals:

Min	1Q	Median	3Q	Max
-1.1689	-0.2896	0.0283	0.3338	1.1057

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-4.3198	2.883444	-1.498	0.1461
medianCCU	-0.01118	0.005275	-2.118	0.0438
pH	0.878975	0.374355	2.348	0.0268
fines	0.003689	0.006063	0.609	0.5481

Residual standard error: 0.576 on 26 degrees of freedom

Multiple R-squared: 0.3969, Adjusted R-squared: 0.3273

F-statistic: 5.703 on 3 and 26 DF, p-value: 0.003873

Table 5: 2010 Invertebrate diversity model

Stepwise AIC model selection results:

Initial Model:
shannonH ~ (medianCCU) + temp + D50 + fines + drain_area

Final Model:
shannonH ~ medianCCU + D50 + fines

Step			Df	Deviance	Resid. Df	Resid. Dev	AIC
1					10	2.655964	-15.9686
2	-	drain_area	0	0	10	2.655964	-15.9686
3	-	temp	1	0.001723	11	2.657686	-17.9589

Model results:

formula = shannonH ~ medianCCU + D50 + fines

Residuals:

Min	1Q	Median	3Q	Max
-0.7319	-0.4064	0.1621	0.2553	0.5937

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-3.34E-16	1.27E-01	0	1
medianCCU	-4.14E-01	1.38E-01	-2.995	0.012198
D50	7.30E-01	2.07E-01	3.527	0.004739
fines	-1.03E+00	2.10E-01	-4.926	0.000452

Residual standard error: 0.4915 on 11 degrees of freedom
Multiple R-squared: 0.8102, Adjusted R-squared: 0.7584
F-statistic: 15.65 on 3 and 11 DF, p-value: 0.0002777

Table 6: 2009 Invertebrate species richness model

Stepwise AIC model selection results:

Initial Model:

richness ~ (medianCCU) + pH + temp + drain_area + D50 + fines

Final Model:

richness ~ medianCCU + pH + temp + fines

Step			Df	Deviance	Resid. Df	Resid. Dev	AIC
1					20	5.748522	-27.7661
2	-	drain_area	1	0.026918	21	5.77544	-29.64
3	-	D50	1	0.388974	22	6.164414	-29.8802

Model results:

formula = richness ~ medianCCU + pH + temp + fines

Residuals:

Min	1Q	Median	3Q	Max
-1.45632	-0.21957	0.02074	0.27710	0.92508

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-2.02E-16	1.02E-01	0	1
medianCCU	-7.86E-01	1.41E-01	-5.572	1.34E-05
pH	3.57E-01	1.25E-01	2.863	0.00903
temp	1.71E-01	1.05E-01	1.633	0.1168
fines	2.92E-01	1.49E-01	1.966	0.062

Residual standard error: 0.5293 on 22 degrees of freedom

Multiple R-squared: 0.7629, Adjusted R-squared: 0.7198

F-statistic: 17.7 on 4 and 22 DF, p-value: 1.25e-06

Table 7: 2010 Invertebrate species richness model

Stepwise AIC model selection results:

Initial Model:

richness ~ (medianCCU) + temp + D50 + fines + drain_area

Final Model:

richness ~ medianCCU + D50 + fines

Step			Df	Deviance	Resid. Df	Resid. Dev	AIC
1					10	3.079165	-13.75088
2	-	drain_area	0	0	10	3.079165	-13.75088
3	-	temp	1	0.042625	11	3.12179	-15.54466

Model results:

formula = richness ~ medianCCU + D50 + fines

Residuals:

Min	1Q	Median	3Q	Max
-0.91202	-0.34187	0.06782	0.29065	0.79503

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	5.91E-17	1.38E-01	0	1
medianCCU	-5.80E-01	1.50E-01	-3.866	0.00262
D50	3.77E-01	2.24E-01	1.68	0.12117
fines	-7.45E-01	2.27E-01	-3.279	0.00734

Residual standard error: 0.5327 on 11 degrees of freedom
Multiple R-squared: 0.777, Adjusted R-squared: 0.7162
F-statistic: 12.78 on 3 and 11 DF, p-value: 0.0006616

Table 8: 2009 Food web complexity model

Stepwise AIC model selection results:

Initial Model:

links ~ (medianCCU) + pH + temp + drain_area + D50 + fines

Final Model:

links ~ medianCCU + pH + drain_area + fines

Step			Df	Deviance	Resid. Df	Resid. Dev	AIC
1					20	6.142828	-25.9749
2	-	D50	1	0.026568	21	6.169396	-27.8584
3	-	temp	1	0.060606	22	6.230003	-29.5944

Model results:

formula = links ~ medianCCU + pH + drain_area + fines

Residuals:

Min	1Q	Median	3Q	Max
-1.08010	-0.29969	0.01648	0.29204	1.05325

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-2.30E-16	1.02E-01	0	1
medianCCU	-8.06E-01	1.42E-01	-5.665	1.07E-05
pH	3.33E-01	1.25E-01	2.669	0.014
drain_area	2.44E-01	1.08E-01	2.246	0.0351
fines	2.56E-01	1.52E-01	1.683	0.1065

Residual standard error: 0.5321 on 22 degrees of freedom

Multiple R-squared: 0.7604, Adjusted R-squared: 0.7168

F-statistic: 17.45 on 4 and 22 DF, p-value: 1.4e-06

Table 9: 2010 Food web complexity model

Stepwise AIC model selection results:

Initial Model:
links ~ medianCCU + temp + D50 + fines + drain_area

Final Model:
links ~ medianCCU + D50 + fines

Step			Df	Deviance	Resid. Df	Resid. Dev	AIC
1					10	3.717125	-10.9265
2	-	drain_area	0	0	10	3.717125	-10.9265
3	-	temp	1	0.000634	11	3.717759	-12.92394

Model results:

formula = links ~ medianCCU + D50 + fines

Residuals:

Min	1Q	Median	3Q	Max
-0.88961	-0.24826	0.02251	0.22504	0.94387

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-2.96E-16	1.50E-01	0	1
medianCCU	-5.42E-01	1.64E-01	-3.312	0.00692
D50	4.72E-01	2.45E-01	1.928	0.08012
fines	-7.76E-01	2.48E-01	-3.132	0.00954

Residual standard error: 0.5814 on 11 degrees of freedom
Multiple R-squared: 0.7344, Adjusted R-squared: 0.662
F-statistic: 10.14 on 3 and 11 DF, p-value: 0.001691

Table 10: 2009 Invertebrate functional feeding group (FFG) proportions model

Model results:

formula = proportion_collector/filterer ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.008529	0.019108	0.446	0.6589
log(medianCCU)	0.015701	0.007288	2.155	0.0403

Residual standard error: 0.05967 on 27 degrees of freedom
(1 observation deleted due to missingness)
Multiple R-squared: 0.1467, Adjusted R-squared: 0.1151
F-statistic: 4.642 on 1 and 27 DF, p-value: 0.04028

formula = proportion_scraper ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.22297	0.03817	5.842	3.21E-06
log(medianCCU)	-0.0384	0.01456	-2.638	0.0137

Residual standard error: 0.1192 on 27 degrees of freedom
(1 observation deleted due to missingness)
Multiple R-squared: 0.205, Adjusted R-squared: 0.1755
F-statistic: 6.96 on 1 and 27 DF, p-value: 0.01366

formula = proportion_omnivore ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.14537	0.02834	5.13	2.15E-05
log(medianCCU)	-0.04183	0.01081	-3.87	0.000623

Residual standard error: 0.0885 on 27 degrees of freedom
(1 observation deleted due to missingness)
Multiple R-squared: 0.3568, Adjusted R-squared: 0.333
F-statistic: 14.98 on 1 and 27 DF, p-value: 0.0006231

Table 11: 2010 Invertebrate functional feeding group (FFG) proportions model

Model results:

formula = proportion_scrapers ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.151638	0.026216	5.784	4.98E-06
log(medianCCU)	-0.03484	0.008897	-3.915	0.000615

Residual standard error: 0.0767 on 25 degrees of freedom
Multiple R-squared: 0.3801, Adjusted R-squared: 0.3553
F-statistic: 15.33 on 1 and 25 DF, p-value: 0.0006154

formula = proportion_shredders ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.04007	0.04506	0.889	0.38236
log(medianCCU)	0.04558	0.01529	2.98	0.00633

Residual standard error: 0.1318 on 25 degrees of freedom
Multiple R-squared: 0.2621, Adjusted R-squared: 0.2326
F-statistic: 8.882 on 1 and 25 DF, p-value: 0.006332

formula = proportion_predators ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.15515	0.05369	2.89	0.00786
log(medianCCU)	0.03985	0.01822	2.187	0.03832

Residual standard error: 0.1571 on 25 degrees of freedom
Multiple R-squared: 0.1606, Adjusted R-squared: 0.127
F-statistic: 4.782 on 1 and 25 DF, p-value: 0.03832

Table 12: 2009 Invertebrate drift category proportions model

Model results:

formula = proportion_weak ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.44526	0.04422	10.069	8.28E-11
log(medianCCU)	-0.08145	0.01644	-4.955	3.14E-05

Residual standard error: 0.1387 on 28 degrees of freedom
Multiple R-squared: 0.4672, Adjusted R-squared: 0.4482
F-statistic: 24.55 on 1 and 28 DF, p-value: 3.137e-05

formula = proportion_strong ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.40951	0.06411	6.387	6.50E-07
log(medianCCU)	0.0806	0.02383	3.382	0.00214

Residual standard error: 0.2011 on 28 degrees of freedom
Multiple R-squared: 0.29, Adjusted R-squared: 0.2646
F-statistic: 11.44 on 1 and 28 DF, p-value: 0.00214

Table 13: 2010 Invertebrate drift category proportions model

Model results:

formula = proportion_weak ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.46465	0.06216	7.475	7.91E-08
log(medianCCU)	-0.0657	0.0211	-3.114	0.00458

Residual standard error: 0.1819 on 25 degrees of freedom
Multiple R-squared: 0.2795, Adjusted R-squared: 0.2507
F-statistic: 9.699 on 1 and 25 DF, p-value: 0.004581

formula = proportion_strong ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.44204	0.06849	6.454	9.30E-07
log(medianCCU)	0.05513	0.02325	2.371	0.0257

Residual standard error: 0.2004 on 25 degrees of freedom
Multiple R-squared: 0.1836, Adjusted R-squared: 0.151
F-statistic: 5.624 on 1 and 25 DF, p-value: 0.02573

Table 14: 2009 Emergent invertebrate proportions model

Model results:

formula = Proportion_emergent ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.80777	0.03233	24.989	2.00E-16
log(medianCCU)	0.04234	0.01202	3.523	0.00148

Residual standard error: 0.1014 on 28 degrees of freedom
Multiple R-squared: 0.3072,   Adjusted R-squared: 0.2824
F-statistic: 12.41 on 1 and 28 DF, p-value: 0.001484

Table 15: 2010 Emergent invertebrate proportions model

Model results:

formula = Proportion_emergent ~ log(medianCCU)

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.81581	0.05773	14.13	2.01E-13
log(medianCCU)	0.03469	0.01959	1.77	0.0889

Residual standard error: 0.1689 on 25 degrees of freedom
Multiple R-squared: 0.1114,   Adjusted R-squared: 0.07586
F-statistic: 3.134 on 1 and 25 DF, p-value: 0.08887

REFERENCES

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. Pages 267–281 *in* B. Petrov and F. Csaki, editors. Second international symposium on information theory. Akaemiai Kiado, Budapest.

Beltman, D. J. B., W. H. Clements, J. Lipton, and D. Cacela. 1999. Benthic invertebrate metals exposure, accumulation, and community-level effects downstream from a hard-rock mine site. *Environmental Toxicology and Chemistry* 18(2):299–307.

Bersier, L. F., C. Banašek-Richter, and M. F. Cattin. 2002. Quantitative descriptors of food-web matrices. *Ecology* 83:2394–2407.

Besser, J. M., W. G. Brumbaugh, T. W. May, S. E. Church, and B. a. Kimball. 2001. Bioavailability of metals in stream food webs and hazards to brook trout (Salvelinus fontinalis) in the upper Animas River watershed, Colorado. *Archives of Environmental Contamination and Toxicology* 40:48–59.

Cain, D. J., S. N. Luoma, J. L. Carter, and S. V Fend. 1992. Aquatic Insects as Bioindicators of Trace Element Contamination in Cobble-Bottom Rivers and Streams. *Canadian Journal of Fisheries and Aquatic Sciences* 49:2141–2154.

Campbell, P. G. C., A. Hontela, J. B. Rasmussen, A. Giguère, A. Gravel, L. Kraemer, J. Kovesces, A. Lacroix, H. Levesque, and G. Sherwood. 2003. Differentiating Between Direct (Physiological) and Food-Chain Mediated (Bioenergetic) Effects on Fish in Metal-Impacted Lakes. *Human and Ecological Risk Assessment: An International Journal* 9(March 2015):847–866.

Carlisle, D. M. 2001. Trophic structure and function of stream food webs along a gradient of metal contamination. Colorado State University.

Chadwick, J. W., S. P. Canton, and R. L. Dent. 1986. Recovery of benthic invertebrate communities in Silver Bow Creek, Montana, following improved metal mine wastewater treatment. *Water, Air, & Soil Pollution* 28:427–438.

Chapman, B. M., D. R. Jones, and R. F. Jung. 1983. Processes controlling metal ion attenuation in acid mine drainage streams. *Geochimica et Cosmochimica Acta* 47:1957–1973.

Clements, W. H., D. M. Carlisle, J. M. Lazorchak, and P. C. Johnson. 2000. Heavy metals structure benthic communities in Colorado mountain streams. *Ecological Applications* 10(2):626–638.

Croteau, M.-N., S. N. Luoma, and a. R. Stewart. 2005. Trophic transfer of metals along freshwater food webs: Evidence of cadmium biomagnification in nature. *Limnology and Oceanography* 50(5):1511–1519.

Currie, R. S., W. L. Fairchild, and D. C. G. Muir. 1997. Remobilization and export of cadmium from lake sediments by emerging insects. *Environmental Toxicology and Chemistry* 16(11):2333–2338.

Farag, A. M., D. Skaar, D. A. Nimick, E. Macconnell, and C. Hogstrand. 2003. Characterizing aquatic health using salmonid mortality, physiology, and biomass estimates in streams with elevated concentrations of Arsenic, Cadmium, Copper, Lead, and Zinc in the Boulder River watershed, Montana. *Transactions of the American Fisheries Society* 132(3):450–467.

Farag, A. M., D. F. Woodward, J. N. Goldstein, W. Brumbaugh, and J. S. Meyer. 1998. Concentrations of Metals Associated with MiningWaste in Sediments, Biofilm, Benthic Macroinvertebrates, and Fish from the Coeur d'Alene River Basin, Idaho. *Archives of Environmental Contamination and Toxicology* 34:119–127.

Gray, N. F. 1997. Environmental impact and remediation of acid mine drainage: A management problem. *Environmental Geology* 30(1):62–71.

Griffith, M. B., P. R. Kaufmann, A. T. Herlihy, and B. H. Hill. 2001. Analysis of macroinvertebrate assemblages in relation to environmental gradients in Rocky Mountain streams. *Ecological Applications* 11(2):489–505.

Hare, L. 1992. Aquatic insects and trace metals: bioavailability, bioaccumulation, and toxicity. *Critical Reviews in Toxicology* 22:327–369.

Hare, L., A. Tessier, and P. G. C. Campbell. 1991. Trace Element Distributions in Aquatic Insects: Variations among Genera, Elements, and Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 48:1481–1491.

Henry, T. B., E. R. Irwin, J. M. Grizzle, M. L. Wildhaber, and W. G. Brumbaugh. 1999. Acute Toxicity of an Acid Mine Drainage Mixing Zone to Juvenile Bluegill and Largemouth Bass. *Transactions of the American Fisheries Society* 128:919–928.

Hilsenhoff, W. L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. *Journal of the North American Benthological Society* 7(1):65–68.

Hogsden, K. L., and J. S. Harding. 2012. Consequences of acid mine drainage for the structure and function of benthic stream communities: a review. *Freshwater Science* 31(1):108–120.

Ikemoto, T., N. P. C. Tu, N. Okuda, A. Iwata, K. Omori, S. Tanabe, B. C. Tuyen, and I. Takeuchi. 2008. Biomagnification of trace elements in the aquatic food web in the Mekong Delta, South Vietnam using stable carbon and nitrogen isotope analysis. *Archives of Environmental Contamination and Toxicology* 54:504–515.

Jezierska, B., and M. Witeska. 2006. The metal uptake and accumulation in fish living in polluted waters. *Soil and Water Pollution Monitoring, Protection and Remediation* 3(23):107–114.

Kapustka, L. a, W. H. Clements, L. Ziccardi, P. R. Paquin, M. Sprenger, and D. Wall. 2004.

Issue paper on the ecological effects of metals. *U.S. Environmental Protection Agency*.

Karr, J. R., K. D. Fausch, P. L. Angermeier, P. R. Yant, and I. J. Schlosser. 1986. Assessing biological integrity in running waters: A method and its rationale. *Illinois Natural History Survey Special Publication 5* (August):28.

Kiffney, P. M., and W. H. Clements. 1993. Bioaccumulation of heavy metals by benthic invertebrates at the Arkansas River, Colorado. *Environmental Toxicology and Chemistry* 12:1507–1517.

Kiffney, P. M., and W. H. Clements. 1994. Effects of heavy metals on a macroinvertebrate assemblage from a Rocky Mountain stream in experimental microcosms. *Journal of North American Benthological Society* 13(4):511–523.

Kraus, J. M., T. S. Schmidt, D. M. Walters, R. B. Wanty, R. E. Zuellig, and R. E. Wolf. 2014. Cross-ecosystem impacts of stream pollution reduce resource and contaminant flux to riparian food webs. *Ecological Applications* 24(2):235–243.

Leland, H. V, S. V Fend, T. L. Dudley, and J. L. Carter. 1989. Effects of copper on species composition of benthic insects in a Sierra Nevada, California stream. *Freshwater Biology* 21:163–179.

MacDonald, D. D., C. G. Ingersoll, and T. A. Berger. 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Archives of Environmental Contamination and Toxicology* 39:20–31.

Martin, D. H. 1992. Acid mine/rock drainage effects on water quality, sediments, invertebrates and fish located in Uncle Sam Gulch, Cataract Creek and the Boulder River, northern Jefferson County, Montana. Montana College of Mineral Science and Technology.

Miller, P. A., R. P. Lanno, M. E. McMaster, and D. G. Dixon. 1993. Relative contributions of dietary and waterborne copper to tissue copper burdens and waterborne-copper tolerance in rainbow trout (Oncorhynchus mykiss). *Canadian Journal of Fisheries and Aquatic Sciences* 50:1683–1689.

Montana Fish Wildlife and Parks. 1997. Effects of the June, 1975 Mike Horse Mine Tailings Dam failure on water quality and aquatic resources of the Upper Blackfoot River, Montana. Helena, MT.

Moore, J. N., S. N. Luoma, and D. Peters. 1991. Downstream effects of mine effluent on an intermontane riparian system. *Canadian Journal of Fisheries and Aquatic Sciences* 48:222–232.

Nakano, S., K. D. Fausch, T. Furukawa-Tanaka, K. Maekawa, and H. Kawanabe. 1992. Resource utilization by bull char and cutthroat trout in a mountain stream in Montana, U.S.A. *Japanese Journal of Ichthyology* 39(3):211–217.

Newman, M. C. 2010. Fundamentals of Ecotoxicology. Sleeping Bear Press, Chelsea, MI.

Pioneer Technical Services, I. 2015. Draft Final Feasibility Study Report Summary: Upper Blackfoot Mining Complex. Helena, MT.

Pioneer Technical Services Inc. 1995. Abandoned hardrock mine priority sites: summary report.

Poff, N. L., J. D. Olden, N. K. M. Vieira, D. S. Finn, M. P. Simmons, and B. C. Kondratieff. 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. *Journal of the North American Benthological Society* 25(4):730–755.

Poulton, B. C., D. P. Monda, D. F. Woodward, M. L. Wildhaber, and W. G. Brumbaugh. 1995. Relations between Benthic Community Structure and Metals Concentrations in Aquatic Macroinvertebrates: Clark Fork River, Montana. *Journal of Freshwater Ecology* 10(3):277–293.

Quinn, M. R., X. Feng, C. L. Folt, and C. P. Chamberlain. 2003. Analyzing trophic transfer of metals in stream food webs using nitrogen isotopes. *Science of the Total Environment* 317:73–89.

R Core Team. 2015. R: A Language and Environment for Statistical Computing. https://www.r-project.org/.

Rader, R. B. 1997. A functional classification of the drift: traits that influence invertebrate availability to salmonids. *Canadian Journal of Fisheries and Aquatic Sciences* 54(6):1211–1234.

Sanchez-Bayo, F., P. J. van den Brink, and R. M. Mann. 2012. Ecological Impacts of Toxic Che. Francisco Sanchez-Bayo.

Schmitz, D., J. Mason, M. Blank, and J. Cahoon. 2010. Channel Response Assessment for the Upper Blackfoot River.

Simon, K. S., M. a. Simon, and E. F. Benfield. 2009. Variation in ecosystem function in Appalachian streams along an acidity gradient. *Ecological Applications* 19(5):1147–1160.

Smock, L. A. 1983a. The influence of feeding habits on whole-body metal concentrations in aquatic insects. *Freshwater Biology* 13:301–311.

Smock, L. A. 1983b. Relationships between metal concentrations and organism size in aquatic insects. *Freshwater Biology* 13:313–321.

TetraTech. 2013. Final Remedial Investigation Report: Upper Blackfoot Mining Complex.

Theobald, P. K., H. W. Lakin, and D. B. Hawkins. 1963. The precipitation of aluminum , iron and manganese at the junction of Deer Creek with the Snake River in Summit County , Colorado. *Geochimica et Cosmochimica Acta* 27(2):121–132.

US BLM (Bureau of Land Management). 2014. Abandoned Mine Land Inventory Study for BLM-Managed Lands in California, Nevada, and Utah: Site and Feature Analysis. Denver, CO.

USEPA (Environmental Protection Agency). 1991. Methods for the determination of metals in environmental samples. Washington, DC.

USFWS (Fish and Wildlife Service). 1993. Milltown endangerment assessment project: effects of metal-contaminated sediment, water, and diet on aquatic organismsNTIS PB932. National Fisheries Contaminant Research Center, Columbia, MO.

Vandeberg, G. S., C. W. Martin, and G. M. Pierzynski. 2011. Spatial distribution of trace elements in floodplain alluvium of the upper Blackfoot River, Montana. *Environmental Earth Sciences* 62:1521–1534.

Walters, D. M., K. M. Fritz, and R. R. Otter. 2008. The Dark Side of Subsidies : Adult Stream Insects Export Organic Contaminants to Riparian Predators. *Ecological Applications* 18(8):1835–1841.

Ward, J. V, and B. C. Kondratieff. 1992. An illustrated guide to the mountain stream insects of Colorado, 2nd edition. University Press of Colorado, Fort Collins.

Wilcox, A. C., H. Langner, L. Eby, and S. Sullivan. 2014. Monitoring linkages among river restoration, physical habitat, ecosystem processes, and water quality: Upper Blackfoot River, Mike Horse Mine, Montana. The River Center, University of Montana.

Winner, R. W., M. W. Boesel, and M. P. Farrell. 1980. Insect Community Structure as an Index of Heavy-Metal Pollution in Lotic Ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 37:647–655.