DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE

FINAL REPORT

Effects of Arsenic on Bull Trout: An Investigation of Mine Cleanup Practices in the Pacific Northwest

ENVIRONMENTAL CONTAMINANTS OFF- REFUGE INVESTIGATION

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I. Abstract

This study evaluated the effects of arsenic and heavy metals on individual salmonids and the aquatic community within Gold Creek, Idaho. Gold Creek is a tributary to Lake Pend Oreille in northern Idaho, and has a long history of hard-rock mining within its headwaters. Initial investigations measured concentrations up to 8500 mg As/kg in mine tailings and up to 1200 mg As/kg within stream sediments. Gold Creek is the second most productive stream in terms of spawning and rearing for threatened bull trout within the Lake Pend Oreille Basin. To determine metals effects on salmonids at the organism level we measured concentrations of As, Cd, Cu, Pb, and Zn in water, sediment, benthic macroinvertebrate tissues, and whole body fish tissues. Furthermore, we analyzed histopathology of major organs in fish to assess physiological impacts. Water concentrations were higher than those in reference areas, but only Cd and Zn in Chloride Gulch was above USEPA water quality criteria for protection of aquatic biota. Labile (i.e. weak acid extracted) sediment metals concentrations were 537 mg As /kg, 3.56 mg Cd /kg, 51 mg Cu /kg, 304 mg Pb /kg, 836 mg Zn /kg in CG below Lakeview Mine. Benthic macroinvertebrate tissue concentrations for all analytes were significantly higher at all sites compared to the reference site. Fish tissues were significantly higher below mine sites in comparison to the reference site, but only Cd and Pb were higher as far down as the Gold Creek delta. The concentrations within sediments and biota were similar to other studies in which adverse effects to salmonids were documented. We observed histopathological changes in livers of bull trout within the study area including inflammation, necrosis, and pleomorphism.

To evaluate effects of metals on the aquatic community we collected benthic macroinvertebrate diversity and abundance, fish assemblage, and habitat data. Benthic macroinvertebrate structure showed a graded response to sediment metals concentrations based on 13 metrics. Eleven of 13 metrics indicated adverse impacts to benthic communities and were statistically different below mine sites in comparison to reference conditions. The numbers of metal sensitive families were rare or absent in areas with elevated sediment metals concentrations in comparison to the reference site. The benthic macroinvertebrate metrics were correlated with sediment metals concentrations. Multivariate analysis showed CG and UGC were well separated and statistically different from the reference site based on benthic macroinvertebrate family structure. Fish density and biomass were lower in CG and LGC in comparison to the reference sites. Bull trout were not observed in areas below mine sites with elevated metals concentrations. Our habitat data suggests that habitat is not a major limiting factor within the drainage, and the changes we observed are most likely metals related.

We conclude that metals may be impacting native salmonids within the study area after remediation of Lakeview Mine. Sediment concentrations have not decreased since remediation, although cleanup may have reduced metals loading. Considerable concentrations of metals still persist in stream sediments below mine sites and will likely persist for some time before attenuation within the Gold Creek Drainage.

Keywords: *Remediation Sediment Arsenic Dietary Histopathology Benthic Macroinvertebrate Community/ Structure Bull Trout Toxicity Assemblage/Distribution Habitat*

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V. List of Acronyms/Abbreviations

ACF	Analytical Control Facility
ANOSIM	Analysis of Similarities
ANOVA	Analysis of Variance
BLM	Biotic Ligand Model
CG	Chloride Gulch
DC	Direct Current
D/E	Diversity/Evenness
DEQ	Department of Environmental Quality
DI	De-Ionized
EPA	U.S. Environmental Protection Agency
EPT	Ephemeroptera, Plecoptera, Trichoptera
IBI	Index of Biotic Integrity
ICP-MS	Inductively Coupled Mass Spectrometry
IDFG	Idaho Department of Fish and Game
LC50	Lethal Concentration (50% mortality)
LGC	Lower Gold Creek
LOD	Large Organic Debris
mg/kg	milligrams/kilogram
NMDS	Non-Metric Multidimensional Scaling
OC	Organochlorine
PCA	Principal Components Analysis

QA/QC	Quality Assurance/ Quality Control
SEM	Simultaneously Extracted Metals
SIMPER	Similarity Percentages
TERL	Trace Element Research Laboratory
UCFWO	Upper Columbia Fish and Wildlife Office
UGC	Upper Gold Creek
µg/g	micrograms/gram
µg/L	mircograms/Liter
USFS	U. S. Forest Service
USFWS	U.S. Fish and Wildlife Service
WG	West Gold Creek
XRF	X-Ray Fluorescence

VI. Introduction

Bull trout (*Salvelinus confluentus*) historically ranged from the McCloud River in California to southern Alaska (Cavender 1978). Due to declines throughout their range, bull trout were listed as threatened under the U.S. Endangered Species Act in 1998 (63 FR 31647). Factors that may have contributed to population decline and fragmentation include loss of habitat, competition with exotic species, and hydropower operations (Goetz 1989; 63 FR 31647). Bull trout require headwater tributaries with cold temperatures, good water quality, and coarse-bottom substrates for spawning and rearing (Thurow and Schill 1996). Juvenile bull trout depend on hard-cobble bottoms and are closely associated with substrates for feeding and cover (Pratt 1992).

From the mid 1800's through the early 1970's, extensive hard-rock mining occurred throughout the western United States. During this period, mining operations commonly disposed of waste rock and tailings into nearby watersheds, leaving extensive metals contamination in water and sediments. Arsenic is a contaminant of particular concern commonly associated with hard-rock mining wastes throughout the inland northwest.

In some cases mine-related contamination may be a significant factor contributing to bull trout population decline. Previously, only laboratory studies investigating aqueous metals exposure to bull trout have been undertaken (Hansen *et al*.2001; Hansen *et al*. 2002). However, even when contaminant concentrations in water are below water quality criteria, impacts from sediment exposure pathways may still occur. To our knowledge, metals exposure to bull trout through a dietary pathway under field conditions has not been evaluated.

Aquatic biota can accumulate metals from sediment exposure through dermal and dietary routes. Macroinvertebrates accumulate metals due to their feeding strategies and close association with sediments (Farag *et al.* 1999). Benthic macroinvertebrates are the primary forage of young-of-year salmonids within mountain streams, and these sensitive early-life stages may receive high doses of contaminants transferred through their trophic interactions (Rainbow 1996). Other studies have used benthic macroinvertebrates from metals-contaminated rivers in laboratory dietary exposure studies and demonstrated adverse effects from the consumption of metals contaminated invertebrates (Woodward *et al.* 1994; Farag *et al.* 1999). Arsenic, for example, is thought to be primarily accumulated in salmonids through the dietary route, but to our knowledge, the effect of arsenic on salmonids is poorly understood (Sorenson 1991; Ghosh *et al.* 2006).

Metals burdens in fish tissue are a common measure of exposure and may be correlated with histopathological effects (Hansen *et al.* 2004). Histological change can occur in various organs as result of chronic stressors and can serve as useful biomarker of exposure to contaminants (Johnson *et al.* 1993). Dietary arsenic has been shown in several laboratory studies to be associated with characteristic gallbladder lesions in salmonids (Cockell and Hilton 1988; McGeachy and Dixon 1990; Cockell *et al.* 1991; Cockell *et al.* 1992; Hansen 2004). Degenerative changes observed in the gallbladder included inflammation, oedema, sloughing of the lamina epithelia, hemorrhages, and cellular debris and bleeding within the lumen (Cockell and Bettger 1993). These changes disrupt digestive mechanisms in young fish and ultimately reduce fitness and survival. Researchers have suggested that gallbladder pathology may be a useful indicator of chronic arsenic exposure in salmonids for field investigations (Pedlar *et al*. 2001).

Mine-related contaminants can disrupt multiple levels of biological organization from molecular to ecosystem alterations. However, the majority of the research is limited to understanding impacts at lower levels of biological organization (i.e. cellular and individual levels). These evaluations are generally conducted under laboratory conditions measuring LC50s (lethal concentration resulting in 50% mortality) or other physiological endpoints (Clements and Kiffney 1994). Field studies generally measure metals concentrations in physical media and relate them to concentrations in biota to determine individual impairment (Kovecses *et al.* 2005). Studies that have attempted to determine metals-related impacts at the community level have generally focused on benthic macroinvertebrates. Consequently, our understanding of the impact that elevated metals and mining remediation can have on entire aquatic communities in the absence of other anthropogenic disruptions is limited.

The impacts of historic mining practices and subsequent remediation efforts extend beyond the chemical environment and include alterations to the physical structure of streams. These physical habitat alterations may be significant and result in population and community level impacts, but are often not accounted for when assessing the dominant chemical impact of a remediated mining environment. However, changes in the physical environment can result in the loss or alteration of food resources and suitable rearing habitat features, which may have major consequences for fish populations, including threatened species such as bull trout. Currently, most mine remediation efforts focus on human health and little effort has been made to address ecological consequences. Ecologically-based remediations that have been attempted generally only address the attenuation of chemical contamination and often do not support post – remediation monitoring efforts.

Gold Creek in the Lake Pend Oreille Basin has a history of mining within its watershed and contains elevated sediment metals concentrations. Lakeview Mine in the headwaters of a Chloride Gulch, a tributary to Gold Creek, was a silver mine that sporadically produced ore for over seventy years. The mine-site was abandoned in the early 1980's, leaving large piles of contaminated waste rock and tailings. In 2003, the USEPA and USFS began remediation of the mine site to decrease risk to human and ecological health. Waste rock and mine tailings containing arsenic concentrations as high as 8500 arsenic (As) mg/kg were removed from below mine sites (Weston 2002). Blanket cleanup guidelines developed in the 1960's of 700 mg As/mg was used for the remediation of sediments. However, substantial amounts of metals-contaminated sediments still persist within the stream sediments over eight km downstream to the Gold Creek Delta in Lake Pend Oreille (Weston 2002).

Gold Creek is the second-most productive stream in the Lake Pend Oreille Basin in terms of spawning and rearing for federally listed bull trout (Rieman and Myers 1997; Downs *et al.* 2003). Bull trout are at risk within the basin due to hydroelectric dams, loss of habitat, and competition with non-native salmonids (Downs *et al.* 2003). Juvenile bull trout migrating into Lake Pend Oreille may have limited survival due to predation and increased competition for food resources; therefore providing adequate habitat for juvenile rearing within the basin will help support and recover this critical population (Downs *et al.* 2006).

Study Objectives

The objectives of this study were to: (1) determine the spatial extent of contamination in water and sediments within the study area; (2) to quantify the extent to which metal burdens within water and sediment are impacting benthic macroinvertebrate communities by comparing community metrics across a potential gradient of metals disturbance; (3) to determine the extent to which metal concentrations in water and sediment are limiting the abundance, distribution or density of resident fish populations, including those of threatened bull trout, and (4) to compare the relative impacts of physical habitat alterations associated with mining and remediation efforts to that of the measured metal impacts for fish and benthic macroinvertebrate communities across a gradient of impacted sites.

Management Focus

Potential management implications based on collected chemical and biological data include: (1) identify any sites within the study area that may need additional cleanup; (2) determine the adequacy of the current USFS and USEPA cleanup target of 700 mg As/kg for bull trout and other salmonids in the Inland Northwest; (3) determine the extent to which contaminants, such as As, limit rearing habitat and food resources for bull trout which may justify cleanup activities in other contaminated areas throughout the inland northwest; (4) establish baseline data for future monitoring to gauge recovery of the study area.

VII. Materials and Methods

A. Site Selection

Gold Creek was partitioned into five sites for sampling to establish a gradient from unimpacted sites to impacted sites, and five 150-m reaches were randomly chosen within each site (Figure 1). The five sites included: (1) lower Gold Creek (LGC) below the confluence with West Gold to the Lake Pend Oreille delta; (2) upper Gold Creek (UGC) above the confluence with Chloride Gulch below Conjecture Mine; (3) Chloride Gulch (CG) below Lakeview mine; (4) West Gold (WG) which has no history of mining and little timber harvest (USFS 2001), to serve as our reference site; and (5) the delta of Gold Creek in Lake Pend Oreille.

B. Sample Collection

Water

Water samples were collected from June through August of 2006 and 2007. Each sample was filtered through a pre-rinsed (5% nitric acid/ nanopure DI water) 0.45µm disc filter attached to an acid-washed 60-ml syringe. The sample was filtered into a pre-cleaned150-ml polypropylene sample bottle and immediately placed on ice. The samples were later (≤ 2 hours) preserved with concentrated nitric acid to a pH < 2. In addition, duplicate water samples were taken at two sites as well as blank samples of nanopure DI water. During water sampling, pH, temperature, and specific conductivity were measured with regularly calibrated YSI 85 (YSI Inc., Yellow Springs, OH) and Orion 265A (Thermo Inc. Baton Rouge, LA) instruments. Samples were analyzed by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for As, cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) and other prevalent metals. To further analyze possible aqueous exposure by taking water chemistry into account, we used the Biotic Ligand Model version 2.2.3 (HydroQual Inc. Mahwah, NJ) for Cd, Cu, and Zn LC50 predictions.

Sediment

Inorganic Sediment Collection

Sediments were collected from June through August of 2006 and 2007. Two sediment samples were taken in depositional zones within each 150-m reach. Samples were collected with polystyrene sediment scoops from stream reaches or a petite ponar sampler within the delta. Sediments were placed in an acid-washed polypropylene bowl and thoroughly mixed, then placed into 250-ml wide-mouth polypropylene jars. Samples were later frozen until they were analyzed. Sediment samples were digested in the laboratory one of two ways. One sample was leached with a weak acid (1N HCl), followed by separation of the leachate by centrifugation. This weak-acid extraction method was equivalent to methods for simultaneously extracted metals (SEM), which is representative of the bioavailable fraction to biological receptors. The other sample was digested using a strong acid extraction of (HNO3/HCl), followed by heating, agitation, and centrifugation. The strong-acid digestion follows EPA method 3050B for an estimation of recoverable metals that may become environmentally available. The sediment sample concentrations from weak-acid extractions were used for reporting and statistical comparisons. In addition, three blanks and field duplicates were also taken. Samples were analyzed with ICP-MS for As, Cd, Cu, Pb, and Zn and other prevalent metals. Results are reported in mg/kg on a dry weight basis.

Organic Sediment Collection

To determine impacts from other mining-associated contaminants, an additional sediment sample was collected from each reach in 2007 for analysis of chlorinated hydrocarbons and polycyclic aromatic hydrocarbons. Samples were collected in depositional areas with a stainless steel sediment scoop and thoroughly homogenized in a stainless steel mixing bowl. Sediment material was removed and the samples were placed into clean 250-ml glass wide-mouth jars. We also collected additional blanks and field duplicates. Samples were immediately placed on ice and frozen within eight hours for preservation. Chlorinated hydrocarbons were analyzed by Gas Chromatography, and polycyclic aromatic hydrocarbons were analyzed by Mass Spectrometry.

Benthic Macroinvertebrates

Macroinvertebrate Tissues

Benthic macroinvertebrates were collected from June through August of 2006. Sampling effort began at the downstream end of each reach and progressed upstream. All suitable habitats were sampled using a 500-µm mesh 1m² kick net. Benthic macroinvertebrates were removed from the net with 5% nitric acid-rinsed forceps. Macroinvertebrates of various taxa and sizes were collected to obtain a representative sample of bull trout diets. Samples were placed into clean 125-ml wide-mouth polypropylene jars and immediately placed on dry ice. Samples were analyzed by ICP-MS for As, Cd, Cu, Pb, and Zn and other prevalent metals. Results are reported in µg/g on a dry weight basis.

Diversity and Abundance

Macroinvertebrate abundance and diversity samples were collected from June-July 2006. Riffles were marked within each reach and randomly selected using a random number generator. A 1m² kick net with 500µm mesh was used to measure the area of collection, and then sediments were disturbed and washed into the net. Large rocks and boulders were thoroughly scrubbed to remove benthic macroinvertebrates from substrates and algae. All contents of the net were placed into glass jars and filled with 70% ethanol.

Macroinvertebrate samples were sorted from organic matter under a dissecting microscope. Samples were then rinsed under water and allowed to soak for at least one hour or until all benthic macroinvertebrates had settled to the bottom of the tray. Samples were placed onto a 500µm mesh subsampler (9cm diameter divided into 8 pie sections). The sections were randomly chosen until at least 300 organisms were counted. Sorted subsamples were placed into sample containers of 70% ethanol. Macroinvertebrates were identified to the family level using keys provided in Merritt and Cummins (1999).

Twelve single metrics that were selected to establish impacts to the benthic community included number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) families, % EPT, EPT richness, taxa richness, % Chironomidae, % Elmidae, % Hydropsychidae, % dominance, density (macroinvertebrates/m²), and family-level Simpson's Diversity/Evenness (Siemann *et al*. 1997). The family level multimetric Index of Biotic Integrity (IBI) was calculated for each site to further quantify benthic macroinvertebrate health (Morley 2000).

Fish

Tissue Residues

Whole-body fish were collected from July through August 2006. Fish were collected using a Smith-Root LR-24 backpack electrofisher incorporating pulsed DC current. Sampling began at the downstream section of each reach and progressed upstream. Juvenile salmonids were collected between 50-75mm. Westslope cutthroat (*Oncorhynchus clarki lewisi)* trout were used as surrogates where bull trout were absent. We considered westslope cutthroat appropriate surrogates for bull trout due to the dietary overlap between these two juvenile salmonid species across this size range in headwater habitats (Pratt 1992). Whole-body samples were immediately placed into 125-ml wide-mouth polystyrene jars and placed on dry ice. Individual-fish samples were analyzed by ICP-MS for As, Cd, Cu, Pb, and Zn and other prevalent metals. Results are reported in µg/g on a dry weight basis.

Histopathology

Fish histology samples were collected along with fish whole-body metals samples. Collection procedures follow those described above. Fish used for histopathological analysis were euthanized with a 1:10 clove oil/ethanol mixture, and then rinsed with ambient water. A ventral incision was made to expose the peritoneal cavity before preserving the fish in Davidson's fixative solution (Hansen *et al.* 2004). The samples were sent to the U.S. Fish and Wildlife Service Fish Health Center (Bozeman, MT, USA) for histopathological analysis. Each fish was embedded in paraffin and sectioned sagitally. Four, 5-µm thick tissue sections were taken at different depths from each block; two were stained with hematoxylin and eosin and the other two with giemsa (Beth MacConnell, U.S. Fish and Wildlife Service, histopathologist, personal communication). Up to three additional hematoxylin and eosin sections were cut from each fish to obtain adequate views of gallbladder tissue. All slides were examined with no knowledge of sample site. A minimum of four tissue sections were examined for each fish.

Fish Population Assessment

Fish population and distribution data was collected in July and August 2006. In areas where bull trout were expected to be at low densities, fish population estimates were assessed using two-pass depletion estimates. Fish were electroshocked with pulsed DC current using a Smith-Root LR24 backpack unit. Fish were identified, measured and then returned below the downstream starting point. In two sites where permission to electroshock would be difficult because of concerns for threatened bull trout, night snorkeling was used as a comparable means of population estimation. Two reaches in West Gold Creek were snorkeled and electofished for population estimate comparisons between the methods. Prior to snorkeling, each snorkeler was tested using fish identification flash cards to calibrate length and species observations underwater at night. Snorkeling began after complete darkness, two lateral snorkelers progressed upstream thoroughly inspecting all habitat types. Fish length classes and species were recorded for each 150-m reach. Fish biomass was estimated using length-weight regressions on electrofishing data collected within Gold Creek in previous years.

C. Habitat Measurements

Habitat quality was measured in August 2006. In each 150-m reach we established three evenly spaced perpendicular transects. Flow was measured using methods described in Bain and Stevenson (1999) at the furthest downstream transect. At each transect we measured bankfull width, bankfull height, and wetted depth at 3, 5, or 7 evenly spaced intervals depending on width across the channel. We measured % canopy cover using a spherical densitometer at left bank facing downstream, right bank facing downstream, and center facing up and downstream. Longitudinal habitat distribution of riffles, runs, and pools were counted throughout the entire length of the reach. We measured four random pools within each reach to determine pool quality in terms of residual depth, average substrate size, percent overhead cover, percent undercut banks, and percent submerged cover. The variables were scored in methods described in Clark (2004) to determine pool quality scores. Large organic debris (LOD) >20cm in diameter was counted throughout the reach. Pebble counts were conducted within each reach following methods described by Wolman (1954) to quantify substrate composition.

D. Quality Assurance/Quality Control

All 2006 and 2007 samples were processed through the USFWS Analytical Control Facility (ACF), Shepherdstown, West Virginia. TDI Brooks and Trace Element Research Laboratory (TERL), College Station, Texas are ACF contract laboratories and meet Quality Assurance/Quality Control (QA/QC) criteria established by ACF. The Service's ACF was responsible for assuring the quality of the chemical analyses it provides through in-house and contract laboratories. The quality of chemical analyses is considered assured when the analysis is performed in a technically competent manner qualified personnel using appropriate methods and equipment, and the precision and accuracy of the measurement are within the expected ranges for the technique. Accuracy and precision of analytical chemistry data were measured by matrix spike recoveries and duplicate sample analysis. Limits of detection were within acceptable limits for abiotic and biotic samples in 2006 and 2007. No spike or duplicate anomalies were reported for As, Cd, Cu, Pb, or Zn analysis.

E. Statistical Analysis

Data for water, sediment, benthic macroinvertebrate tissues, fish tissues, benthic macroinvertebrate metrics, and fish assemblages were tested for normality and homogeneity using Anderson-Darling and F-tests. If data did not meet distributional assumptions it was transformed using log and square root transformations. We then tested the data using a one-way ANOVA. Tests that were statistically significant were then compared using Dunnet's multiple comparison procedure comparing all sites to the reference site. All tests were considered statistically significant at $\alpha < 0.05$. To test for differences in sediment concentrations between years, data were log transformed to meet assumptions. A t-test was used to compare water and sediment data in 2006 and 2007. Benthic macroinvertebrate metrics were correlated with sediment metals concentrations using Spearman's Rank Correlation.

To quantify general impacts and assess the relative effects of habitat and metals concentrations, we simplified multivariate effects using Principal Components Analysis (PCA). Principal component scores for all of the habitat variables, as well as for As, Cd, Cu, Pb, and Zn sediment metals concentrations were used as predictor variables in a multiple regression model with benthic macroinvertebrate metrics, fish density, and biomass as the response variables to determine if metals impacts were significant after habitat was accounted for in the model**.** Data for water, sediment, benthic macroinvertebrate metrics, fish assemblages, and habitat was analyzed using Minitab® Version 15 Statistical Software (State College, PA, Minitab Inc**.).**

We used non-parametric multivariate techniques to help identify community structure and families contributing to differences between sites. Non-parametric multivariate statistics are robust and not constrained by distributional assumptions. Statistical analysis was performed using Primer 6.0 (Plymouth Marine Laboratory, Plymouth, UK). Non-metric multidimensional scaling (NMDS) was performed to ordinate the data by site in multivariate space to visualize separation. Statistical differences were determined using analysis of similarity (ANOSIM); this procedure compared benthic macroinvertebrate family composition between the sites. The test calculated the global R statistic (R) to determine similarity based on a range of 0-1, with 0 being completely similar, and 1 being completely dissimilar (Clarke and Gorley 2001). The statistic was derived from 1000 non-parametric permutations based on a Bray-Curtis (Bray and Curtis 1957) similarity matrix. Interpretation followed the guidelines set by Clarke and Warwick (2001) of $r > 0.75$ well separated, $r > 0.5$ overlapping but different, and $r < 0.25$ barely separable. Lastly, the SIMPER (similarity percentages) routine within PRIMER was used to determine the families that were contributing to the observed differences. Benthic macroinvertebrate data was $\log_{10}(x + 1)$ transformed to mask large effects of the few dominant families and allow low count or rare families to be revealed in the analysis.

VIII. Results

A. Metals Concentrations in Water and Sediment

Dissolved metals concentrations within water (µg/L) were elevated at all sites compared to the reference site (Table 1). Chloride Gulch was significantly higher than the reference site for As, Cd, and Zn. Upper Gold Creek was significantly higher for As, Cd, Pb, and Zn. However, all measured concentrations except Cd and Zn in CG, were below U.S. Environmental Protection Agencies chronic criteria for the protection of aquatic life. Biotic Ligand Model LC50 predictions for Cd and Cu were below our measured concentrations, but measured zinc concentrations exceeded LC50 model predictions in two reaches within CG below Lakeview Mine (Figure 2).

Concentrations of As, Cd, Cu, Pb, and Zn in sediments (mg/kg) were significantly higher at all sites compared to the reference site (Table 2). In all cases, sediment metals concentrations followed the general trend of CG>UGC>LG>Delta>WG. Arsenic concentrations at CG were up to 200 times greater than WG. Arsenic sediment concentrations decreased by 92% from a mean of 537 mg As/kg in CG below Lakeview mine to a mean of 27 mg As/kg at the Delta over a stream distance of 8.2 km. The graded extent of sediment contamination was clear and followed a consistent downstream decreasing pattern for all analytes. Concentrations of organic contaminants in sediment were low throughout the study area, and below levels thought to impact stream biota.

B. Benthic Macroinvertebrates

Tissue Residues

Macroinvertebrate metals tissue concentrations (µg/g dry weight) were elevated throughout the study compared to the reference site (Table 3). The concentrations in tissues were correlated with those in sediment, and decreased as a function of distance from mining sites. Chloride Gulch had the highest mean benthic macroinvertebrate tissue concentrations for all metals except Pb, which was greater at UGC despite lower Pb sediment concentrations. Mean benthic macroinvertebrate As concentrations were 13 times higher at CG compared to the reference site.

Diversity and Abundance

Metrics/Correlations

We observed 35 families of insects within the study area with 22 families of mayflies, stoneflies, and caddisflies. Dipterans comprised ten families and we identified three families of aquatic beetles. Ten non-insect families were observed which included aquatic oligachaetes, flatworms, and molluscs.

The benthic macroinvertebrate EPT metrics were statistically different below mine sites in comparison to the reference site (Figure 7). The number of Ephemeroptera families were significantly fewer across all sites in comparison to the reference site ($p < 0.0001$ to $p = 0.0004$). Only CG ($p = 0.04$) and UGC ($p = 0.02$) had significantly fewer Trichoptera families. No differences were observed for the number of Plecoptera families throughout the study area. The % EPT of the sample was significantly lower at CG ($p = 0.01$) compared to the reference site. Lastly, EPT richness was significantly lower in CG ($p = 0.005$) and UGC ($p = 0.01$) in comparison to the reference site.

Several additional metrics of benthic macroinvertebrate community structure were suggestive of significant impacts from mine-related changes (Figure 7). Chironomids were the dominant taxa at contaminated sites, and the % Chironomidae in CG was up to 4-times higher than the reference site. The % Elmidae was significantly lower for all sites in comparison to the reference site ($p < 0.0001$ to $p = 0.0007$), and this metric showed a graded response to metals concentrations throughout the study area. The total invertebrate taxa richness was lower below mine sites, but not statistically lower than the reference site. However, benthic macroinvertebrate density (total benthic macroinvertbrates/m²) in CG was less than half of that observed in the reference site. We also observed a decline in family-level IBI scores for all sites in comparison to the reference, but only CG ($p = 0.0002$) scores were statistically lower. Lastly, family-level Simpsons D/E was statistically lower in CG ($p = 0.05$), suggesting a lack of evenness for the benthic macroinvertebrate assemblages below Lakeview Mine.

Of the 13 benthic macroinvertebrate metrics we evaluated, nine were correlated with sediment metals concentrations (Table 5). The significant correlations between benthic macroinvertrbrate metrics and sediment metals concentrations further supported metals impacts on benthic macroinvertebrate assemblages. The number of Ephemeroptera and Trichoptera taxa, as well as EPT richness, was negatively correlated with As, Cd, Cu, Pb, and Zn sediment concentrations. Furthermore, % Chironomidae showed a strong positive correlation ($r^2 > 0.77$) for As, Cd, Cu, Pb, and Zn sediment concentrations. The strongest negative correlation ($r^2 > 0.80$) for all metals was % Elmidae. Lastly, IBI and Simpsons D/E were negatively correlated with all sediment metals concentrations.

Community Structure

Two-dimensional NMDS ordination of raw macroinvertebrate data were grouped according to site and showed a clear separation with a stress level of 0.12, indicating a reliable model (Figure 3). Chloride Gulch and UGC which had the highest metal concentrations in sediment had the greatest separation from the reference site. The separation of sites based on benthic macroinvertebrate data followed a graded pattern of sediment metals contamination. The ANOSIM one-way analysis showed a statistical difference between the sites (Table 6). The global R value was 0.75 ($\alpha = 0.05$) indicating a statistically significant difference between sites. Chloride Gulch below Lakeview Mine ($R = 0.90$) and UGC below Conjecture Mine ($R = 0.82$) were statistically well separated from the reference site. Lower Gold Creek was statistically different, but overlapping with the reference site. The SIMPER procedure showed the percentage of families that were contributing to site differences. Families contributing to 75% of the site samples composition were; (WG) 20% Elmidae, 14% Baetidae, 13% Chloroperlidae, 13% Heptageniidae, 11% Chironomidae, and 5% Ephemerellidae; (LGC) 21% Heptageniidae, 17% Ephemerellidae, 15% Baetidae, 13% Chloroperlidae, and 8% Perlodidae; (UGC) 25% Heptageniidae, 22% Baetidae, 19% Chironomidae, 6% Chloroperlidae, and 4% Nemouridae; (CG) 53% Chironomidae, 15% Heptageniidae, and 5% Baetidae.

C. Fish

Fish Tissues

Metals concentrations in fish tissues (µg/g dry weight) followed a concentration gradient covarying with concentrations found in sediment and benthic macroinvertebrate tissues (Figure 4). Fish tissue metals concentrations were significantly higher compared to the reference site for all analytes in CG and UGC (Table 7). Below Lakeview mine in CG, fish tissue As concentrations were up to 10-times higher than those in the reference site. Concentrations of Cd, Cu, Pb, and Zn in LGC were significantly higher compared to the reference site. In the delta, fish tissue metals concentrations were significantly higher for Cd and Zn compared to reference site fish tissues.

Histopathology

Livers from westslope cutthroat in CG below Lakeview Mine showed mild glycogen vacuolation and ceroid-like cytoplasmic inclusions, and one fish had moderately enlarged nuclei and individual cell necrosis in hepatocytes. Westslope cutthroat livers in UGC below Conjecture Mine showed moderate glycogen vacuolation and scattered inflammation, and one fish had moderate scattered degeneration of hepatocytes. Bull trout livers in LGC showed moderate nuclear enlargement, scattered degeneration, and necrosis of hepatocytes with scattered foci of inflammation. Bull trout in the delta had the most numerous and severe liver alterations, including moderate vasculitis in one fish, moderately severe pleomorphism and moderate degeneration and necrosis in three fish (Figures 5 and 6).

Population Assessment

Fish densities across all sites were lower compared to the reference site (Table 8), and the community composition varied across all sites. West Gold had the highest fish densities (0.42 fish/m^2) and was comprised of both westslope cutthroat and bull trout. Lower Gold Creek had the lowest fish densities (0.05 fish/m^2) within the study area and was significantly lower ($p = 0.0004$) than WG. Lower Gold Creek was comprised mainly of bull trout and few westslope cutthroat. Chloride Gulch had the second lowest fish density (0.20 fish/m^2) and was significantly lower ($p = 0.04$) in comparison to the reference site. Only westslope cutthroat were observed within CG. Upper Gold Creek had lower fish densities (0.25 fish/m^2) than the reference site and no bull trout were observed at this site. Fish lengths were not statistically different across sites. Biomass estimates were significantly lower for LGC (0.42 g/m^2) and CG (1.54 g/m^2) from WG (4.8 g/m^2). Upper Gold Creek biomass estimates (4.1 g/m^2) were not statistically different from the reference site.

D. Pathway Analysis

Metals distributions in various physical and biological ecosystem pools showed a clear and consistent pattern throughout the study area. Metals concentrations in benthic macroinvertebrates and fish were proportional to concentrations found in sediments. The concentration gradient followed sediments > benthic macroinvertebrates > fish > water for As, Cu, Pb, and Zn and benthic macroinvertebrates > sediment > fish > water for Cd. Metals concentrations were greatest below mine sites and decreased downstream to the Gold Creek Delta. The ratio of metals within sediments and biotic tissues increased further downstream of mine sites indicating that metals were more bioavailable at downstream sites.

E. Habitat Analysis

Habitat variables were reduced to three principal components explaining over 68% of the variation within the data. The measured chemical factors loaded strongly and appeared to explain the greatest amount of variation. Dissolved oxygen differed among sites, but was greater than 8 mg/L for all sites. The pH also differed and ranged from 6.9-8.2 in CG and the LGC delta respectively. Conductivity differed across sites and ranged from 64-165 µS. Temperature loaded strongly on PC2 and ranged from 7.7-15.3ºC. Physical characteristics that loaded strongly on PC1 included the number of riffles which was lower in LGC in comparison to the other sites. The % boulders were higher in the furthest downstream site in LGC. The % canopy cover was loaded strongly on PC2 and was also reduced in the furthest downstream site in LGC. Lastly, the gradient loaded strongly on PC2 reflecting the higher gradient in UGC compared to the rest of the sites. The sediment metals variables were reduced to two principal components explaining over 94% of the variation, and all variables loaded strongly on PC1.

Habitat PC1-3 and metals PC1-2 scores explaining over 68% and 94% of the data variation respectively was used in a multiple regression analysis as predictor variables (Table 9). The regression model used benthic macroinvertebrate metrics, fish density, and fish biomass as dependant variables. The metals PC1-2 scores (As, Cd, Cu, Pb, and Zn sediment concentrations) had a significant influence after habitat was accounted for in the model for the dependant benthic macroinvertebrate variables: number Ephemeroptera taxa, % Chironomidae, % Hydropsychidae, % EPT, % Elmidae, % dominance, Simpsons D/E, and IBI. Metals PC1-2 had a significant influence on fish density and biomass.

IX. Discussion

Our observations suggest that bull trout are exposed and impacted by mining-related metals pollution. Bull trout collected as far as 8 km downstream of source areas had structural liver damage consistent with contaminant exposure including cell necrosis, degeneration, and pleomorphism. These findings, along with low concentrations of dissolved metals in water and high concentrations of metals in sediment, benthic macroinvertebrates and fish tissues, suggest that salmonids are exposed to toxic concentrations of metals through a dietary pathway. These results are consistent with other studies in which dietary exposure of metals resulted in cellular damage and reduced health of salmonids (Vighi 1981; Cockell *et al.* 1991; Woodward *et al.* 1994; Farag *et al.* 1999, Pedlar *et al.* 2002; Hansen *et al.* 2004). However, to our knowledge, this is the first field study to assess sediment-driven dietary exposure and effects of metals to threatened bull trout.

The impacts of mining and subsequent remediation efforts have multiple interacting effects on stream structure and function. In our study, we addressed these combined impacts on the aquatic community by assessing changes in benthic macroinvertebrate community structure, fish density, biomass, and distribution, as a function of proximity to mine sites. Studies frequently focus on a narrow set of assessment endpoints (e.g. body burdens) without taking changes to habitat quality into consideration. Through multiple lines of evidence we were able to determine metals-associated impacts while accounting for biological response due to environmental variables. Our data suggests elevated metals concentrations in sediment are structuring benthic macroinvertebrate communities, and may be suppressing fish densities, biomass, and distributions within the study area. Of the thirteen benthic macroinvertebrate biological metrics we examined, eleven metrics below mine sites showed a significant negative response in comparison to the reference community. The response of benthic macroinvertebrate communities followed a graded pattern covarying with metals concentrations in sediment, and benthic biological response was highly correlated with sediment metals concentrations. Benthic macroinvertebrate biological response was also evident through non-parametric multivariate analysis, which showed a significant statistical separation in benthic community structure between metals contaminated and reference sites. Fish densities and biomass were generally lower in contaminated sites, and bull trout distribution was limited to areas furthest downstream of mine sites and in the reference site. We coupled biological response with habitat measurements, and were able to conclude changes in physical structure could not account for the biological differences we observed between contaminated and reference sites.

A. Pathways of Exposure

Aqueous Exposure

Dissolved metals concentrations in water at our sites did not appear to pose a major risk to salmonid health within the study area. All metal concentrations in water, except Cd and Zn, were lower than U.S. Environmental Protection Agency acute and chronic criteria for protection of aquatic life throughout the study area (USEPA 2006). Incorporating the Biotic Ligand Model into our analysis, we were able to predict rainbow trout LC50's for Cd, Cu, and Zn based on measured stream chemical parameters. Model predictions for LC50 concentrations were much higher than our measured metals concentrations in water for all metals with the exception of Zn directly below Lakeview Mine.

Sediment Exposure

Sediment metals concentrations measured in the Gold Creek drainage may be high enough to reduce health of native salmonids, including bull trout within Gold Creek drainage. The sediment metals concentrations measured in CG and UGC were similar to sediment metals concentrations in some areas of the Clark Fork and Coeur d' Alene Rivers of Montana and Idaho. These rivers have extensive mining-related metals contamination, and the adverse effects on biota were well documented (Farag *et al.* 1995; Woodward *et al.* 1995; Farag *et al.* 1999). However, sediment concentrations of As in Chloride Gulch sediments are higher than that measured in most areas of these other river systems (Farag *et al.* 1998; Hansen *et al.* 2004). Researchers have noted that As uptake through the dietary pathway resulted in elevated fish tissue metals burdens, and characteristic physical effects are highly correlated with sediment concentrations of As (Farag *et al.* 2007; Hansen *et al.* 2004).

Macroinvertebrates within Gold Creek accumulated sediment-derived metals, and may provide a potential risk of dietborne exposure of metals to salmonids. Several studies have noted the dietary effects to salmonids from elevated metals within benthic macroinvertebrate tissues. Farag *et al.* (1999) used benthic macroinvertebrates collected from the Coeur d'Alene River and fed them to cutthroat trout in a laboratory study. The metals concentrations within the diet were 13.5 µg As/g, 29.1 µg Cd/g, 43.8 µg Cu/g, 452 µg Pb/g, and 2119 µg Zn/g. Although the levels of Cd and Pb were greater in this diet compared to our field collected benthic macroinvertebrates, levels of Cu and Zn were similar, and As within Gold Creek benthic macroinvertebrate tissues were up to eight times greater. The Coeur d' Alene River study found that metals incorporated into benthic macroinvertebrate tissues efficiently transferred across the gut and resulted in elevated metals within fish tissues. The diet resulted in reduced survival, growth, and histopathological alterations to fish tissues (Farag *et al*. 1999). The results of our study are concordant with laboratory studies in which transfer of metals through the dietary pathway resulted in deleterious effects to salmonids, including increased metals tissue burdens resulting in destructive cellular alterations within the liver (Vighi 1981; Woodward *et al.* 1994; Woodward *et al.* 1995; Hansen *et al.* 2004).

Field Observations

Fish below mine sites showed symptoms indicative of metal exposure including large distended stomachs and pronounced darkened skin coloration. Woodward *et al.* (1995) observed more than 50% of brown trout (*Salmo trutta*) fed benthic macroinvertebrates from the Clark Fork River, MT exhibited swollen abdomens, and constipation. The authors suggested that the swollen stomachs were a result of gut impaction, and attributed the observed physiological effects to exposure of dietary metals. Arsenic has been shown to induce melanin production, and increased chromatophores may be suitable biomarkers of As toxicity (Allen *et al.* 2004). Farag *et al.* (2003) observed fish held in cages in the Boulder River, MT below mine sites with elevated As concentrations developed darkened skin coloration.

B. Tissue Metals Burdens and Histopathology

Fish Tissue Metals Burdens

Westslope cutthroat and bull trout are accumulating metals within their tissues in Gold Creek, and concentrations may be high enough to compromise fish health. This study found a dry weight average in fish tissues of 10.9 µg As/g, 1.1 µg Cd/g, 7 µg Cu/g, 1.4 µg Pb/g, and 349 µg/g in CG and 8.3 µg As/g, 0.89 µg Cd/g, 6.3 µg Cu/g, 3.1 µg Pb/g, and 312 µg Zn/g in UGC, which are consistent with other studies in which similar fish tissue metals burdens resulted in deleterious effects to salmonids (Woodward *et al.* 1995; Farag *et al.* 1999). However, As concentrations in this study were higher than those measured in the previous two studies (Table 7). Farag et al. (2003) measured 8 µg As/g dry weight whole-body concentrations in rainbow trout (*Onchorynchus mykiss*) in the

Boulder River, (MT) below mine sites. These were the highest whole-body As concentrations observed within the entire river drainage. Whole-body burdens were highly correlated with sediment and thought to primarily have been derived through dietary exposure. Although other metals were elevated within tissues, these body burdens led to decreased health including increased metallothionein, lipid peroxidation, reduced growth, and depressed fish densities.

Histopathological Response

The histopathological alterations we observed were consistent with other findings in which dietary exposure to metals resulted in cellular damage. Pedlar *et al.* (2002) fed lake whitefish a diet of 1-100 µg As/g and observed nuclear and architectural alterations to livers. Woodward *et al.* (1994) observed nuclear pleomorphism, cytoplasmic vacuolation, and scattered degenerate hepatocytes in rainbow trout fed a metals-contaminated diet with concentrations in benthic macroinvertebrates similar to those measured concentrations in our study. Many studies have noted the liver as the primary site of toxic action for metals in fish (Lage *et al.* 2006). We observed histological aberrations in other tissues, but hepatic tissues were the most consistent and damaged tissues. Our study supports previous work in which liver histopathological alterations may serve as a useful biomarker of dietary metals exposure and adverse effects. Although structural liver alterations were observed in westslope cutthroat with higher metals concentrations in tissues, liver alterations were more numerous and severe in bull trout with lower tissue metals concentrations. The original study plan sought to collect age-0 bull trout throughout the study area and collect age-0 westslope cutthroat as surrogates, where bull trout were absent. We were unable to collect age-0 salmonids in CG or UGC, and therefore collected age-1 westslope cutthroat. The mean length of westslope cutthroat and bull trout collected for histology samples was 70 and 56 mm, respectively. This size and age difference could have affected the degree of histopathological abnormalities we observed (Bernet *et al.* 1999). It is well documented that young juvenile fish are more susceptible to metals exposure than larger older fish (Chapman 1978; Eaton *et al.*1978; Buhl and Hamilton 1990). Older fish with a history of exposure may develop mechanisms for more efficient metal excretion, thus limiting cellular damage (Lage *et al.* 2006). Alternatively, the most sensitive individuals may have been lethally removed from the population, and we were only able to collect the strongest, most resistant individuals that demonstrated less severe effects. Lastly, bull trout may be more susceptible to metals due to physiological differences between the two species. It is well known that species can have vastly different tolerances to metals insults (Buhl and Hamilton 1990; Luoma and Rainbow 2005). These interspecies differences may account for the degree of histopathological alterations we observed between groups.

Several laboratory studies have noted gallbladder lesions in salmonids associated with dietary arsenic, and suggested that gallbladder pathology may be a useful indicator of arsenic exposure in field studies (Pedlar *et al.* 2002). Interestingly, we did not observe any of these characteristic lesions in our samples despite high As concentrations in benthic prey items. However, most laboratory studies are less than 90 days in duration, and those studies documented initial acute stages of gallbladder pathology (Cockell and

Hilton 1988; Cockell *et al.* 1991; Cockell and Bettger 1993). Individuals that are more susceptible, may develop these characteristic lesions, and be removed from the population at critical early life stages, thus making it difficult to document in field studies from surviving individuals. Our findings suggest that gallbladder pathology may not be a useful indicator of chronic dietary arsenic exposure under complex field conditions.

Arsenic speciation may also play a major role in affecting toxicological responses to aquatic organisms. Frankenberger (1990) suggests that to assess risk from arsenic to aquatic biota, arsenic speciation must be taken into account. Differing forms and species of arsenic can have vastly differing toxicological results to organisms (Lage *et al.* 2006). However, determining arsenic species in sediments and tissues has proven challenging. Jankong (2007) reported extraction efficiencies for As in fish tissues as low as 60% in snakehead (*Channa argus*) fish tissues. Furthermore, little is known about the varying effects of each As species to fish in general, and salmonids specifically. Arsenic speciation could have explained some of the variation we observed in histopathological endpoints between sites. Further research on arsenic speciation and toxicological effects in fish is warranted.

Field versus Laboratory Studies

Laboratory studies may underestimate effects of dietborne contaminants to salmonids. Other research suggests that in laboratory exposures, the diets may be different than natural food items. Some laboratory study diets were supplemented with vitamins and minerals, thus potentially modifying the diet form what would normally be consumed (Woodward *et al.* 1994). Other studies exposed live-diets to metals under brief or limited exposure times (Hansen *et al.* 2004). Under field conditions, metals may be bound to tissues in prey items quite differently than laboratory food items (Farag *et al.*1999). For example, benthic macroinvertebrates exposed to metals for their entire life cycle may incorporate metals within internal tissues differently than short-term laboratory exposures. These differences could have vastly different toxicological effects, and natural dietborne exposure may be more toxic than laboratory diets (Woodward *et al.* 1994). Furthermore, laboratory studies are conducted in relatively benign environments. Gauthier *et al.* (2006) found that fathead minnows (*Pimephales promelas*) under field conditions showed a biological response to low levels of aqueous Cd and Ni, including prolonged hatching time and increased mortality, whereas no effects were observed in laboratory counterparts. The authors attributed the differences to higher stress associated with natural environments which may sensitize fish, thus making fish more susceptible to the toxic effects of contaminants under stressful field conditions. Additional insults from metals exposure may greatly reduce young salmonids' ability to compete for food and cover, elude predators, and cope with additional stressors (e.g. increasing thermal regimes). Therefore, site-specific field data can be invaluable in evaluating risks to threatened species such as bull trout.

C. Benthic Macroinvertebrate Community Impacts

Community Response

Dissolved metals concentrations in water at our sites did not appear to pose a major risk to salmonid health for most of the study area. All metals concentrations in water, except Cd and Zn in two reaches directly below Lakeview Mine in CG, were lower than U.S. Environmental Protection Agency acute and chronic criteria for protection of aquatic life throughout the study area (USEPA 2006). Sediment metals concentrations were elevated below mine sites, and exceeded concentrations known to adversely impact aquatic biota. The sediment metals concentrations were highest below mine sites, and followed a decreasing concentration-gradient with distance downstream. Large datasets compiled from nationwide studies have been evaluated in terms of biological impacts from sediment metals concentrations to develop sediment quality guidelines. These evaluations have determined total-recoverable sediment metals concentrations exceeding 33µg As/g, 5µg Cd/g, 149µg Cu/g, 128 µg Pb/g, and 459µg Zn/g would likely cause adverse impacts to benthic macroinvertebrates (Ingersoll *et al.* 2001; Macdonald *et al.* 2000). The sediment metals concentrations below mine sites in our study exceeded these values for As, Pb, and Zn, and total-recoverable As sediment concentrations were up to 50-times greater below mine sites than reported sediment quality guidelines. Macdonald *et al.* (2000) evaluated sediment quality guidelines for As concentrations from 150 sediment samples, and found that 76% of those samples showed adverse impacts to benthic macroinvertbrate communities when As sediment concentrations exceeded 33µg As/g.

Metrics

Other studies have reported that EPT indices are a reliable indicator of benthic macroinvertebrate changes in relation to stressors (Plafkin *et al.* 1989; Lenat and Barbour 1994). Benthic macroinvertebrate EPT indices have been shown to be useful in determining impacts from mining-related metals contamination in Rocky Mountain streams. Clements *et al.* (2000) observed a significant reduction in EPT taxa and abundance collected from 95 stations throughout southern Colorado mountain streams contaminated with metals. Our data was concordant with other studies in which EPT taxa, %EPT, and EPT richness were reduced within sites with elevated metals concentrations (Figure 6), (Kiffney and Clements 1996; Clements *et al.* 2000). However, this metric can be misleading, and does not account for the sensitivity of the families that comprise the sample. For example, if large numbers of metals tolerant Baetidae or Hydropsychidae exist within a sample, interpretation of % EPT may be misleading in areas with metals impacts (Clements 1994). To minimize the impact of potentially tolerant taxa within EPT families, we used additional common metrics to better define community patterns.

In general, benthic macroinvertebrate communities below mine sites were dominated by Chironomids, showed less biological evenness, showed a reduction of sensitive families, had lower densities, and showed a reduction in biotic integrity (Figure 7). A high percentage of Chironomids within a sample has been shown to be a useful indicator of disturbance (MacCoy 2004; Mize and Deacon 2002; Voshell 2002). Our data suggests that a high percentage of Chironomids may be an indication of metals related impacts, below Lakeview Mine in Chloride Gulch, Chironomidae families comprised up to 42% of the sample, 4 times higher than the reference site. Furthermore, we found the metric % Elmidae to be very sensitive to metals pollution. Elmidae are very sensitive to disturbance due to its life history characteristics, and may be completely eradicated from areas with only moderate amounts of pollution (Young 1961). We also evaluated the density of our sample to determine overall reduction within our study area. Benthic macroinverterbrate density was significantly lower despite high numbers of Chironomids in Chloride Gulch in comparison to the reference site. However, we recognize our density estimates may have been biased due to the inherent variability of kicknet sampling. Nevertheless, benthic macroinvertebrate densities were reduced as much as 60% below mine sites compared to reference site densities. Lastly, we used the multimetric family level IBI, and found IBI scores were reduced within sites with elevated metals concentrations. The multimetric IBI is a reliable indicator of benthic health, and many studies have shown the relation of IBI scores and compromised benthic health (Plafkin *et al.*1989; Keran and Karr 1994; Barbour *et al.* 1996).

Community Structure

Benthic macroinvertebrate metrics have been useful to determine deleterious effects within benthic community structure; however, metrics focus on specific groups of taxa with a range of tolerances. Therefore, it is useful to use other techniques such as multivariate analysis which analyzes all of the taxa within the entire community. Multivariate analysis such as NMDS, ANOSIM, and SIMPER coupled with benthic metrics, will yield a more statistically robust investigation of benthic community structural impacts. These techniques allows for a community to be analyzed by ordination by site to determine separation (NMDS), testing for a statistical separation of sites (ANOSIM), and determining which taxa contributed to the differences (SIMPER). For example, in our analysis sensitive families were rare or absent in both CG and UGC, and more tolerant taxa were in greater abundance at these sites in comparison to the reference site.

Mayflies are very sensitive to metals and may be the first to respond to metals contamination in Rocky Mountain streams (Leland *et al.* 1989; Nelson and Roline 1993; Clements 1994; Clements and Kiffney 1995). Similarly, we observed community mayfly differences between reference sites, and sites with elevated sediment metals burdens. The mayfly family Leptophlebidae was only found within the reference site. This family is characterized by large branched filamentous gills, and is considered to be very intolerant of metals (McGuire 2001). Also the metals-sensitive family Ephemerellidae was more common in the reference site than in both CG and UGC. Interestingly, Heptageniidae was common throughout the study sites, and numbers were not reduced below mine sites; Heptageniidae is considered a metals sensitive family, and other studies have noted that lower abundance of Heptageniid mayflies is a useful indicator of metals pollution in Rocky Mountain streams (Nelson and Roline 1993; Kiffney and Clements 1994;

Clements 1994; Clements *et al.* 2000). In the present study, Heptageniidae did not prove a useful indicator of metals impacts. However, other studies have noted sensitivity can be variable within the same species due to life histories and population genetics (Poff and Ward 1990). Furthermore, Canivet *et al*. (2001) conducted toxicity studies with *Heptagenia sulphurea* and found that this species of Heptageniidae were very tolerant of As, but molted nearly twice as much as controls. The authors suggested that the higher molt rate was a mechanism for As loss, and allowed this species to cope with the chemical insult.

We observed only slight structural differences in stonefly families between contaminated and reference sites. This is in agreement with Plecoptera metrics which were not different across sites. However, multivariate analysis did reveal slight differences in family composition contributing to separation between contaminated and reference sites. For example, the stonefly family Perlidae was rare below mine sites; Perlidae are considered a metals-sensitive family (McGuire 2001). Interestingly, the small roach stonefly belonging to the family Peltoperlidae was more common throughout the metals contaminated sites, indicating this family may be tolerant to elevated sediment metals concentrations and may have replaced metals intolerant Plecoptera families.

The caddisfly community was structurally different across metals impacted sites in relation to the reference site. Hydropsychidae were more abundant in UGC and comprised nearly 50% of Trichoptera at those sites. Hydropsychidae are considered metals tolerant, and other studies have noted increased abundance of these taxa in metals contaminated sites (Cain *et al*. 1992; McGuire 2001). Lastly, the caddisfly Philopomatidae was found only within the reference site. This family is considered metals intolerant and may not be able to tolerate even low levels of metals (McGuire 2001).

Families other than those in the EPT orders that are contributing to site differences may be due to varying degrees of metals tolerance. We only found the dipterans Pelecorhnchidae within reference sites. This family is considered to be very sensitive to pollution and may serve as a useful indicator species (Adams and Vaughan 2003). Dance and black flies of the families Empidae and Simulidae were more common in UGC and CG. These dipteran families are considered to be very tolerant to metals (McGuire 2001).

Researchers have reported that course level (i.e. family level taxonomic resolution) is suitable to detect structural changes in benthic macroinvertebrate communities related to effects from pollutants (Warwick 1993; Ferraro and Cole 1995; Vanderklift *et al.*1996; Bailey *et al.* 2001; Rhea *et al.* 2006). We used family level resolution, and our results support the use of family level taxonomy may adequately define adverse impacts. Course-level identification was desirable because of the lesser degree of taxonomic expertise, and lower costs than species-level identification.

This study is valuable in terms of establishing valuable baseline data in the headwaters of Gold Creek. We were able to collect baseline data for post-remediation below Lakeview Mine and pre-remediation below Conjecture Mine. By employing practical family-level identification, it will be possible for cost effective and repeatable macroinvertebrate monitoring to gauge recovery of the benthic community below these remediated mine sites. By monitoring the recovery of these sites, it will deepen our understanding of recovery processes, thus allowing us to better understand impairment overall.

D. Fish Community Impacts

Fish assemblage data suggest that sediment metals concentrations may be limiting native salmonid densities within the Gold Creek Drainage. Other research has shown that elevated metals due to mining have suppressed salmonid densities. Farag *et al*. (2003) found that salmonid density at contaminated sites was only 36% of the densities found within reference streams in the Boulder River, MT. However, we recognize our data must be interpreted with caution. We used both snorkeling and electrofishing techniques to estimate fish densities, and avoid electroshocking threatened bull trout that may be stressed due to sublethal metals concentrations within the drainage. By using non-uniform techniques, it is difficult to compare among sites where different techniques were used with reasonable certainty. However, our comparisons between the two methods showed density estimates were very similar for both techniques (Table 10). The lowest density estimates we observed were in LGC, but this site was comprised almost entirely of bull trout and few westslope cutthroat. The species composition may have contributed to the low densities we observed in LGC. For example, we sampled during early fall when young-of-year bull trout may have been migrating to Lake Pend Oreille, thus reducing our fish density estimates (Scott Deeds, U.S. Fish and Wildlife Service, fisheries biologist, personal communication). Chloride Gulch had the second-lowest fish densities, and interestingly the lowest density for westslope cutthroat observed within the study area was directly below the mine site with the highest metals concentrations among all sites. Furthermore, the age class structure was different in comparison to the reference stream for both CG and UGC. Both of these tributaries had reduced numbers of fish less than 50mm. In Chloride Gulch we only observed one fish less than 50mm, and in UGC we found less than 10 young-of-year in comparison to several hundred in the reference stream. These low numbers may be due to outmigration and/or poor survival of young-of-year fish as a consequence of sediment metals contamination below mine sites.

We observed a reduction in biomass within contaminated sites and this may be due to a loss of individual fish mass associated with mining-related stressors. Fish lengths were not different across sites, but biomass was reduced in Chloride Gulch and lower Gold Creek. This may have been a function of lower fish density within contaminated sites. However, Farag *et al.* (2003) in the Boulder River, MT found tributaries with elevated metals concentrations had decreased fish mass, but not decreased length. Clements and Rees (1997) found condition factors for brown trout were negatively correlated with metals concentrations in the liver. Reductions in fish condition may be due to metabolic costs associated with acclimation to metals. For example, metallothioneins bind metals in livers, but increased metallothionein induction comes with physiological costs, thus reducing fish growth (Stegeman *et al.* 1992; Marr *et al.* 1995; Sprague 1981).

Metals can have both direct and indirect effects to salmonid communities resulting from benthic macroinvertebrates exposed to elevated metals concentrations. Numerous laboratory studies have used artificial and natural diets exposed to metals, causing reduced growth in salmonids (Woodward *et al.* 1995; Farag *et al.* 1999; Hansen *et al.* 2004). Woodward *et al.* (1995) showed a 40-50% reduction in fish weight fed a metals-contaminated benthic macroinvertebrate diet from the Clark Fork River, MT. Metals may also reduce the biomass of benthic fauna which serves as the primary food source to fluvial salmonids. This reduction in food would ultimately reduce the carrying capacity of the stream for salmonids, thus reducing fish densities and biomass within the contaminated system.

The absence of bull trout within CG and UGC where habitat parameters were suitable may be due to elevated metals concentrations within the sediments. We sampled below mine sites using two-pass depletion estimates, and this method has been shown to reliably determine bull trout presence/absence within small mountain streams (Thurow and Schill 1996). Other systems with elevated sediment metals concentrations have experienced bull trout reductions or extirpation. In the Coeur d'Alene Basin, ID bull trout distributions have been greatly reduced from their historical basin range, and their decline is most likely due to metals pollution (Mauser *et al.* 1988). Another example of bull trout decline attributable to metals contamination is in the Thutade Watershed, in northwestern B.C. In 1994, the Kemess Mine began operations, and within a six year period the number of spawning bull trout adults was significantly lower, and age-1 juvenile bull trout densities were greatly reduced within the watershed (Paul *et al.* 2004).

Bull trout distribution may also be limited by intermittent summer flows through portions of Gold Creek. Above the confluence with West Gold Creek, the mainstem of Gold Creek (approximately 2-km) flows subsurface from mid-summer through early winter. This dry section segregates the drainage and results in a potential loss of approximately five km of headwater habitat to bull trout for spawning and rearing. However, it is unclear if subsurface flows through this section are a result of natural geologic features or are a consequence of extensive underground hard-rock mines and sedimentation from mine sites. The USFS estimated that over 25,000 cubic yards of sediment were deposited within the stream bed potentially resulting in subsurface flows under this large aggregation of mine-related deposits. Intermittent flows would obviously constrain spawning migrations of adfluvial bull trout to the lower section of Gold Creek and West Gold. Currently, the majority of bull trout spawn in the lower section of Gold Creek with few fish (i.e. less than five redds per year) spawning in the lowest 0.25-km of West Gold Creek. However, we observed juvenile bull trout as far up as 2-km in West Gold Creek, indicating that juveniles migrate a considerable distance upstream in this tributary to rearing areas. In spring and early summer when flows are adequate it is possible for juvenile bull trout to migrate into the headwaters of Gold Creek and Chloride Gulch where habitat and temperatures provide suitable rearing areas. It is unclear if bull trout once used these headwaters or if metals and/or mining-related hydrologic changes are limiting distributions.

E. Habitat Influence

Suitable instream physical habitat was present throughout the wetted reaches in the study area, and was determined to provide little additional explanatory power to changes in the aquatic community based upon its relative contribution to a multiple regression. We developed a robust habitat assessment for both chemical and physical stream qualities. Our results indicate that sites contained chemical attributes (e.g. temperature and dissolved oxygen) that were within Idaho DEQ (Department of Environmental Quality) coldwater quality criteria for aquatic life. Physical habitat was also suitable, including adequate instream cover, pool complexity, substrate composition, overhead cover, and diverse geomorphologic units within reaches. Based on our habitat measurements, study sites were capable of supporting healthy aquatic communities, and environmental variables were not able to fully explain all the differences we observed between sites.

Our statistical analysis indicated that although habitat did play a role, metals were the most consistent driver of biological response within the study area. Of the thirteen benthic macroinvertebrate metrics we analyzed, 8 of those were significantly influenced by sediment metals concentrations after habitat had been accounted for in the model Furthermore, metals were the primary driver of both fish density and biomass within the study area (Table 10).

X. Management Implications

From 2003 through 2007, USEPA and USFS conducted an ecological cleanup of Lakeview and Conjecture Mines in the headwaters of Gold Creek, Idaho. These remedial actions removed over 56,000 cubic yards of waste rock and mine tailings from headwater riparian zones. Efforts were made to remediate stream sediments as well as restore instream structures to create valuable aquatic habitat while establishing clean migration corridors. Grasses were planted and large woody debris were placed on disturbed slopes to reduce surface runoff. The cleanup efforts will prevent future influx and transport of metals contaminated mine wastes into the Gold Creek Watershed, while reducing metals exposure to aquatic receptors.

We did not observe any reductions in aqueous and sediment metals concentrations below Lakeview Mine, as of 2007. However, due to the small drainage area, and seasonal subsurface flows, that limit flushing capacity, a significant attenuation in metals concentrations may be a slow process.

Target cleanup concentrations were 700 mg As/kg in sediment at remediation sites and our data suggests that this level may not be protective of aquatic organisms for an ecologically based cleanup. The present study showed a biological gradient of impacts covarying with metals contamination. We documented direct and indirect impacts to threatened bull trout in areas with sediment concentrations as low as 100 mg As/kg. Although it is challenging to attribute impacts to one specific metal when a metals mixture is present, our data suggests elevated sediment As concentrations were primarily contributing to our results. Our data is concordant with other studies in which adverse impacts were documented with similar As concentrations. Based on large nationwide datasets, the probable effect concentration for individual sediment concentrations is 33µg As/g, 5µg Cd/g, 149µg Cu/g, 128 µg Pb/g, and 459µg Zn/g. Arsenic concentrations exceeded 33 µg As/g throughout the length of the system. In the lower sections of Gold Creek, only As concentrations exceeded these probable effect concentrations, where we documented a loss of sensitive benthic macroinvertbrate families, reduced fish densities and histopatholgical abnormalities in the majority of bull trout. These impacts to bull trout are of a chronic non-lethal nature, however it is unknown if any acute response may occur in this population at other times during stream residency. Our data suggests blanket guidelines (e.g. 700 mg As/kg) are not sufficient to adequately protect aquatic organisms, and it is recommended that these guidelines be reconsidered.

In 2007, Shoshone Silver Mining Company began exploring former mining sites within the Lakeview Mining district including Lakeview, Conjecture, and Weber Mines with the intention to extract ore bodies that had not previously been recovered. These operations may contribute additional metals loads into the Gold Creek Drainage negating additional remediation of stream sediments below mine sites.

This study has established cost effective and reproducible chemical and biological baseline data. We recommend that a sampling plan be drafted to monitor the post remediation efforts within the Gold Creek Drainage. The ecological significance of this watershed warrants long-term monitoring in light of the extensive remediation efforts and continued ore exploration and extraction activities.

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XII. Appendix A. Tables

Table 1.Dissolved metals concentrations (µg/L) in Gold Creek, Idaho (mean with standard deviation in parentheses).

Parameter	CG	UGC	LGC	Delta	WG
pH	7.1 (0.52)	8.0 (0.07)	8.2 (0.02)	8.3 (0.01)	8.1 (0.06)
Hardness as mg/L $CaCO_3$	19 (3.2)	55 (10)	58 (9)	68 (11)	57 (12)
Arsenic	12 (6.4)*	5.5 (1.4)*	1.2 (0.24)	1.1 (0.40)	0.91 (0.23)
Cadmium	0.12 (0.08)*	0.06 (0.01)*	<0.006	<0.006	<0.006
Copper	<2.5	<2.5	<2.5	<2.5	<2.5
Lead	0.11 (0.06)	0.13 (0.04)*	0.07 (0.03)	0.08 (0.03)	0.04 (0.02)
Zinc	85 (59)*	31 (9.6)*	7.8 (3.0)	10 (4.5)	7.8 (2.6)

Significant differences were (denoted by an asterisk *) derived by comparison to the reference site (WG). All tests were considered statistically significant at $\alpha \leq 0.05$.

Table 2. Weak-acid extracted sediment concentrations (mg/kg dry wt.) in Gold Creek, Idaho (mean with standard deviation in parentheses).

Parameter	CG	UGC	LGC	Delta	WG
Arsenic	537 (168)*	50.2 (11.9)*	38.2 (10.4)*	27.7 (12.6)*	2.62 (0.59)
Cadmium	3.56 (1.07)*	2.08 (0.25)*	0.65 (0.19)*	0.57 (0.18)*	0.06 (0.03)
Copper	51.3 (22.4)*	32.3 (7.30)*	9.6 (1.9)*	11.2 (4.6)*	2.78 (1.6)
Lead	304 (97.1)*	249 (67.2)*	56.1 (13.9)*	60.9 (19.1)*	5.1 (1.5)
Zinc	836 (355)*	688 (111)*	232 (54.3)*	247 (41.8)*	7.5 (2.0)

Significant differences were (denoted by an asterisk *) derived by comparison to the reference site (WG). All tests were considered statistically significant at $\alpha \leq 0.05$.

Table 3. Benthic macroinvertebrate tissue metals concentrations (µg/g dry wt.) collected in Gold Creek, Idaho (mean with standard deviation in parentheses).

Parameter	CG	UGC	LGC	Delta	WG
Arsenic	96.7 (35.9)*	41.1 (18.3)*	20.6 (5.10)*	28.2 (1.91)*	5.40 (1.60)
Cadmium	5.81 (0.94)*	3.23 (0.35)*	1.60 (0.14)*	1.68 (0.05)*	1.15 (0.33)
Copper	64.1 (10.1)*	43.9 (2.55)*	23.0 (1.69)*	26.1 (2.26)*	19.5 (2.64)
Lead	41.1 (6.49)*	68.5 (13.2)*	12.5 (2.01)*	21.2 (6.92)*	2.55 (1.34)
Zinc	1870 (431)*	990 (157)*	746 (62.5)*	719 (52.3)*	206 (26.5)

Significant differences were (denoted by an asterisk *) derived by comparison to the reference site (WG). All tests were considered statistically significant at $\alpha \leq 0.05$.

Table 4.Benthic macroinvertebrate metrics measured within Gold Creek, Idaho (mean with standard deviation in parentheses).

	Site			
Metric	WG	LGC	UGC	CG
# Taxa E ↓	5 (0)	3.2 (0.4)*	4 (0)*	3.8 (0.4)*
# Taxa P ↓	4.8 (0.8)	5.4 (0.5)	5.2 (1.3)	4.8 (0.8)
# Taxa T ↓	4.6 (0.8)	4 (1)	2.8 (0.4)*	3 (1)*
% EPT ↓	66 (7)	80 (7)	73 (4)	48 (14)*
EPT Richness ↓	14 (1)	13 (1)	12 (1)*	11 (1)*
% Chironomidae ↑	10 (5)	8 (3)	18 (5)	42 (15)*
% Hydropsychidae ↑	17 (12)	6 (3)	50 (15)*	8 (9)
% Elmidae ↓	18 (7)	5 (3)*	3 (1)*	1 (1)*
Taxa Richness ↓	21 (3)	19 (3)	21 (3)	19 (3)
% Dominance ↑	24 (5)	21 (3)	26 (5)	41 (15)*
Density ↓	2190 (702)	1488 (523)	1763 (523)	962 (531)*
IBI ↓	22 (1)	19 (0.8)	19 (0.5)	18 (0.8)*
Simpson’s D/E ↓	0.39 (0.07)	0.46 (0.04)	0.34 (0.06)	0.27 (0.12)*

Significant differences were (denoted by an asterisk *) derived by comparison to the reference site (WG). All tests were considered statistically significant at $\alpha \leq 0.05$.

Table 5. Spearman's correlation between sediment metals concentrations and benthic macroinvertebrate community indices in Gold Creek, Idaho.

Benthic Metric	Sediment Analyte				
	Arsenic	Cadmium	Copper	Lead	Zinc
	-0.454*	-0.466*	-0.478*	-0.466*	-0.466*
Taxa E	(0.044)	(0.038)	(0.033)	(0.038)	(0.038)
	0.057	-0.034	0.048	0.014	-0.062
Taxa P	(0.811)	(0.866)	(0.839)	(0.952)	(0.795)
	-0.551*	-0.511*	-0.498*	-0.494*	-0.536*
Taxa T	(0.012)	(0.021)	(0.025)	(0.027)	(0.015)
	-0.358	-0.392	-0.330	-0.402	-0.328
% EPT	(0.121)	(0.087)	(0.155)	(0.079)	(0.158)
	-0.535*	-0.567*	-0.524*	-0.527*	-0.611*
EPT Richness	(0.015)	(0.009)	(0.018)	(0.017)	(0.004)
	-0.036	-0.104	0.022	-0.042	-0.061
Taxa Richness	(0.880)	(0.662)	(0.926)	(0.860)	(0.797)
	0.777*	0.791*	0.777*	0.788*	0.786*
% Chironomidae	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
	-0.836*	-0.862*	-0.844*	-0.817*	-0.820*
% Elmidae	(0.000)	(0.000)	(0.000)	(0.000)	(0.000)
	0.038	0.047	0.109	0.068	0.029
% Hydropsychidae	(0.872)	(0.845)	(0.647)	(0.776	(0.905)
	0.453*	0.518*	0.450*	0.496*	0.456*
% Dominance	(0.045)	(0.019)	(0.047)	(0.026)	(0.043)
	-0.582*	-0.586*	-0.550*	-0.626*	-0.547*
Density	(0.007)	(0.007)	(0.012)	(0.003)	(0.012)
	-0.544*	-0.577*	-0.562*	-0.595*	-0.591*
Shannon's D(E)	(0.013)	(0.008)	(0.010)	(0.006)	(0.006)
	-0.685*	-0.697*	-0.634*	-0.666*	-0.675*
IBI	(0.001)	(0.001)	(0.003)	(0.001)	(0.001)

Correlations were considered statistically significant (denoted by an asterisk) at $\alpha \leq 0.05$.

Table 6. Results of ANOSIM on contaminated sites versus the reference site in Gold Creek, Idaho

Pairwise Tests

Sites	Statistic
WG vs LGC	0.606
WG vs UGC	0.828*
WG vs CG	0.904*
Global R	0.746

Asterisks indicate well separated sites.

Table 7. Fish tissue metals concentrations (µg/g dry wt.) collected in Gold Creek, Idaho (mean with standard deviation in parentheses).

Parameter	CG	UGC	LGC	Delta	WG
Arsenic	10.9 (4.96)*	8.38 (1.59)*	2.10 (0.694)	1.61 (0.426)	1.15 (0.33)
Cadmium	1.10 (0.354)*	0.899 (0.236)*	0.473 (0.105)*	0.267 (0.103)*	0.129 (0.0287)
Copper	7.03 (1.89)*	6.31 (0.873)*	4.33 (0.832)*	2.92 (0.428)	2.58 (0.355)
Lead	1.43 (0.684)*	3.10 (2.51)*	1.14 (1.37)*	0.738 (0.382)*	0.112 (0.067)
Zinc	349 (77.4)*	312 (98.1)*	216 (29.7)*	169 (12.5)	128 (10.6)

Significant differences were (denoted by an asterisk *) derived by comparison to the reference site (WG). All tests were considered statistically significant at $\alpha \leq 0.05$.

Table 8. Fish lengths, density, and biomass in Gold Creek, Idaho.

Site	Sample Method	Species	Fish Length	Density (fish/m²)	Biomass (g/m²)
WG	Snorkeling	BLT and WCT	111 (11)	0.42 (0.18)	4.8 (2.2)
LGC	Snorkeling	BLT and WCT	94 (17)	0.05 (0.01)*	0.42 (0.15)*
UGC	Electrofishing	WCT	114 (6.8)	0.25 (0.05)	4.1 (0.83)
CG	Electrofishing	WCT	98 (12)	0.20 (0.10)*	1.54 (0.51)*

Data were compared to the reference site (WG) for statistical tests. Statistical differences (denoted by an asterisk) were considered statistically different at $\alpha \leq 0.05$.

Table 9. Multiple regression analysis of habitat variables and metals concentrations versus benthic macroinvertebrate metrics and fisheries estimates in Gold Creek, Idaho.

Dependant Variable	T-value	P-value	R-squared (adj.)
# Ephemeroptera families	-2.36	0.03*	80%
# Plecoptera families	0.78	0.44	0%
# Trichoptera families	-1.59	0.14	17%
% EPT families	-3.42	0.006*	69%
EPT family richness	-1.35	0.21	27%
Taxa family richness	0.79	0.44	20%
% Chironomidae	3.13	0.01*	62%
% Hydropsychidae	3.88	0.003*	45%
% Elmidae	-3.02	0.01*	70%
% Dominance	3.43	0.006*	56%
Density (number/m^2)	-1.34	0.21	9%
Simpson's D/E	-2.68	0.02*	50%
Family level IBI	-2.35	0.03*	60%
Fish density	-3.20	0.009*	66%
Fish biomass	-3.63	0.004*	93%

Regressions were considered statistically significant (denoted by an asterisk) at $\alpha \leq 0.05$.

Table 10. Fish sampling methods for each site within Gold Creek, Idaho 2006.

Site	Sample Method	Species	Mean Size (mm)	Density (fish/m²)	Biomass (g/m²)
WG 1.95	Snorkeling	WCT and BLT	93	0.48	3.10
WG 1.35	Snorkeling	WCT and BLT	112	0.72	8.30
WG 1.2	Snorkeling	WCT	118	0.34	4.80
WG 1.05	Snorkeling	WCT	124	0.33	5.30
WG 1.05	Electrofishing	WCT and BLT	120	0.33	5.40
WG 0.90	Snorkeling	WCT and BLT	109	0.23	2.60
WG 0.90	Electrofishing	WCT	108	0.17	2.10
LGC 1.05	Snorkeling	WCT and BLT	113	0.04	0.51
LGC 0.75	Snorkeling	WCT and BLT	93	0.07	0.49
LGC 0.60	Snorkeling	WCT and BLT	97	0.05	0.41
LGC 0.45	Snorkeling	WCT and BLT	104	0.05	0.54
LGC 0.3	Snorkeling	WCT and BLT	65	0.06	0.15
UGC 1.5	Electrofishing	WCT	124	0.28	5.40
UGC 1.35	Electrofishing	WCT	112	0.32	4.30
UGC 0.90	Electrofishing	WCT	106	0.20	3.64
UGC 0.75	Electrofishing	WCT	112	0.28	4.00
UGC 0.45	Electrofishing	WCT	118	0.20	3.20
CG 1.5	Electrofishing	WCT	113	0.08	1.03
CG 1.05	Electrofishing	WCT	91	0.29	2.06
CG 0.9	Electrofishing	WCT	91	0.22	1.54
CG 0.3	N/A	N/A	N/A	N/A	N/A
CG 0.15	N/A	N/A	N/A	N/A	N/A

XIII. Appendix B. Figures

Figure 1. Map showing the mine sites, sampling sites, and reaches within the study area in Bonner County, northern Idaho, USA. Sampling sites include Chloride Gulch (CG) below Idaho Lakeview Mine, upper Gold Creek (UGC) below Conjecture Mine, West Gold Creek (WG) the reference site, lower Gold Creek (LG), and the Gold Creek delta in Lake Pend Oreille (Delta).

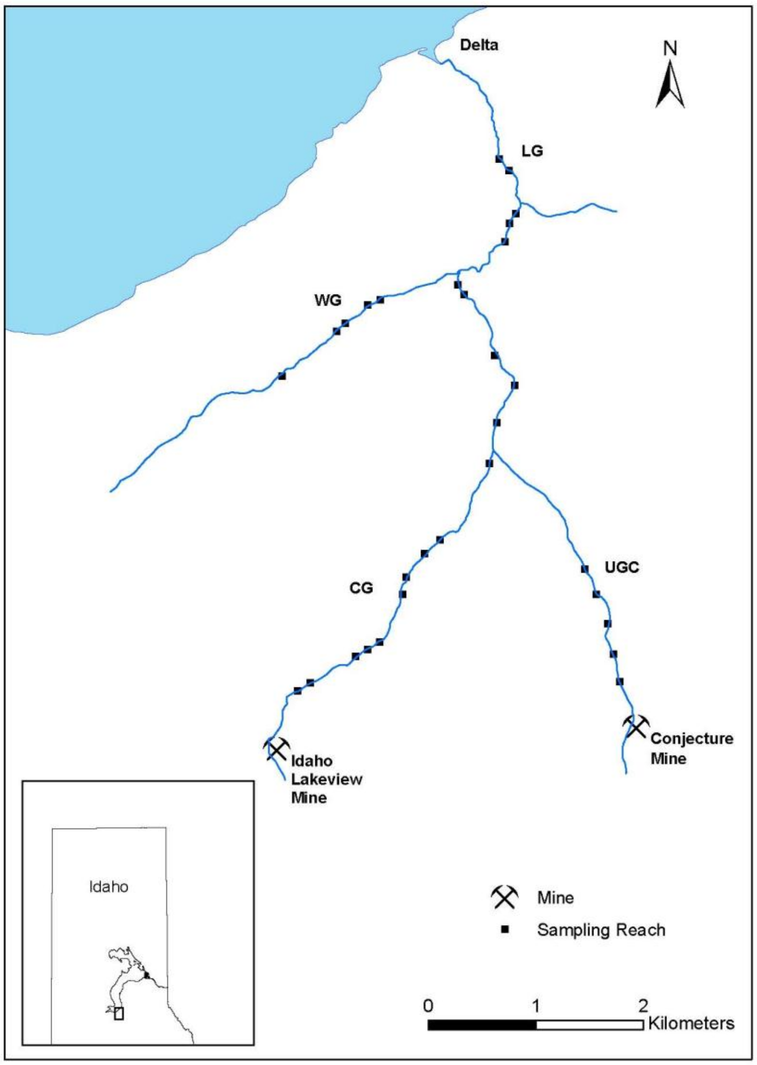
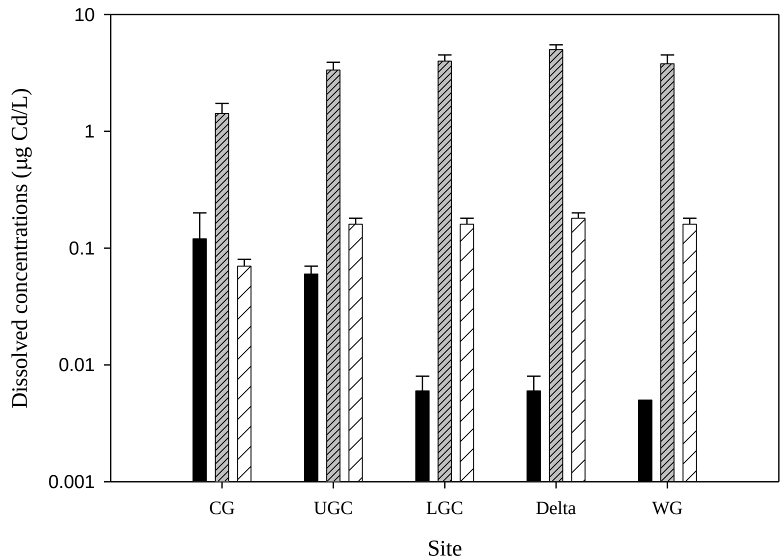


Figure 2. Dissolved concentrations (µg/L) of measured (solid), predicted LC50's (fine), and U.S. EPA chronic water quality criteria (course) for Cd and Zn in Gold Creek, Idaho.



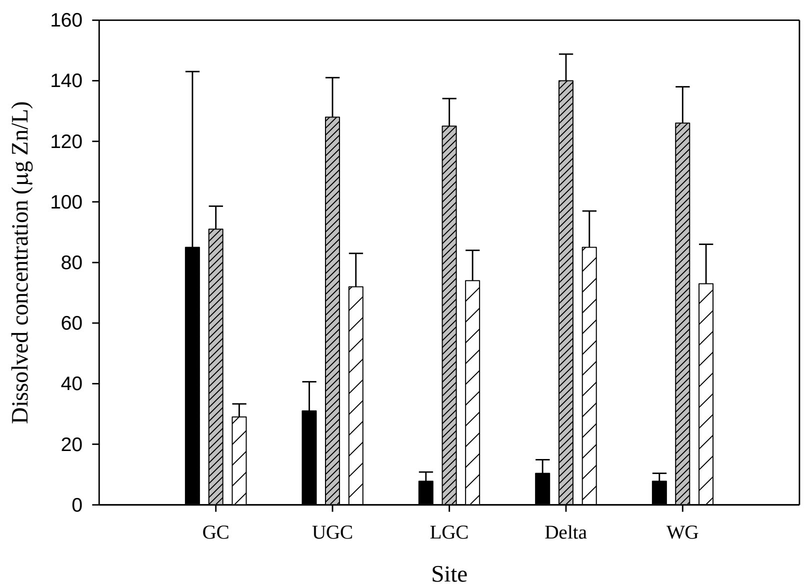


Figure 3. Ordination by non-metric multidimensional scaling of benthic macroinvertebrate community data in Gold Creek, Idaho.

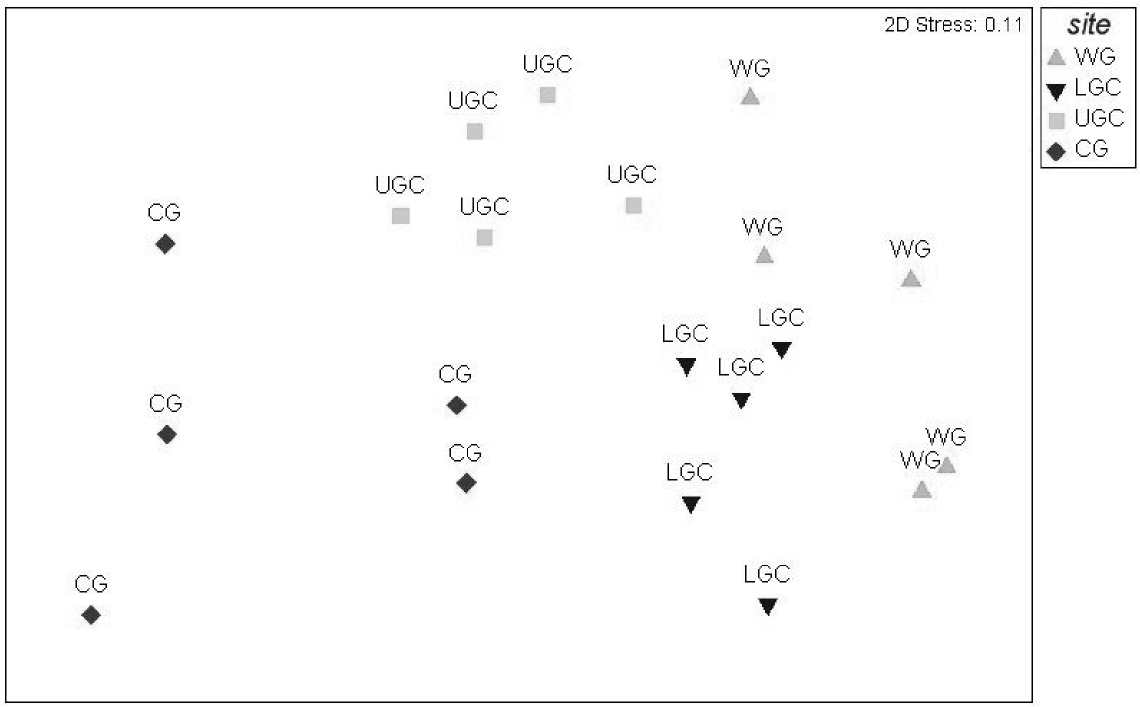


Figure 4. Scatterplot of relationship between As concentrations in benthic macroinvertebrate tissues and fish whole-body tissues in Gold Creek, Idaho.

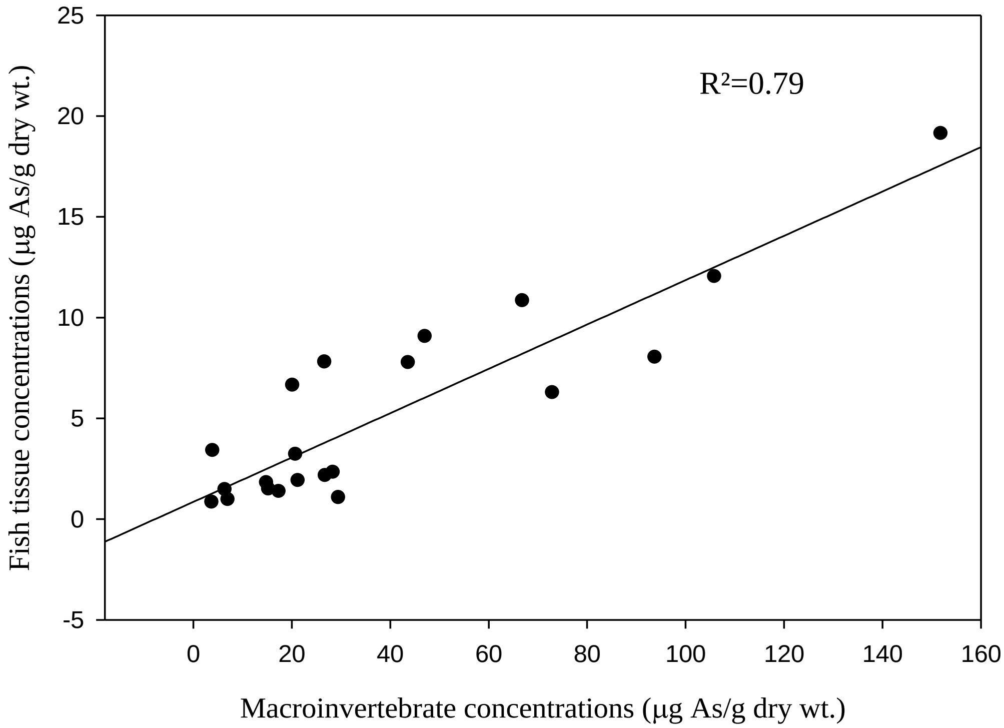


Figure 5. Illustration of bull trout liver nuclear pleomorphism from the Gold Creek Drainage, Idaho. Light arrow indicates single nuclear pleomorphism and dark arrow indicates normal nucleus.

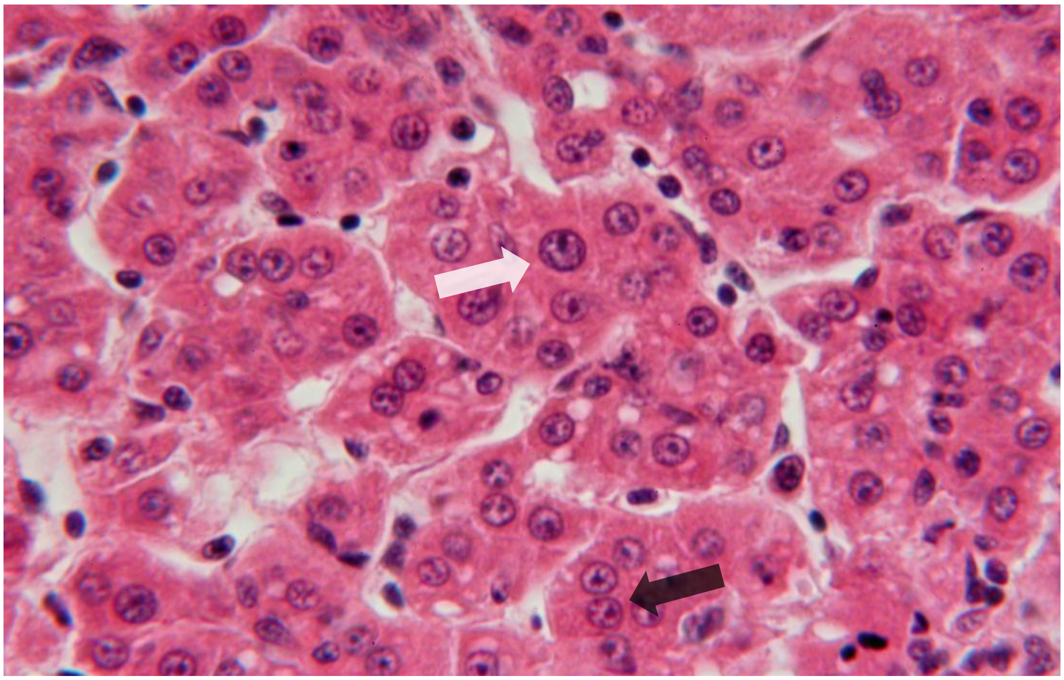


Figure 6. Illustration of scattered degeneration and single-cell necrosis (light arrows) of bull trout hepatocytes from Gold Creek, Idaho.

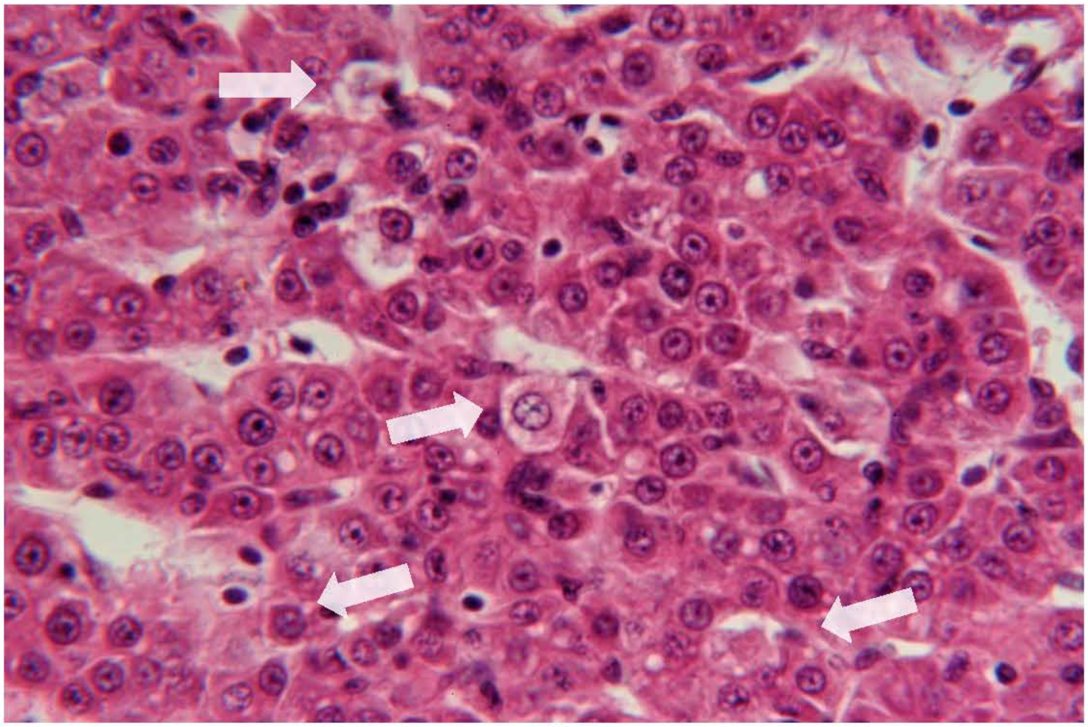
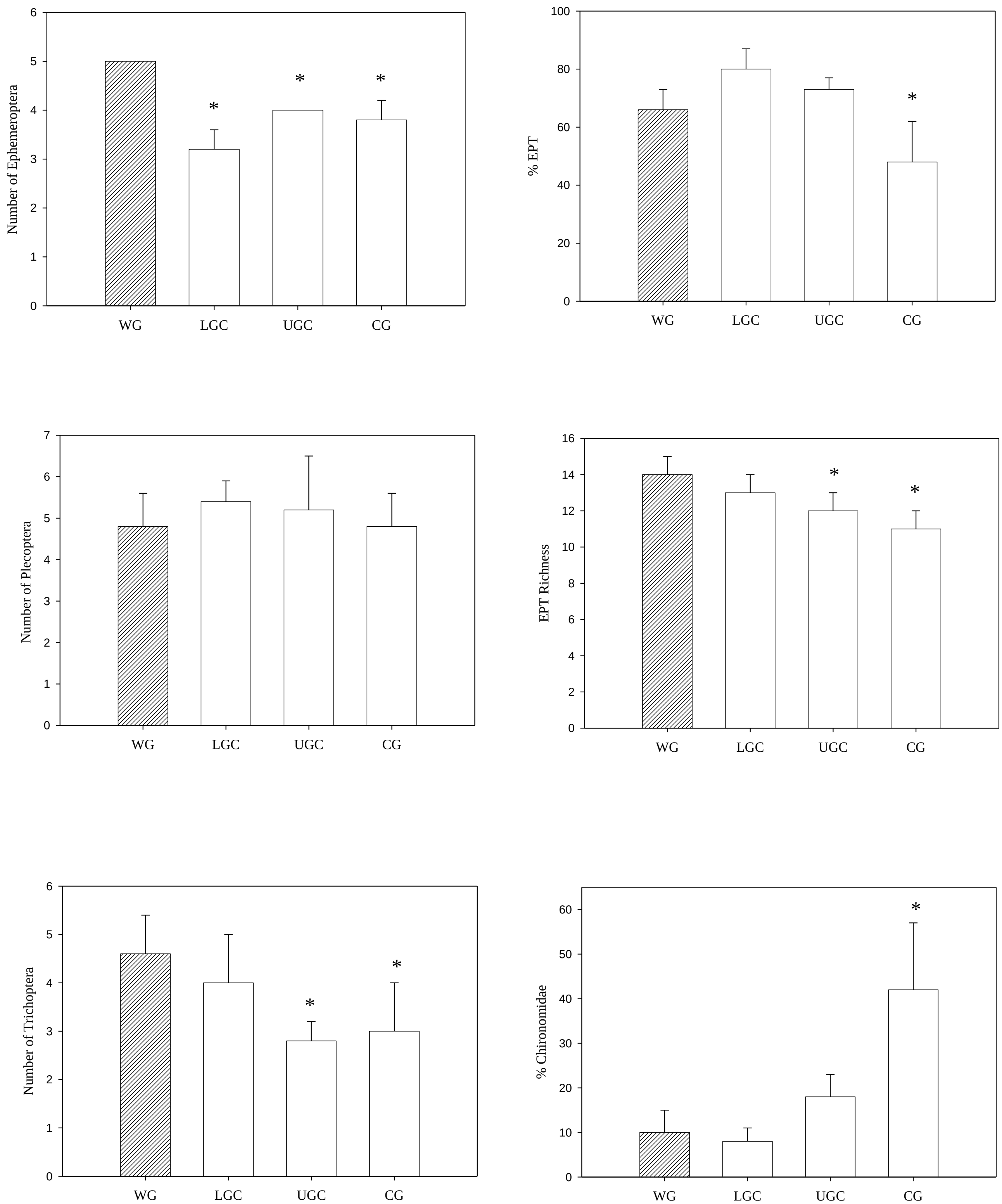
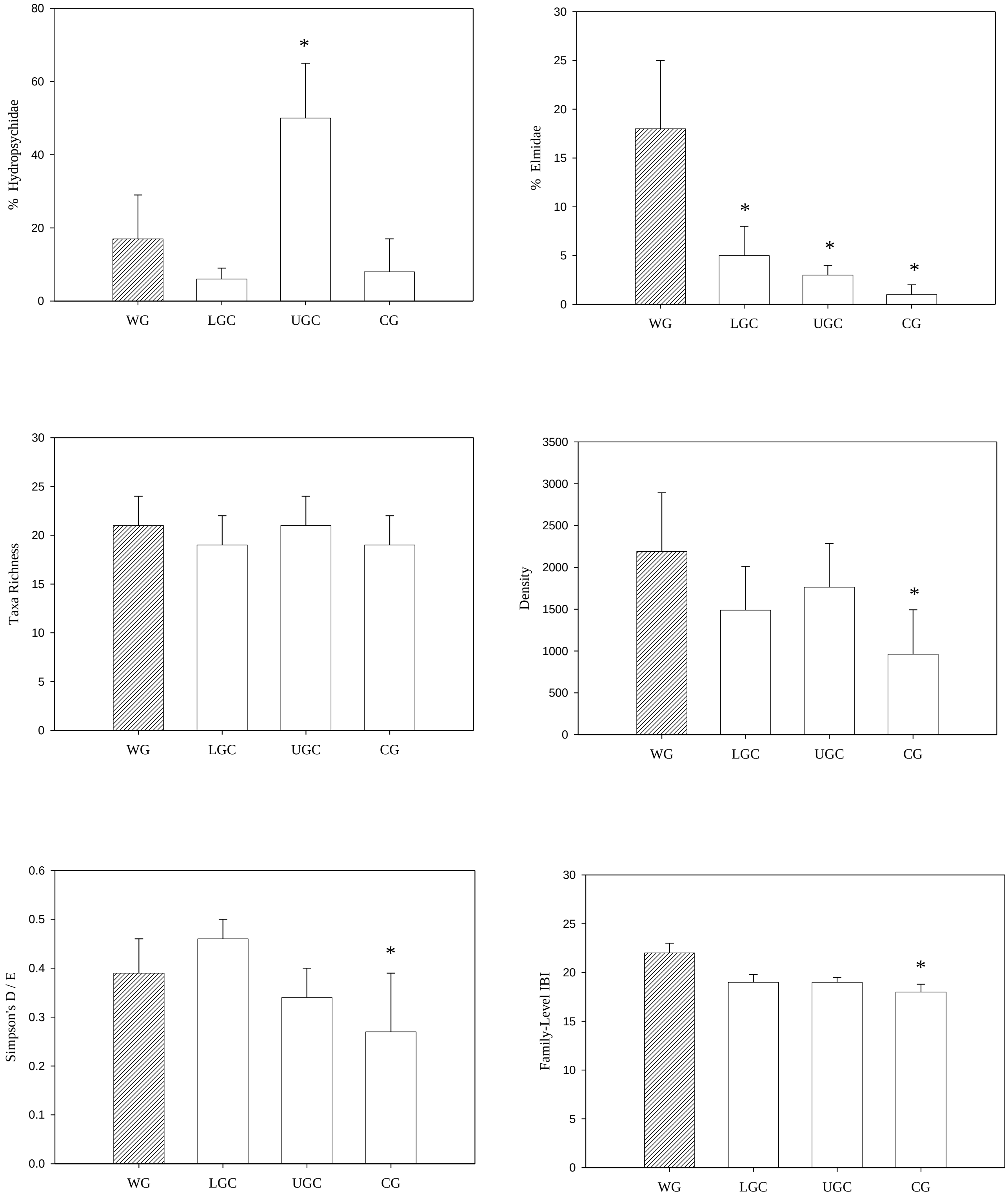


Figure 7. Bar graphs of benthic macroinvertebrate metrics by site in Gold Creek, Idaho 2006 (significant differences are denoted by an asterisk).




% Hydropsychidae
0
20
40
60
80
*
WG
LGC
UGC
CG
% Elmidae
0
5
10
15
20
25
30
*
*
*
WG
LGC
UGC
CG
Taxa Richness
0
5
10
15
20
25
30
WG
LGC
UGC
CG
Density
0
500
1000
1500
2000
2500
3000
3500
*
WG
LGC
UGC
CG
Simpson's D / E
0.0
0.1
0.2
0.3
0.4
0.5
0.6
*
WG
LGC
UGC
CG
Family-Level IBI
0
5
10
15
20
25
30
*
WG
LGC
UGC
CG