Bonita Peak Mining District
2016 Benthic Macroinvertebrate Assessment

August, 2017

Prepared For:
Environmental Protection Agency
Region 8
1595 Wynkoop Street
Denver, Colorado 80202

Prepared By:
Scott Roberts, Aquatic Ecologist
Mountain Studies Institute
1309 E. Third Avenue #106
Durango, Colorado 81301

Table of Contents

List of Tables

List of Figures

List of Abbreviations and Acronyms

BMI	Benthic Macroinvertebrate
CCU	Cumulative Criteria Unit *(see table 26)*
CDPHE	Colorado Department of Public Health and Environment
EDAS	Ecological Data Application System
EPA	Environmental Protection Agency
EPT	Ephemeroptera, Plecoptera, and Trichoptera *(see table 2)*
HBI	Hilsenhoff Biotic Index *(see table 2)*
HQ	Hazard Quotients
MetalSensRA	Relative abundance of Ephemerellidae, Heptageniidae, and Taeniopterygidae families *(see table 2)*
MetalSensRich	Richness of Ephemerellidae, Heptageniidae, and Taeniopterygidae families *(see table 2)*
MMI	Multi-metric index *(see table 2)*
MSI	Mountain Studies Institute
NMS	Non-metric multi-dimensional scaling ordination
SWDI	Shannon-Weaver Diversity Index *(see table 2)*

<u>Metals and Minerals:</u>

Al	aluminum
Sb	antimony
As	arsenic
Ba	barium
Be	beryllium
Cd	cadmium
Ca	calcium
Cl-	chloride
Cr	chromium
Cu	copper
Fe	iron
F-	fluoride
Hg	mercury
Pb	lead
Mo	molybdenum
Mg	magnesium
Mn	manganese
Ni	nickel
Se	selenium
SiO_2	silica
SO_4	sulfate
Ag	silver
Sr	strontium
Tl	thallium
U	uranium
V	vanadium
Zn	zinc

1. Executive Summary

The Bonita Peak Mining District (BPMD) was designated by the Environmental Protection Agency (EPA) as a Superfund site on the National Priorities List in 2016. The EPA identified 48 distinct contaminant sources related to mining activities that warrant further investigation. The 48 sources are scattered throughout the Upper Animas River and tributaries in San Juan County, Colorado. BPMD is located in a highly-mineralized zone of the San Juan Mountains where high metal concentrations from natural and mine-related sources have contributed to a long history of degraded water quality in the Upper Animas River and tributaries. Numerous studies have documented the impacts to benthic macroinvertebrates (BMI) from metal contamination in this region, particularly for the Animas River and its tributaries, but the last spatially comprehensive evaluation of BMI distribution in the Upper Animas watershed was conducted in the mid-1990s by Anderson (2007a). While this historical information is valuable, an updated evaluation was warranted, given that remediation projects that have been completed in the watershed during the past 20 years. Furthermore, several of the contaminant sources identified by the EPA for inclusion in the Superfund site are located in subwatersheds where benthic communities were not surveyed by Anderson in the mid-1990s.

For this research, we conducted surveys throughout the Upper Animas River and Mineral Creek watersheds across a gradient of metal exposure to BMI through water and sediment to accomplish the following objectives: 1) Provide a spatially comprehensive evaluation of the current condition of BMI communities throughout BPMD; 2) Document metal concentrations of BMI tissue, which are reflective of overall metal bioavailability; 3) Characterize the physical habitat for aquatic life at surveyed sites; and 4) Examine what environmental factors are most responsible for differences in benthic communities across BPMD.

Data from our 2016 surveys suggest the following conclusions:

- BMI communities within BPMD vary from reach to reach, ranging from sparsely populated sites with substantial metal contamination to sites with abundant, diverse communities that reflect minimal exposure to metal contamination.

- The most robust, diverse benthic communities were observed at sites located on tributaries (Cunningham Creek, Maggie Gulch, Picayne Gulch, Bear Creek, and Mill Creek), on the South Fork of Mineral Creek, and on the uppermost reaches of the South and North Forks of the Animas River. We observed the lowest benthic diversity and abundance in two general areas: 1) at sites on Mineral Creek from the Middle Fork of Mineral Creek to the Animas River; 2) on the South Fork of the Animas River immediately upstream of the confluence with Eureka Gulch; and 3) at sites near Animas Forks and throughout the West Fork of the Animas River.

- In some locations, healthy BMI communities sharply decline in diversity and abundance over a relatively short distance.

- Several sites that currently do not have an aquatic life use designation had benthic communities that, based on Multi-metric index (MMI) (CDPHE 2010b) scores, would be in attainment of a class two aquatic life use designation. Many sites in BPMD have benthic communities that would not meet an aquatic life use designation.

- We found a gradient of sensitivity among the three metal-sensitive BMI families that directly corresponds with increases in several metals and minerals in BMI tissue, pore water, and surface water. The loss or addition of each of the three metal-sensitive families over time could be a valuable indicator for assessing the successfulness of remediation efforts.

- We used statistical correlation and non-metric multi-dimensional scaling ordination (NMS) to examine the correlations between environmental factors and benthic communities in BPMD. There was broad agreement between the two methods that surface water and pore water metal and mineral concentrations more strongly influenced BMI communities than other environmental parameters. There was a weaker relationship between BMI communities and concentrations of metals and minerals in sediment.

- Multiple lines of evidence, including statistical correlation, NMS, and hazard quotients, suggest that surface water concentrations of Al, Cd, Cu, and Zn likely shape the distribution and community composition of BMI populations across BPMD. Further research, such as experimental bioassays, could confirm if these and other metals that correlated with BMI metrics have a direct causative effect on BMI communities.

Long-term monitoring of benthic communities has been demonstrated as an effective tool for detecting improvements in water quality and the health of aquatic life following mining-related remediation efforts (Clements et al. 2010). To assess the success of remediation efforts in BPMD, we recommend an annual, long-term monitoring program that targets a subset of the 2016 sites across a gradient of metal exposure. BMI monitoring programs that are long-term, and ideally occur on an annual frequency, are more effective at isolating the direct effects of anthropogenic activities from natural variability of communities. Sites selected for continued monitoring should be located in close downstream proximity to remedial actions. In addition, we recommend that Animas River sites downstream of Silverton be included in a long-term monitoring plan to determine if remediation efforts translate to down-canyon improvement in the health of aquatic life. Monitoring assessment should focus on BMI metrics that most strongly correlated with metal exposure, which include total richness, density, EPT, MMI, and the richness of metal sensitive families.

2. Introduction

In 2016, the Environmental Protection Agency (EPA) designated the Bonita Peak Mining District (BPMD) as a Superfund site on the National Priorities List. The EPA identified 48 distinct contaminant sources related to mining activities that warrant further investigation. The 48 sources are scattered throughout the Upper Animas River and tributaries in San Juan County, Colorado. BPMD is located in a highly-mineralized zone of the San Juan Mountains where high metal concentrations from natural and mine-related sources have contributed to a long history of degraded water quality. The extent to which metal contamination impacts aquatic life varies throughout the district with some stream reaches supporting abundant and diverse aquatic life while other stream reaches are largely devoid of aquatic life (Besser, Finger, and Church 2007).

Numerous studies have documented the impacts to benthic macroinvertebrates (BMI) from metal contamination in the Animas River and tributaries (Anderson 2007a; Anderson 2007b; Besser and Brumbaugh 2007; Courtney and Clements 2002; EPA 2015; Roberts 2015; Roberts 2016; Smith 1976; etc.), but the bulk of this research has focused on the Animas River downstream of Silverton, Colorado. The last spatially comprehensive evaluation of BMI distribution in the Upper Animas watershed was conducted in the mid-1990s by Anderson (2007a). While this historic information is valuable, an updated evaluation is warranted, given the number of significant remediation projects that have been completed in the watershed during the past 20 years. Furthermore, several of the contaminant sources identified by the EPA for inclusion in the Superfund site are located in subwatersheds where benthic communities were not surveyed by Anderson in the mid-1990s.

Therefore, we conducted surveys throughout the Upper Animas River and Mineral Creek watersheds across a gradient of metal contamination to accomplish the following objectives:

1) Provide a spatially comprehensive evaluation of the current condition of BMI communities throughout BPMD.
2) Document metal concentrations of BMI tissue, which are reflective of overall metal bioavailability.
3) Characterize the physical habitat for aquatic life at surveyed sites.
4) Examine what environmental factors are most responsible for differences in benthic communities across BPMD.

3. Methods

3.1 Monitoring Locations

EPA designated nineteen exposure units and six reference areas along streams in the Mineral Creek and upper Animas watersheds within BPMD (EPA 2016). We collected BMI community composition data, BMI tissue data (when available), and physical habitat data in each of the 19 exposure units and at five reference locations – a total of 28 locations (Table 1 and Maps 1-2 in Appendix A).

3.2 Field Survey Methodology

3.2.1 BMI Community Samples

To allow direct comparison to historical BMI data from the Animas River watershed, we replicated a BMI sampling method (to the greatest extent possible) that was developed by Chester Anderson and used previously within the Animas River watershed (Anderson 2007a; personal communication). Anderson's method utilized and modified protocols developed by the EPA (Barbour et al. 1999) and CDPHE (CDPHE 2010a). Anderson (2000) assessed a variety of BMI sampling methods and determined that the most appropriate method for use in the Animas River was a targeted riffle method that utilized a modified rectangular dip net coupled with a dolphin bucket. We altered Anderson's (2007a) methodology by increasing the amount of habitat sampled per site to 1.15 m^2, which more closely follows the methodology outlined by CDPHE (2010a) and provides a better representation of the spatial heterogeneity of BMI communities.

At each site we collected ten samples at equal intervals along a 150-meter-long stream reach. We collected each sample by placing the net securely on the bottom of the river with the net opening facing upstream. Standing downstream of the net, we disturbed the substrate on the river bottom that is immediately upstream of the net. We lifted and scrubbed rocks and gravel by hand for approximately 30 seconds to ensure that BMIs were dislodged and drifted downstream into the net opening. For each sample, we disturbed an area of approximately 0.115 m^2 of substrate, which was estimated in the field by using the size of the net opening as a guide (*net opening is 46 cm by 25 cm; area of 0.115 m^2*). We then composited the ten samples into a single sample container representing 1.15 m^2 (1782 in^2) of habitat at each site.

3.2.2 BMI Tissue Sampling

After community sampling was complete, we collected a second BMI sample from each site to analyze for tissue metal concentrations. We collected specimens in a similar manner as described in section 3.2.1. Using forceps and a fine mesh net, we triple rinsed each specimen in deionized water before combining all specimens into a community composite sample for each site. To meet laboratory analysis requirements, we attempted to collect at least four grams of wet weight BMI tissue for each site. To accommodate laboratory quality control procedures, we increased the BMI tissue mass at 10% of the sites to at least eight grams of wet weight BMI tissue. We kept samples cold (between 2-6 degrees Celsius) in DigiTube™ sample containers and shipped them on ice for analysis at the Environmental Services Assistance Team (ESAT) Analytical Chemistry Department within the EPA Region 8 laboratory. We delivered samples within specified hold times of 180 days for total recoverable metal analysis and 28 days for mercury analysis.

3.2.3 Physical Habitat Evaluation

We evaluated physical habitat using CDPHE's (2015) "105-Count Procedure" along twenty-one cross-sectional transects equidistantly located within the 150-meter reach delineated for BMI community sampling. At five points along each of the twenty-one cross-section transects, we selected a substrate at random and measured the intermediate axis length in millimeters, estimated percent embeddedness, and noted the presence/absence of algae, moss, or other vegetative cover. In total, we evaluated 105 substrates at each site. We estimated embeddedness as the percent surface area of cobble sized substrate (i.e., substrate with intermediate axix longer than 64 mm in length) that was covered by fine sediment or biofilm mats.

We evaluated the condition of habitat using a habitat assessment protocol developed for high-gradient streams by Barbour and others (1999) in which the following habitat parameters were visually assessed and given a numerical score: epifaunal substrate/available cover; embeddedness; velocity/depth regime; sediment deposition; channel flow status; channel alteration; frequency of riffles; bank stability; vegetation; and riparian vegetative zone.

At each site we photographed the downstream and upstream perspective at 50m intervals along the reach (0m, 50m, 100m, and 150m). We also photographed typical substrate at each site for reference. These photos can be used to ensure that subsequent surveys are conducted within the same reach.

Site photos are included in Appendix B and the physical habitat field data sheet is included in Appendix C.

3.3 Laboratory Methods

3.3.1 BMI Community Samples

Samples were identified by Scott Roberts (aquatic ecologist, Mountain Studies Institute) and Dr. Michael Bogan (University of Arizona). We sub-sampled each field sample using a rotating drum splitter until a minimum of 500 organisms was obtained. Using a 10x microscope, we identified organisms to the lowest practical taxonomic level based on Merrit and Cummings (1996). Dr. Bogan identified all Chironomidae and Acari taxa and served as a second taxonomist for our quality assurance program by independently verifying at least 10% of all taxa.

3.3.2 BMI Tissue Samples

Tissue metal concentrations were analyzed by the EPA Region 8 laboratory in Golden, Colorado using EPA analytical methods EPA 200.2, 200.7, and 200.8. The entire body of BMIs were analyzed, including metals in internal organs, on gill surfaces, and elsewhere in the body.

3.4 Data Analysis

3.4.1 BMI Metrics

Several metrics have been developed to assess the composition and health of BMI communities (Table 2). Many of these metrics can also be used as evidence of the overall condition of the habitat and water quality of an aquatic system. Table 2 presents BMI metrics in order of their applicability to BMI communities that are exposed to elevated metal concentrations.

3.4.2 Ecological Data Application System (EDAS)

We utilized the Ecological Data Application System (EDAS) developed by CDPHE to calculate the Colorado Multi-metric index (MMI). MMI scores are based on a fixed count of 300 individuals per sample.

3.4.3 Standardizing Sample Size

To eliminate potential bias from differing sample sizes, we employed an algorithm to subsample all samples to a fixed count of 500 individuals. All metrics discussed in this report are based on the 500 count subsampled data, except MMI, which is based on a fixed count of 300 organisms per sample (see section 3.4.2).

3.4.4 Statistical Analysis

Using JMP statistical software (JMP 2013), we calculated spearman correlation coefficients between BMI metrics, physical habitat variables, and metal and mineral concentrations in BMI tissue, sediment, surface water, and pore water.

We applied non-metric multi-dimensional scaling ordination (NMS) within PC-ORD software (McCune & Mefford 1999) to assess differences in benthic communities across BPMD and to determine which environmental explanatory variables (e.g., embeddedness; dissolved aluminum concentrations in surface water) may drive the variability in community composition among sites. Our NMS analysis was based on Bray-Curtis distance measures of species abundance per site. To eliminate bias from rare taxa in the NMS, we only used species that occurred in more than 5% of samples. Two sites had benthic communities that were substantially different from all other sites. Hermosa Creek had different community composition due to it being located at a lower elevation than all other sites. Burrows Creek was unique in that it's benthic community was dominated by Chironomidae taxa, perhaps due to a smaller substrate size distribution than all other sites. We excluded these two sites from NMS analysis because, as outliers, they skewed ordination results and obscured interpretation. The approach of excluding outliers is consistent with NMS procedures (Peck 2016).

3.4.5 EPA chemistry data

In the fall of 2016, EPA collected sediment, pore water, and surface water samples from numerous sites within BPMD, including most of the same sites where BMI samples were collected (Table 1). Samples were analyzed for metal and mineral concentrations. We acquired the data from EPA's online SADIE database and have included data source information and chemistry data as Appendix E.

4. Results

Note: Sites are referred to by site IDs found in Table 1.

4.1 Physical Habitat

4.1.1 Substrate

Physical habitat surveys revealed relative differences among sites (Tables 3 and 4). While most sites were dominated by cobble sized substrates, a few sites had abundant gravel and pebble (BUaNFA; SFMbC; MILaM; and BEaM). Conversely, several higher elevation sites had abundant boulder sized substrate (AaEU; NFAaBU; WFAaA; WFAaPL). Fine and sand sized particles made up a small proportion of habitat at all sites; only five sites had more than 5% of substrate that was fine and sand. There was a large gradient of embeddedness across sites, mostly driven by metal precipitates and biofilm mats. Embeddedness ranged from 50% at the MFMaM to less than 1% at the AaARR.

4.1.2 Aquatic vegetative cover

We report aquatic vegetative cover as the proportion of the 105 substrates observed during pebble counts that had algae, moss, other aquatic vegetation present (Table 3). Aquatic vegetation was relatively sparse at most sites; 23 of the 28 sites had less than 10% cover of algae, moss, or other aquatic vegetation. Exceptions include MaBG, AaMINN, and MILaM, which had abundant algae, and HERaD and BUaNFA, which had abundant algae and moss.

4.1.3 Bank stability and riparian zone

Most sites had stable banks and intact, undisturbed riparian zones (Table 4). A few sites had eroding banks and/or sparsely vegetated riparian zones (e.g., BEaM; SFMaM; MaMFM) that could be the result of the flood plain geomorphology or metal contamination of overbank sediments. We found that in these high gradient systems, the presence of eroding banks and/or sparsely vegetated riparian zones did not appear to translate to in-channel sediment storage (i.e., sites with low scoring bank stability did not have higher embeddedness or higher percent fines than sites with high scoring bank stability) (Tables 3 and 4).

4.2 BMI Community Composition

4.2.1 Spatial overview

Benthic community composition and abundance varied greatly across BPMD. Metrics of benthic community health and structure revealed high spatial heterogeneity among sites, ranging from sites with diverse communities comprised of more than 25 species (e.g., BEaM) to sparsely populated sites inhabited by only a couple of benthic species (e.g., PLaWFA) (Figures 1-21; Maps 3-10). The Hermosa Creek site (HERaD) will be discussed in a forthcoming EPA document focusing on aquatic risk of the lower reach of the Animas River.

Most BMI metrics, including taxa richness, EPT richness, Hilsenhoff Biotic Index, and density generally depicted a similar spatial pattern. The most robust, diverse benthic communities were observed at sites located on tributaries (Cunningham Creek, Maggie Gulch, Picayne Gulch, Bear Creek, and Mill Creek), on the South Fork of Mineral Creek, and on the uppermost reaches of the South and North Forks of the Animas River. We observed the lowest benthic diversity and abundance in three general areas: 1) at sites on Mineral Creek from the Middle Fork of Mineral Creek to the Animas River; 2) at sites near Animas Forks including the AaEU, NFAaWFA, WFAaA, WFAaPL, PLaWFA, and BUaNFA; and 3) at SFAaEU.

In some cases, we detected large changes in the benthic community within short distances. For example, the benthic community in the South Fork of the Animas River above the Avalanche Zone had sixteen EPT taxa (members of the Ephemeroptera, Plecoptera, and Trichoptera orders, see Table 2), but approximately ½ a mile downstream, immediately above the confluence with Eureka Gulch, there were only eight EPT taxa (Figures 4-6).

4.2.2 Metal-sensitive BMI families

Three families of BMI have been documented as being particularly sensitive to elevated metals: Heptageniidae, Ephemerellidae, and Taeniopterygidae (Courtney and Clements 2002) (Table 2). The presence and diversity of these families varied across sites in BPMD. At some sites, multiple species of the three metal-sensitive families were present (e.g., AaCU) while other sites had no representation by any of the metal-sensitive families (e.g., NFAaWFA) (Figures 10-12; Maps 5-6).

Data revealed a pattern that may indicate a gradient of sensitivity among the three metal-sensitive families. Across all sites, when Ephemerellidae was present, Heptageniidae and Taeniopterygidae were also present. Taeniopterygidae was always present if Heptageniidae was present, but Taeniopterygidae could be present when Heptageniidae was absent (Figures 10-12). This pattern suggests that within BPMD, Ephemerellidae may be more sensitive than Heptageniidae and Heptageniidae may be more sensitive than Taeniopterygidae. There appears to be a strong relationship between the richness of metal sensitive families and the concentration of some metals and minerals across environmental media. We examined patterns of metal concentrations along the following gradient of metal sensitive family richness:

a) *Richness of 5: Sites where all three metal sensitives families are present with some families represented by more than one species;*

b) *Richness of 3: Sites where all three metal sensitive families are present, but each family is only represented by one species;*

c) *Richness of 2: Sites where only two of the three metal sensitive families are present (Heptageniidae and Taeniopterygidae);*

d) *Richness of 1: Sites where only one of the three metal sensitive families are present (Taeniopterygidae);*

e) *Richness of 0: Sites where none of the three metal sensitive families are present.*

We calculated the average metal concentration among sites that shared the same richness of metal sensitive families and found a distinct corresponding increase in the concentration of several metals with each reduction in metal sensitive family richness. For example, we found that aluminum in BMI tissue, dissolved copper in pore water, and dissolved copper in surface water all increase with decreasing metal sensitive family richness (Figures 22-24). Metals and minerals that had higher concentrations with each reduction of metal sensitive family richness included Al, Sb, Be, Hg, Ni, SiO_2, Ag, and Tl in BMI tissue; dissolved Cu, total Fl-, and total and dissolved Mn in pore water; and total and dissolved Al, dissolved Cu, total Fl-, and dissolved Ni in surface water (Tables 5-8). Although we observed this pattern for many metals, it is possible that only a subset of metals that exhibit this pattern are directly causing the reduction in metal sensitive family richness. Several other metals and minerals, such as dissolved zinc in surface water, did not consistently increase with metal sensitive family richness but did increase substantially between a richness of 1 and a richness of 0 (Figure 25), which is consistent with previous research that documented a non-linear relationship between zinc concentrations and BMI community composition (Clements and Kiffney 1995). There was a much weaker relationship between metals and minerals in sediment and metal sensitive family richness.

4.2.3 Functional Feeding Groups

The feeding behaviors exhibited by members of a benthic community can be an indicator of habitat conditions. For example, the absence of 'scraper' taxa (insects that feed on biofilm and/or algae using mouthparts to scrap material from the surface of rocks) can be an indication of metal precipitates coating rock surfaces, preventing growth of algae, and reducing food availability (Clements et al 2000; Hogsden and Harding 2012a). We found higher relative abundances of scrapers at sites located on tributaries (Picayne Gulch, Bear Creek, and Mill Creek), on the Animas River above Cunningham, and on the uppermost reaches of the South and North Forks of the Animas River (Figure 13-15).

4.2.4 Multi-metric Index (MMI)

The MMI was developed by CDPHE to assess the extent to which biological communities may have been altered by environmental stressors and to evaluate whether a water body is in attainment or impairment of designated aquatic life use (CDPHE 2010b). Of the twenty-eight sites where BMI surveys were conducted in 2016, only ten sites are located on reaches that currently have an aquatic life use designation (CDPHE 2016). 2016 MMI scores indicate that three of these sites may be in impairment of their aquatic life use designation: AaARR; MaA; and SFMaM (Figure 16-18 and Maps 7-8).
Using MMI scores we can assess whether the eighteen sites that are located on reaches that currently do not have an aquatic life use designation would be in attainment or impairment, if theoretically, they did have a designation. Three of these sites had MMI scores that would indicate attainment of a class one or class two designation (NFAaBU; EUaSFA; and SFAaAV). Four sites had MMI scores that would indicate attainment of a class two designation, but not a class one designation (AaMINN; SFAaA; MaBG; and MaMIL). The remaining eleven sites had MMI scores that would indicate impairment if they had an aquatic life use designation.

4.3 BMI Tissue Metal Concentrations

The level of metals in macroinvertebrate tissue reflect the overall bioavailability of metals in a system (Kiffney and Clements 1993). Benthic tissue concentrations varied greatly across BPMD, indicating an uneven spatial distribution of metal bioavailability (Table 9). For most metals and minerals, we found the highest concentrations in benthic tissue from the following sites: PLaWFA; SFAaA; BUaNFA; and MaSFM. There were also high levels of Cd at CUaA and at NFAaBU, and high levels of Cu at MaMIL and at EUaSFA. Lead concentrations were highest in benthic tissue from MaMIL, EUaSFA, and MaBG. Zinc concentrations were highest in benthic tissue from SFAaA, AaCU, and AaMINN. Concentrations of metals and minerals were lowest in benthic tissue from SFAaAV, MAGaA, and AaARR.

4.4 Relationship between physical habitat metrics, BMI metrics, and concentrations of metals and minerals in surface water, pore water, and sediment.

We calculated Spearman correlation coefficients between BMI metrics and physical habitat characteristics, such as substrate size distribution and embeddedness (Table 10). Most measures of substrate size distribution (e.g., average substrate size, D50, percent cobble) were poorly correlated with BMI metrics. Exceptions included the percent of substrate composed of fines size class, which had a strong positive correlation with HBI, and the percent of substrate composed of boulder size class, which had a strong negative correlation with collector-gatherers (cg) and a strong positive correlation with shredders (sh).

Several BMI metrics had significant negative correlations with percent embeddedness. Embeddedness of reaches within BPMD was often associated with the visible presence of metal precipitates and biofilm mats, which covered substrate, filled interstitial spaces, and altered benthic habitat (see images 10, 79, 80, 129, 130, 160, 169, 170, 179, 180, 199, 200, 209, 210 in Appendix B). Although metal concentrations of biofilm were not measured in this study, Farag (2007) suggests that biofilm serves as an important exposure pathway for metal uptake by aquatic life.

We found significant positive correlations between percent embeddedness and total and dissolved concentrations of several metals and minerals in surface water, but weaker relationships between percent embeddedness and metals and minerals in sediment and pore water (Table 11). This suggest that one source of embeddedness at these sites could be metal precipitates or biofilm mats derived from surface water metal concentrations.

4.5 Relationship between BMI metrics and concentrations of metal and mineral concentrations in BMI tissue, sediment, pore water, and surface water.

We observed wide variability in BMI community composition throughout BPMD. Although the specific environmental drivers of this variability are unknown, BMI communities are known to be influenced by metal concentrations (Hogsden and Harding 2012b). We calculated Spearman correlation coefficients between BMI metrics and metal concentrations observed in BMI tissue, stream sediment, pore water, and surface water (Tables 12-17). We examined the overall strength of these relationships using cumulative measures across media, such as the average strength of correlations and the number of statistically significant correlations. We assessed which BMI metrics were most strongly correlated with metal conditions, which media (e.g., pore water, surface water, sediment, tissue) were most strongly correlated with BMI metrics, and which metals or minerals were most strongly correlated with BMI metrics (Figures 26-28).

We found statistically significant correlations between BMI metrics and several different metals in BMI tissue, sediment, pore water, and surface water. Although most metals and minerals were negatively correlated with BMI metrics, there were a few exceptions that were positively correlated with BMI metrics (e.g., calcium in sediment was positively correlated with total richness). The strength of correlations differed among the different media and metals. Metals in pore water and surface water were more strongly correlated with BMI metrics than metals in BMI tissue or stream sediment. Similarly, researchers have documented that the primary mode of impairment to BMI communities in the Upper Powell River in Virginia (Schmidt et al. 2002) and the Animas River (Courtney and Clements 2002) was from metals in surface water rather than metal contaminated sediments.

Results from correlation analysis also revealed that some BMI metrics were more strongly correlated with metals in BMI tissue, stream sediment, pore water, and surface water than others. The strongest correlations with metals and minerals across environmental media were found with the following BMI metrics: Total richness, density, MMI, EPT, Metal sensitive family richness, and relative abundance of scraper taxa. We found that HBI, SWDI, and Functional Feeding Groups other than scrapers (cf, cg, o, p, and sh) had weaker correlations with metals.

Of the 17 metals and minerals that were consistently analyzed across all media, Al, Cd, Cu, Fe, Mn, Ni, Pb, and Zn had the strongest correlations with BMI metrics. Silica also had strong correlations with BMI metrics in tissue, pore water, and surface water, but was not analyzed in sediment samples.

4.6 Non-metric multidimensional scaling ordination (NMS)

To further examine the variability in benthic communities across BPMD, we used non-metric multidimensional scaling ordination (NMS), a statistical technique that plots each sample along axes in ordination space that represent gradients in community composition. Thus, points closer together in the plot represent samples that have more similar communities than points further apart. Not all environmental variables were collected at every site, so we produced separate NMS ordinations for each group of sites where an environmental measure was collected consistently. For example, benthic tissue was collected at a subset of sites due to low BMI abundance, so for plots that include benthic tissue concentrations as orthogonal vector lines, we only included those sites where BMI tissue was collected (Table 1; Table 18). For all ordinations, we found that a two-dimensional solution provided the optimal ordination. NMS ordination revealed similarities and differences in benthic community composition among sampled sites (Figures 29-37). It is apparent from ordination that some benthic communities are quite similar in composition despite being located in different watersheds (e.g., MaBG is plotted in close proximity to several Upper Animas sites) (Figure 29). Conversely, benthic communities at some Upper Animas sites are clearly distinct from any Mineral Creek sites (e.g., WFAaWFA, WFAaPL, and NFAaWFA).

Reference sites (BaM, PICaA, MAGaA, MILaM, and NAaBU), thought to be minimally influenced by mining-related metals, were clustered in the lower left corner of the NMS plot, indicating similarity in benthic community composition (Figure 30). Interestingly, SAaAV and MaBG are plotted closer to the reference sites than to other South Fork Animas or Mineral Creek sites. This suggests that the benthic communities of these two locations are more similar to benthic communities found at the reference sites than to the benthic communities found just downstream at SAaEU or MaMF. Of the mainstem Animas River sites, AaCU had a benthic community most similar to those found at the reference sites. Benthic communities at Cunningham Creek, CUaA, were also similar to those from reference sites.

As discussed in section 4.2.4, not all sites sampled are located within reaches that are designated for aquatic life use. However, coding the sample sites in ordination space by whether they would or would not meet MMI attainment of class two aquatic life use indicates a relatively clear separation between sites. Benthic communities from impaired sites, except for SMaM, are clearly dissimilar to those from attainment sites, as evidenced by the separation between the groups in ordination space (Figure 31). Ordination plots also reveal that sites with less than two taxa from metal sensitive families have benthic communities that differ from sites with two or more taxa from metal sensitive families (Figure 32).

Environmental variables, such as surface water metal concentrations, can be added to ordination plots as orthogonal vector lines to visualize correlations between these variables and benthic community composition (Figures 33-37). The angle and length of the orthogonal vector lines reflect the direction and strength of the relationship between the variable and ordination axes (Peck 2016). The orientation of vector lines can reveal the relationship between environmental variables. Vector lines that are parallel to one another and are pointing in the same direction as one another are likely correlated.

Several environmental variables had strong correlations (>=0.4 r^2) with NMS ordination, which suggests that these factors may be directly or indirectly responsible for the differences in benthic communities we observed in BPMD (Figures 33-37; Table 19). Among physical habitat variables, only embeddedness had a strong correlation with NMS ordination (Figure 33). The concentration of calcium in sediment had a strong correlation with NMS ordination, and the orthogonal vector line indicates that higher calcium in sediment was more associated with the healthier benthic communities that are depicted on the left-hand side of the ordination plot (Figure 34). There were several metals and minerals in surface water, pore water, and BMI tissue that correlated strongly with NMS ordination, separating most of the impaired sites from all other sites along axis one (Figures 35-37). Interestingly, copper and cadmium had strong correlations with BMI community composition in surface water and pore water, but not in BMI tissue. This could either indicate that BMI uptake of copper and cadmium is limited or that the copper and cadmium body burden of individuals does not scale up to population level effects that could shift the composition of benthic communities.

In summary, NMS ordination described the differences in benthic communities across BPMD along two axes. Most of the observed variability among benthic communities was explained by axis one (Table 18), which was strongly correlated with vectors of several environmental parameters (Table 19; Figures 33-37). Axis two accounted for a much smaller proportion of the variability in benthic communities and the separation of sites along axis two was not correlated with any measured environmental parameter (Table 18).

5. Conclusions, Research Recommendations, and Further Questions

We found that BMI communities within BPMD vary from reach to reach, ranging from sparsely populated sites with substantial metal contamination to sites with abundant, diverse communities that reflect minimal exposure to metal contamination. In several locations, we documented a shift from a healthy BMI community to a community diminished in diversity and abundance, occurring over a relatively short distance. For example, BMI metrics and NMS indicate, respectively, that there is an abrupt decline in the health of BMI communities and an abrupt shift in community composition from SFAaAV to SFAaEU, from MaBG to MaMFM, and from NFAaBU to NFAaWFA.

We used two approaches to examine the influence of environmental factors on benthic communities in BPMD. The first approach assessed statistical correlations between BMI metrics and measured environmental variables (e.g. physical habitat, metal and mineral concentrations of BMI tissue, sediment, pore water, and surface water). The second approach used NMS ordination to examine the relationship between benthic community composition (taxa counts per site) and the same set of measured environmental variables. There was broad overlap in the results between the two approaches. We found that both approaches suggested that metal and mineral concentrations in surface water were more strongly correlated with benthic communities than other environmental variables, suggesting that of the variables we assessed, surface water may be the most important exposure pathway to BMIs. Concentrations of several metal and minerals in pore water were correlated with BMI metrics, but NMS analysis indicated that only pore water concentrations of cadmium and copper were strongly correlated with BMI community composition. Both approaches indicated that metal and mineral concentrations in sediment may be less influential on benthic communities than surface water or pore water, which is consistent with previous research (Courtney and Clements 2002; Schmidt et al 2002). The two approaches indicated that the concentrations of metals in BMI tissue were strongly tied with BMI metrics and benthic community composition. Among physical habitat variables, both approaches indicated that embeddedness may have the greatest influence on benthic community composition.

Through NMS and statistical correlation, we found strong relationships between the concentration of several metals and minerals and the composition of BMI communities. However, we also found strong correlations among metals within each media, suggesting that many metals co-occur with one another (Tables 20-25). The co-occurrence of metals may limit our ability to determine which metals are driving the distribution and structure of benthic communities. For example, in surface water, both cadmium and nickel were strongly correlated with BMI metrics (Tables 16-17) and BMI community composition (Table 19 and Figure 36), but were also strongly correlated to one another (Tables 24-25). This suggests a number of possible scenarios including: a) cadmium and nickel are both occurring at levels that negatively affect benthic communities; b) cadmium and nickel are co-occurring, but only cadmium is occurring at levels that negatively affect benthic communities; or c) cadmium and nickel are co-occurring, but only nickel is occurring at levels that negatively affect benthic communities. One approach that can be used to determine whether a metal is occurring at a level that has potential ecological consequence is to compare the observed concentration to water quality standards. CDPHE has developed water quality standards that are designed to protect aquatic life from acute (brief, short-term) and chronic (persistent, long-term) exposure to many metals in surface water (CDPHE 2017). In table 26, we present a comparison of observed metal concentrations to water quality standards for each site, expressed as a hazard quotient (HQ). We calculated HQs as the ratio of measured exposure (observed metal concentration) to CDPHE basic water quality standards. HQ values equal to or greater than 1.0 indicate a potential for ecological risk and HQ values below 1.0 indicate a low probability of ecological risk. The results of HQ analysis suggest that there is potential for ecological risk at several sites for Al, Cd, Cu, Fe, Pb, Mn, and Zn, but not for As, Cr, Ni, Ag, or Tl. When considered in conjunction with results from statistical correlation and NMS, it appears that surface water concentrations of Al, Cd, Cu, and Zn likely shape the distribution and community composition of BMI populations in BPMD. Surface water concentrations of Fe, Mn, and Pb were correlated with BMI metrics, and at some sites had HQ values indicating a potential for ecological risk, but were not identified by NMS as having as strong a relationship with BMI community composition as Al, Cd, Cu, and Zn did. Maps depicting summed acute and chronic hazard quotients for each site are included as Maps 11-14.

The use of hazard quotients to determine whether a metal that correlated with BMI metrics may be ecological significant is a limited approach in that CDPHE water quality standards for aquatic life have only been developed for surface water and do not include all parameters assessed in this study (e.g., Be, F-, and SiO_2). Further research, such as experimental bioassays, could confirm if these and other metals that correlated with BMI metrics have a direct causative effect on BMI communities.
The potential sensitivity gradient of metal sensitive families (Heptageniidae, Ephemerellidae, and Taeniopterygidae), as discussed in Section 4.2.2 of this report, may have implications for future monitoring. The loss or addition of each of the three metal-sensitive families over time could be a valuable indicator for assessing the successfulness of remediation efforts. For example, Ephemerellidae and Heptageniidae were absent from MaMFM in 2016, but Taeniopterygidae was present. If future surveys found that Heptageniidae was present in addition to Taenipterygidae, it would suggest improving conditions. However, if future surveys found that Taeniopterygidae was no longer present, it would suggest deteriorating conditions.

Monitoring of BMI communities across a gradient of metal exposure will be an important component of assessing the successfulness of remediation efforts. Long-term, annual monitoring of BMI communities is essential in order to differentiate the direct effects of remediation from natural variability of communities (Anderson 2007a; Chapman 1999; Mazor et al. 2009; Resh et al. 2013). Clements and others (2010) documented the ability to use long-term monitoring to detect improvements in the health of benthic communities following the implementation of remediation projects designed to reduce metal exposure to aquatic life. We recommend an annual, long-term monitoring program in BPMD that targets a subset of the 2016 sites across a gradient of metal exposure. Sites selected for continued monitoring should be located in close downstream proximity to substantial remediation activities. In addition, we recommend that Animas River sites downstream of Silverton be included in a long-term monitoring plan to determine if remediation efforts translate to down-canyon improvement in the health of aquatic life. Monitoring assessment should focus on BMI metrics that most strongly correlated with metal exposure, which include total richness, density, EPT, MMI, and the richness of metal sensitive families.

In Summary:

- BMI communities within BPMD vary from reach to reach, ranging from sparsely populated sites with substantial metal contamination to sites with abundant, diverse communities that reflect minimal exposure to metal contamination.

- The most robust, diverse benthic communities were observed at sites located on tributaries (Cunningham Creek, Maggie Gulch, Picayne Gulch, Bear Creek, and Mill Creek), on the South Fork of Mineral Creek, and on the uppermost reaches of the South and North Forks of the Animas River. We observed the lowest benthic diversity and abundance in three general areas: 1) at sites on Mineral Creek from the Middle Fork of Mineral Creek to the Animas River; 2) on the South Fork of the Animas River immediately upstream of the confluence with Eureka Gulch; and 3) at sites near Animas Forks and throughout the West Fork of the Animas River.

- In some locations, healthy BMI communities sharply decline in diversity and abundance over a relatively short distance.

- Several sites that currently do not have an aquatic life use designation had benthic communities that, based on MMI scores, would be in attainment of a class two aquatic life use designation. Many sites in BPMD have benthic communities that would not meet an aquatic life use designation.

- We found a gradient of sensitivity among the three metal-sensitive BMI families that directly corresponds with increases in several metals and minerals in BMI tissue, pore water, and surface water. The loss or addition of each of the three metal-sensitive families over time could be a valuable indicator for assessing the successfulness of remediation efforts.

- We used statistical correlation and NMS to examine the correlations between environmental factors and benthic communities in BPMD. There was broad agreement between the two methods that surface water and pore water metal and mineral concentrations more strongly influenced BMI communities than other environmental parameters. There was a weaker relationship between BMI communities and concentrations of metals and minerals in sediment.

- Multiple lines of evidence, including statistical correlation, NMS, and hazard quotients, suggest that surface water concentrations of Al, Cd, Cu, and Zn likely shape the distribution and community composition of BMI populations across BPMD. Further research, such as experimental bioassays, could confirm if these and other metals that correlated with BMI metrics have a direct causative effect on BMI communities.

6. Works Cited

Anderson, C. 2000. Monitoring macroinvertebrates to assess the effectiveness of mine site remediation. *In* Use Attainability Analysis for the Animas River Watershed - Appendix 6-B. 2001. Animas River Stakeholders Group.

Anderson, C. 2007a. Effects of mining on benthic macroinvertebrate communities and monitoring strategy. *In* S. Church, P. von Guerard, and S. Finger (Eds.), Integrated Investigations of Environmental Effects of Historical Mining in the Animas River Watershed, San Juan County, Colorado. USGS Professional Paper 1651.

Anderson, C. 2007b. Nutrient loading, periphyton biomass, and macroinvertebrate community composition, Animas River Colorado, Southern Ute Indian Tribe, New Mexico. Prepared for the Animas River Nutrient Workgroup, Durango, CO.

Anderson, C. 2013. Personal communication, 10/8/2013.

Barbour, M., J. Gerritsen, B. Snyder, and J. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, second edition. EPA 841-B-99-002. US Environmental Protection Agency; Office of Water; Washington, D.C.

Besser, J. and W. Brumbaugh. 2007. Status of stream biotic communities in relation to metal exposure. *In* S. Church, P. von Guerard, and S. Finger (Eds.), Integrated Investigations of Environmental Effects of Historical Mining in the Animas River Watershed, San Juan County, Colorado. USGS Professional Paper 1651.

Besser, J., S. Finger, and S. Church. 2007. Impacts of historical mining on aquatic ecosystems—An ecological risk assessment. *In* S. Church, P. von Guerard, and S. Finger (Eds.), Integrated Investigations of Environmental Effects of Historical Mining in the Animas River Watershed, San Juan County, Colorado. USGS Professional Paper 1651.

Besser, J. and K. Leib. 2007. Toxicity of metals in water and sediment to aquatic biota. *In* S. Church, P. von Guerard, and S. Finger (Eds.), Integrated investigations of environmental effects of historical mining in the Animas River watershed, San Juan County, Colorado. USGS Professional Paper 1651.

Carlisle, D, and W. Clements. 2003. Growth and secondary production of aquatic insects along a metal pollution gradient in Rocky Mountain streams. Journal of North American Benthological Society, 22: 582-597.

Carlisle, D., and W. Clements. 2005. Leaf litter breakdown, microbial respiration, and shredder production in metal-polluted streams. Freshwater Biology, 50: 380-390.

Chapman, M. 1999. Improving sampling design for measuring restoration in aquatic habitats. Journal of Aquatic Ecosystem Stress and Recovery, 6: 235–251.

Clark, J., and W. Clements. 2006. The use of in situ and stream microcosm experiments to assess population- and community-level responses to metals. Environmental Toxicology and Chemistry, 25(9):2306-2312.

Clements, W., D. Carlisle, J. Lazorchak, and P. Johnson. 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecological Applications, 10:626-638.

Clements, W. and P. Kiffney. 1995. The influence of elevation on benthic community responses to heavy metals in Rocky Mountain streams. Canadian Journal of Fisheries and Aquatic Sciences, 52: 1966-1977.

Clements, W., N. Vieira, and S. Church. 2010. Quantifying restoration success and recovery in a metal-polluted stream: a 17-year assessment of physiocochemical and biological responses. Journal of Applied Ecology, 47(4): 899-910.

Colorado Department of Public Health and Environment (CDPHE). 2010a. Benthic macroinvertebrate sampling protocols. Water Quality Control Division – standard operation procedure. WQCDSOP-001.

CDPHE. 2010b. Aquatic life use attainment, methodology to determine use attainment for rivers and streams. Policy Statement 10-1.

CDPHE. 2015. Standard operating procedure for the collection of pebble counts. Water Quality Control Division. https://www.colorado.gov/pacific/sites/default/files/WQ_NonpointSoruce-SOP-Collection-of-Pebble-Counts-051215.pdf. Accessed 9/20/2016.

CDPHE. 2016. Regulation No. 34 – Classifications and Numeric Standards for San Juan River and Dolores River Basins (5 CCR 1002-34), effective 6/30/2016.

Colorado Department of Public Health and Environment (CDPHE). 2017. Regulation No. 31 - The Basic Standards and Methodologies for Surface Water (5 CCR 1002-31), effective 3/1/2017.

Courtney, L. and W. Clements. 2002. Assessing the influence of water and substratum quality on benthic macroinvertebrate communities in a metal-polluted stream: an experimental approach. Freshwater Biology, 47: 1766-1778.

Environmental Protection Agency (EPA). 2015. DRAFT baseline ecological risk assessment. US Environmental Protection Agency Region 8. DCN: EP8-2-1222.

EPA. 2016. Sampling and Analysis Plan/Quality Assurance Project Plan, Fall 2016/Sampling Event, Bonita Peak Mining District. DCN: EP8-4-1081.

Farag, A., D. Nimick, B. Kimball, S. Church, D. Harper, and William Brumbaugh. 2007. Concentrations of metals in water, sediment, biofilm, benthic macroinvertebrates, and fish in the Boulder River watershed, Montana. Archives of Environmental Contamination and Toxicology, 52: 397-409.

Hilsenhoff, W. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomologist, 20(1): 31-39.

Hogsden, K. and J. Harding. 2012a.. Anthropogenic and natural sources of acidity and metals and their influence on the structure of stream food webs. Environmental Pollution, 162: 466–472.

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

JMP, Version 10. 2013. SAS Institute Inc., Cary, NC.

Kiffney, P., and W. Clements. 1993. Bioaccumulation of heavy metals by benthic invertebrates at the Arkansas River, Colorado. Environmental Toxicology and Chemistry, 12(8): 1507-1517.

Mazor, R., A. Purcell, and V. Resh. 2009. Long-term variability in bioassessments: a twenty-year study from two northern California streams. Environmental Management, 43(6): 1269-1286.

Maret, T, D. Cain, D. MacCoy, and T. Short. Response of benthic macroinvertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA. Journal of North American Benthological Society, 22 (4): 598-620.

McCune, B., Mefford, M.J. 1999. PC-ORD. Multivariate analysis of ecological data. Version 4.0. MjM Software, Gleneden Beach, Oregon, USA.

Merrit, R., and K. Cummings. 1996. An introduction to the aquatic insects of North America (3rd edition). Kendall/Hunt Publ. Co., Dubuque, Iowa. USA. 862 p.

Peck, J. 2016. Multivariate Analysis for Ecologists: Step-by-Step, Second edition. MjM Software Design, Gleneden Beach, OR. 192 pp.

Resh, V., L. Beche, J. Lawrence, R. Mazor, E. McElravy, A. O'Dowd, D. Rudnick, and S. Carlson. 2013. Long-term population and community patterns of benthic macroinvertebrates and fishes in Northern California Mediterranean-climate streams. Hydrobiologia, 719(1): 93-118.

Roberts, S. 2015. Lower Animas Bioassessment: 2014 BMI data in context of historical data. Prepared for Trout Unlimited, 5 Rivers Chapter, Durango, CO.

Roberts, S. 2016. Animas River 2015 benthic macroinvertebrate (BMI) report, Gold King Mine release monitoring. Mountain Studies Institute.

Schmidt, T., D. Soucek, and D. Cherry. 2002. Integrative assessment of macroinvertebrate community impairment from metal contaminated waters in tributaries of the Upper Powell River, Virginia, USA. Environmental Toxicology and Chemistry, 21(10): 2233-2241.

Shannon, C. 1948. A mathematical theory of communication. The Bell System Technical Journal 27: 379-423 and 623-656.

Smith, N. 1976. Aquatic inventory: Animas La Plata Project. Denver, Colorado. Colorado Division of Wildlife.

7. Tables

Table 1. Benthic macroinvertebrate monitoring sites surveyed in 2016.

ID	EU	Stream Name	Site Name	Lat	Long	Date Collected	BMI Sample Type	2016 EPA Data
Mineral Cr								
MaA	EU1	Mineral Cr	Above Animas River	37.80289	-107.6724994	10/12/16	C+T	SW, PW, Sed
MaSFM	EU2	Mineral Cr	Above SF Mineral	37.82191	-107.7194105	10/13/16	C+T	SW, PW, Sed
MaMFM	EU3	Mineral Cr	Above MF Mineral	37.84647	-107.7285496	10/14/16	C+T	SW, PW, Sed
MaBG	EU3	Mineral Cr	Above Browns Gulch	37.85640	-107.72626	10/17/16	C+T	-
MaMIL	EU4	Mineral Cr	Above Mill Creek	37.87201	-107.7239838	10/17/16	C+T	SW, PW, Sed
SFMaM	EU5	SF Mineral Cr	Above Mineral Creek	37.81831	-107.7194105	10/13/16	C+T	SW, PW, Sed
SFMbC	EU5	SF Mineral Cr	Below Campground	37.80470	-107.76956	10/13/16	C+T	-
MFMaM	EU6	MF Mineral Cr	Above Mineral Creek	37.84529	-107.7413355	10/14/16	C	SW, PW, Sed
Upper Animas								
AaARR	EU7	Animas River	Above Arrastra Creek	37.82765	-107.624209	10/2/16	C+T	SW, PW, Sed
CUaA	EU8	Cunningham Creek	Above Animas River	37.83516	-107.595011	10/4/16	C+T	SW, PW, Sed
AaCU	EU9	Animas River	Above Cunningham	37.84303	-107.589894	10/4/16	C+T	SW, PW, Sed
AaMINN	EU10	Animas River	Above Minnie Gulch	37.86332	-107.571627	10/11/16	C+T	SW, PW, Sed
SFAaEU	EU11	SF Animas River	Above Eureka Gulch	37.88293	-107.592597	10/8/16	C	SW, PW, Sed
SFAaAV	EU11	SF Animas River	Above Avalanche Zone	37.87758	-107.59838	10/8/16	C+T	SW
EUaSFA	EU12	Eureka Gulch	Above SF Animas	37.88483	-107.591341	10/6/16	C+T	SW, PW, Sed
SFAaA	EU13	SF Animas River	Above Animas River	37.87948	-107.567451	10/6/16	C+T	SW, PW, Sed
AaEU	EU14	Animas River	Above Eureka	37.88174	-107.564966	10/11/16	C+T	SW, PW, Sed
WFAaA	EU15	WF Animas River	Above Animas River	37.93191	-107.570513	10/7/16	C	SW, PW, Sed
PLaWFA	EU16	Placer Gulch	Above WF Animas River	37.92819	-107.58869	10/10/16	C+T	SW, PW, Sed
WFAaPL	EU17	WF Animas River	Above Placer Gulch	37.93148	-107.589986	10/10/16	C	SW, PW, Sed
NFAaWFA	EU18	NF Animas River	Above WF Animas River	37.93179	-107.569852	10/7/16	C	SW, PW, Sed
BUaNFA	EU19	Burrows Creek	Above NF Animas River	37.94568	-107.575561	10/7/16	C+T	SW, PW, Sed
Reference Sites								
MILaM	-	Mill Creek	Above Mineral Creek	37.87266	-107.7360692	10/17/16	C+T	SW, PW, Sed
BEaM	-	Bear Creek	Above Mineral Creek	37.81317	-107.6964562	10/12/16	C+T	SW, PW, Sed
MAGaA	-	Maggie Gulch	Above Animas River	37.85424	-107.57163	10/4/16	C+T	SW, PW, Sed
PICaA	-	Picayne Gulch	Above Animas River	37.91139	-107.555845	10/5/16	C+T	SW, PW, Sed
NFAaBU	-	NF Animas River	Above Burrows Creek	37.94917	-107.573613	10/7/16	C+T	SW, PW, Sed
HERaD	-	Hermosa Creek	Above Ditch	37.42196	-107.845217	9/30/16	C+T	SW, PW, Sed

Note: ID = Site ID used in this document based on Stream Name and Site Name; EU = Exposure Unit; Lat/Long in NAD83; C+T = indicates sites where BMI community and tissue data was collected; C = sites where BMI community data was collected and tissue data was not collected; SW=surface water, PW=pore water, and Sed=sediment data was collected by EPA in the fall of 2016 (see Appendix E). Physical habitat data was collected at all sites.

Table 2. BMI metrics.

BMI Metric	Metric Description	Justification and Source
Metal Sensitive Families	Heptageniidae Richness : Total # of unique taxa units (richness) that are members of the Heptageniidae family of mayflies.	Heptageniid mayflies are particularly sensitive to elevated metals in the Animas River (Courtney and Clements 2002) and elsewhere in Colorado and the Rocky Mountains (Kiffney and Clements 1993; Clements and Kiffney 1995; Clements et al. 2000; Besser and Leib 2007; Carlisle and Clements 2003). *Epeorus* occurs at lower abundances on contaminated substrate from the Animas River (Courtney and Clements 2002).
	Ephemerellidae Richness: Total # of unique taxa units (richness) that are members of the Ephemerellidae family of mayflies.	Ephemerellid mayflies are particularly sensitive to elevated metals in Animas River water and contaminated substrate, especially *Drunella doddsi* (Courtney and Clements 2002), and at other locations (Kiffney and Clements 1993; Besser and Leib 2007; Clark and Clements 2006).
	Taeniopterygidae Richness: Total # of unique taxa units (richness) that are members of the Taeniopterygidae family of winter stoneflies.	Taeniopterygid stoneflies are particularly sensitive to elevated metals in Animas River water and contaminated substrate (Courtney and Clements 2002) and elsewhere in Colorado (Carlisle and Clements 2005).
EPT Richness	Total # of unique taxa units that are members of the orders Ephemeroptera (mayfly), Plecoptera (stonefly), and Trichoptera (caddisfly).	EPT taxa are generally considered to be sensitive to degraded water quality, including elevated metals (Maret et al. 2003). Ephemeroptera are more sensitive to metals than Plecoptera or Trichoptera (Clements et al. 2000).
Taxa Richness	Total # of distinct taxa units.	Taxa richness has been found to be reduced in streams with elevated metal concentrations (Maret et al. 2003).

Table 2 (cont.)

<table>
<tr><th>BMI Metric</th><th colspan="2">Metric Description, Justification, and Source</th></tr>
<tr><td>Functional Feeding Groups - Relative abundance of scraper taxa</td><td>Proportion of BMI community composed of scraper taxa</td><td>Functional Feeding Groups include collector-filterers (cf), collector-gatherers (cg), omnivores (o), predators (p), scrapers (sc), and shredders (sh).

The absence of scraper taxa, insects that feed on biofilm and/or algae using mouthparts to scrap material from the surface of rocks, can be an indication of metal precipitates that coat rock surfaces, prevent the growth of algae, and reduce food availability (Carlisle and Clements 2005; Clements et al 2000; Hogsden and Harding 2012a).</td></tr>
<tr><td>Multi-metric Index (MMI)</td><td colspan="2">MMI is a bioassessment tool developed by Colorado Water Quality Control Division and the Environmental Protection Agency (CDPHE 2010b). MMI quantifies the extent to which biological communities may have been altered by environmental stressors. MMI scores are evaluated in context to MMI scores from known reference sites and stressed sites in Colorado. CDPHE (2010b) provides MMI thresholds that can be used to evaluate whether a water body is in attainment or impairment of designated aquatic life use. A MMI score that is below the attainment threshold is evidence that the site is not supportive of aquatic life use. Additional metrics (e.g., HBI) are used to determine whether a site with a MMI score that falls between the attainment and impairment threshold should be considered impaired. The attainment threshold varies according to the biotype and class that the water body is located in. See CDPHE 2010b for more details.</td></tr>
<tr><td>Hilsenhoff Biotic Index (HBI)</td><td colspan="2">HBI is an index of the overall tolerance of a community to degraded water quality and is based on taxon-specific tolerance values and their relative abundance within the sample (Hilsenhoff 1987). The index value ranges from 0 (more sensitive) to 10 (more tolerant).</td></tr>
<tr><td>Shannon-Weaver Diversity Index (SWDI)</td><td colspan="2">SWDI is a measure of the diversity and evenness of a community (Shannon 1948).</td></tr>
</table>

Table 3. Physical habitat results – substrate size, embeddedness, and vegetative cover.

Site				Substate Size (mm)						Substrate Class Size (%)						Embeddedness	Proportion of substrates with vegetative cover (%)		
ID	**EU**	**Stream Name**	**Site Name**	**Average size**	**Standard Deviation**	**25th Percentile**	**50th Percentile**	**75th Percentile**	**90th Percentile**	**Fines**	**Sand**	**Gravel**	**Pebble**	**Cobble**	**Boulder**	**Average Embeddedness**	**Algae**	**Moss**	**Veg**
Mineral Cr																			
MaA	EU1	Mineral Cr	Above Animas River	130.81	84.66	70	105	190	250	0.00	0.00	0.00	22.86	67.62	9.52	19.25	0.00	0.00	0.00
MaSFM	EU2	Mineral Cr	Above SF Mineral	112.93	80.57	50	100	180	210	10.48	0.00	0.95	16.19	65.71	6.67	40.43	0.00	0.00	0.00
MaMFM	EU3	Mineral Cr	Above MF Mineral	191.48	164.70	80	130	250	399	0.95	0.00	2.86	15.24	57.14	23.81	11.10	0.00	0.00	0.00
MaBG	EU3	Mineral Cr	Above Browns	202.15	201.24	70	140	290	410	0.95	0.00	4.76	18.10	50.48	25.71	1.83	52.38	0.00	0.00
MaMIL	EU4	Mineral Cr	Above Mill Creek	101.87	71.50	50	80	130	200	2.86	0.00	3.81	22.86	66.67	3.81	23.47	0.00	0.00	0.00
SFMaM	EU5	SF Mineral Cr	Above Mineral Creek	123.99	101.78	45	95	180	290	2.86	0.00	5.71	23.81	53.33	14.29	23.93	0.00	0.00	0.00
SFMbC	EU5	SF Mineral Cr	Blw Campground	99.31	84.98	45	80	125	190	0.00	0.00	4.95	32.67	57.43	4.95	10.69	0.00	0.00	0.00
MFMaM	EU6	MF Mineral Cr	Above Mineral Creek	104.08	75.83	45	100	150	210	8.57	0.00	4.76	21.90	61.90	2.86	50.00	0.00	0.00	0.00
Upper Animas																			
AaARR	EU7	Animas River	Above Arrastra Creek	161.29	118.22	80	130	210	346	1.90	0.00	0.95	18.10	61.90	17.14	0.49	5.71	0.00	0.00
CUaA	EU8	Cunningham Creek	Above Animas River	198.67	163.49	80	145	300	396	0.00	0.00	3.81	15.24	51.43	29.52	9.07	0.95	0.00	0.95
AaCU	EU9	Animas River	Above Cunningham	92.67	49.19	60	90	120	150	1.90	0.00	2.86	20.95	73.33	0.95	2.81	5.71	0.00	0.95
AaMINN	EU10	Animas River	Above Minnie Gulch	131.16	96.73	60	110	200	246	13.33	0.00	0.00	12.38	64.76	9.52	30.14	33.33	0.00	0.00
SFAaEU	EU11	SF Animas River	Above Eureka Gulch	203.06	143.64	110	180	290	380	5.71	0.00	0.00	11.43	52.38	30.48	22.98	0.00	0.00	0.00
SFAaAV	EU11	SF Animas River	Above Avalanche Zone	155.86	121.14	70	130	200	306	0.00	0.00	5.71	17.14	61.90	15.24	0.91	0.00	0.00	0.00
EUaSFA	EU12	Eureka Gulch	Above SF Animas	262.06	277.79	90	170	320	648	6.67	0.00	0.95	7.62	52.38	32.38	15.98	0.95	0.00	0.00
SFAaA	EU13	SF Animas River	Above Animas River	131.86	79.93	75	120	180	250	1.90	0.00	0.00	16.19	72.38	9.52	16.76	0.00	0.00	0.00
AaEU	EU14	Animas River	Above Eureka Gulch	271.43	216.89	100	210	350	573	0.00	0.00	0.00	6.67	51.43	41.90	35.45	0.00	0.00	0.00
WFAaA	EU15	WF Animas River	Above Animas River	302.15	321.27	80	160	330	1000	1.90	0.00	0.00	10.48	48.57	39.05	26.76	0.00	0.00	0.00
PLaWFA	EU16	Placer Gulch	Above WF Animas River	179.57	232.28	50	95	200	342	1.90	0.00	4.76	28.57	44.76	20.00	38.20	0.00	0.00	0.00
WFAaPL	EU17	WF Animas River	Above Placer Gulch	358.00	396.51	70	140	1000	1000	0.95	0.00	1.90	14.29	45.71	37.14	26.94	0.00	0.00	0.00
NFAaWFA	EU18	NF Animas River	Above WF Animas River	294.33	357.31	70	110	290	1000	0.95	0.00	0.95	19.05	49.52	29.52	28.07	0.00	0.00	0.95
BUaNFA	EU19	Burrows Creek	Above NF Animas River	46.14	34.32	20	35	60	90	0.00	0.00	14.29	60.95	24.76	0.00	3.75	46.67	10.48	0.00
Reference Sites																			
MILaM	-	Mill Creek	Above Mineral Creek	113.69	91.51	45	90	160	250	0.00	0.00	7.62	29.52	54.29	8.57	6.36	21.90	0.00	0.00
BEaM	-	Bear Creek	Above Mineral Creek	114.36	84.57	45	100	170	226	0.00	0.00	9.52	24.76	58.10	7.62	2.02	0.00	0.00	0.00
MAGaA	-	Maggie Gulch	Above Animas River	224.76	162.27	90	190	330	420	0.00	0.00	0.95	12.38	50.48	36.19	0.98	0.00	0.00	0.00
PICaA	-	Picayne Gulch	Above Animas River	192.95	131.24	70	170	290	376	0.95	0.00	0.00	16.19	51.43	31.43	3.63	0.00	0.00	0.00
NFAaBU	-	NF Animas River	Above Burrows Creek	451.20	640.99	85	140	1000	1000	3.81	0.00	0.95	15.24	40.00	40.00	4.02	5.71	0.00	0.00
HERaD	-	Hermosa Creek	Above Ditch	164.93	106.19	100	140	220	292	4.76	0.95	0.00	4.76	77.14	12.38	22.06	47.62	30.48	0.00

Table 4. Physical habitat results – habitat evaluation.

Site				Habitat Evaluation Parameters										
				Available Cover	Embedded -ness	Velocity/ Depth	Sediment	Channel Flow	Channel Alteration	Riffle Frequency	Bank Stability	Vegetative Protection	Riparian Zone	Average Score
ID	EU	Stream Name	Site Name	1	2	3	4	5	6	7	8	9	10	-
Mineral Cr														
MaA	EU1	Mineral Cr	Above Animas River	15	14	12	15	15	18	19	18	18	10	15
MaSFM	EU2	Mineral Cr	Above SF Mineral	17	7	14	10	12	18	19	16	16	10	14
MaMFM	EU3	Mineral Cr	Above MF Mineral	17	16	16	17	15	19	19	10	12	18	16
MaBG	EU3	Mineral Cr	Above Browns	17	19	19	19	16	19	18	15	16	17	18
MaMIL	EU4	Mineral Cr	Above Mill Creek	14	15	10	15	10	19	18	14	14	14	14
SFMaM	EU5	SF Mineral Cr	Above Mineral Creek	19	14	19	16	11	20	19	9	16	16	16
SFMbC	EU5	SF Mineral Cr	Blw Campground	17	18	16	18	11	16	19	16	14	11	16
MFMaM	EU6	MF Mineral Cr	Above Mineral Creek	15	11	16	15	14	14	19	18	18	18	16
Upper Animas														
AaARR	EU7	Animas River	Above Arrastra Creek	16	18	13	18	18	20	18	16	16	15	17
CUaA	EU8	Cunningham Creek	Above Animas River	13	18	8	18	18	18	18	14	11	8	14
AaCU	EU9	Animas River	Above Cunningham	17	16	18	15	12	18	18	13	15	15	16
AaMINN	EU10	Animas River	Above Minnie Gulch	10	10	11	10	10	18	18	12	12	12	12
SFAaEU	EU11	SF Animas River	Above Eureka Gulch	17	11	16	16	14	20	19	20	18	18	17
SFAaAV	EU11	SF Animas River	Above Avalanche Zone	17	20	16	19	17	20	19	14	12	20	17
EUaSFA	EU12	Eureka Gulch	Above SF Animas	8	15	8	16	17	18	13	14	10	18	14
SFAaA	EU13	SF Animas River	Above Animas River	13	20	11	13	20	20	13	20	20	20	17
AaEU	EU14	Animas River	Above Eureka Gulch	16	15	18	18	14	20	19	18	16	16	17
WFAaA	EU15	WF Animas River	Above Animas River	17	12	15	16	15	19	17	18	18	20	17
PLaWFA	EU16	Placer Gulch	Above WF Animas River	16	12	15	16	14	19	17	18	18	20	17
WFAaPL	EU17	WF Animas River	Above Placer Gulch	15	9	10	16	15	19	19	18	18	20	16
NFAaWFA	EU18	NF Animas River	Above WF Animas River	15	11	12	13	9	19	18	20	18	16	15
BUaNFA	EU19	Burrows Creek	Above NF Animas River	16	19	15	18	16	20	15	18	20	16	17
Reference Sites														
MILaM	-	Mill Creek	Above Mineral Creek	18	18	16	17	15	19	18	10	17	14	16
BEaM	-	Bear Creek	Above Mineral Creek	19	20	15	19	15	20	19	4	16	20	17
MAGaA	-	Maggie Gulch	Above Animas River	13	18	13	18	18	18	13	20	18	20	17
PICaA	-	Picayne Gulch	Above Animas River	16	16	16	15	15	20	19	17	20	19	17
NFAaBU	-	NF Animas River	Above Burrows Creek	15	16	18	16	16	20	20	20	20	20	18
HERaD	-	Hermosa Creek	Above Animas River	16	13	14	10	10	20	18	17	20	16	15

Note: See Barbour and others (1999) for a definition of habitat evaluation parameters. Numerical scores are scaled from 1 (poor) to 20 (good).

Table 5. Average metal or mineral concentration of BMI tissue among sites with the same metal-sensitive family richness.

		Average metal or mineral concentration of sites with a metal-sensitive family richness of…					Was there an incremental increase in concentration with each reduction of metal-sensitive family richness?
		5	3	2	1	0	
Tissue (mg/kg)	Aluminum	232.32	323.32	548.83	640.00	960.50	**Yes**
	Antimony	0.11	0.13	0.16	0.69	1.26	**Yes**
	Arsenic	0.80	0.37	0.75	1.50	2.48	-
	Beryllium	0.53	0.63	0.75	3.45	6.29	**Yes**
	Cadmium	0.76	0.45	0.63	0.20	0.32	-
	Calcium	329.00	548.67	176.33	131.00	314.00	-
	Chromium	0.55	0.58	0.49	1.54	2.51	-
	Copper	11.32	12.60	31.35	9.78	17.85	-
	Iron	325.67	741.28	613.47	3520.50	735.50	-
	Lead	5.12	6.79	36.19	8.11	14.20	-
	Magnesium	214.17	233.08	146.83	260.00	318.50	-
	Manganese	114.97	43.93	86.09	25.40	171.55	-
	Mercury	0.05	0.05	0.07	0.28	0.50	**Yes**
	Nickel	0.21	0.24	0.34	0.69	1.26	**Yes**
	Selenium	0.57	0.48	0.35	1.41	2.51	-
	Silica (SiO_2)	306.83	334.38	398.33	836.00	1288.50	**Yes**
	Silver	0.13	0.14	0.16	0.69	1.12	**Yes**
	Strontium	2.52	4.85	2.15	7.29	12.59	-
	Thallium	0.20	0.28	0.32	1.38	2.51	**Yes**
	Uranium	0.08	0.04	0.17	0.15	2.35	-
	Zinc	120.38	129.32	122.45	37.70	45.15	-

Note: Number of sites with metal-sensitive family richness of 5 (n=6); 4 (n=1, so not included); 3 (n= 6); 2 (n= 7); 1 (n= 3); and 0 (n= 5).

Table 6. Average metal or mineral concentration of sediment among sites with the same metal-sensitive family richness.

		Average metal or mineral concentration of sites with a metal-sensitive family richness of...					Was there an incremental increase in concentration with each reduction of metal-sensitive family richness?
		5	3	2	1	0	
Sediment (mg/kg)	Aluminum	10501.67	12587.50	14948.33	10593.33	13714.00	-
	Antimony	1.34	1.20	5.44	0.76	3.70	-
	Arsenic	28.43	9.55	43.53	42.60	37.68	-
	Barium	79.22	71.50	79.33	40.93	53.64	-
	Beryllium	0.85	0.67	1.53	0.29	1.94	-
	Cadmium	3.73	2.71	5.30	1.09	8.08	-
	Calcium	3443.33	11712.50	2728.33	1386.67	1402.00	-
	Chromium	2.95	8.83	4.23	1.93	2.74	-
	Cobalt	9.75	10.63	11.55	8.33	18.80	-
	Copper	67.58	75.75	200.60	47.43	204.22	-
	Iron	23566.67	27000.00	36933.33	60866.67	24040.00	-
	Lead	425.13	345.43	764.00	174.40	1370.40	-
	Magnesium	5203.33	6867.50	5630.00	4346.67	4220.00	-
	Manganese	3441.00	2398.25	5980.83	895.33	9428.00	-
	Mercury	0.08	0.23	0.04	0.03	0.20	-
	Molybdenum	3.87	3.10	7.27	3.77	7.50	-
	Nickel	5.22	9.93	5.75	2.23	4.58	-
	Selenium	1.47	1.80	2.90	1.60	2.01	-
	Silver	1.21	1.00	7.56	0.71	2.35	-
	Thallium	0.55	0.68	0.52	0.65	1.08	-
	Vanadium	15.42	18.28	20.13	18.63	14.42	-
	Zinc	709.83	754.98	2397.83	619.00	1641.60	-

Note: Number of sites with metal-sensitive family richness of 5 (n=6); 4 (n=1, so not included); 3 (n= 6); 2 (n= 7); 1 (n= 3); and 0 (n= 5).

Table 7. Average metal or mineral concentration of pore water among sites with the same metal-sensitive family richness.

		Average metal or mineral concentration of sites with a metal-sensitive family richness of…					Was there an incremental increase in concentration with each reduction of metal-sensitive family richness?
		5	3	2	1	0	
Pore Water (total) (ug/L)	Aluminum	1469.00	2279.55	682.10	12466.67	7088.00	-
	Arsenic	3.73	2.50	2.74	13.55	4.19	-
	Beryllium	2.00	2.00	2.00	2.00	5.20	-
	Cadmium	1.25	1.05	1.50	1.29	8.82	-
	Calcium	39983.33	74925.00	47283.33	75966.67	28540.00	-
	Chloride	0.50	1.50	0.72	1.43	0.42	-
	Copper	16.76	8.10	14.84	64.02	48.50	-
	Fluoride	0.23	0.40	0.47	0.90	2.22	Yes
	Iron	2009.67	7367.25	1036.17	41233.33	1943.00	-
	Lead	76.31	14.14	17.13	165.80	122.12	-
	Magnesium	3878.33	6935.00	3670.00	7986.67	4520.00	-
	Manganese	386.50	640.10	945.87	1446.67	6708.00	Yes
	Nickel	2.50	4.31	2.50	7.94	7.50	-
	Nitrate/Nitrite as N	0.12	0.15	0.12	0.30	0.18	-
	Silica (SiO2)	9071.67	21207.50	6706.67	39166.67	10946.00	-
	Strontium	391.38	830.75	494.33	830.00	134.08	-
	Sulfate as SO4	78.80	144.25	119.00	315.00	120.78	-
	Thallium	6.07	7.16	6.61	6.27	5.00	-
	Total Alkalinity	34.47	52.58	18.46	5.45	6.98	-
	Zinc	190.88	263.25	439.43	374.33	1998.00	-
Pore Water (dissolved) (ug/L)	Aluminum	26.35	1788.65	34.95	6905.70	4451.80	-
	Arsenic	0.52	0.50	0.50	1.17	0.50	-
	Beryllium	2.00	2.00	2.00	2.00	4.71	-
	Cadmium	0.29	0.81	1.34	0.91	7.97	-
	Calcium	37366.67	70550.00	44766.67	70600.00	27200.00	-
	Chromium	1.09	1.49	1.00	2.33	1.00	-
	Copper	0.97	2.74	4.59	8.40	23.19	Yes
	Hardness	107.17	203.00	126.00	205.67	85.00	-
	Iron	100.00	6148.00	100.00	25366.67	101.80	-
	Lead	0.14	1.62	0.51	29.40	6.19	-
	Magnesium	3340.00	6497.50	3495.00	7213.33	4174.00	-
	Manganese	17.51	517.17	808.63	1013.77	6151.80	Yes
	Nickel	0.50	2.52	0.64	5.58	5.60	-
	Silica (SiO2)	4840.00	20350.00	5960.00	31860.00	7354.00	-
	Strontium	363.73	775.50	474.67	778.33	129.66	-
	Thallium	1.00	1.26	1.00	2.33	1.00	-
	Zinc	70.28	227.50	399.12	247.33	1879.80	-

Note: Number of sites with metal-sensitive family richness of 5 (n=6); 4 (n=1, so not included); 3 (n= 6); 2 (n= 7); 1 (n= 3); and 0 (n= 5).

Table 8. Average metal or mineral concentration of surface water among sites with the same metal-sensitive family richness.

		Average metal or mineral concentration of sites with a metal-sensitive family richness of...					Was there an incremental increase in concentration with each reduction of metal-sensitive family richness?
		5	3	2	1	0	
Surface Water (total) (ug/L)	Aluminum	122.67	190.00	707.77	5166.67	6449.20	**Yes**
	Beryllium	2.00	2.60	2.00	2.00	6.68	-
	Cadmium	0.65	0.68	1.50	0.88	9.61	-
	Calcium	38733.33	55200.00	45250.00	74600.00	35020.00	-
	Chloride	0.45	0.60	0.63	1.20	0.42	-
	Copper	2.88	4.65	15.00	12.88	30.72	-
	Fluoride	0.22	0.32	0.50	0.60	2.88	**Yes**
	Iron	100.00	206.40	498.33	7493.67	155.00	-
	Lead	0.88	0.79	7.28	6.00	6.55	-
	Magnesium	3475.00	4972.00	3588.33	6396.67	5302.00	-
	Manganese	129.36	123.49	895.67	563.00	8856.00	-
	Nickel	2.50	3.00	2.50	2.76	7.44	-
	Nitrate/Nitrite as N	0.12	0.14	0.12	0.23	0.10	-
	Silica (SiO2)	4868.33	6326.00	6193.33	14586.67	7930.00	-
	Strontium	372.50	676.60	467.17	817.67	166.86	-
	Sulfate as SO4	76.12	110.50	113.15	247.67	148.36	-
	Thallium	5.00	6.00	6.35	6.05	5.77	-
	Total Alkalinity	36.00	57.48	19.48	6.19	5.00	-
	Zinc	90.67	102.54	421.70	187.67	2300.00	-
Surface Water (dissolved) (ug/L)	Aluminum	36.00	54.24	57.20	3119.00	5235.20	**Yes**
	Arsenic	0.51	0.80	0.50	1.17	0.50	-
	Beryllium	2.00	2.60	2.00	2.00	5.94	-
	Cadmium	0.37	0.34	1.36	0.98	9.08	-
	Calcium	36866.67	52680.00	43133.33	70066.67	33260.00	-
	Chromium	1.18	1.52	1.00	2.33	1.00	-
	Copper	1.12	1.79	4.91	8.05	25.91	**Yes**
	Hardness	105.83	151.20	121.83	200.33	104.40	-
	Iron	100.00	168.80	312.50	5885.33	119.80	-
	Lead	0.19	0.13	2.05	2.03	5.22	-
	Magnesium	3346.67	4728.00	3490.00	6116.67	5158.00	-
	Manganese	116.96	120.07	792.67	546.00	8698.20	-
	Nickel	0.50	0.60	0.66	1.35	7.13	**Yes**
	Silica (SiO2)	4803.33	6248.00	5968.33	13986.67	7828.00	-
	Strontium	353.15	640.00	449.50	771.33	162.30	-
	Zinc	87.90	98.36	393.43	189.67	2280.00	-

Note: Number of sites with metal-sensitive family richness of 5 (n=6); 4 (n=1, so not included); 3 (n= 6); 2 (n= 7); 1 (n= 3); and 0 (n= 5).

Table 9. BMI tissue metal concentrations.

Site				BMI Tissue																				
				Al	Sb	As	Be	Cd	Ca	Cr	Cu	Fe	Pb	Mg	Mn	Hg	Ni	Se	SiO_2	Ag	Sr	Tl	U	Zn
ID	EU	Stream Name	Site Name	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Mineral Cr																								
MaA	EU1	Mineral Cr	Above Animas River	580	0.13	0.18	0.63	0.11	124	0.50	11.40	1790	1.67	120	7.8	0.05	0.07	0.40	744	0.13	1.4	0.15	0.17	40
MaSFM	EU2	Mineral Cr	Above SF Mineral Cr	558	1.26	2.52	6.3	0.25	135	2.52	5.46	6400	8.20	315	23	0.5	1.26	2.52	1260	1.26	13	2.52	0.25	24
MaMFM	EU3	Mineral Cr	Above MF Mineral	722	0.12	0.48	0.61	0.14	127	0.57	14.10	641	8.02	205	28	0.05	0.11	0.30	412	0.12	2	0.24	0.05	52
MaBG	EU3	Mineral Cr	Above Browns Gulch	378	0.06	0.73	0.58	0.24	190	0.54	18.90	415	28.50	145	25	0.05	0.10	0.31	298	0.12	2.8	0.23	0.03	99
MaMIL	EU4	Mineral Cr	Above Mill Cr	304	0.14	2.55	0.67	0.37	170	0.44	58.70	646	166.00	134	49	0.05	0.20	0.18	285	0.07	3.7	0.27	0.07	110
SFMaM	EU5	SF Mineral Cr	Above Mineral Cr	471	0.12	0.35	0.58	0.11	243	0.79	7.92	1510	0.70	156	10	0.05	0.27	0.49	508	0.12	1.8	0.23	0.04	42
SFMbC	EU5	SF Mineral Cr	Below CG, Below Clear Lake Cr	569	0.22	0.34	1.12	0.32	252	0.51	3.91	216	1.62	151	72	0.09	0.88	0.54	377	0.22	1.7	0.45	0.09	91
Upper Animas																								
AaARR	EU7	Animas River	Above Arrastra Cr	171	0.12	0.18	0.14	0.59	142	0.55	11.60	160	3.46	157	64	0.05	0.19	0.41	159	0.12	1.8	0.24	0.05	196
CUaA	EU8	Cunningham Gulch	Above Animas River	197	0.12	0.43	0.62	1.34	276	0.53	29.00	545	8.52	266	39	0.05	0.10	0.67	309	0.28	2.6	0.25	0.02	134
AaCU	EU9	Animas River	Above Cunningham	334	0.13	0.45	0.21	0.97	181	0.62	19.70	304	10.60	206	167	0.05	0.25	0.35	248	0.10	2	0.25	0.12	213
AaMINN	EU10	Animas River	Above Minnie	952	0.08	0.90	0.79	1.12	177	0.58	30.70	324	10.50	185	133	0.05	0.30	0.23	528	0.11	1.9	0.27	0.40	212
SFAaAV	EU11	SF Animas River	Above Avalanche	17.1	0.12	0.08	0.6	0.12	150	0.31	1.89	57.7	0.33	89	8.9	0.05	0.17	0.48	43.3	0.12	1.5	0.24	0.02	47
EUaSFA	EU12	Eureka Gulch	Above SF Animas River	319	0.12	0.33	0.61	1.09	190	0.48	57.20	620	34.40	188	224	0.05	0.34	0.23	254	0.19	2.9	0.24	0.01	199
SFAaA	EU13	SF Animas River	Above Animas River	840	0.25	0.75	1.24	1.62	687	0.72	31.50	2220	7.64	519	147	0.1	0.62	0.32	807	0.25	8.6	0.50	0.04	345
AaEU	EU14	Animas River	Above Eureka	569	0.27	0.21	0.68	0.77	145	0.41	26.20	84.8	2.96	103	31	0.11	0.27	0.53	202	0.27	1.3	0.53	0.30	83
PLAaWF	EU16	Placer Gulch	Above WF Animas River	1200	1.92	3.83	9.59	0.34	479	3.83	19.80	241	15.10	479	35	0.77	1.92	3.83	1920	1.92	19	3.83	1.32	52
BUaNFA	EU19	Burrow Creek	Above NF Animas River	721	0.60	1.12	2.98	0.31	149	1.19	15.90	1230	13.30	158	308	0.24	0.60	1.19	657	0.31	6	1.19	3.37	39
Reference Sites																								
MILaM	-	Mill Cr	Above Mineral Cr	370	0.13	0.31	0.63	0.26	222	0.49	5.18	157	4.42	161	76	0.05	0.15	0.34	279	0.13	4.1	0.13	0.15	56
BEaM	-	Bear Cr	Above Mineral Cr	96.9	0.12	0.29	0.62	0.29	722	0.46	5.16	118	0.20	180	42	0.05	0.25	1.10	190	0.12	2.7	0.25	0.14	62
MAGaA	-	Maggie Gulch	Above Animas River	51.5	0.12	0.23	0.61	0.22	205	0.50	4.27	96.6	0.54	193	30	0.05	0.11	0.90	136	0.12	2.5	0.24	0.00	71
PICaA	-	Picayne Gulch	Above Animas River	128	0.10	1.26	0.48	0.18	225	0.55	3.43	291	1.02	201	290	0.04	0.24	0.54	246	0.06	2.5	0.19	0.02	72
NFAaBU	-	NF Animas River	Above Burrow Creek	268	0.07	2.07	0.63	1.52	348	0.67	5.46	539	5.97	271	76	0.05	0.27	0.42	569	0.09	1.2	0.16	0.05	185
HERaD	-	Hermosa Cr	Above Ditch	62.8	0.12	0.14	0.61	0.03	1880	0.56	3.78	85	0.08	333	8.4	0.03	0.12	0.86	191	0.12	13	0.25	0.02	48

Note: BMI tissue was not collected for 5 sites due to very low BMI abundance: MFMaM; NFAaWFA; WFAaPLA; WFAaaA; and SFAaEU.

Table 10. Spearman correlation coefficients for BMI and physical habitat metrics. Yellow highlight indicates statistically significant correlations at $p<0.05$.

BMI Metric	Phab													
	AvgSubSize	StDevSubSize	D25	D50	D75	D90	Fines(%)	Sand(%)	Gravel(%)	Pebble(%)	Cobble(%)	Boulder(%)	Embeddedness(%)	AvgHabitatScore
Total Richness	-0.09	-0.10	0.13	0.09	-0.02	-0.11	-0.05	0.30	0.17	-0.08	0.26	-0.06	-0.63	0.04
Density (#/m^2)	-0.12	-0.20	0.10	0.16	-0.06	-0.12	-0.32	0.11	0.18	0.02	0.14	-0.05	-0.81	0.39
HBI	-0.25	-0.27	-0.27	-0.29	-0.24	-0.26	0.54	0.25	0.00	0.03	0.14	-0.34	0.29	-0.32
MMI	-0.04	-0.04	0.15	0.12	0.02	-0.04	-0.17	0.32	0.17	-0.09	0.27	-0.01	-0.62	0.07
SWDI	-0.03	0.01	-0.02	-0.05	0.01	0.00	0.05	0.13	0.24	-0.02	0.21	-0.05	-0.34	-0.20
EPT	-0.09	-0.08	0.10	0.12	-0.02	-0.09	-0.24	0.17	0.18	0.00	0.23	-0.01	-0.66	0.11
MetalSensRA	-0.16	-0.18	-0.04	0.04	-0.10	-0.13	-0.32	-0.18	0.22	0.15	0.21	-0.07	-0.68	0.27
MetalSensRich	-0.08	-0.15	0.12	0.14	-0.02	-0.08	-0.20	0.10	0.07	0.01	0.25	0.00	-0.67	0.26
cf	0.04	-0.03	0.25	0.22	0.12	0.02	-0.09	0.35	-0.03	-0.14	-0.08	0.06	-0.56	0.12
cg	-0.33	-0.33	-0.35	-0.28	-0.29	-0.30	0.20	0.25	0.28	0.12	0.13	-0.38	0.00	-0.24
o	-0.15	-0.09	-0.19	-0.28	-0.16	-0.15	-0.06	0.26	0.32	0.16	-0.11	-0.20	-0.17	-0.07
p	0.29	0.25	0.29	0.26	0.30	0.30	-0.03	-0.12	-0.22	-0.34	-0.01	0.25	0.17	-0.35
sc	-0.07	-0.11	0.14	0.10	-0.04	-0.09	-0.15	0.18	0.06	-0.01	0.31	-0.03	-0.70	0.25
sh	0.33	0.33	0.20	0.25	0.27	0.30	-0.15	-0.32	-0.26	-0.03	-0.25	0.38	0.05	0.30

Note: See table 2 for explanation of BMI metrics. MetalSensRA = relative abundance of Ephemerellidae, Heptageniidae, and Taeniopterygidae families; MetalSensRich = richness of Ephemerellidae, Heptageniidae, and Taeniopterygidae families. Functional Feeding Groups include collector-filterers (cf), collector-gatherers (cg), omnivores (o), predators (p), scrapers (sc), and shredders (sh). AvgSubSize = average substrate size; StDevSubSize = standard deviation of substrate size; D25 = 25th percentile of substrate size; D50 = 50th percentile of substrate size; D75 = 75th percentile of substrate size; D90 = 90th percentile of substrate size; AvgHabitatScore = based on Barbour et al. 1999.

Table 11. Spearman correlation coefficients between percent embeddedness and metal concentrations in sediment, pore water, and surface water. Yellow highlight indicates statistically significant correlations at $p<0.05$.

	Sediment	Pore Water (Total)	Pore Water (Dissolved)	Surface Water (Total)	Surface Water (Dissolved)
Alkalinity	-	-0.09	-	-0.62	-
Hardness	-	-	-0.23	-	0.25
Al	0.14	0.00	-0.03	0.64	0.62
Sb	-0.03	-	-	-	-
As	0.35	-0.24	0.07	-	-0.08
Ba	-0.24	-	-	-	-
Be	-0.06	-0.10	-0.11	-0.05	-0.05
Cd	-0.02	0.23	0.20	0.39	0.56
Ca	-0.47	-0.18	-0.19	0.18	0.19
Cl-	-	-	-0.15	0.07	-
Cr	-0.07	-	0.08	-	-0.18
Co	-0.16	-	-	-	-
Cu	0.22	-0.01	0.17	0.54	0.52
F-	-	0.09	-	0.44	-
Fe	0.24	-0.19	-0.09	0.43	0.45
Pb	0.16	-0.03	0.23	0.33	0.51
Mg	-0.12	-0.18	-0.19	0.50	0.51
Mn	-0.02	0.14	0.05	0.54	0.55
Hg	-0.02	-	-	-	-
Mo	0.34	-	-	-	-
Ni	-0.25	0.05	0.19	0.24	0.49
Nitrate/ Nitrite	-	-0.10	-	0.24	-
Se	0.23	-	-	-	-
SiO_2	-	-0.19	-0.26	0.41	0.38
Ag	0.12	-	-	-	-
Sr	-	-0.15	-0.15	0.03	0.02
SO4	-	-0.31	-	0.46	-
Tl	0.07	-0.10	0.18	-0.12	0.04
V	0.10	-	-	-	-
Zn	0.27	0.07	0.12	0.47	0.47

Table 12. Spearman correlation coefficients for BMI metrics and concentrations in BMI tissue. Yellow highlight indicates statistically significant correlations at $p<0.05$.

BMI Metric	BMI Tissue																				
	Al	Sb	As	Be	Cd	Ca	Cr	Cu	Fe	Pb	Mg	Mn	Hg	Ni	Se	SiO_2	Ag	Sr	Tl	U	Zn
Total Richness	-0.75	-0.44	-0.27	-0.37	0.09	0.52	-0.34	-0.39	-0.40	-0.33	0.10	0.15	-0.51	-0.18	-0.01	-0.54	-0.39	-0.15	-0.30	-0.60	0.33
Density (#/m^2)	-0.70	-0.45	-0.34	-0.57	-0.04	0.32	-0.33	-0.36	-0.46	-0.31	-0.02	0.22	-0.58	-0.48	0.00	-0.64	-0.35	0.00	-0.50	-0.55	0.23
HBI	0.02	-0.04	0.15	0.03	-0.11	-0.14	0.43	-0.01	0.37	0.06	0.12	0.10	-0.04	0.30	0.04	0.16	-0.15	0.19	0.17	0.21	-0.16
MMI	-0.72	-0.40	-0.38	-0.39	0.04	0.51	-0.43	-0.41	-0.49	-0.37	0.06	0.06	-0.52	-0.32	-0.08	-0.57	-0.34	-0.15	-0.41	-0.58	0.29
SWDI	-0.64	-0.39	-0.29	-0.30	0.23	0.32	-0.46	-0.15	-0.32	-0.19	-0.08	0.20	-0.38	-0.07	-0.27	-0.53	-0.41	-0.19	-0.28	-0.40	0.42
EPT	-0.72	-0.44	-0.36	-0.47	0.05	0.53	-0.37	-0.46	-0.46	-0.48	0.00	0.12	-0.55	-0.24	0.09	-0.58	-0.35	-0.32	-0.37	-0.59	0.31
MetalSensRA	-0.47	-0.42	-0.14	-0.53	0.10	0.28	-0.35	-0.21	-0.26	-0.16	-0.11	0.21	-0.42	-0.38	-0.22	-0.43	-0.42	-0.19	-0.52	-0.50	0.41
MetalSensRich	-0.67	-0.47	-0.26	-0.56	0.08	0.48	-0.24	-0.34	-0.35	-0.40	0.12	0.15	-0.54	-0.41	-0.02	-0.50	-0.46	-0.21	-0.56	-0.50	0.35
cf	-0.57	-0.22	-0.21	-0.36	0.08	0.22	0.05	-0.18	-0.22	-0.16	0.28	0.31	-0.42	-0.18	0.34	-0.42	-0.07	0.10	-0.12	-0.37	0.13
cg	-0.33	-0.02	-0.16	-0.12	-0.10	0.07	0.21	-0.28	-0.01	-0.16	0.15	0.18	-0.15	0.13	0.24	-0.18	0.02	0.26	0.01	0.03	-0.19
o	-0.04	0.14	0.06	0.12	0.07	0.15	-0.06	0.00	-0.02	0.28	-0.08	0.36	0.08	0.09	-0.16	-0.01	-0.01	0.25	-0.01	0.06	0.06
p	-0.04	-0.14	-0.17	0.00	0.40	-0.13	-0.57	0.41	-0.09	0.15	-0.25	0.13	0.00	-0.23	-0.51	-0.25	-0.14	-0.33	0.00	-0.14	0.52
sc	-0.67	-0.43	-0.34	-0.44	0.13	0.45	-0.36	-0.36	-0.45	-0.35	0.07	0.24	-0.48	-0.23	-0.17	-0.54	-0.42	-0.16	-0.49	-0.48	0.39
sh	0.40	-0.09	0.22	0.05	-0.07	0.13	0.16	0.01	0.17	-0.03	0.05	-0.19	0.07	-0.01	-0.01	0.39	0.02	-0.24	-0.12	-0.08	0.02

Note: See table 2 for explanation of BMI metrics. MetalSensRA = relative abundance of Ephemerellidae, Heptageniidae, and Taeniopterygidae families; MetalSensRich = richness of Ephemerellidae, Heptageniidae, and Taeniopterygidae families. Functional Feeding Groups include collector-filterers (cf), collector-gatherers (cg), omnivores (o), predators (p), scrapers (sc), and shredders (sh).

Table 13. Spearman correlation coefficients for BMI metrics and concentrations in sediment. Yellow highlight indicates statistically significant correlations at $p<0.05$.

BMI Metric	Sediment																					
	Al	Sb	As	Ba	Be	Cd	Ca	Cr	Co	Cu	Fe	Pb	Mg	Mn	Hg	Mo	Ni	Se	Ag	Tl	V	Zn
Total Richness	-0.08	-0.08	-0.44	0.37	-0.19	-0.09	0.73	0.17	-0.25	-0.37	-0.12	-0.28	0.47	-0.17	0.01	-0.43	0.15	-0.09	-0.13	-0.06	0.07	-0.27
Density (#/m^2)	-0.16	0.08	-0.49	0.43	-0.06	-0.06	0.65	0.17	0.00	-0.32	-0.22	-0.21	0.33	-0.02	0.10	-0.28	0.24	-0.06	-0.10	-0.10	0.00	-0.29
HBI	0.16	-0.29	-0.03	-0.42	-0.20	-0.30	-0.23	-0.21	-0.38	-0.15	0.03	-0.26	0.02	-0.23	-0.28	0.04	-0.27	-0.11	-0.26	0.17	-0.09	-0.17
MMI	-0.13	-0.07	-0.49	0.35	-0.13	-0.07	0.76	0.16	-0.23	-0.37	-0.25	-0.23	0.47	-0.13	0.12	-0.47	0.15	-0.11	-0.17	-0.19	0.06	-0.23
SWDI	-0.19	-0.31	-0.44	-0.04	-0.21	-0.12	0.50	-0.11	-0.48	-0.29	-0.31	-0.15	0.32	-0.13	-0.09	-0.59	-0.20	-0.38	-0.41	-0.29	-0.13	-0.08
EPT	-0.13	-0.03	-0.47	0.42	-0.10	-0.10	0.82	0.24	-0.12	-0.39	-0.22	-0.26	0.50	-0.11	0.11	-0.38	0.26	-0.07	-0.11	-0.11	0.09	-0.27
MetalSensRA	-0.31	-0.03	-0.32	0.36	-0.16	0.00	0.64	0.04	-0.02	-0.25	-0.11	-0.03	0.25	0.01	0.18	-0.39	0.12	-0.14	-0.11	-0.37	-0.04	-0.06
MetalSensRich	-0.21	-0.02	-0.40	0.48	-0.10	-0.07	0.78	0.20	-0.06	-0.38	-0.20	-0.19	0.38	-0.10	0.20	-0.31	0.26	0.00	-0.07	-0.20	0.05	-0.22
cf	0.18	0.22	-0.40	0.26	0.17	0.00	0.47	0.15	-0.04	-0.24	-0.23	-0.21	0.29	-0.03	0.10	-0.24	0.27	-0.16	-0.08	0.24	0.05	-0.29
cg	0.12	-0.31	-0.38	-0.31	-0.21	-0.39	0.04	-0.31	-0.33	-0.38	-0.18	-0.39	0.16	-0.21	-0.17	-0.16	-0.27	-0.17	-0.42	0.12	-0.10	-0.31
o	0.24	0.02	-0.01	-0.13	0.17	0.14	0.09	-0.15	-0.14	0.05	-0.11	-0.04	0.02	0.11	-0.21	-0.20	-0.07	-0.18	-0.17	-0.05	-0.15	0.08
p	-0.38	-0.13	-0.07	-0.31	-0.18	0.07	0.01	-0.14	-0.34	0.12	-0.17	0.19	-0.04	-0.04	-0.31	-0.23	-0.40	-0.53	-0.09	-0.42	-0.08	0.11
sc	-0.11	0.09	-0.36	0.55	0.01	0.13	0.76	0.27	-0.07	-0.22	-0.25	-0.05	0.39	0.05	0.22	-0.26	0.29	-0.01	0.06	-0.21	0.03	-0.05
sh	0.11	0.25	0.28	0.36	0.25	0.13	-0.05	0.40	0.50	0.19	0.25	0.09	0.01	0.20	0.26	0.35	0.40	0.50	0.45	0.12	0.27	0.06

Note: See table 2 for explanation of BMI metrics. MetalSensRA = relative abundance of Ephemerellidae, Heptageniidae, and Taeniopterygidae families; MetalSensRich = richness of Ephemerellidae, Heptageniidae, and Taeniopterygidae families. Functional Feeding Groups include collector-filterers (cf), collector-gatherers (cg), omnivores (o), predators (p), scrapers (sc), and shredders (sh).

Table 14. Spearman correlation coefficients for BMI metrics and total concentrations in pore water. Yellow highlight indicates statistically significant correlations at p<0.05.

BMI Metric	Pore Water (Total)																			
	Al	As	Be	Cd	Ca	Cl-	Cu	F-	Fe	Pb	Mg	Mn	Ni	Nitrate/Nitrite	SiO_2	Sr	SO4	Tl	Alkalinity	Zn
Total Richness	-0.50	-0.05	-0.37	-0.49	0.13	0.05	-0.62	-0.53	-0.16	-0.43	-0.08	-0.64	-0.53	-0.17	-0.17	0.28	-0.27	0.42	0.75	-0.58
Density (#/m^2)	-0.52	-0.18	-0.31	-0.55	0.01	-0.10	-0.62	-0.41	-0.27	-0.47	-0.25	-0.62	-0.57	-0.36	-0.30	0.17	-0.29	0.05	0.75	-0.61
HBI	0.40	-0.04	0.41	0.26	0.27	0.34	0.15	0.48	0.34	0.10	0.37	0.41	0.52	0.36	0.51	0.11	0.49	0.23	-0.32	0.20
MMI	-0.55	-0.04	-0.38	-0.48	0.08	-0.02	-0.62	-0.57	-0.27	-0.46	-0.09	-0.64	-0.58	-0.19	-0.27	0.24	-0.32	0.33	0.72	-0.56
SWDI	-0.33	-0.11	-0.14	-0.15	-0.03	0.06	-0.41	-0.34	-0.21	-0.33	-0.13	-0.40	-0.30	-0.10	-0.13	0.08	-0.32	0.38	0.40	-0.23
EPT	-0.48	-0.06	-0.47	-0.50	0.14	0.03	-0.63	-0.54	-0.18	-0.44	-0.07	-0.58	-0.60	-0.19	-0.22	0.21	-0.33	0.33	0.79	-0.62
MetalSensRA	-0.44	0.00	-0.51	-0.50	0.07	0.05	-0.50	-0.48	-0.18	-0.35	-0.23	-0.57	-0.56	-0.24	-0.28	0.24	-0.35	0.16	0.59	-0.55
MetalSensRich	-0.49	-0.01	-0.52	-0.58	0.12	0.02	-0.64	-0.54	-0.16	-0.41	-0.12	-0.60	-0.64	-0.16	-0.24	0.21	-0.34	0.19	0.72	-0.67
cf	-0.20	-0.07	0.00	-0.16	-0.04	0.07	-0.26	-0.18	-0.06	-0.15	-0.06	-0.25	-0.30	-0.17	-0.05	-0.02	-0.24	0.20	0.66	-0.25
cg	0.31	-0.12	0.30	0.05	0.16	0.12	-0.10	0.36	0.23	-0.08	0.27	0.25	0.42	0.23	0.35	0.09	0.30	0.14	-0.14	-0.06
o	-0.12	-0.04	0.42	0.17	-0.22	-0.18	-0.01	-0.09	-0.21	-0.12	0.00	-0.08	0.06	-0.12	-0.13	-0.06	0.04	0.22	-0.05	0.13
p	-0.16	-0.02	-0.03	0.17	-0.25	-0.03	0.11	-0.13	-0.30	-0.07	-0.25	-0.12	-0.29	-0.04	-0.22	-0.12	-0.37	0.02	0.15	0.16
sc	-0.65	-0.08	-0.44	-0.55	0.00	-0.13	-0.66	-0.57	-0.35	-0.48	-0.22	-0.68	-0.73	-0.23	-0.41	0.14	-0.40	0.23	0.73	-0.59
sh	0.03	0.27	-0.16	-0.13	0.02	-0.25	0.07	-0.15	0.15	0.18	-0.03	0.00	-0.10	-0.11	-0.08	-0.14	-0.06	-0.20	-0.06	-0.05

Note: See table 2 for explanation of BMI metrics. MetalSensRA = relative abundance of Ephemerellidae, Heptageniidae, and Taeniopterygidae families; MetalSensRich = richness of Ephemerellidae, Heptageniidae, and Taeniopterygidae families. Functional Feeding Groups include collector-filterers (cf), collector-gatherers (cg), omnivores (o), predators (p), scrapers (sc), and shredders (sh).

Table 15. Spearman correlation coefficients for BMI metrics and dissolved concentrations in pore water. Yellow highlight indicates statistically significant correlations at p<0.05.

BMI Metric	Pore Water (Dissolved)																
	Al	As	Be	Cd	Ca	Cr	Cu	Hardness	Fe	Pb	Mg	Mn	Ni	SiO_2	Sr	Tl	Zn
Total Richness	-0.67	0.02	-0.22	-0.62	0.13	0.30	-0.65	0.11	-0.11	-0.66	-0.12	-0.65	-0.68	-0.37	0.28	-0.11	-0.65
Density (#/m^2)	-0.67	0.03	-0.15	-0.66	0.00	0.21	-0.72	-0.04	-0.17	-0.73	-0.24	-0.63	-0.65	-0.41	0.17	-0.21	-0.67
HBI	0.54	0.09	0.41	0.33	0.27	0.08	0.36	0.32	0.65	0.38	0.44	0.46	0.47	0.66	0.09	0.33	0.29
MMI	-0.73	-0.03	-0.24	-0.60	0.09	0.29	-0.69	0.05	-0.20	-0.69	-0.14	-0.62	-0.69	-0.46	0.25	-0.15	-0.63
SWDI	-0.43	-0.04	-0.05	-0.19	-0.02	0.12	-0.30	-0.04	-0.15	-0.37	-0.13	-0.36	-0.39	-0.25	0.09	0.00	-0.23
EPT	-0.69	0.07	-0.34	-0.65	0.13	0.32	-0.74	0.09	-0.19	-0.68	-0.13	-0.67	-0.68	-0.43	0.21	-0.05	-0.71
MetalSensRA	-0.67	0.13	-0.41	-0.56	0.05	0.12	-0.62	0.01	-0.27	-0.60	-0.28	-0.62	-0.62	-0.46	0.24	-0.01	-0.60
MetalSensRich	-0.73	0.06	-0.42	-0.70	0.09	0.22	-0.80	0.06	-0.20	-0.72	-0.16	-0.70	-0.69	-0.44	0.21	-0.06	-0.75
cf	-0.36	0.06	0.10	-0.35	-0.04	0.36	-0.39	-0.06	0.04	-0.44	-0.08	-0.42	-0.35	-0.16	-0.03	-0.23	-0.35
cg	0.29	0.11	0.39	0.07	0.15	0.22	0.10	0.18	0.64	0.14	0.30	0.25	0.38	0.39	0.09	0.31	0.01
o	-0.15	-0.20	0.57	0.13	-0.20	-0.05	0.09	-0.16	0.08	-0.14	0.03	0.08	0.02	-0.07	-0.04	-0.20	0.16
p	-0.15	-0.07	-0.16	0.18	-0.22	-0.05	0.13	-0.26	-0.43	0.04	-0.32	-0.06	-0.24	-0.30	-0.11	-0.11	0.16
sc	-0.75	-0.02	-0.35	-0.65	-0.01	0.12	-0.79	-0.03	-0.30	-0.82	-0.19	-0.68	-0.76	-0.52	0.15	-0.23	-0.65
sh	0.01	-0.01	-0.28	-0.16	0.00	-0.21	-0.11	0.01	-0.38	-0.04	-0.06	-0.13	-0.09	-0.16	-0.15	-0.05	-0.12

Note: See table 2 for explanation of BMI metrics. MetalSensRA = relative abundance of Ephemerellidae, Heptageniidae, and Taeniopterygidae families; MetalSensRich = richness of Ephemerellidae, Heptageniidae, and Taeniopterygidae families. Functional Feeding Groups include collector-filterers (cf), collector-gatherers (cg), omnivores (o), predators (p), scrapers (sc), and shredders (sh).

Table 16. Spearman correlation coefficients for BMI metrics and total concentrations in surface water. Yellow highlight indicates statistically significant correlations at $p<0.05$.

BMI Metric	Surface Water (Total)																		
	Al	Be	Cd	Ca	Cl-	Cu	F-	Fe	Pb	Mg	Mn	Ni	Nitrate /Nitrite	SiO_2	Sr	SO4	Tl	Alkalinity	Zn
Total Richness	-0.89	-0.22	-0.51	0.07	0.08	-0.74	-0.64	-0.50	-0.52	-0.33	-0.78	-0.47	0.11	-0.50	0.24	-0.47	0.10	0.79	-0.64
Density (#/m^2)	-0.77	-0.18	-0.60	-0.04	-0.10	-0.73	-0.53	-0.54	-0.59	-0.47	-0.69	-0.45	-0.07	-0.54	0.16	-0.49	0.05	0.82	-0.68
HBI	0.40	0.23	0.24	0.18	0.21	0.22	0.38	0.22	0.09	0.45	0.35	0.29	0.12	0.58	0.05	0.44	0.17	-0.29	0.22
MMI	-0.88	-0.18	-0.49	0.05	0.07	-0.73	-0.66	-0.54	-0.52	-0.36	-0.78	-0.44	0.13	-0.58	0.22	-0.50	0.03	0.77	-0.62
SWDI	-0.58	-0.02	-0.15	-0.11	0.14	-0.45	-0.41	-0.46	-0.24	-0.28	-0.42	-0.17	0.08	-0.39	0.04	-0.46	0.14	0.41	-0.27
EPT	-0.90	-0.29	-0.59	0.02	0.00	-0.82	-0.65	-0.56	-0.58	-0.41	-0.79	-0.53	0.07	-0.57	0.15	-0.58	-0.02	0.88	-0.73
MetalSensRA	-0.69	-0.35	-0.56	-0.06	-0.05	-0.72	-0.60	-0.42	-0.46	-0.52	-0.61	-0.57	-0.07	-0.60	0.19	-0.60	-0.08	0.70	-0.60
MetalSensRich	-0.80	-0.41	-0.68	-0.03	-0.10	-0.86	-0.66	-0.53	-0.60	-0.39	-0.73	-0.65	0.01	-0.56	0.14	-0.60	-0.21	0.82	-0.74
cf	-0.52	-0.10	-0.33	-0.06	-0.02	-0.48	-0.22	-0.61	-0.29	-0.09	-0.36	-0.22	-0.06	-0.22	0.01	-0.28	0.12	0.64	-0.36
cg	0.14	0.22	-0.01	0.06	0.07	-0.15	0.20	-0.08	-0.21	0.14	0.02	0.25	0.20	0.32	0.01	0.15	0.32	-0.03	-0.08
o	0.02	0.28	0.18	-0.21	-0.04	0.06	-0.09	-0.26	0.02	0.05	0.04	0.14	-0.02	-0.09	-0.10	-0.01	0.22	-0.07	0.24
p	0.02	-0.11	0.21	-0.15	0.05	0.28	-0.03	-0.23	0.27	-0.03	0.16	-0.11	-0.08	-0.21	-0.01	-0.15	-0.03	-0.03	0.22
sc	-0.84	-0.22	-0.50	0.01	-0.11	-0.73	-0.59	-0.54	-0.50	-0.38	-0.71	-0.48	0.07	-0.61	0.14	-0.51	0.02	0.76	-0.59
sh	0.07	-0.14	-0.13	0.07	-0.30	0.02	-0.05	0.24	-0.07	0.02	-0.01	-0.12	-0.28	-0.03	-0.14	0.04	-0.39	-0.04	-0.02

Note: See table 2 for explanation of BMI metrics. MetalSensRA = relative abundance of Ephemerellidae, Heptageniidae, and Taeniopterygidae families; MetalSensRich = richness of Ephemerellidae, Heptageniidae, and Taeniopterygidae families. Functional Feeding Groups include collector-filterers (cf), collector-gatherers (cg), omnivores (o), predators (p), scrapers (sc), and shredders (sh).

Table 17. Spearman correlation coefficients for BMI metrics and dissolved concentrations in surface water. Yellow highlight indicates statistically significant correlations at $p<0.05$.

BMI Metric	Surface Water (Dissolved)															
	Al	As	Be	Cd	Ca	Cr	Cu	Hardness	Fe	Pb	Mg	Mn	Ni	SiO_2	Sr	Zn
Total Richness	-0.78	0.13	-0.22	-0.70	0.08	0.39	-0.71	-0.01	-0.49	-0.52	-0.33	-0.80	-0.70	-0.49	0.26	-0.64
Density (#/m^2)	-0.65	0.16	-0.18	-0.74	-0.02	0.38	-0.72	-0.11	-0.52	-0.57	-0.46	-0.71	-0.66	-0.53	0.18	-0.67
HBI	0.41	-0.05	0.23	0.30	0.16	-0.10	0.27	0.19	0.18	0.25	0.46	0.34	0.30	0.58	0.03	0.23
MMI	-0.80	0.14	-0.18	-0.69	0.06	0.40	-0.72	-0.02	-0.50	-0.52	-0.36	-0.78	-0.67	-0.57	0.25	-0.62
SWDI	-0.49	0.06	-0.02	-0.31	-0.10	0.15	-0.38	-0.16	-0.42	-0.16	-0.27	-0.42	-0.36	-0.37	0.07	-0.26
EPT	-0.82	0.19	-0.29	-0.78	0.04	0.42	-0.83	-0.05	-0.48	-0.61	-0.40	-0.80	-0.74	-0.56	0.16	-0.73
MetalSensRA	-0.64	0.21	-0.35	-0.67	-0.05	0.26	-0.69	-0.13	-0.40	-0.51	-0.52	-0.63	-0.68	-0.58	0.20	-0.61
MetalSensRich	-0.76	0.09	-0.41	-0.79	-0.02	0.29	-0.84	-0.09	-0.46	-0.69	-0.39	-0.75	-0.79	-0.55	0.14	-0.74
cf	-0.44	-0.05	-0.10	-0.41	-0.05	0.33	-0.37	-0.11	-0.58	-0.30	-0.10	-0.39	-0.38	-0.23	0.02	-0.35
cg	0.19	0.09	0.22	-0.01	0.06	0.17	-0.04	0.05	0.00	0.12	0.14	0.03	0.15	0.33	0.03	-0.07
o	0.05	-0.24	0.28	0.17	-0.21	-0.13	0.16	-0.21	-0.36	0.10	0.05	0.03	0.07	-0.09	-0.05	0.25
p	-0.10	-0.17	-0.11	0.24	-0.12	-0.10	0.25	-0.12	-0.34	0.27	-0.03	0.17	-0.04	-0.25	0.01	0.23
sc	-0.79	0.16	-0.22	-0.68	0.01	0.25	-0.74	-0.05	-0.49	-0.63	-0.38	-0.72	-0.67	-0.58	0.15	-0.58
sh	0.00	-0.05	-0.14	-0.08	0.04	-0.16	-0.07	0.10	0.30	-0.31	0.02	-0.02	-0.12	-0.03	-0.17	-0.04

Note: See table 2 for explanation of BMI metrics. MetalSensRA = relative abundance of Ephemerellidae, Heptageniidae, and Taeniopterygidae families; MetalSensRich = richness of Ephemerellidae, Heptageniidae, and Taeniopterygidae families. Functional Feeding Groups include collector-filterers (cf), collector-gatherers (cg), omnivores (o), predators (p), scrapers (sc), and shredders (sh).

Table 18. Percent of variation explained by Axis one and Axis two for each ordination group. Since environmental variables were not consistently sampled at all sites, ordination had to be performed separately for each environmental variable using the group of sites where that variable was sampled consistently.

Ordination Group	# of sites	% of variation		Coresponding Figure #s
		Axis 1	Axis 2	
All sites*	26	67.1	16.8	25-29
Sediment	23	65.8	14.1	30
Pore Water	23	65.8	14.1	31
Surface Water	24	66.3	14.5	32
BMI Tissue	21	70.7	13.5	33

Table 19. Correlation of environmental variables with NMS axes (r^2>=0.4 reported). Direction of relationship noted in parenthesis. Correlations with Axis two were all below r^2 of 0.4.

Environmental Media	Variable	Axis 1	Axis 2
Physical Habitat	Embeddedness	(+) 0.48	-
Sediment	Calcium	(-) 0.40	-
Pore Water	Dissolved copper	(+) 0.53	-
	Dissolved cadmium	(+) 0.44	-
	Total cadmium	(+) 0.40	-
Surface Water	Dissolved copper	(+) 0.72	-
	Dissolved cadmium	(+) 0.64	-
	Total aluminum	(+) 0.64	-
	Total cadmium	(+) 0.60	-
	Dissolved nickel	(+) 0.59	-
	Dissolved aluminum	(+) 0.56	-
	Total zinc	(+) 0.54	-
	Dissolved zinc	(+) 0.54	-
	Total alkalinity	(+) 0.50	-
	Dissolved magnesium	(+) 0.45	-
	Total copper	(+) 0.44	-
	Total magnesium	(+) 0.44	-
BMI Tissue	Silica	(+) 0.60	-
	Thallium	(+) 0.58	-
	Mercury	(+) 0.58	-
	Antimony	(+) 0.57	-
	Silver	(+) 0.57	-
	Beryllium	(+) 0.57	-
	Chromium	(+) 0.55	-
	Nickel	(+) 0.50	-
	Selenium	(+) 0.50	-
	Aluminum	(+) 0.40	-

Table 20. Spearman correlation coefficients between metal concentrations in BMI tissue. Yellow highlight indicates statistically significant correlations at $p<0.05$.

	BMI Tissue																				
	Al	Sb	As	Be	Cd	Ca	Cr	Cu	Fe	Pb	Mg	Mn	Hg	Ni	Se	SiO_2	Ag	Sr	Tl	U	Zn
Al	-	0.42	0.46	0.61	0.23	-0.22	0.48	0.52	0.51	0.48	0.09	0.13	0.68	0.46	-0.17	0.80	0.40	0.13	0.44	0.65	-0.02
Sb	0.42	-	0.16	0.69	0.13	-0.06	0.11	0.21	0.15	0.18	0.13	0.04	0.77	0.43	0.38	0.35	0.74	0.43	0.71	0.57	-0.27
As	0.46	0.16	-	0.44	0.35	0.08	0.58	0.39	0.56	0.65	0.42	0.40	0.48	0.50	-0.01	0.67	-0.05	0.39	0.36	0.27	0.12
Be	0.61	0.69	0.44	-	0.33	0.08	0.26	0.27	0.28	0.27	0.20	0.11	0.87	0.62	0.25	0.67	0.63	0.33	0.66	0.62	-0.17
Cd	0.23	0.13	0.35	0.33	-	0.16	0.10	0.66	0.14	0.54	0.28	0.65	0.47	0.42	-0.25	0.21	0.11	-0.02	0.37	0.15	0.79
Ca	-0.22	-0.06	0.08	0.08	0.16	-	0.14	-0.16	-0.23	-0.25	0.49	0.16	-0.16	0.19	0.29	-0.03	0.03	0.31	0.04	-0.31	0.22
Cr	0.48	0.11	0.58	0.26	0.10	0.14	-	0.11	0.49	0.26	0.64	0.15	0.27	0.48	0.27	0.67	0.14	0.33	0.30	0.27	-0.09
Cu	0.52	0.21	0.39	0.27	0.66	-0.16	0.11	-	0.47	0.80	0.08	0.31	0.41	0.19	-0.48	0.38	0.14	0.19	0.40	0.22	0.52
Fe	0.51	0.15	0.56	0.28	0.14	-0.23	0.49	0.47	-	0.51	0.20	0.10	0.30	0.22	-0.23	0.77	0.11	0.22	0.10	0.13	-0.04
Pb	0.48	0.18	0.65	0.27	0.54	-0.25	0.26	0.80	0.51	-	0.13	0.40	0.43	0.24	-0.40	0.47	0.09	0.35	0.32	0.26	0.36
Mg	0.09	0.13	0.42	0.20	0.28	0.49	0.64	0.08	0.20	0.13	-	0.22	0.12	0.29	0.27	0.34	0.20	0.53	0.26	-0.13	0.17
Mn	0.13	0.04	0.40	0.11	0.65	0.16	0.15	0.31	0.10	0.40	0.22	-	0.26	0.44	-0.19	0.06	-0.10	0.12	0.17	0.09	0.58
Hg	0.68	0.77	0.48	0.87	0.47	-0.16	0.27	0.41	0.30	0.43	0.12	0.26	-	0.64	0.14	0.62	0.55	0.18	0.70	0.73	-0.02
Ni	0.46	0.43	0.50	0.62	0.42	0.19	0.48	0.19	0.22	0.24	0.29	0.44	0.64	-	0.23	0.47	0.34	0.23	0.66	0.43	0.08
Se	-0.17	0.38	-0.01	0.25	-0.25	0.29	0.27	-0.48	-0.23	-0.40	0.27	-0.19	0.14	0.23	-	0.01	0.50	0.23	0.33	0.14	-0.55
SiO_2	0.80	0.35	0.67	0.67	0.21	-0.03	0.67	0.38	0.77	0.47	0.34	0.06	0.62	0.47	0.01	-	0.37	0.25	0.31	0.48	-0.14
Ag	0.40	0.74	-0.05	0.63	0.11	0.03	0.14	0.14	0.11	0.09	0.20	-0.10	0.55	0.34	0.50	0.37	-	0.40	0.55	0.35	-0.34
Sr	0.13	0.43	0.39	0.33	-0.02	0.31	0.33	0.19	0.22	0.35	0.53	0.12	0.18	0.23	0.23	0.25	0.40	-	0.41	0.05	-0.16
Tl	0.44	0.71	0.36	0.66	0.37	0.04	0.30	0.40	0.10	0.32	0.26	0.17	0.70	0.66	0.33	0.31	0.55	0.41	-	0.47	0.03
U	0.65	0.57	0.27	0.62	0.15	-0.31	0.27	0.22	0.13	0.26	-0.13	0.09	0.73	0.43	0.14	0.48	0.35	0.05	0.47	-	-0.25
Zn	-0.02	-0.27	0.12	-0.17	0.79	0.22	-0.09	0.52	-0.04	0.36	0.17	0.58	-0.02	0.08	-0.55	-0.14	-0.34	-0.16	0.03	-0.25	-

Table 21. Spearman correlation coefficients between metal concentrations in sediment. Yellow highlight indicates statistically significant correlations at $p<0.05$.

	Sediment																					
	Al	Sb	As	Ba	Be	Cd	Ca	Cr	Co	Cu	Fe	Pb	Mg	Mn	Hg	Mo	Ni	Se	Ag	Tl	V	Zn
Al	-	0.41	0.14	0.28	0.56	0.12	0.09	0.46	0.41	0.13	0.43	-0.20	0.30	0.13	0.07	0.24	0.53	0.60	0.25	0.47	0.55	0.05
Sb	0.41	-	0.27	0.60	0.79	0.60	-0.03	0.48	0.25	0.62	0.00	0.47	0.06	0.63	0.31	0.53	0.34	0.36	0.72	0.12	0.19	0.43
As	0.14	0.27	-	-0.01	0.27	0.50	-0.51	-0.20	0.24	0.50	0.34	0.37	-0.58	0.24	-0.19	0.54	-0.13	0.11	0.35	0.16	-0.19	0.56
Ba	0.28	0.60	-0.01	-	0.38	0.33	0.46	0.62	0.26	0.13	0.09	0.07	0.21	0.25	0.37	0.24	0.55	0.58	0.62	0.13	0.30	0.09
Be	0.56	0.79	0.27	0.38	-	0.43	0.04	0.43	0.43	0.46	-0.14	0.23	0.00	0.57	0.21	0.42	0.44	0.37	0.43	0.22	0.18	0.36
Cd	0.12	0.60	0.50	0.33	0.43	-	-0.15	-0.03	0.30	0.74	-0.12	0.78	-0.28	0.72	0.16	0.38	0.12	-0.08	0.63	0.01	-0.42	0.80
Ca	0.09	-0.03	-0.51	0.46	0.04	-0.15	-	0.44	0.05	-0.43	-0.16	-0.36	0.55	-0.15	0.15	-0.32	0.52	0.16	-0.03	-0.04	0.25	-0.26
Cr	0.46	0.48	-0.20	0.62	0.43	-0.03	0.44	-	0.28	0.16	0.20	-0.11	0.64	0.15	0.18	0.16	0.73	0.67	0.47	0.09	0.73	-0.09
Co	0.41	0.25	0.24	0.26	0.43	0.30	0.05	0.28	-	0.19	0.15	-0.01	-0.11	0.26	0.14	0.22	0.72	0.38	0.28	0.44	0.11	0.18
Cu	0.13	0.62	0.50	0.13	0.46	0.74	-0.43	0.16	0.19	-	0.12	0.81	-0.18	0.70	-0.04	0.37	0.01	0.08	0.59	-0.12	-0.14	0.80
Fe	0.43	0.00	0.34	0.09	-0.14	-0.12	-0.16	0.20	0.15	0.12	-	-0.18	0.05	-0.25	-0.25	0.02	0.15	0.49	0.10	0.14	0.55	0.02
Pb	-0.20	0.47	0.37	0.07	0.23	0.78	-0.36	-0.11	-0.01	0.81	-0.18	-	-0.30	0.63	0.27	0.22	-0.18	-0.27	0.46	-0.37	-0.46	0.82
Mg	0.30	0.06	-0.58	0.21	0.00	-0.28	0.55	0.64	-0.11	-0.18	0.05	-0.30	-	0.09	0.09	-0.14	0.40	0.29	0.07	0.09	0.58	-0.29
Mn	0.13	0.63	0.24	0.25	0.57	0.72	-0.15	0.15	0.26	0.70	-0.25	0.63	0.09	-	0.10	0.46	0.14	0.00	0.57	0.06	-0.27	0.61
Hg	0.07	0.31	-0.19	0.37	0.21	0.16	0.15	0.18	0.14	-0.04	-0.25	0.27	0.09	0.10	-	-0.09	0.29	0.15	0.12	-0.06	0.01	0.20
Mo	0.24	0.53	0.54	0.24	0.42	0.38	-0.32	0.16	0.22	0.37	0.02	0.22	-0.14	0.46	-0.09	-	0.09	0.33	0.68	0.43	-0.01	0.22
Ni	0.53	0.34	-0.13	0.55	0.44	0.12	0.52	0.73	0.72	0.01	0.15	-0.18	0.40	0.14	0.29	0.09	-	0.62	0.37	0.41	0.44	-0.05
Se	0.60	0.36	0.11	0.58	0.37	-0.08	0.16	0.67	0.38	0.08	0.49	-0.27	0.29	0.00	0.15	0.33	0.62	-	0.42	0.43	0.63	-0.06
Ag	0.25	0.72	0.35	0.62	0.43	0.63	-0.03	0.47	0.28	0.59	0.10	0.46	0.07	0.57	0.12	0.68	0.37	0.42	-	0.21	0.13	0.37
Tl	0.47	0.12	0.16	0.13	0.22	0.01	-0.04	0.09	0.44	-0.12	0.14	-0.37	0.09	0.06	-0.06	0.43	0.41	0.43	0.21	-	0.13	-0.16
V	0.55	0.19	-0.19	0.30	0.18	-0.42	0.25	0.73	0.11	-0.14	0.55	-0.46	0.58	-0.27	0.01	-0.01	0.44	0.63	0.13	0.13	-	-0.37
Zn	0.05	0.43	0.56	0.09	0.36	0.80	-0.26	-0.09	0.18	0.80	0.02	0.82	-0.29	0.61	0.20	0.22	-0.05	-0.06	0.37	-0.16	-0.37	-

Table 22. Spearman correlation coefficients between total metal concentrations in pore water. Yellow highlight indicates statistically significant correlations at $p<0.05$.

	Pore Water (Total)																			
	Al	As	Be	Cd	Ca	Cl-	Cu	F-	Fe	Pb	Mg	Mn	Ni	Nitrate/ Nitrite	SiO_2	Sr	SO4	Tl	Alkalinity	Zn
Al	-	0.50	0.47	0.56	-0.01	0.18	0.68	0.53	0.76	0.72	0.37	0.80	0.76	0.33	0.73	-0.30	0.32	-0.19	-0.67	0.51
As	0.50	-	0.22	0.12	-0.06	0.28	0.50	-0.04	0.62	0.67	0.18	0.26	0.08	0.22	0.46	-0.10	-0.04	-0.15	-0.11	0.15
Be	0.47	0.22	-	0.51	-0.32	-0.20	0.49	0.48	0.22	0.29	-0.04	0.52	0.37	0.08	0.29	-0.49	0.10	-0.23	-0.35	0.52
Cd	0.56	0.12	0.51	-	-0.28	-0.12	0.75	0.58	0.08	0.53	0.10	0.74	0.46	0.06	0.18	-0.54	0.04	-0.25	-0.53	0.94
Ca	-0.01	-0.06	-0.32	-0.28	-	0.49	-0.21	0.25	0.28	-0.18	0.69	0.05	0.15	0.25	0.31	0.78	0.77	0.41	0.12	-0.30
Cl-	0.18	0.28	-0.20	-0.12	0.49	-	0.15	-0.06	0.45	0.30	0.31	0.02	0.14	0.32	0.53	0.61	0.34	0.31	-0.02	-0.14
Cu	0.68	0.50	0.49	0.75	-0.21	0.15	-	0.50	0.42	0.83	0.02	0.70	0.44	0.04	0.48	-0.35	0.11	-0.41	-0.52	0.82
F-	0.53	-0.04	0.48	0.58	0.25	-0.06	0.50	-	0.25	0.24	0.30	0.73	0.52	0.26	0.38	-0.12	0.49	-0.31	-0.36	0.59
Fe	0.76	0.62	0.22	0.08	0.28	0.45	0.42	0.25	-	0.68	0.32	0.41	0.46	0.40	0.91	0.09	0.33	0.06	-0.33	0.06
Pb	0.72	0.67	0.29	0.53	-0.18	0.30	0.83	0.24	0.68	-	0.00	0.50	0.34	0.09	0.61	-0.28	0.00	-0.30	-0.45	0.58
Mg	0.37	0.18	-0.04	0.10	0.69	0.31	0.02	0.30	0.32	0.00	-	0.44	0.47	0.42	0.30	0.35	0.76	0.33	-0.23	-0.02
Mn	0.80	0.26	0.52	0.74	0.05	0.02	0.70	0.73	0.41	0.50	0.44	-	0.68	0.37	0.47	-0.37	0.38	-0.20	-0.62	0.67
Ni	0.76	0.08	0.37	0.46	0.15	0.14	0.44	0.52	0.46	0.34	0.47	0.68	-	0.22	0.51	-0.02	0.58	0.00	-0.81	0.44
Nitrate/Nitrite	0.33	0.22	0.08	0.06	0.25	0.32	0.04	0.26	0.40	0.09	0.42	0.37	0.22	-	0.40	0.14	0.23	0.35	-0.24	-0.03
SiO_2	0.73	0.46	0.29	0.18	0.31	0.53	0.48	0.38	0.91	0.61	0.30	0.47	0.51	0.40	-	0.12	0.42	-0.03	-0.34	0.16
Sr	-0.30	-0.10	-0.49	-0.54	0.78	0.61	-0.35	-0.12	0.09	-0.28	0.35	-0.37	-0.02	0.14	0.12	-	0.53	0.47	0.22	-0.50
SO4	0.32	-0.04	0.10	0.04	0.77	0.34	0.11	0.49	0.33	0.00	0.76	0.38	0.58	0.23	0.42	0.53	-	0.20	-0.38	0.06
Tl	-0.19	-0.15	-0.23	-0.25	0.41	0.31	-0.41	-0.31	0.06	-0.30	0.33	-0.20	0.00	0.35	-0.03	0.47	0.20	-	0.11	-0.37
Alkalinity	-0.67	-0.11	-0.35	-0.53	0.12	-0.02	-0.52	-0.36	-0.33	-0.45	-0.23	-0.62	-0.81	-0.24	-0.34	0.22	-0.38	0.11	-	-0.55
Zn	0.51	0.15	0.52	0.94	-0.30	-0.14	0.82	0.59	0.06	0.58	-0.02	0.67	0.44	-0.03	0.16	-0.50	0.06	-0.37	-0.55	-

Table 23. Spearman correlation coefficients between dissolved metal concentrations in pore water. Yellow highlight indicates statistically significant correlations at $p<0.05$.

Pore Water (Dissolved)																	
	Al	As	Be	Cd	Ca	Cr	Cu	Hardness	Fe	Pb	Mg	Mn	Ni	SiO_2	Sr	Tl	Zn
Al	-	0.21	0.43	0.60	0.17	-0.01	0.68	0.21	0.52	0.71	0.44	0.88	0.86	0.59	-0.12	0.35	0.54
As	0.21	-	-0.09	-0.14	0.43	0.71	-0.07	0.41	0.23	-0.02	0.37	0.11	0.07	0.06	0.41	0.50	-0.27
Be	0.43	-0.09	-	0.47	-0.15	-0.13	0.47	-0.09	0.15	0.23	0.21	0.47	0.50	0.35	-0.36	-0.09	0.47
Cd	0.60	-0.14	0.47	-	-0.21	-0.33	0.87	-0.19	0.11	0.73	0.11	0.71	0.70	0.24	-0.43	0.12	0.95
Ca	0.17	0.43	-0.15	-0.21	-	0.46	-0.11	0.99	0.45	-0.12	0.67	0.18	0.01	0.41	0.78	0.45	-0.23
Cr	-0.01	0.71	-0.13	-0.33	0.46	-	-0.27	0.41	0.34	-0.09	0.33	-0.03	-0.09	0.04	0.46	0.34	-0.47
Cu	0.68	-0.07	0.47	0.87	-0.11	-0.27	-	-0.08	0.23	0.83	0.11	0.69	0.78	0.30	-0.23	0.13	0.86
Hardness	0.21	0.41	-0.09	-0.19	0.99	0.41	-0.08	-	0.48	-0.12	0.74	0.20	0.07	0.44	0.74	0.45	-0.21
Fe	0.52	0.23	0.15	0.11	0.45	0.34	0.23	0.48	-	0.43	0.57	0.48	0.56	0.64	0.37	0.61	-0.03
Pb	0.71	-0.02	0.23	0.73	-0.12	-0.09	0.83	-0.12	0.43	-	0.10	0.68	0.80	0.40	-0.19	0.30	0.64
Mg	0.44	0.37	0.21	0.11	0.67	0.33	0.11	0.74	0.57	0.10	-	0.50	0.43	0.35	0.29	0.39	0.01
Mn	0.88	0.11	0.47	0.71	0.18	-0.03	0.69	0.20	0.48	0.68	0.50	-	0.80	0.46	-0.06	0.26	0.63
Ni	0.86	0.07	0.50	0.70	0.01	-0.09	0.78	0.07	0.56	0.80	0.43	0.80	-	0.51	-0.25	0.38	0.61
SiO_2	0.59	0.06	0.35	0.24	0.41	0.04	0.30	0.44	0.64	0.40	0.35	0.46	0.51	-	0.19	0.43	0.24
Sr	-0.12	0.41	-0.36	-0.43	0.78	0.46	-0.23	0.74	0.37	-0.19	0.29	-0.06	-0.25	0.19	-	0.29	-0.41
Tl	0.35	0.50	-0.09	0.12	0.45	0.34	0.13	0.45	0.61	0.30	0.39	0.26	0.38	0.43	0.29	-	-0.08
Zn	0.54	-0.27	0.47	0.95	-0.23	-0.47	0.86	-0.21	-0.03	0.64	0.01	0.63	0.61	0.24	-0.41	-0.08	-

Table 24. Spearman correlation coefficients between total metal concentrations in surface water. Yellow highlight indicates statistically significant correlations at $p<0.05$.

Surface Water (Total)																			
	Alkalinity	Al	Be	Cd	Ca	Cl-	Cu	F-	Fe	Pb	Mg	Mn	Ni	Nitrate/ Nitrite	SiO_2	Sr	SO4	Tl	Zn
Alkalinity	-	-0.88	-0.46	-0.73	0.15	-0.08	-0.72	-0.49	-0.61	-0.66	-0.31	-0.72	-0.70	-0.13	-0.45	0.31	-0.46	-0.20	-0.70
Al	-0.88	-	0.31	0.58	-0.02	-0.03	0.75	0.69	0.50	0.48	0.45	0.86	0.51	-0.05	0.62	-0.23	0.59	0.03	0.70
Be	-0.46	0.31	-	0.57	-0.01	0.01	0.43	0.48	0.23	0.11	0.18	0.34	0.80	0.00	0.29	-0.32	0.42	0.36	0.41
Cd	-0.73	0.58	0.57	-	-0.14	-0.08	0.80	0.64	0.12	0.67	0.33	0.76	0.70	0.13	0.15	-0.40	0.39	0.22	0.88
Ca	0.15	-0.02	-0.01	-0.14	-	0.49	-0.04	0.27	0.31	-0.30	0.51	-0.02	-0.06	0.41	0.32	0.76	0.70	0.09	-0.17
Cl-	-0.08	-0.03	0.01	-0.08	0.49	-	-0.01	-0.13	0.46	0.14	0.17	-0.09	0.02	0.38	0.23	0.66	0.31	0.12	-0.21
Cu	-0.72	0.75	0.43	0.80	-0.04	-0.01	-	0.67	0.35	0.70	0.30	0.81	0.50	-0.06	0.35	-0.18	0.55	0.12	0.87
F-	-0.49	0.69	0.48	0.64	0.27	-0.13	0.67	-	0.22	0.28	0.46	0.81	0.60	0.07	0.59	-0.16	0.66	0.21	0.68
Fe	-0.61	0.50	0.23	0.12	0.31	0.46	0.35	0.22	-	0.17	0.16	0.20	0.30	0.17	0.64	0.28	0.54	0.17	0.12
Pb	-0.66	0.48	0.11	0.67	-0.30	0.14	0.70	0.28	0.17	-	0.03	0.60	0.25	-0.04	0.06	-0.23	0.08	0.00	0.71
Mg	-0.31	0.45	0.18	0.33	0.51	0.17	0.30	0.46	0.16	0.03	-	0.46	0.31	0.42	0.35	0.13	0.72	-0.12	0.26
Mn	-0.72	0.86	0.34	0.76	-0.02	-0.09	0.81	0.81	0.20	0.60	0.46	-	0.51	-0.14	0.40	-0.28	0.51	-0.03	0.87
Ni	-0.70	0.51	0.80	0.70	-0.06	0.02	0.50	0.60	0.30	0.25	0.31	0.51	-	0.09	0.36	-0.37	0.46	0.44	0.52
Nitrate/Nitrite	-0.13	-0.05	0.00	0.13	0.41	0.38	-0.06	0.07	0.17	-0.04	0.42	-0.14	0.09	-	0.09	0.30	0.29	0.28	-0.17
SiO_2	-0.45	0.62	0.29	0.15	0.32	0.23	0.35	0.59	0.64	0.06	0.35	0.40	0.36	0.09	-	0.07	0.63	0.11	0.22
Sr	0.31	-0.23	-0.32	-0.40	0.76	0.66	-0.18	-0.16	0.28	-0.23	0.13	-0.28	-0.37	0.30	0.07	-	0.34	0.11	-0.36
SO4	-0.46	0.59	0.42	0.39	0.70	0.31	0.55	0.66	0.54	0.08	0.72	0.51	0.46	0.29	0.63	0.34	-	0.19	0.38
Tl	-0.20	0.03	0.36	0.22	0.09	0.12	0.12	0.21	0.17	0.00	-0.12	-0.03	0.44	0.28	0.11	0.11	0.19	-	0.09
Zinc	-0.70	0.70	0.41	0.88	-0.17	-0.21	0.87	0.68	0.12	0.71	0.26	0.87	0.52	-0.17	0.22	-0.36	0.38	0.09	-

Table 25. Spearman correlation coefficients between dissolved metal concentrations in surface water. Yellow highlight indicates statistically significant correlations at $p<0.05$.

Surface Water (Dissolved)																
	Al	As	Be	Cd	Ca	Cr	Cu	Hardness	Fe	Pb	Mg	Mn	Ni	SiO_2	Sr	Zn
Al	-	0.01	0.33	0.64	-0.03	-0.29	0.70	0.05	0.37	0.52	0.38	0.72	0.68	0.47	-0.12	0.58
As	0.01	-	0.17	-0.19	0.47	0.71	-0.24	0.46	0.32	-0.06	0.18	-0.13	0.17	0.02	0.39	-0.31
Be	0.33	0.17	-	0.40	-0.02	0.02	0.38	0.09	0.23	0.27	0.19	0.35	0.63	0.32	-0.29	0.41
Cd	0.64	-0.19	0.40	-	-0.12	-0.48	0.91	-0.05	0.13	0.77	0.37	0.90	0.73	0.30	-0.33	0.96
Ca	-0.03	0.47	-0.02	-0.12	-	0.45	-0.08	0.98	0.29	-0.22	0.51	-0.02	-0.10	0.29	0.75	-0.17
Cr	-0.29	0.71	0.02	-0.48	0.45	-	-0.46	0.43	0.07	-0.20	0.18	-0.47	-0.07	-0.11	0.47	-0.58
Cu	0.70	-0.24	0.38	0.91	-0.08	-0.46	-	-0.02	0.13	0.80	0.29	0.83	0.70	0.33	-0.14	0.90
Hardness	0.05	0.46	0.09	-0.05	0.98	0.43	-0.02	-	0.34	-0.21	0.63	0.06	0.01	0.34	0.67	-0.11
Fe	0.37	0.32	0.23	0.13	0.29	0.07	0.13	0.34	-	0.11	0.19	0.18	0.44	0.66	0.12	0.01
Pb	0.52	-0.06	0.27	0.77	-0.22	-0.20	0.80	-0.21	0.11	-	0.08	0.66	0.66	0.15	-0.14	0.71
Mg	0.38	0.18	0.19	0.37	0.51	0.18	0.29	0.63	0.19	0.08	-	0.42	0.43	0.32	0.13	0.25
Mn	0.72	-0.13	0.35	0.90	-0.02	-0.47	0.83	0.06	0.18	0.66	0.42	-	0.69	0.43	-0.28	0.87
Ni	0.68	0.17	0.63	0.73	-0.10	-0.07	0.70	0.01	0.44	0.66	0.43	0.69	-	0.45	-0.31	0.63
SiO_2	0.47	0.02	0.32	0.30	0.29	-0.11	0.33	0.34	0.66	0.15	0.32	0.43	0.45	-	0.05	0.24
Sr	-0.12	0.39	-0.29	-0.33	0.75	0.47	-0.14	0.67	0.12	-0.14	0.13	-0.28	-0.31	0.05	-	-0.35
Z	0.58	-0.31	0.41	0.96	-0.17	-0.58	0.90	-0.11	0.01	0.71	0.25	0.87	0.63	0.24	-0.35	-

Table 26. Hazard quotients for metals.

Site				HQ																									
				Aluminum		Arsenic		Cadmium			Chromium VI		Copper		Iron	Lead		Manganese		Nickel		Selenium		Silver	Thalium	Zinc		Cumulative Criteria Unit	
ID	EU	Stream Name	Site Name	Acute	Chronic	Acute	Chronic	Acute	Acute (trout)	Chronic	Acute	Chronic	Acute	Chronic	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic	Acute	Chronic
Mineral Cr																													
MaA	EU1	Mineral Cr	Above Animas River	0.38	2.63	0.00	0.00	0.14	0.23	0.95	0.06	0.09	0.10	0.15	2.04	0.00	0.03	0.07	0.13	0.00	0.01	0.05	0.22	0.18	0.07	0.52	0.69	1.75	7.01
MaSFM	EU2	Mineral Cr	Above SF Mineral	0.51	3.58	0.00	0.00	0.22	0.36	1.54	0.06	0.09	0.54	0.85	4.70	0.04	1.10	0.13	0.24	0.00	0.01	0.05	0.22	0.09	0.07	0.81	1.07	2.82	13.46
MaMFM	EU3	Mineral Cr	Above MF Mineral	0.23	1.59	0.00	0.00	0.27	0.44	1.81	0.06	0.09	0.43	0.66	0.58	0.01	0.13	0.07	0.12	0.00	0.01	0.05	0.22	0.18	0.07	1.15	1.52	2.89	6.79
MaMIL	EU4	Mineral Cr	Above Mill Creek	0.07	0.47	0.00	0.00	0.62	0.99	3.90	0.06	0.09	1.00	1.47	0.20	0.21	5.45	0.09	0.16	0.00	0.01	0.05	0.22	0.35	0.07	3.60	4.75	7.05	16.79
SFMaM	EU5	SF Mineral Cr	Above Mineral Creek	0.20	1.39	0.00	0.00	0.06	0.09	0.35	0.06	0.09	0.04	0.06	0.45	0.00	0.04	0.03	0.05	0.00	0.01	0.05	0.22	0.29	0.07	0.12	0.15	0.94	2.89
MFMaM	EU6	MF Mineral Cr	Above Mineral Creek	0.69	4.82	0.01	0.02	0.15	0.23	1.07	0.31	0.45	0.12	0.20	17.20	0.00	0.06	0.22	0.40	0.00	0.02	0.27	0.22	0.19	0.33	0.30	0.39	2.49	25.19
Upper Animas																													
AaARR	EU7	Animas River	Above Arrastra Creek	0.03	0.18	0.00	0.00	0.19	0.31	1.26	0.06	0.09	0.13	0.20	0.10	0.00	0.06	0.12	0.22	0.00	0.01	0.05	0.22	0.18	0.07	1.09	1.44	2.17	3.84
CUaA	EU8	Cunningham Creek	Above Animas River	0.01	0.07	0.00	0.00	0.06	0.10	0.38	0.07	0.10	0.10	0.15	0.10	0.01	0.19	0.00	0.00	0.00	0.01	0.05	0.22	0.26	0.07	0.17	0.22	0.82	1.51
AaCU	EU9	Animas River	Above Cunningham	0.05	0.32	0.00	0.00	0.25	0.41	1.68	0.06	0.09	0.13	0.20	0.10	0.00	0.03	0.15	0.27	0.00	0.01	0.05	0.22	0.17	0.07	1.57	2.07	2.84	5.06
AaMINN	EU10	Animas River	Above Minnie Gulch	0.14	0.97	0.00	0.00	0.55	0.89	3.69	0.06	0.09	0.18	0.28	0.10	0.01	0.18	0.35	0.64	0.00	0.01	0.05	0.22	0.15	0.07	2.75	3.63	5.14	9.88
SFAaEU	EU11	SF Animas River	Above Eureka Gulch	0.07	0.46	0.00	0.00	0.05	0.09	0.38	0.06	0.09	0.13	0.20	0.45	0.00	0.02	0.04	0.07	0.00	0.01	0.05	0.22	0.11	0.07	0.18	0.24	0.79	2.20
SFAaAV	EU11	SF Animas River	Above Avalanche Zone	0.01	0.04	0.01	0.01	0.06	0.05	0.19	0.06	0.09	0.05	0.08	0.10	0.00	0.06	0.00	0.00	0.00	0.01	0.05	0.22	0.15	0.07	0.18	0.23	0.61	1.09
EUaSFA	EU12	Eureka Gulch	Above SF Animas	0.02	0.14	0.00	0.00	0.19	0.30	1.23	0.06	0.09	0.33	0.51	0.10	0.00	0.09	0.03	0.06	0.00	0.01	0.05	0.22	0.19	0.07	1.35	1.78	2.53	4.29
SFAaA	EU13	SF Animas River	Above Animas River	0.03	0.20	0.00	0.00	0.17	0.27	1.14	0.06	0.09	0.24	0.37	0.13	0.00	0.02	0.04	0.07	0.00	0.01	0.05	0.22	0.12	0.07	0.95	1.25	1.93	3.58
AaEU	EU14	Animas River	Above Eureka Gulch	0.28	1.93	0.00	0.00	1.09	1.75	7.20	0.06	0.09	0.36	0.56	0.10	0.00	0.10	0.91	1.64	0.00	0.02	0.05	0.22	0.18	0.07	4.85	6.40	9.53	18.32
WFAaA	EU15	WF Animas River	Above Animas River	1.15	8.09	0.00	0.00	2.22	3.57	14.72	0.06	0.09	1.49	2.28	0.20	0.05	1.32	3.93	7.11	0.01	0.10	0.05	0.22	0.16	0.07	13.23	17.46	25.93	51.68
PLaWFA	EU16	Placer Gulch	Above WF Animas River	0.33	2.29	0.00	0.00	1.09	1.75	6.94	0.06	0.09	0.78	1.17	0.19	0.09	2.29	0.34	0.62	0.01	0.05	0.05	0.22	0.30	0.07	8.39	11.08	13.19	25.00
WFAaPL	EU17	WF Animas River	Above Placer Gulch	1.80	12.58	0.00	0.00	2.62	4.22	17.79	0.06	0.09	1.18	1.84	0.17	0.00	0.10	6.92	12.52	0.02	0.16	0.05	0.22	0.12	0.07	14.65	19.34	31.64	64.88
NFAaWFA	EU18	NF Animas River	Above WF Animas River	1.08	7.54	0.00	0.00	3.02	4.86	19.14	0.06	0.09	1.49	2.20	0.10	0.20	5.14	0.47	0.85	0.01	0.07	0.05	0.22	0.33	0.07	8.64	11.41	20.23	46.84
BUaNFA	EU19	Burrows Creek	Above NF Animas River	5.90	41.33	0.00	0.00	9.04	14.54	55.80	0.06	0.09	5.80	8.41	0.11	0.13	3.37	1.78	3.22	0.03	0.31	0.05	0.22	0.48	0.07	25.92	34.22	63.73	147.15
Reference Sites																													
MILaM	-	Mill Creek	Above Mineral Creek	0.17	1.20	0.00	0.00	0.13	0.21	0.80	0.06	0.09	0.13	0.18	0.10	0.01	0.19	0.03	0.06	0.00	0.02	0.05	0.22	0.67	0.07	0.39	0.52	1.86	3.44
BEaM	-	Bear Creek	Above Mineral Creek	0.03	0.22	0.00	0.00	0.05	0.08	0.30	0.06	0.09	0.05	0.08	0.10	0.00	0.06	0.00	0.00	0.00	0.01	0.05	0.22	0.42	0.07	0.08	0.11	0.84	1.25
MAGaA	-	Maggie Gulch	Above Animas River	0.02	0.15	0.00	0.00	0.04	0.06	0.24	0.07	0.11	0.07	0.10	0.10	0.00	0.04	0.00	0.00	0.00	0.01	0.05	0.22	0.25	0.07	0.06	0.08	0.63	1.12
PICaA	-	Picayne Gulch	Above Animas River	0.01	0.07	0.00	0.00	0.02	0.03	0.15	0.12	0.18	0.03	0.05	0.10	0.00	0.02	0.03	0.06	0.00	0.01	0.05	0.22	0.09	0.07	0.04	0.05	0.44	0.98
NFAaBU	-	NF Animas River	Above Burrows Creek	0.02	0.16	0.00	0.00	0.28	0.45	1.81	0.06	0.09	0.07	0.10	0.10	0.00	0.04	0.00	0.00	0.00	0.01	0.05	0.22	0.25	0.07	0.85	1.12	2.04	3.72
HERaD	-	Hermosa Creek	Above Animas River	0.00	0.02	0.00	0.00	0.02	0.03	0.12	0.16	0.23	0.02	0.03	0.10	0.00	0.01	0.00	0.00	0.00	0.00	0.05	0.22	0.05	0.07	0.03	0.03	0.36	0.84

Note: CDPHE water quality standards for aquatic life are based on dissolved concentrations, except for aluminum and iron, which are based on total concentrations (CDPHE 2017). Surface water samples were not collected at Mineral Creek above Browns and SF Mineral Cr below campground, and are excluded from this table. We were unable to calculate a chronic HQ for silver since the silver CDPHE chronic water quality standard was below the method detection limit. The Cumulative Criteria Unit (CCU) is the sum of HQs for each site (Clements 2000).

8. Figures

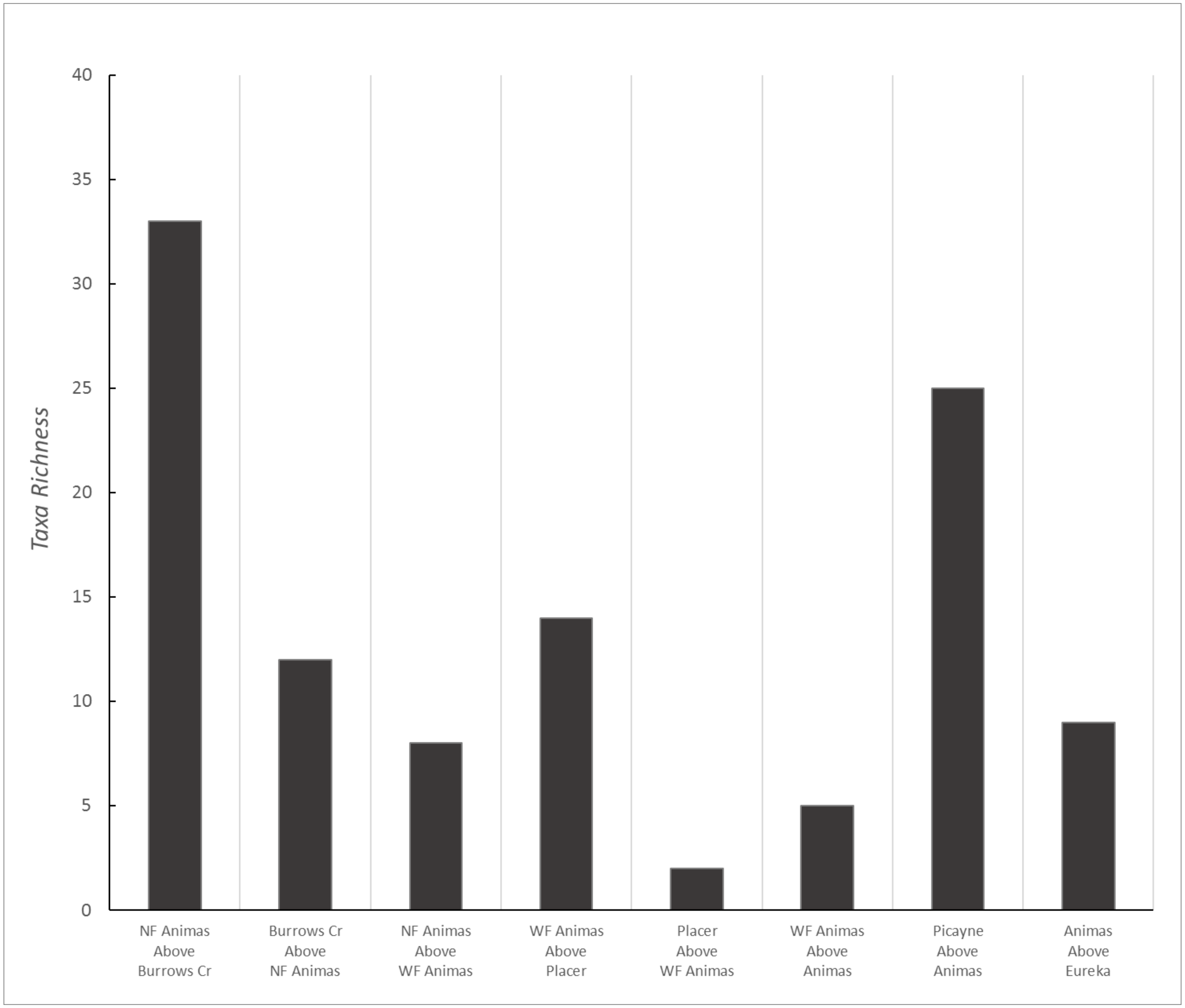

Figure 1: Taxa richness – Upper Animas Group A

Note: see table 2 for an explanation of BMI metrics.

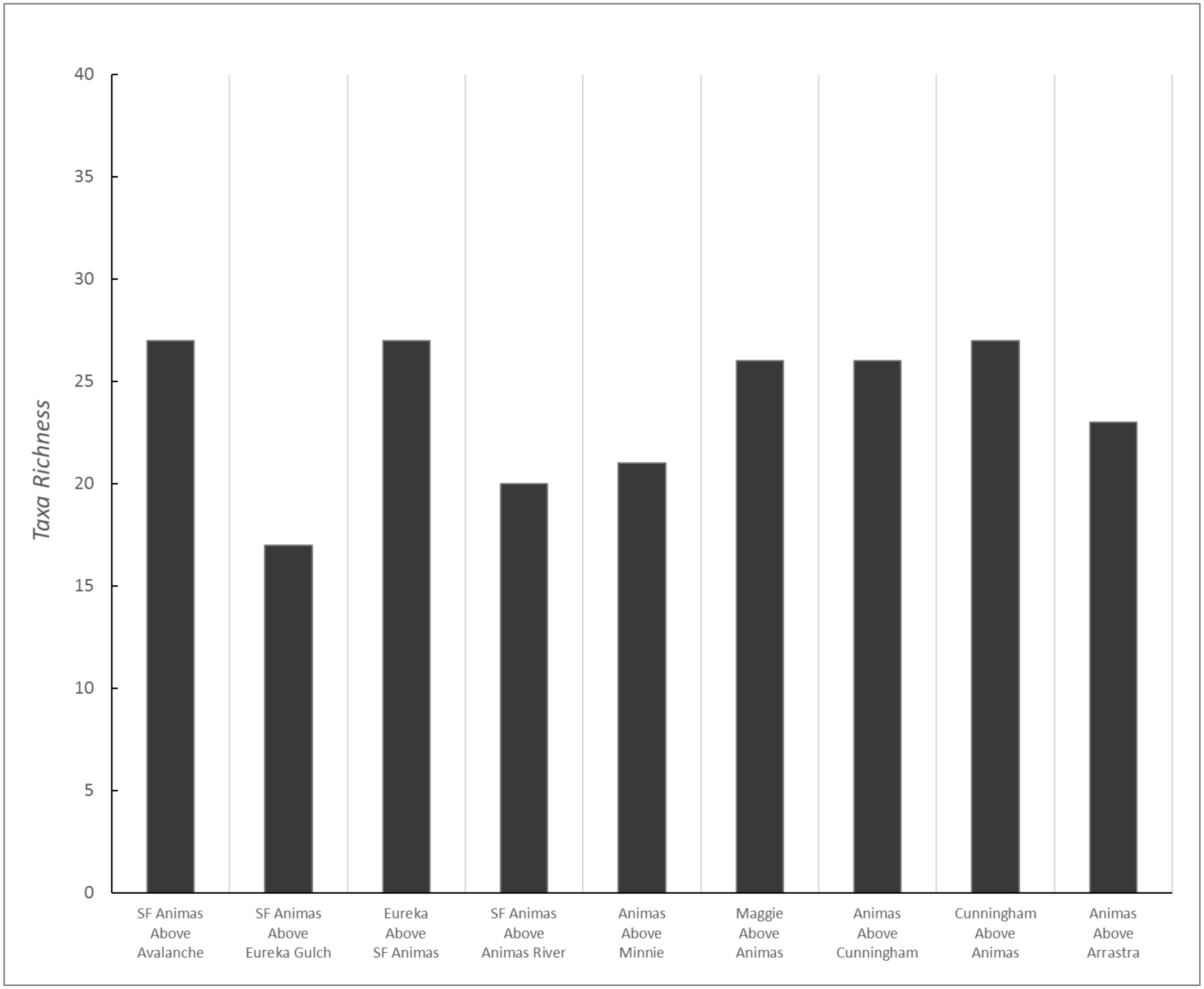

Figure 2: Taxa richness – Upper Animas Group B

Note: see table 2 for an explanation of BMI metrics.

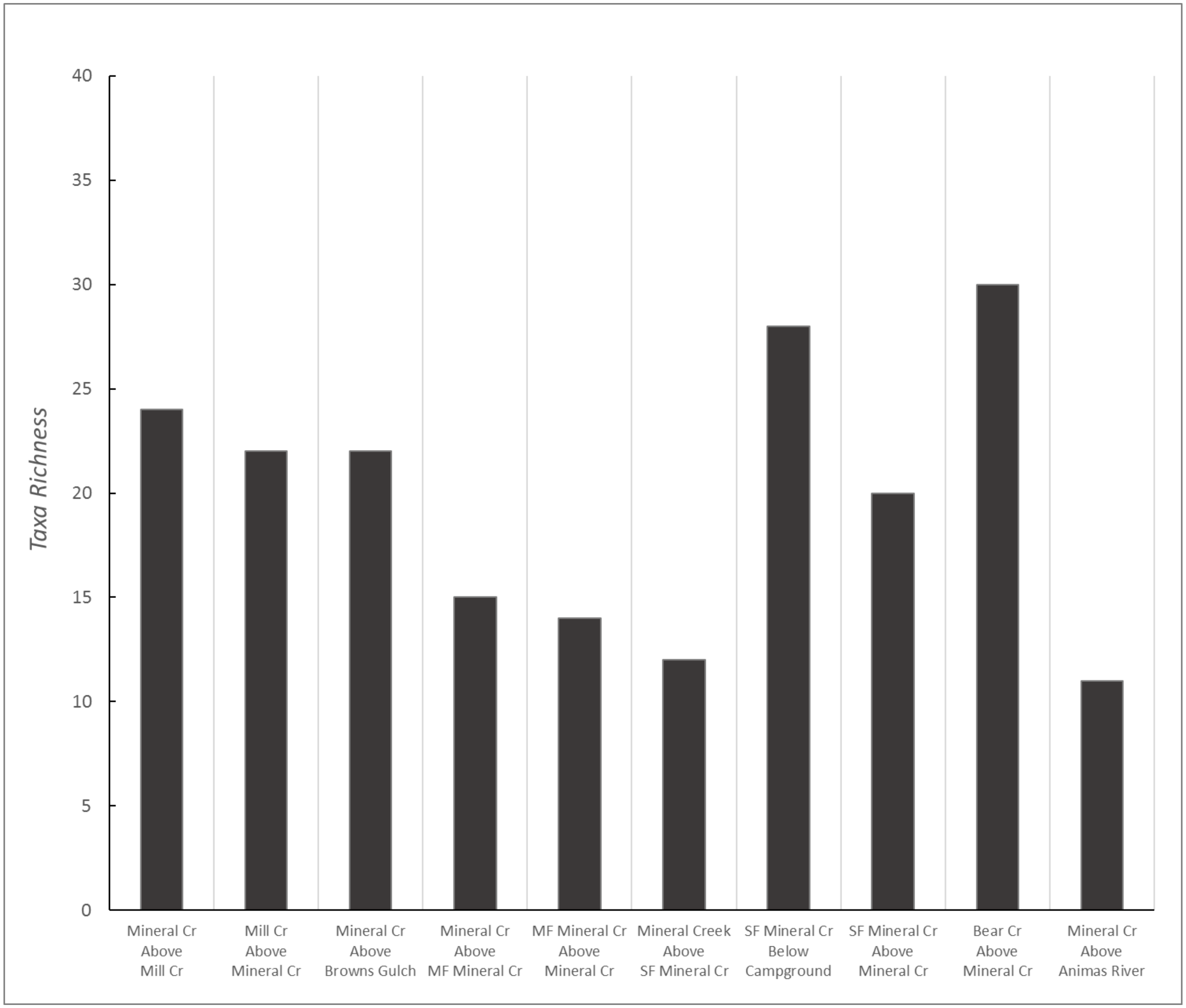

Figure 3: Taxa richness – Mineral Creek Group

Note: see table 2 for an explanation of BMI metrics.

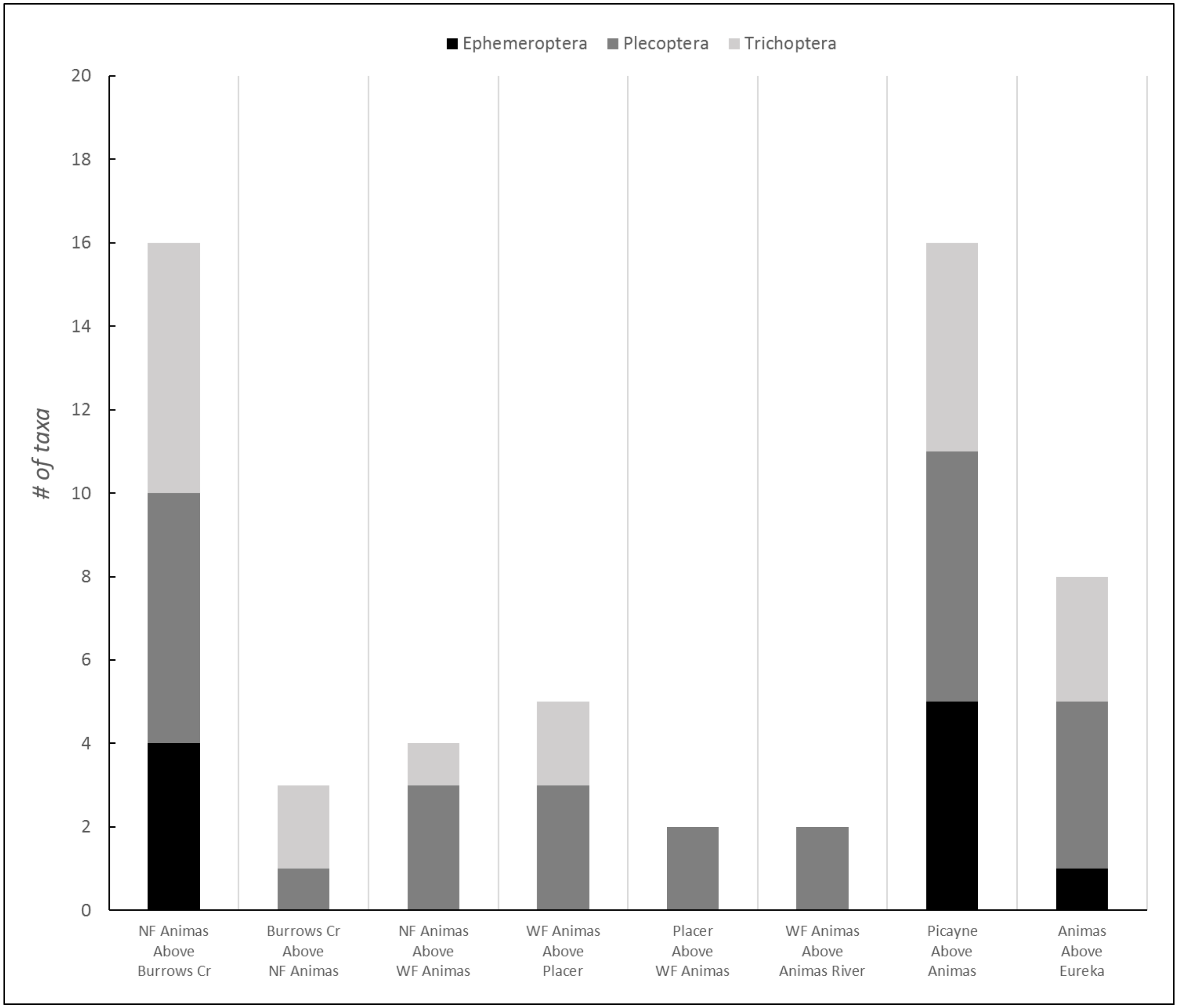

Figure 4: EPT (Ephemeroptera, Plecoptera, and Trichoptera) richness – Upper Animas Group A

Note: see table 2 for an explanation of BMI metrics.

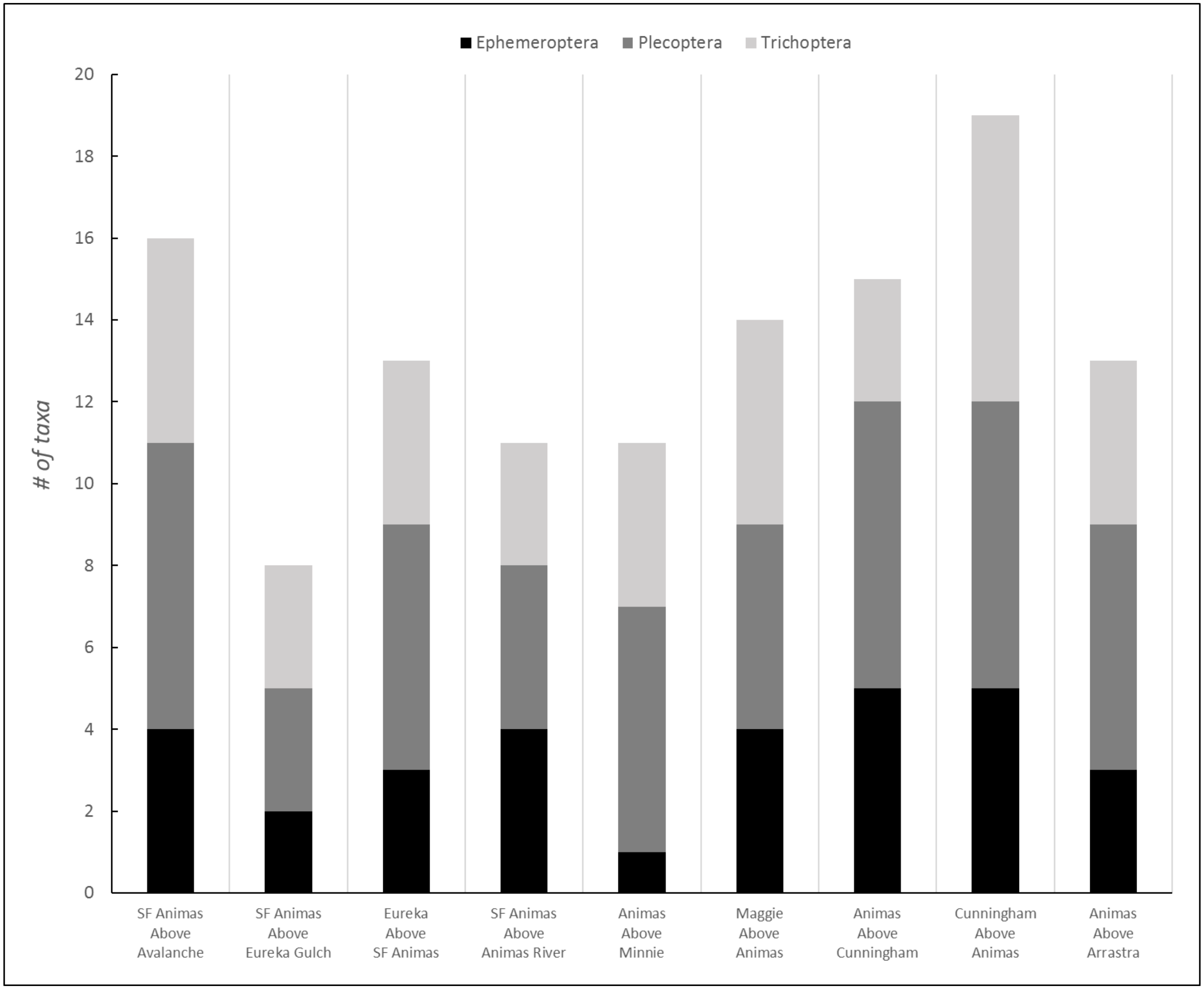

Figure 5: EPT (Ephemeroptera, Plecoptera, and Trichoptera) richness – Upper Animas Group B

Note: see table 2 for an explanation of BMI metrics.

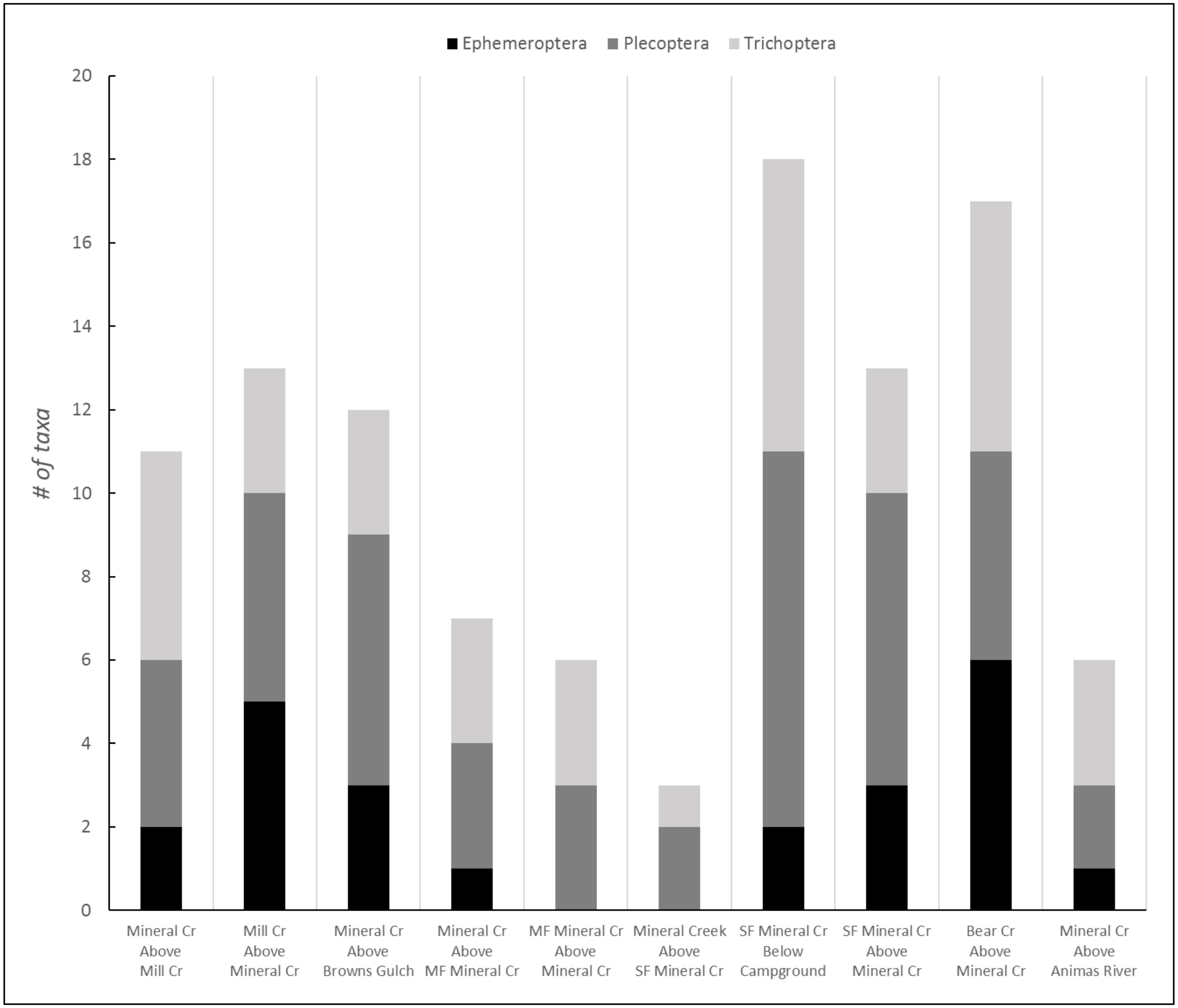

Figure 6: EPT (Ephemeroptera, Plecoptera, and Trichoptera) richness – Mineral Creek Group

Note: see table 2 for an explanation of BMI metrics.

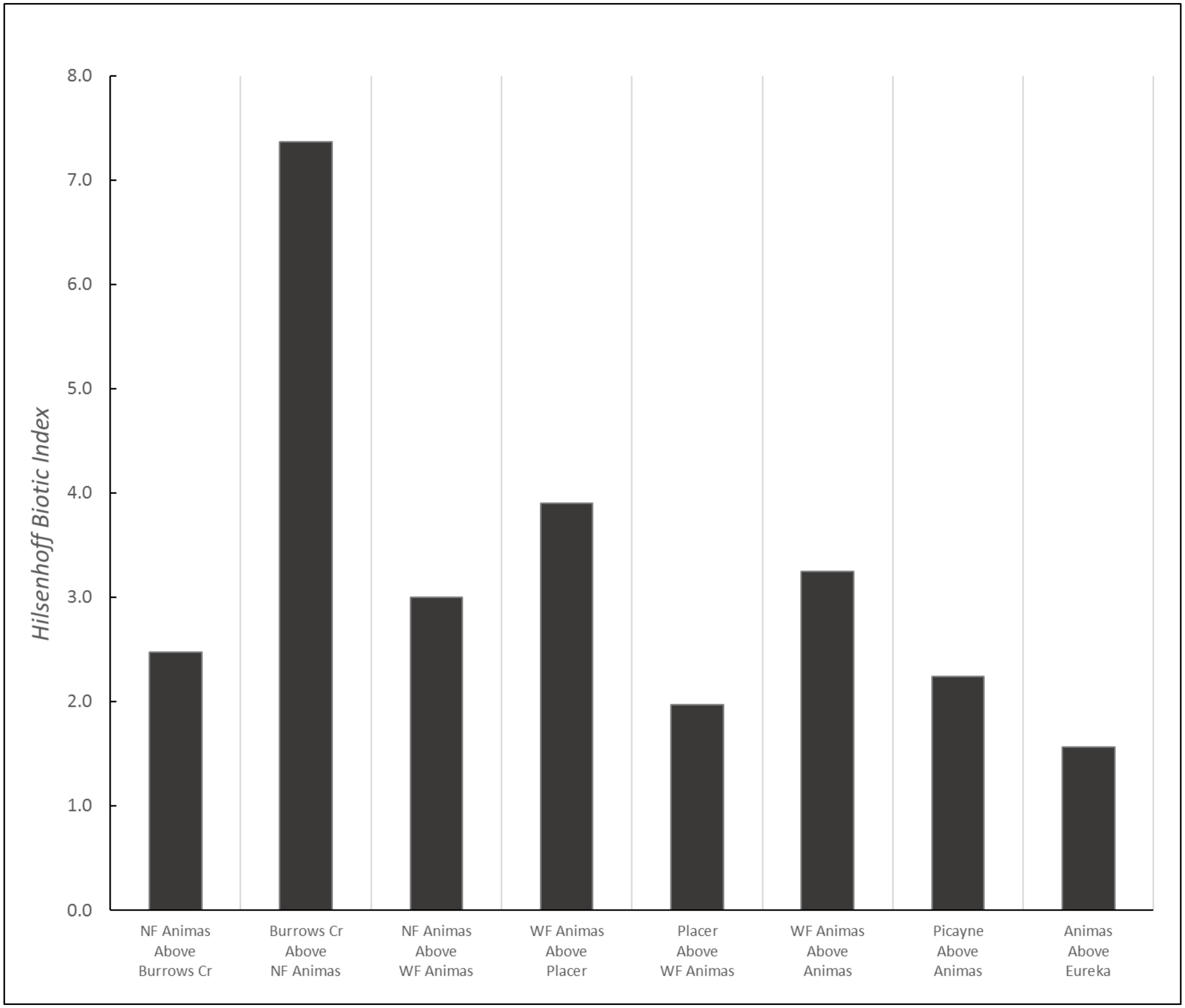

Figure 7: Hilsenhoff Biotic Index (HBI) – Upper Animas Group A

Note: see table 2 for an explanation of BMI metrics.

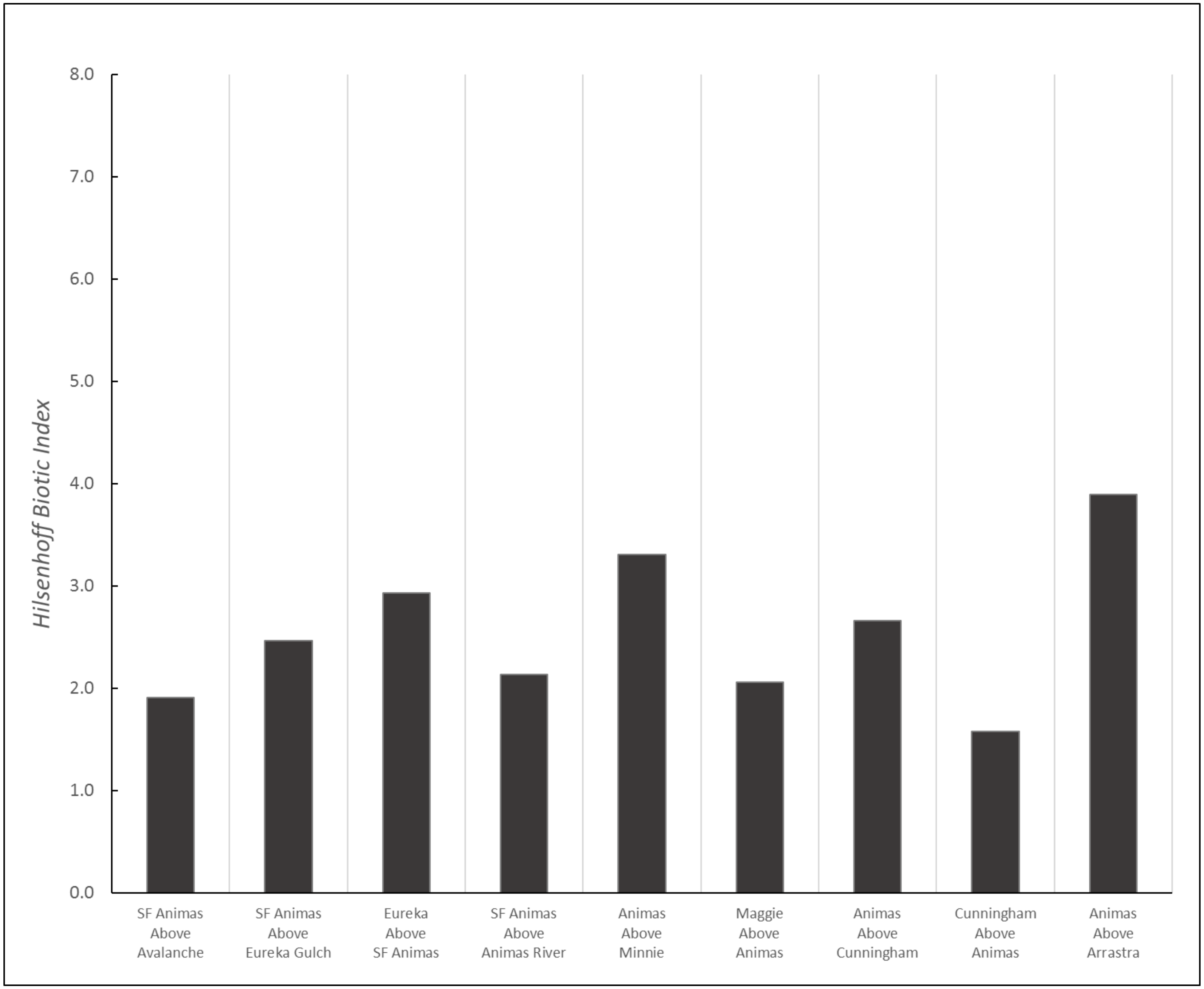

Figure 8: Hilsenhoff Biotic Index (HBI) – Upper Animas Group B
Note: see table 2 for an explanation of BMI metrics.

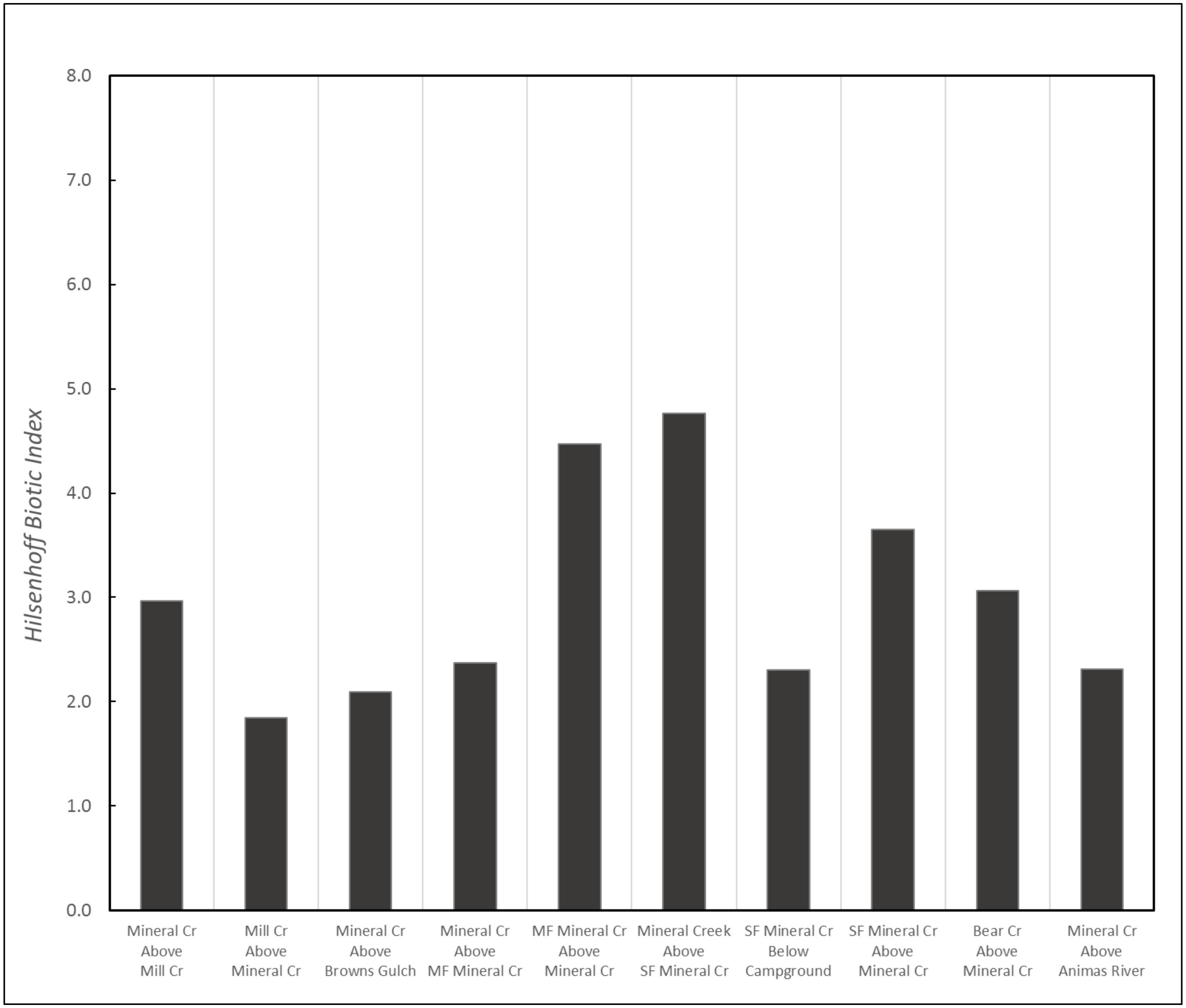

Figure 9: Hilsenhoff Biotic Index (HBI) – Mineral Creek Group

Note: see table 2 for an explanation of BMI metrics.

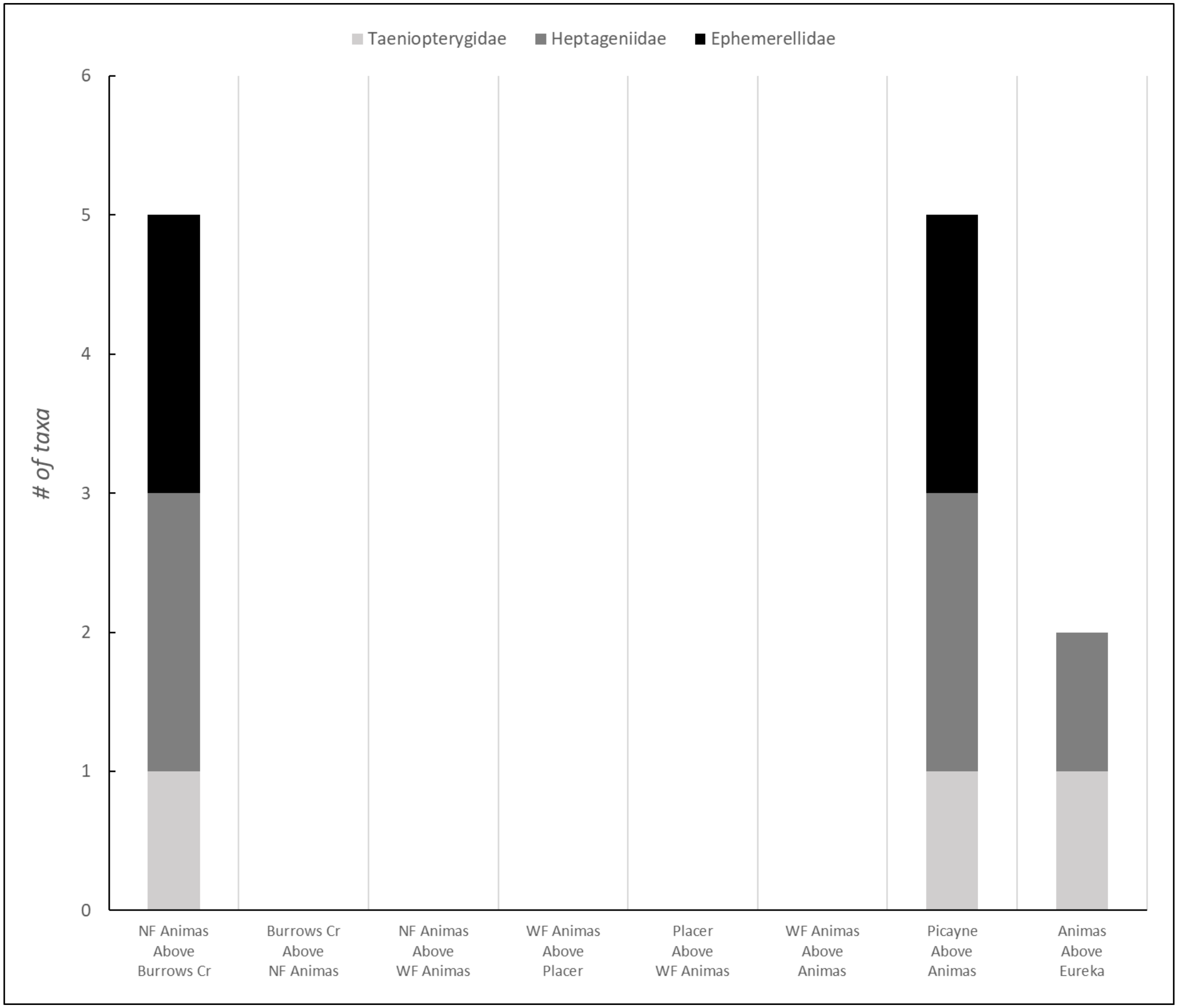

Figure 10: Richness of metal-sensitive families (Ephemerellidae; Heptageniidae; Taeniopterygidae) – Upper Animas Group A

Note: see table 2 for an explanation of BMI metrics.

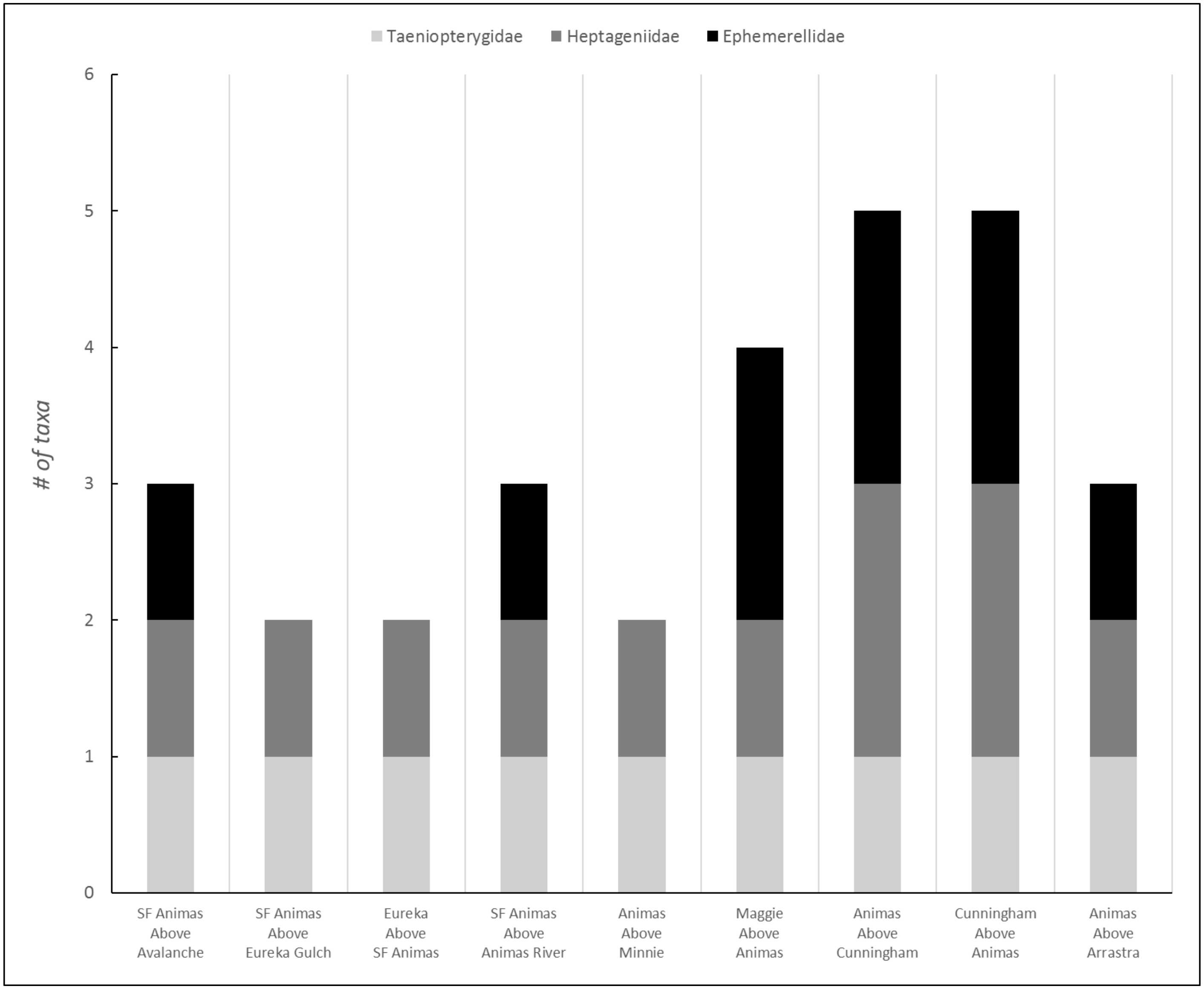

Figure 11: Richness of metal-sensitive families (Ephemerellidae; Heptageniidae; Taeniopterygidae) – Upper Animas Group B

Note: see table 2 for an explanation of BMI metrics.

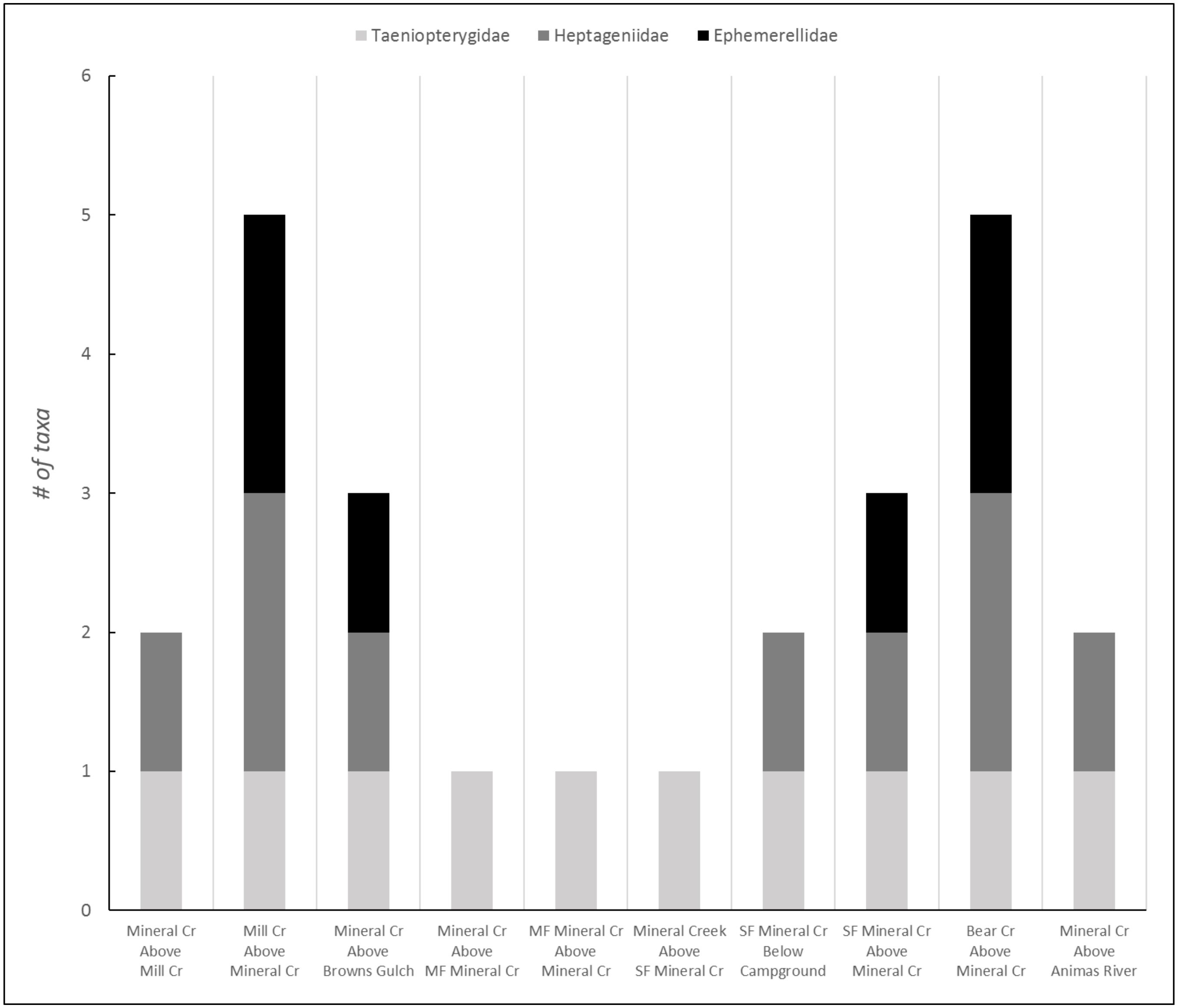

Figure 12: Richness of metal-sensitive families (Ephemerellidae; Heptageniidae; Taeniopterygidae) – Mineral Creek Group

Note: see table 2 for an explanation of BMI metrics.

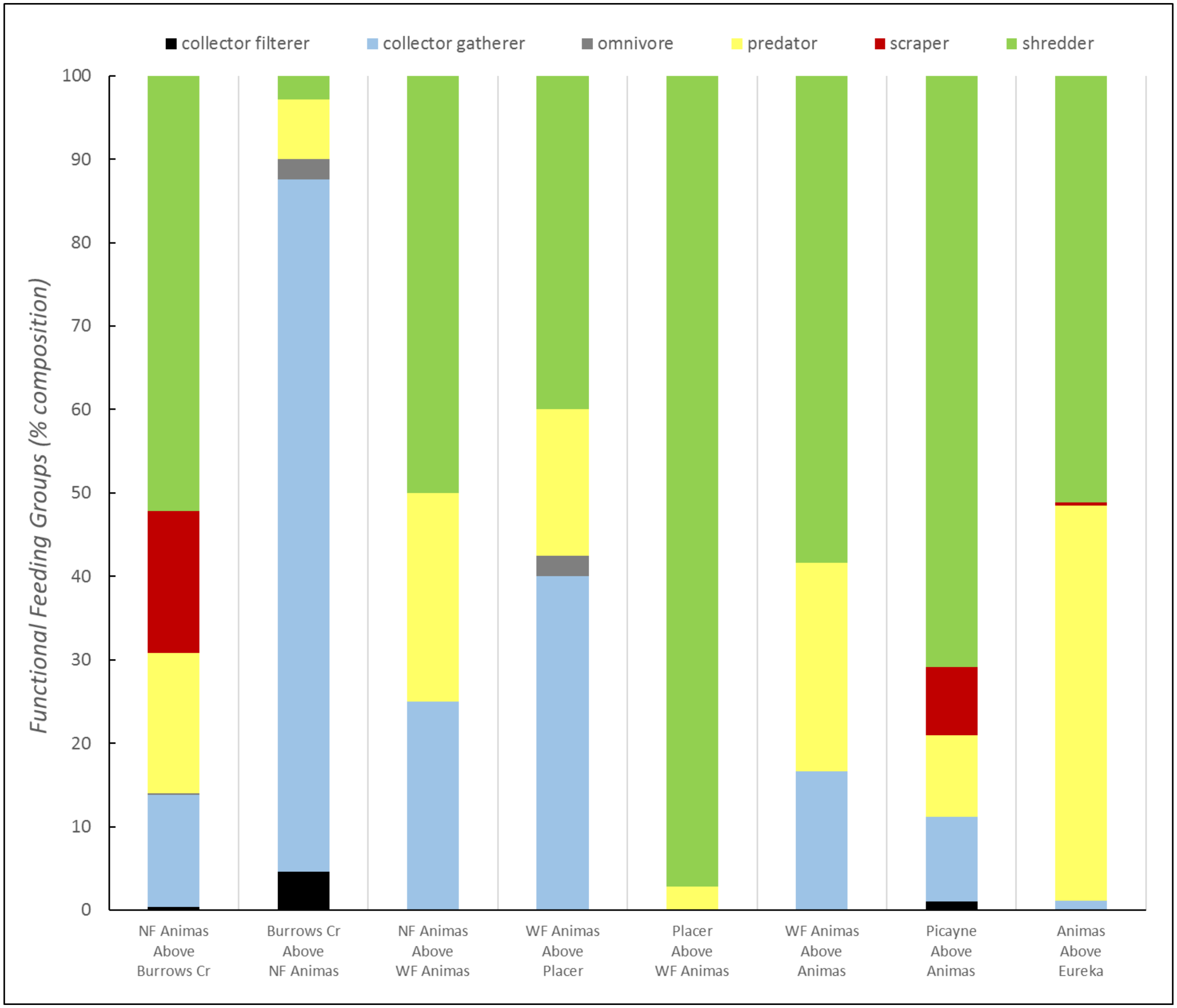

Figure 13: Relative abundance of functional feeding groups – Upper Animas Group A

Note: see table 2 for an explanation of BMI metrics.

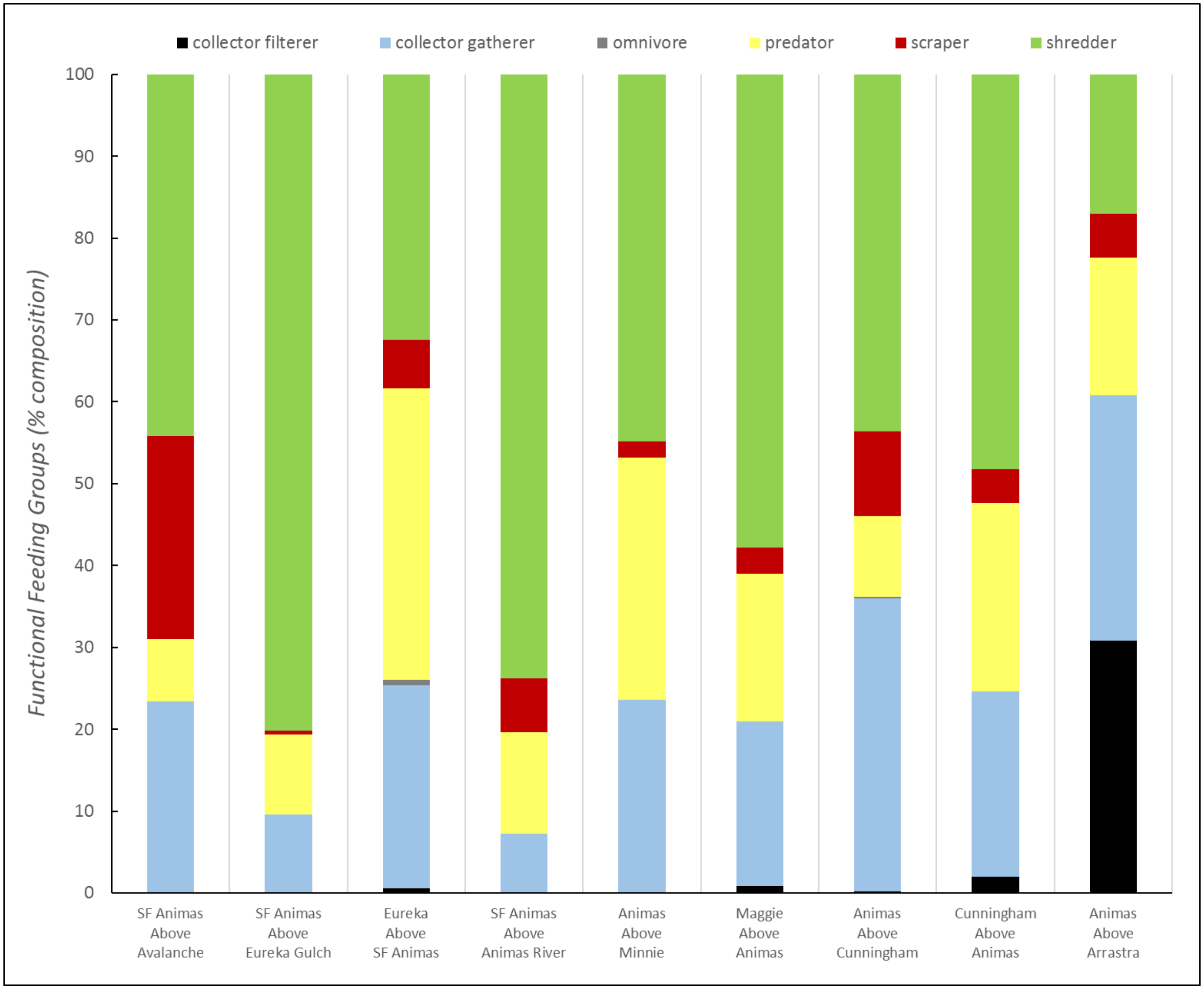

Figure 14: Relative abundance of functional feeding groups – Upper Animas Group B

Note: see table 2 for an explanation of BMI metrics.

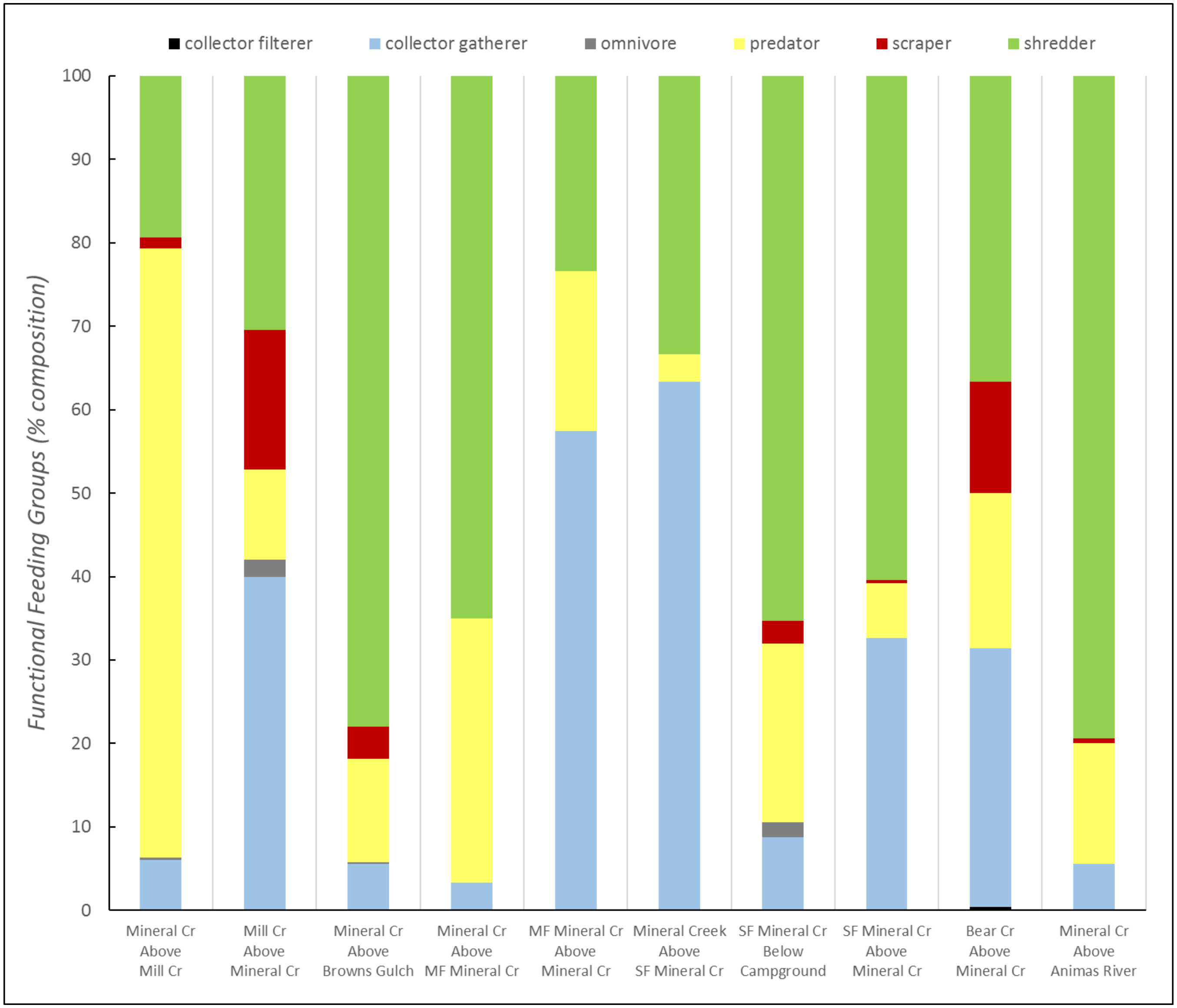

Figure 15: Relative abundance of functional feeding groups – Mineral Creek Group

Note: see table 2 for an explanation of BMI metrics.

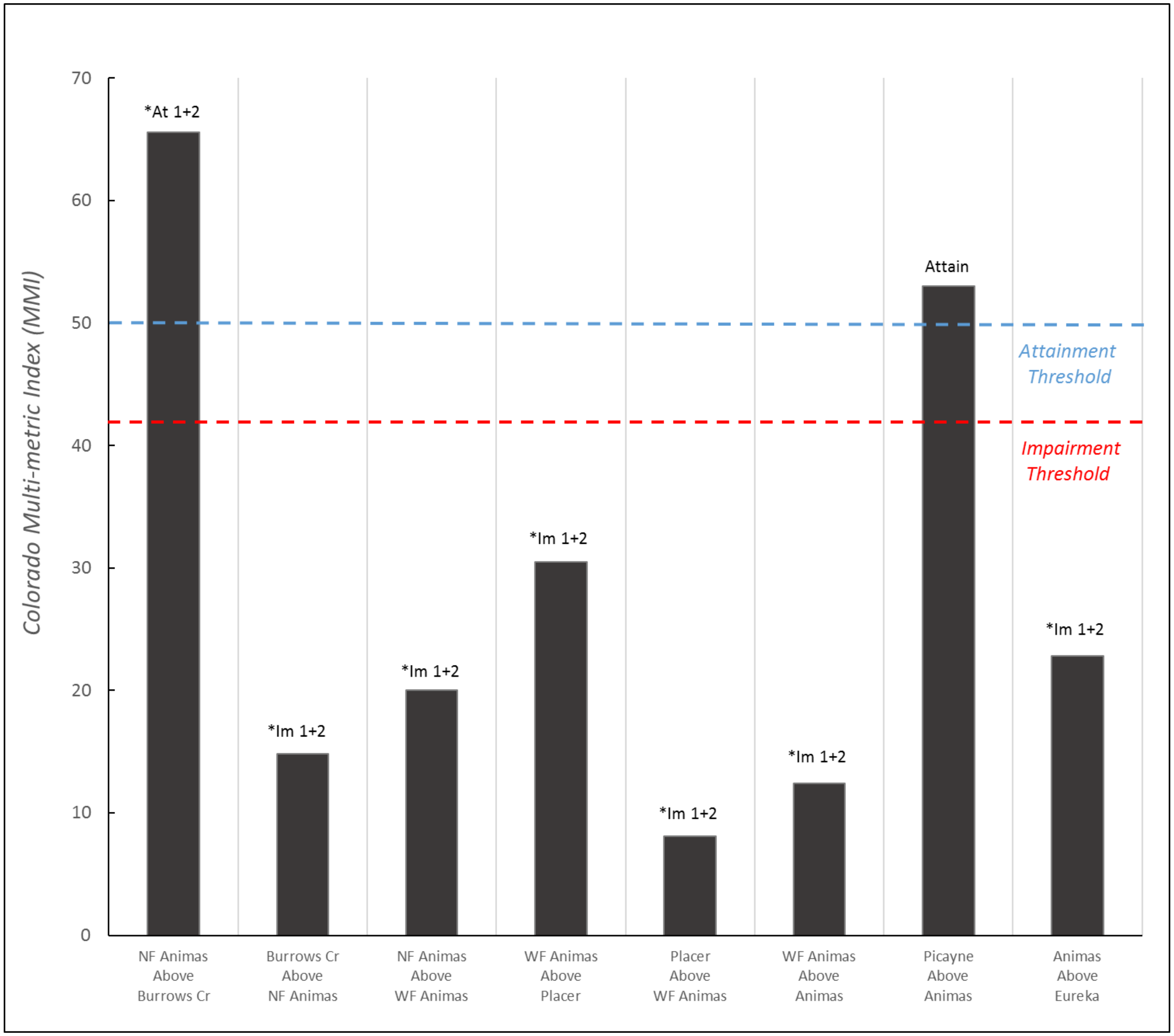

Figure 16: Multi-metric Index (MMI) – Upper Animas Group A

*Note: **Attain** = attainment of aquatic life use designation; **Impaired** = impairment of aquatic life use designation; ***At 1+2** = No aquatic life use designation, but theoretically would be in attainment of class 1 and class 2; ***At 2, Im1** = No aquatic life use designation, but theoretically would be in attainment of class 2, but not class 1; ***Im 1+2** = No aquatic life use designation, but theoretically would be in impairment of class 1 and class 2.*

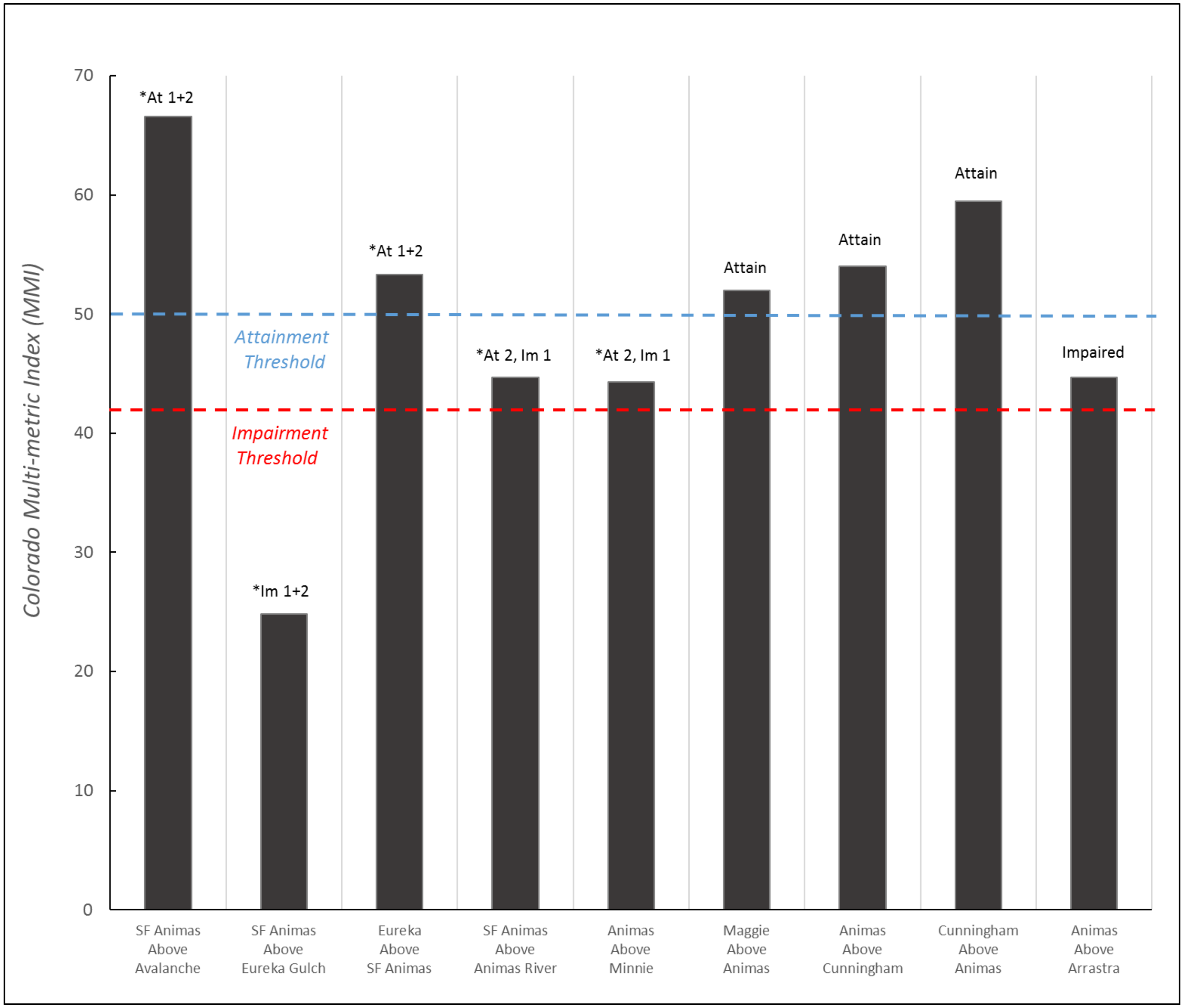

Figure 17: Multi-metric Index (MMI) – Upper Animas Group B

*Note: **Attain** = attainment of aquatic life use designation; **Impaired** = impairment of aquatic life use designation; ***At 1+2** = No aquatic life use designation, but theoretically would be in attainment of class 1 and class 2; ***At 2, Im1** = No aquatic life use designation, but theoretically would be in attainment of class 2, but not class 1; ***Im 1+2** = No aquatic life use designation, but theoretically would be in impairment of class 1 and class 2.*

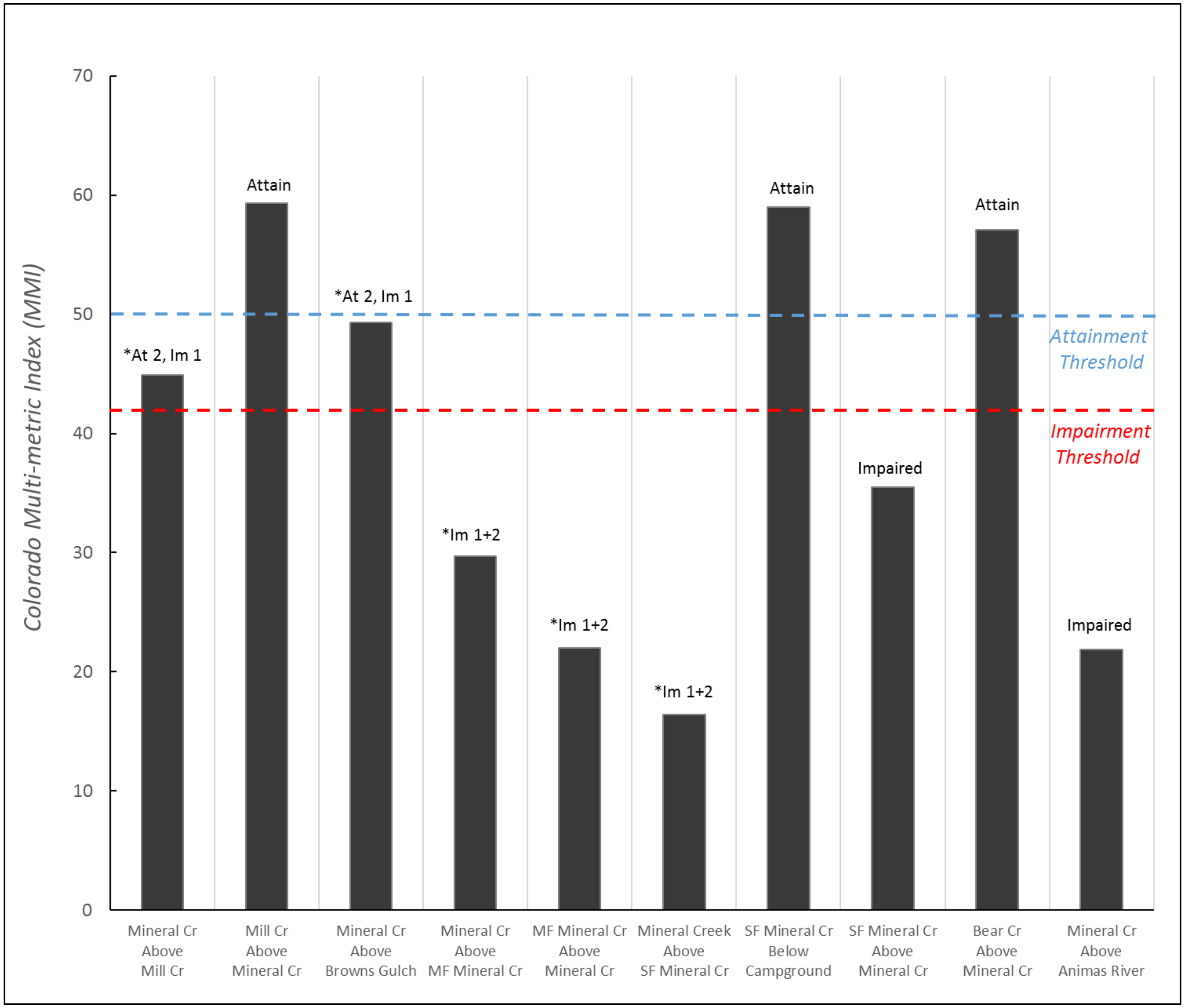

Figure 18: Multi-metric Index (MMI) – Mineral Creek Group

*Note: **Attain** = attainment of aquatic life use designation; **Impaired** = impairment of aquatic life use designation; ***At 1+2** = No aquatic life use designation, but theoretically would be in attainment of class 1 and class 2; ***At 2, Im1** = No aquatic life use designation, but theoretically would be in attainment of class 2, but not class 1; ***Im 1+2** = No aquatic life use designation, but theoretically would be in impairment of class 1 and class 2.*

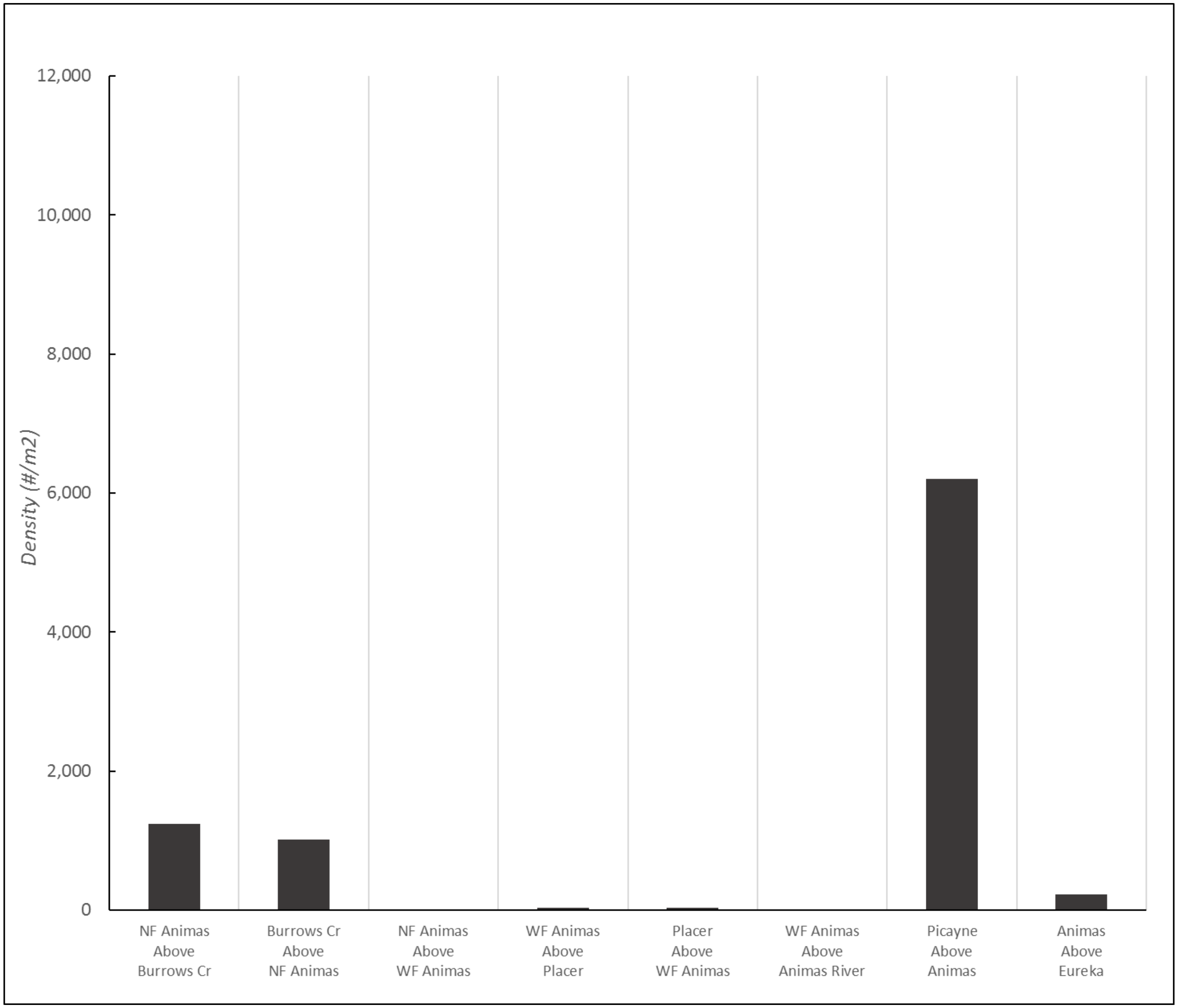

Figure 19: Density (#/m²) – Upper Animas Group A

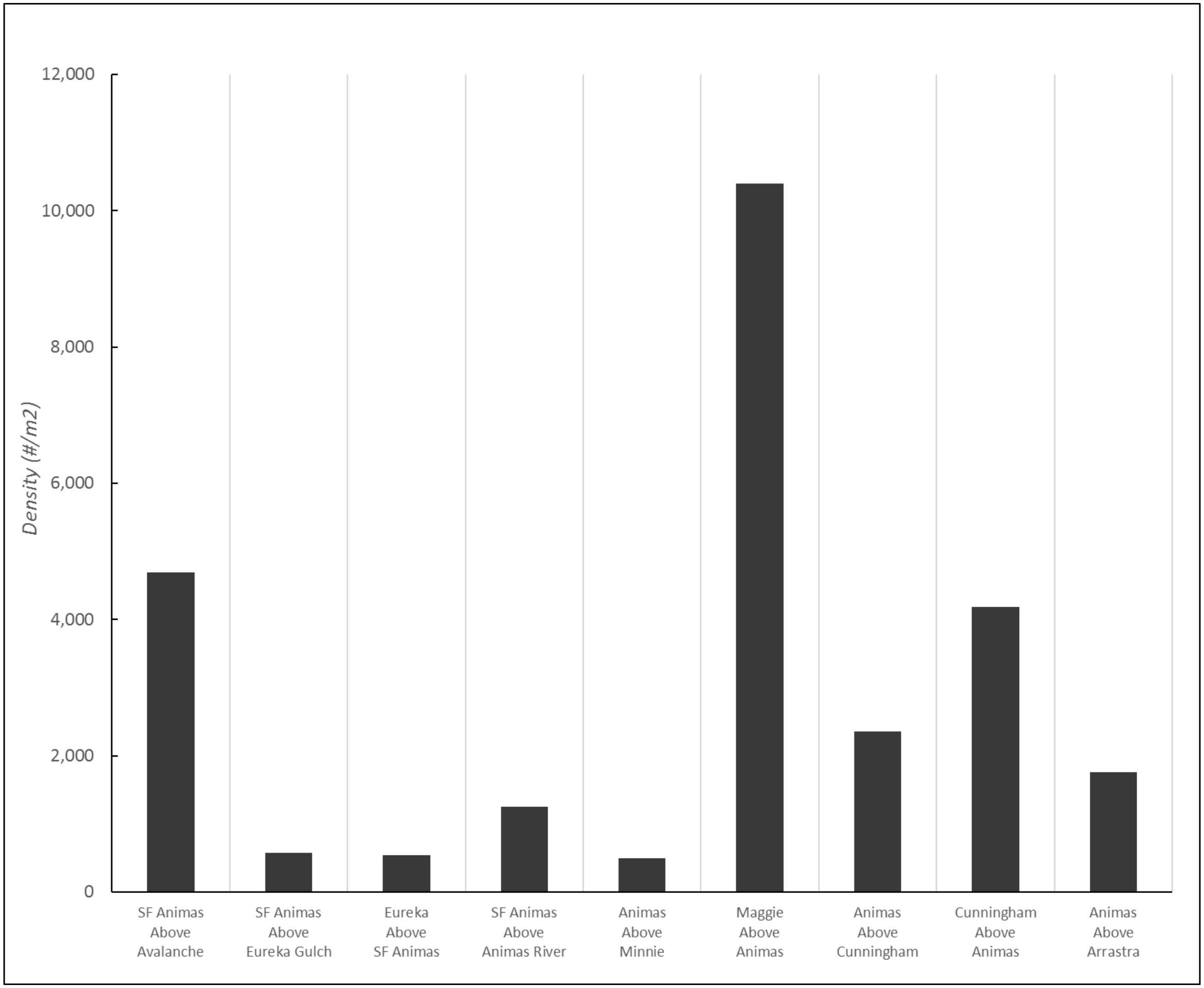

Figure 20: Density (#/m²) – Upper Animas Group B

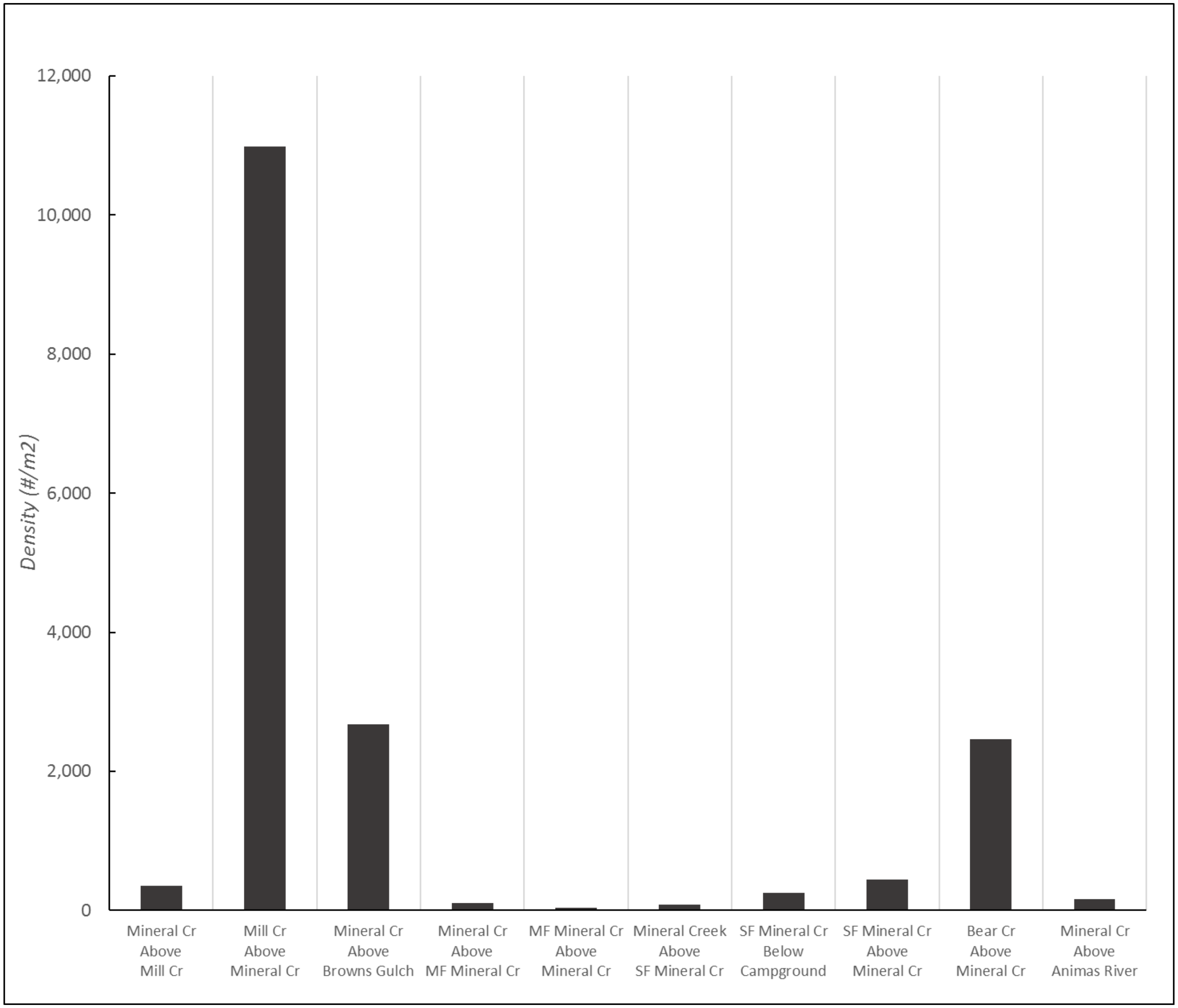

Figure 21: Density (#/m²) – Mineral Creek Group

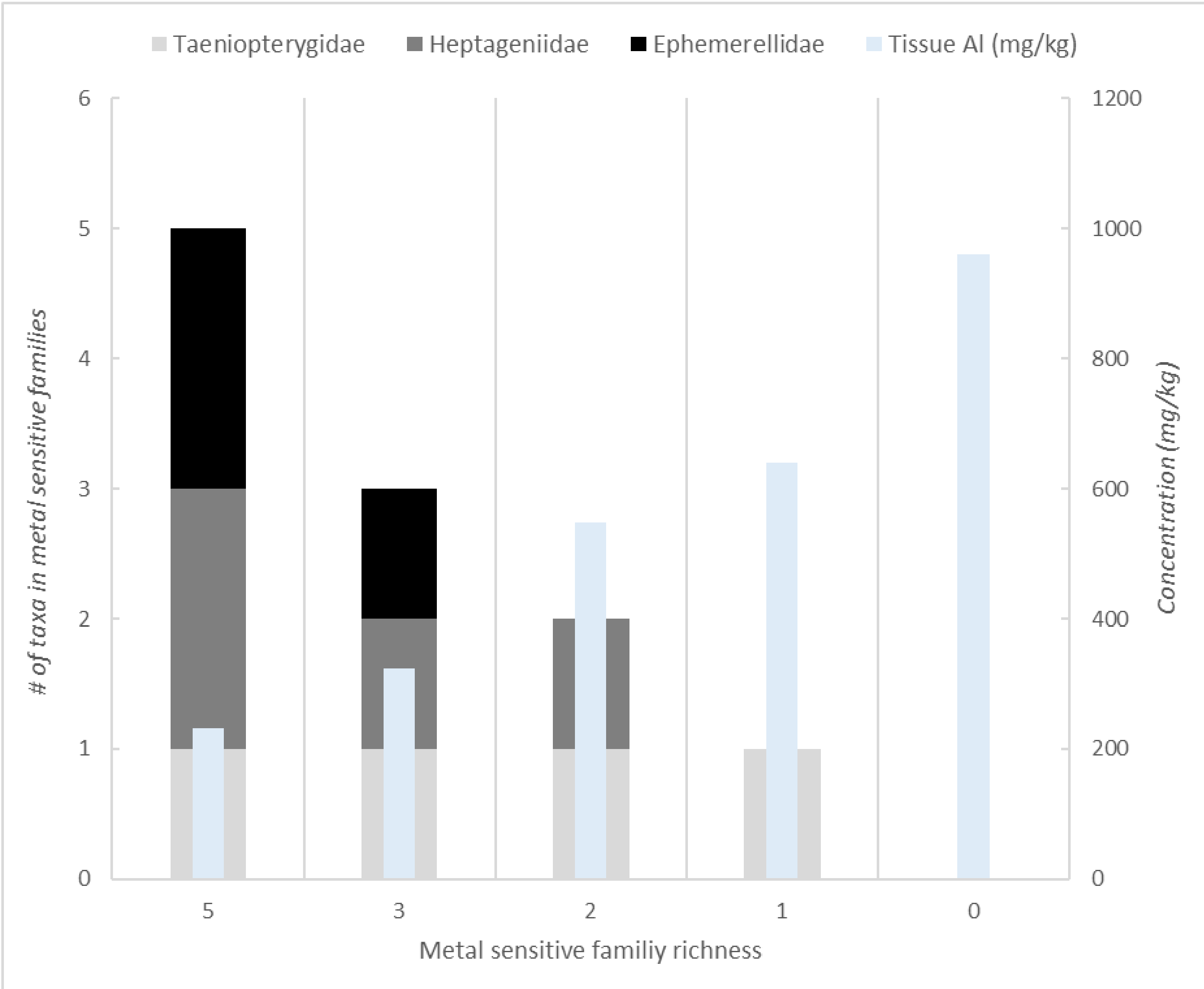

Figure 22: Concentration of aluminum in BMI tissue along a gradient of decreasing metal sensitive family richness

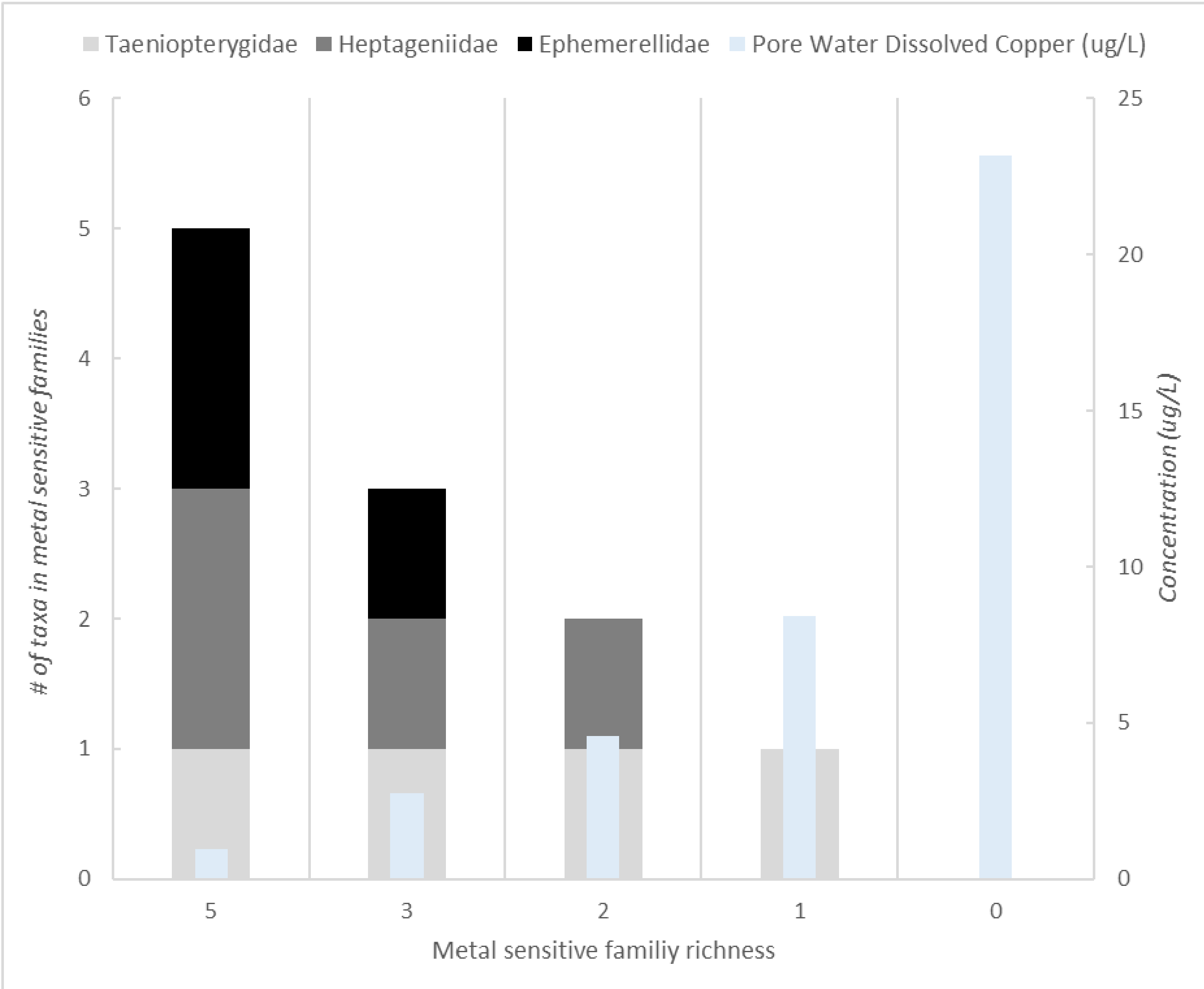

Figure 23: Concentration of dissolved copper in pore water along a gradient of decreasing metal sensitive family richness

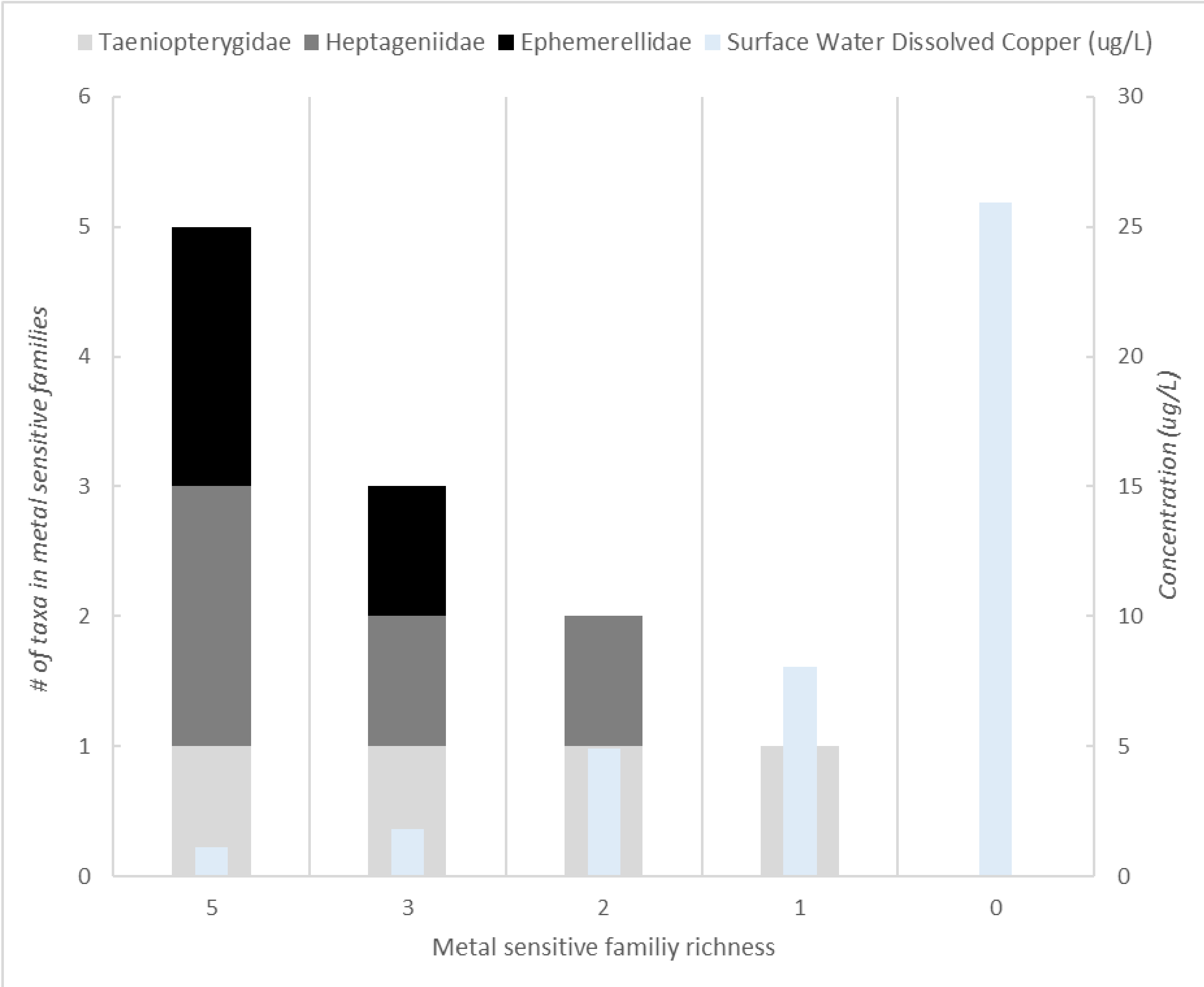

Figure 24: Concentration of dissolved copper in surface water along a gradient of decreasing metal sensitive family richness

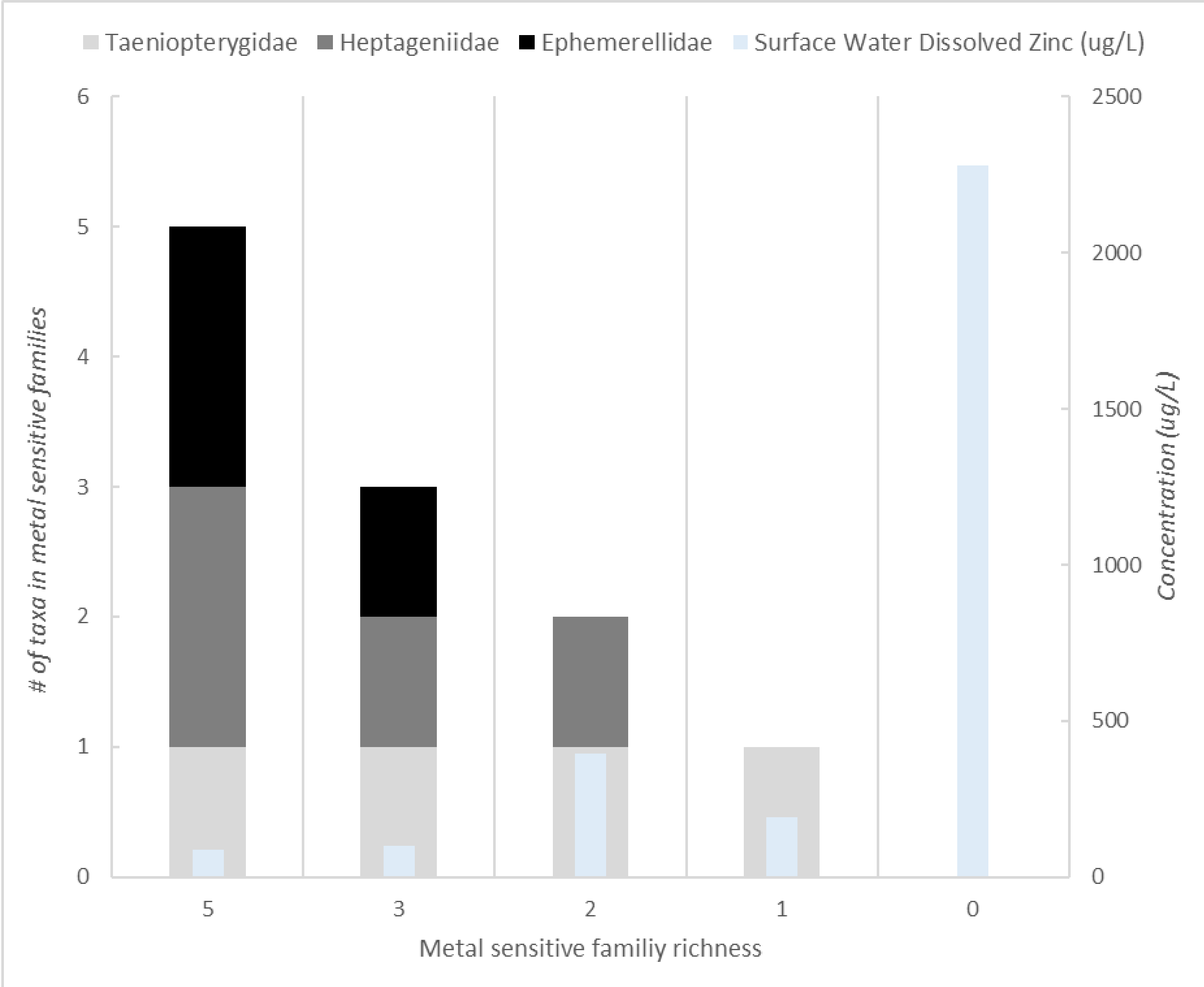

Figure 25: Concentration of dissolved zinc in surface water along a gradient of decreasing metal sensitive family richness

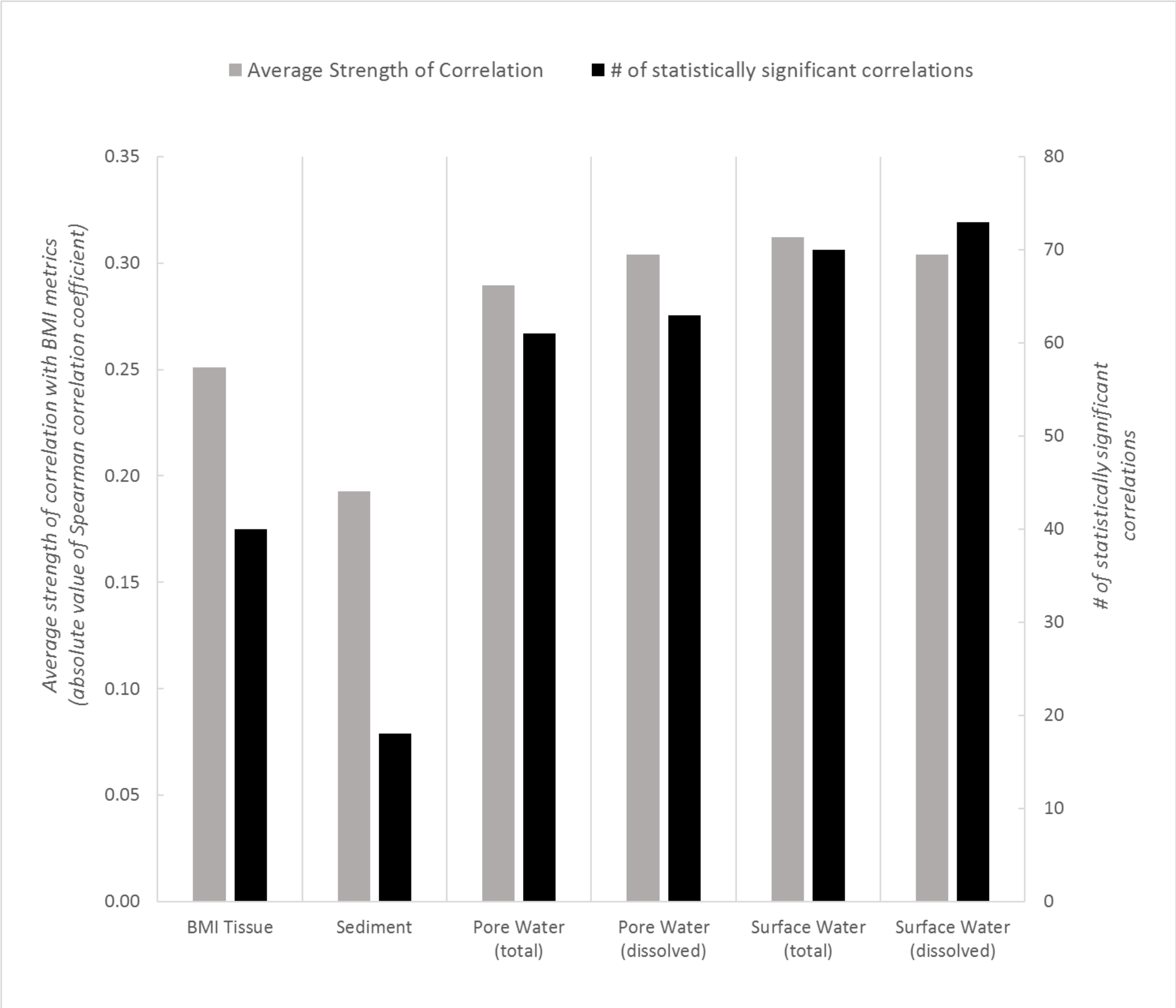

Figure 26: Correlations for each media between BMI metrics and metals and minerals

Note: Correlation analysis included all metals and minerals that were analyzed consistently across all media and sites (Al, Sb, As, Be, Cd, Cr, Cu, Fe, Pb, Mg, Mn, Ni, Se, Ag, Tl, and Zn).

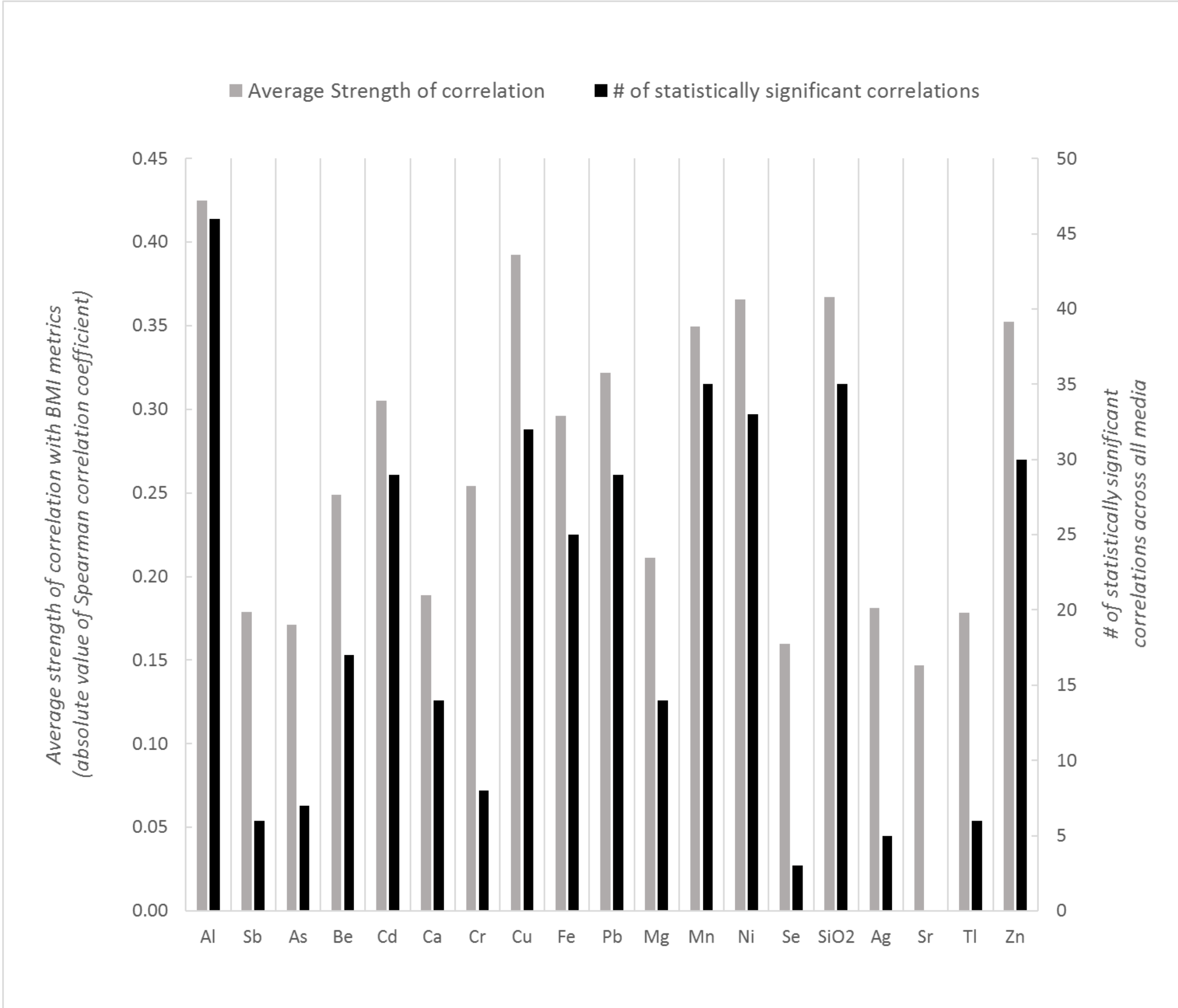

Figure 27: Correlations between BMI metrics and each metal and mineral across all media

Note: Correlation analysis included all metals and minerals that were analyzed consistently across all media and sites (Al, Sb, As, Be, Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Ni, Se, Ag, Tl, and Zn). In addition, Sr and SiO_2 were included even though they were not analyzed for sediment.

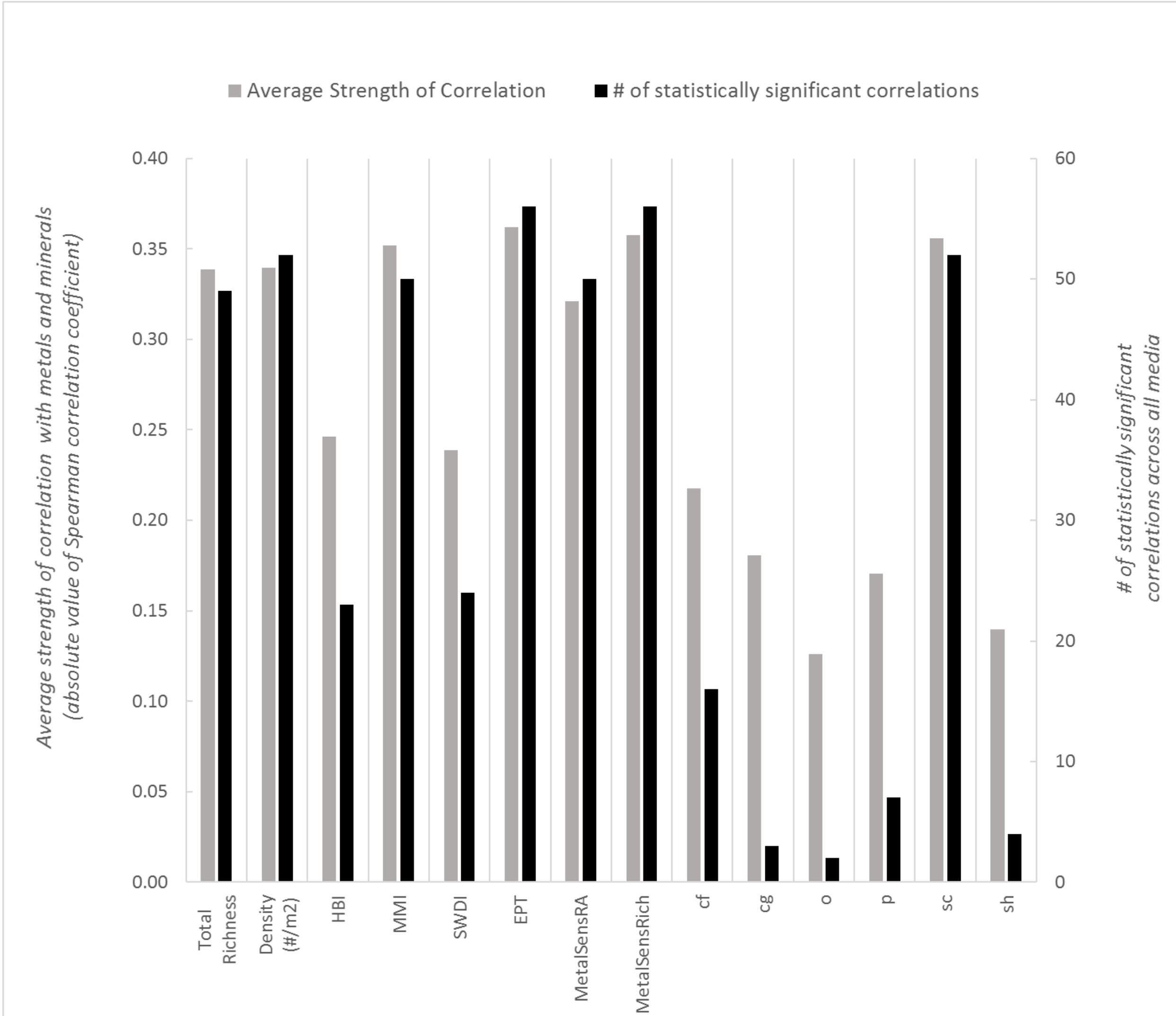

Figure 28: Correlations between BMI metrics and metals and minerals for each BMI metric

Note: See table 2 for explanation of BMI metrics. MetalSensRA = relative abundance of Ephemerellidae, Heptageniidae, and Taeniopterygidae familes; MetalSensRich = richness of Ephemerellidae, Heptageniidae, and Taeniopterygidae families. Functional Feeding Groups include collector-filterers (cf), collector-gatherers (cg), omnivores (o), predators (p), scrapers (sc), and shredders (sh).

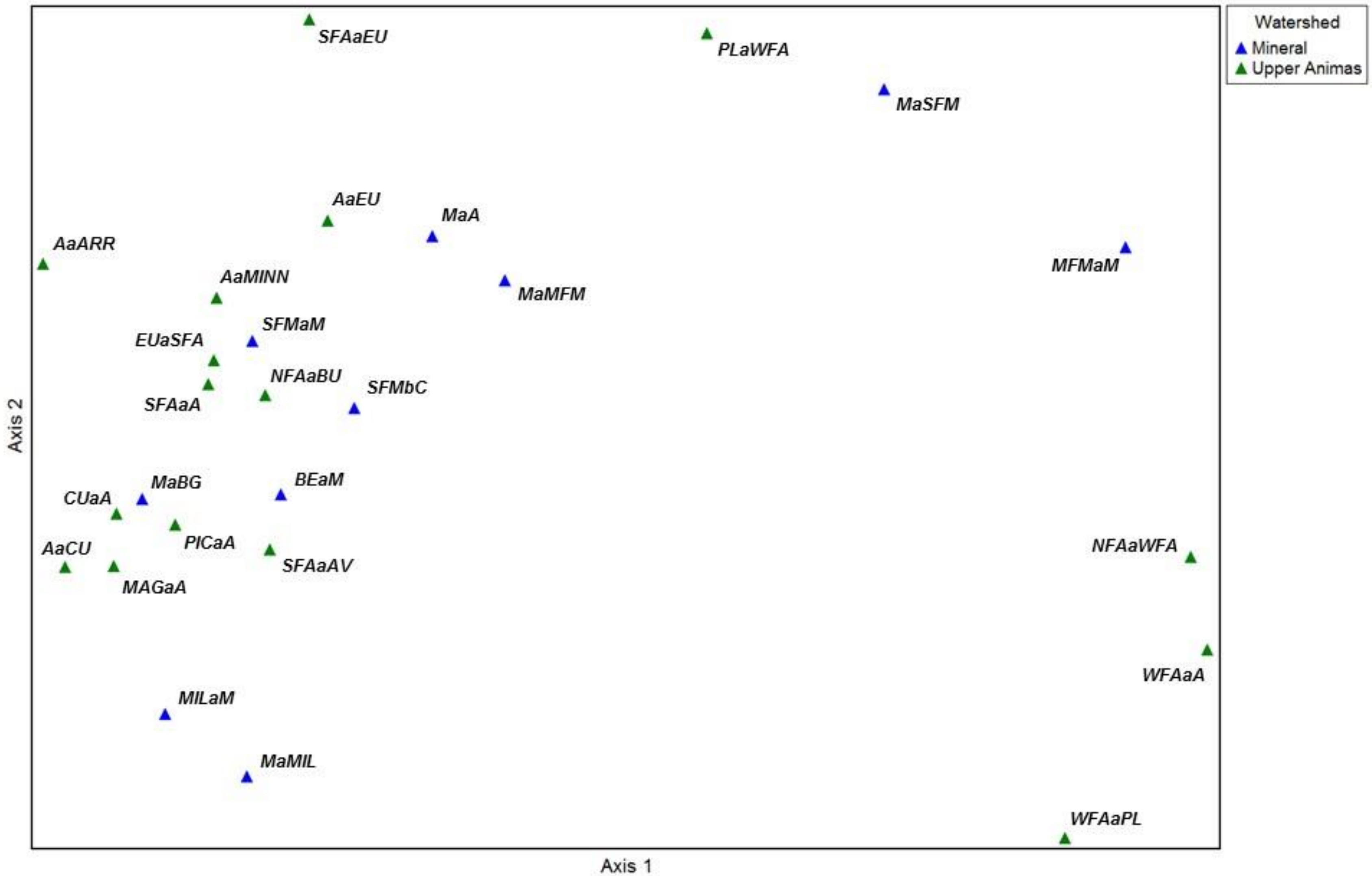

Figure 29: NMS ordination. Sites grouped by watershed (Animas River, Mineral Cr). See Table 13 for ordination details

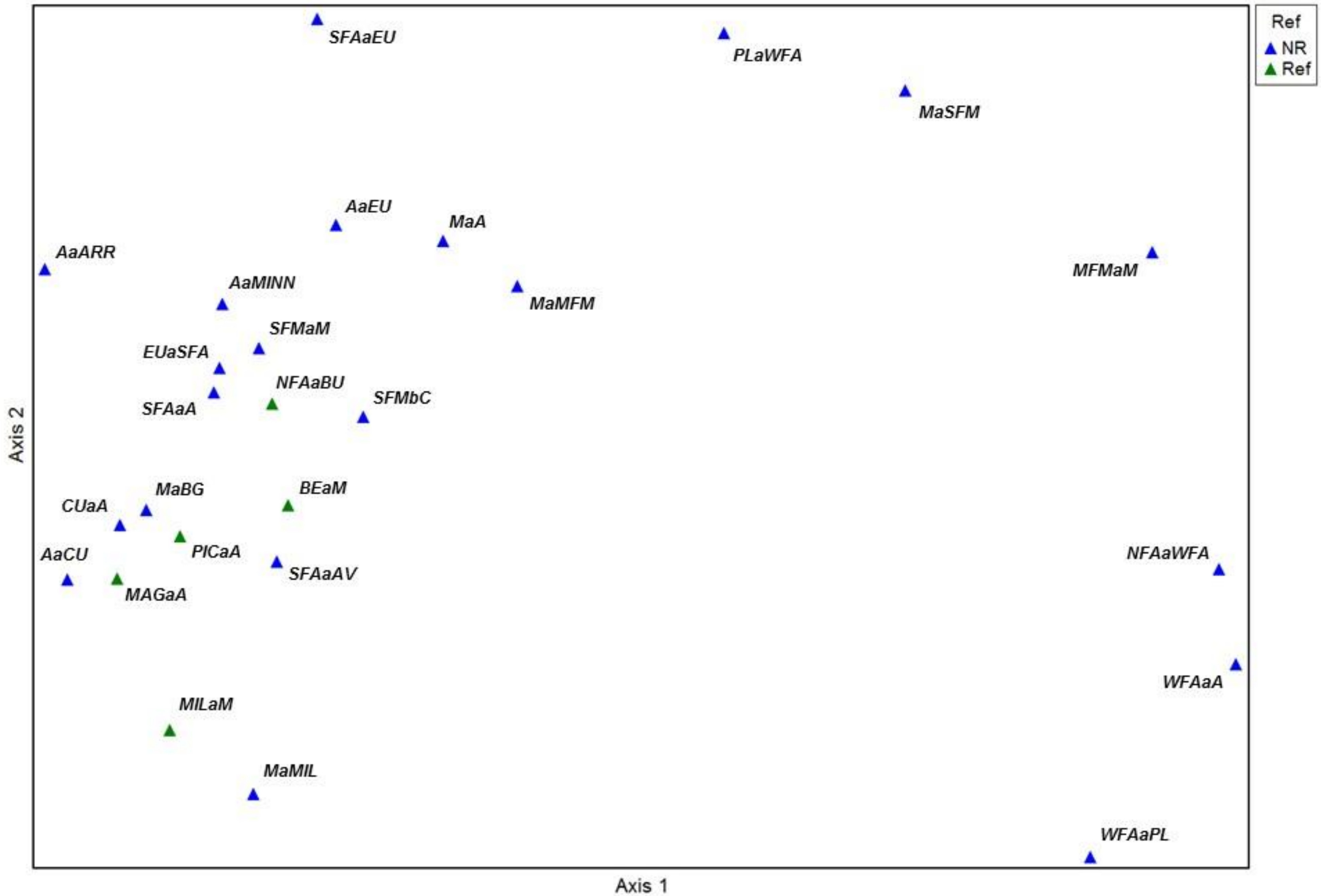

Figure 30: NMS ordination. Sites grouped by reference (Ref) and non-reference (NR). See Table 13 for ordination details

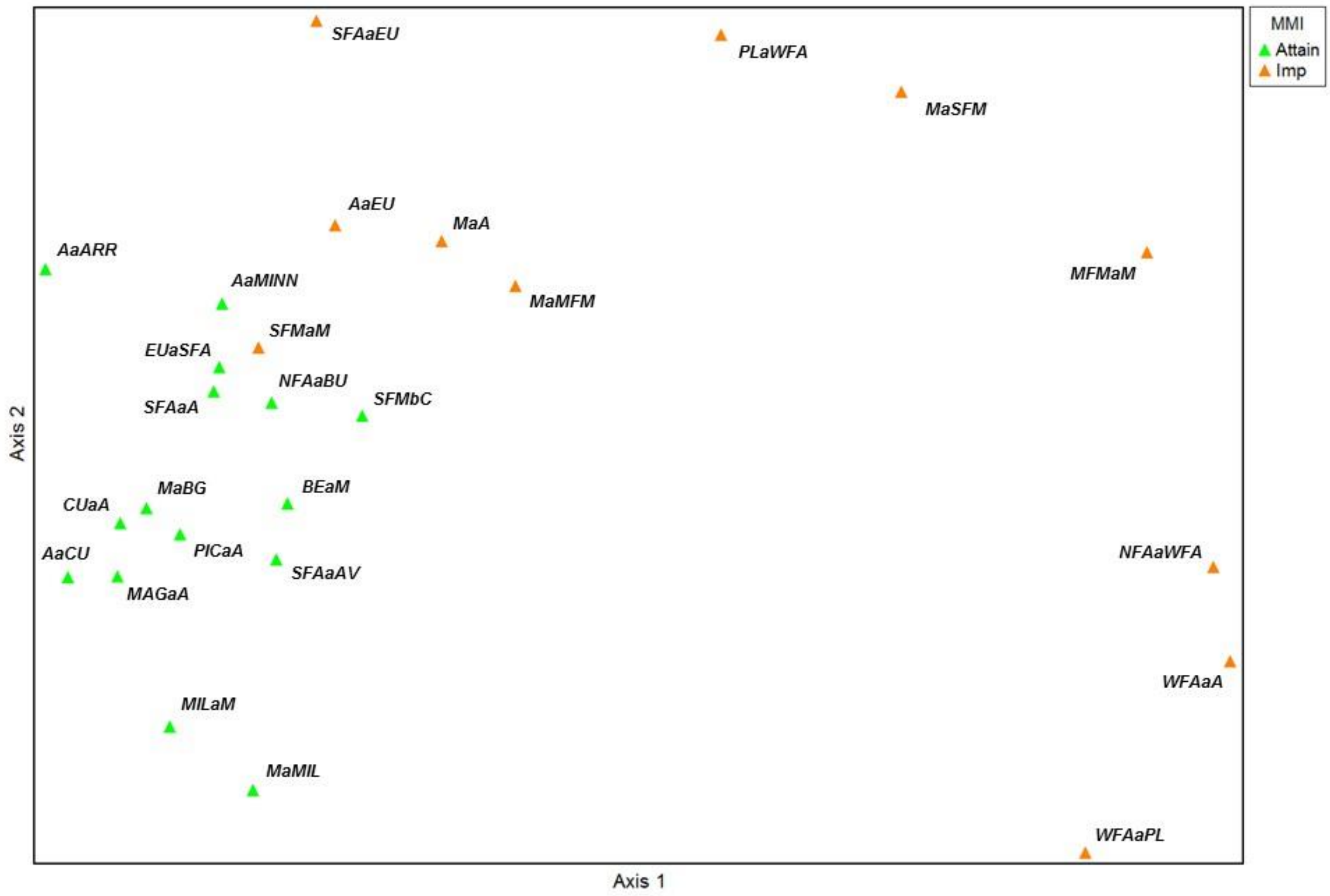

Figure 31: NMS ordination. Sites grouped by aquatic life use attainment (Attain) and impairment (Imp) of class two waters based on MMI scores. The assessment of attainment/impairment is for illustration only since it is based on a theoretical assumption that all sites are designated as having a class two aquatic life use designation. See Table 13 for ordination details

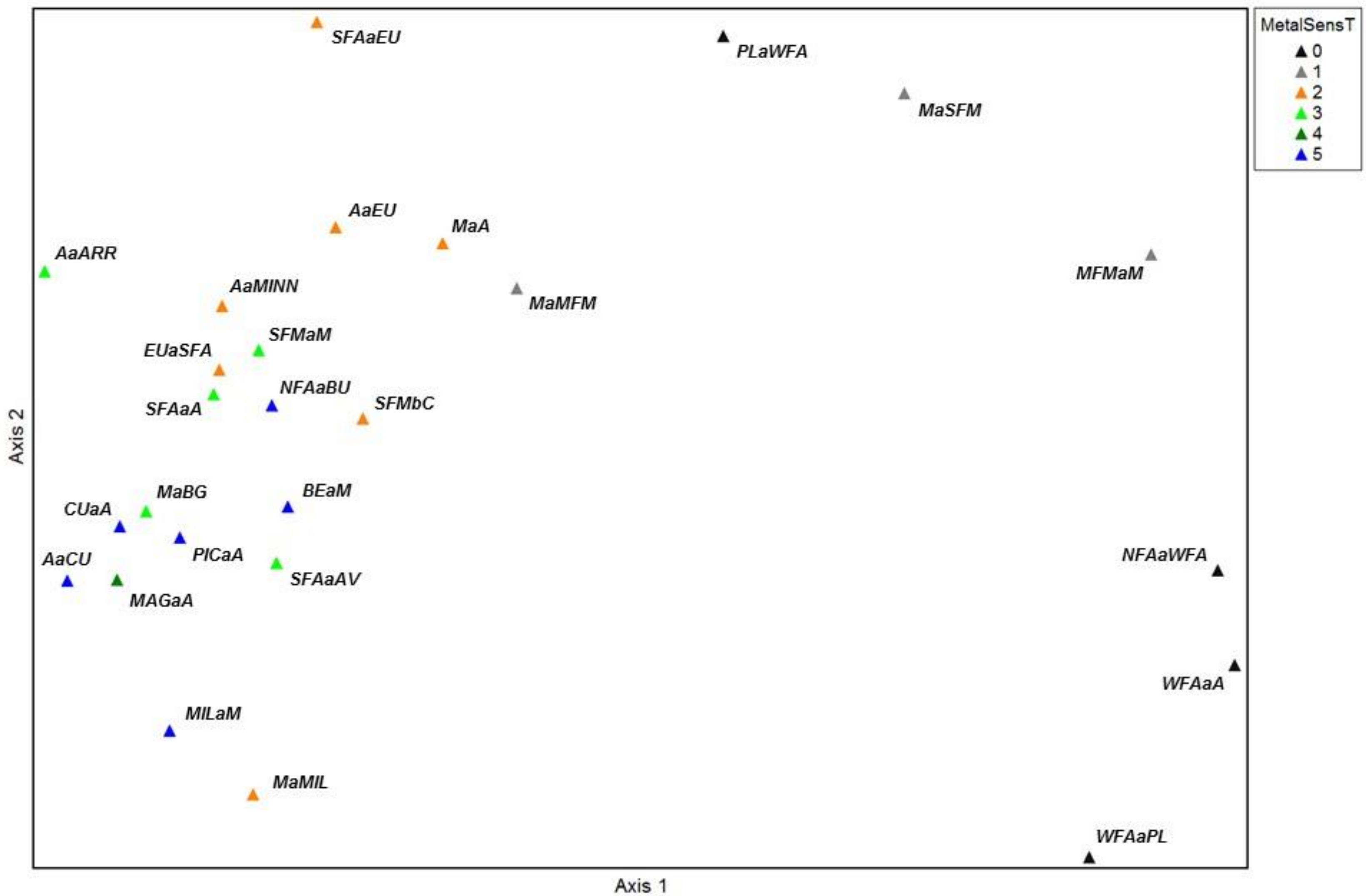

Figure 32: NMS ordination. Sites grouped by richness of metal-sensitive families (Ephemerellidae; Heptageniidae; Taeniopterygidae. See Table 13 for ordination details

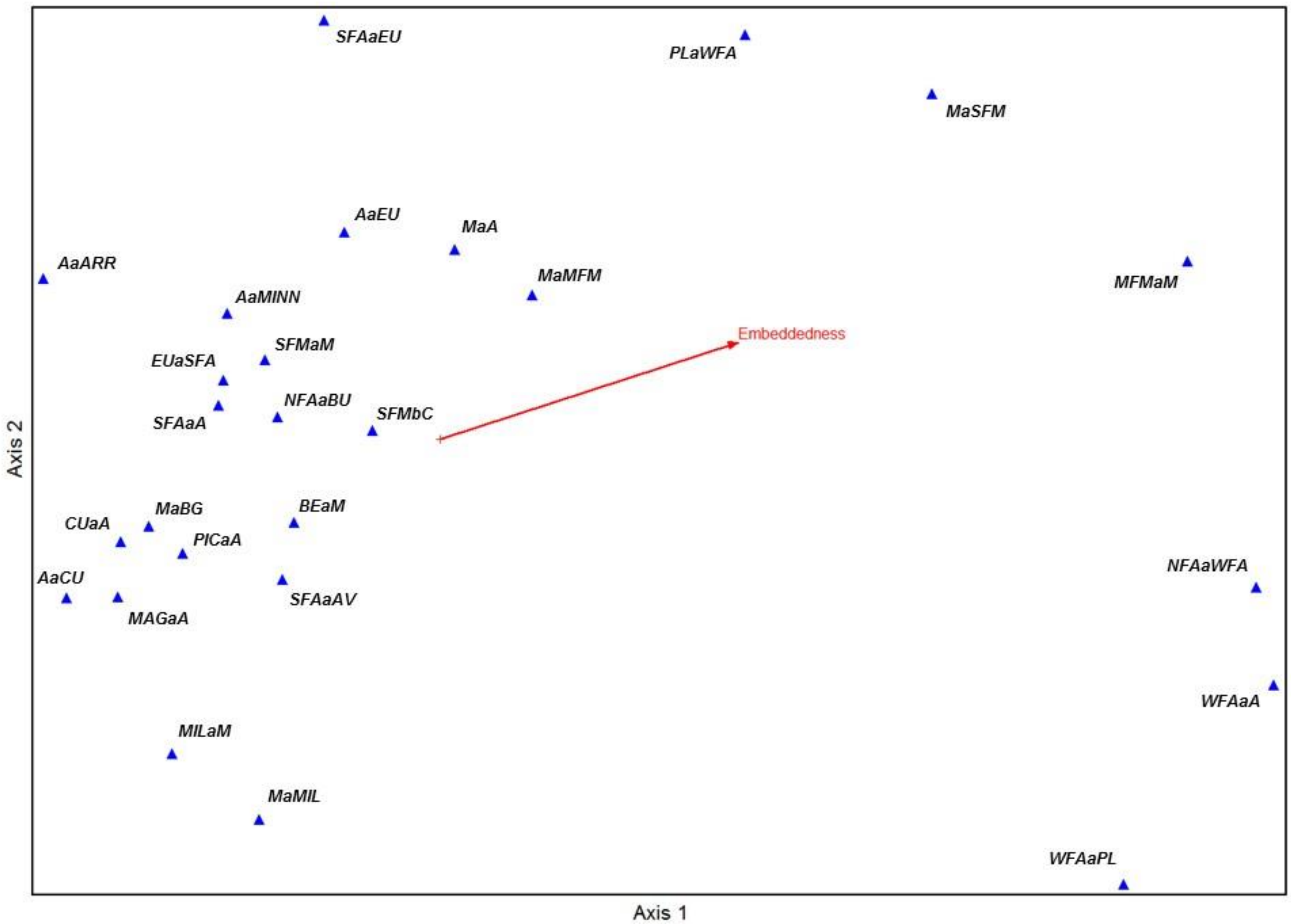

Figure 33: NMS ordination with orthogonal vector lines for physical habitat variables (r^2 correlation greater than 0.4). The angle and length of the lines reflect the direction and strength of the relationship between the variable and ordination axes. See Table 13 for ordination details

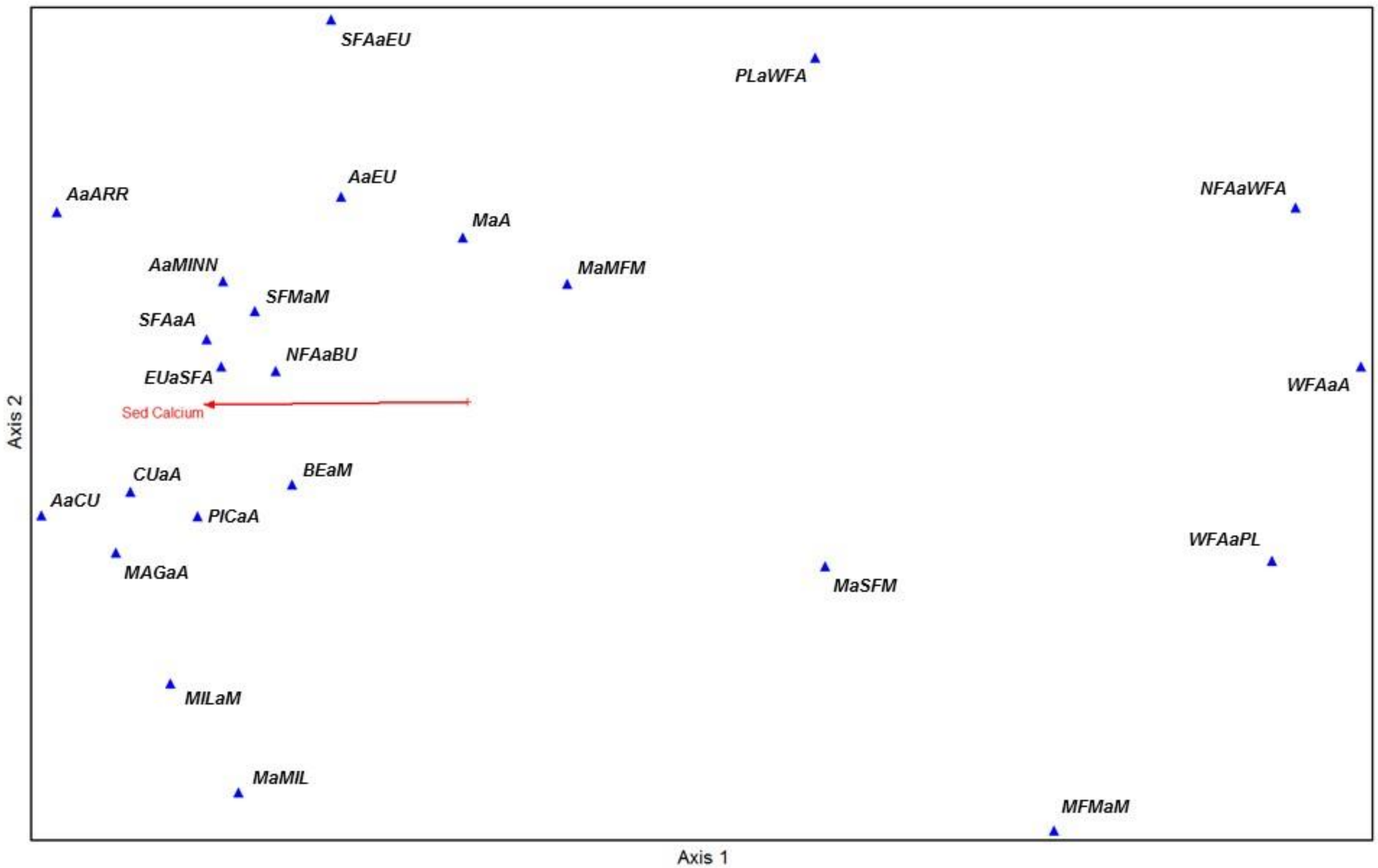

Figure 34: NMS ordination with orthogonal vector lines for metal and mineral concentrations in sediment (r^2 correlations greater than 0.4). The angle and length of the lines reflect the direction and strength of the relationship between the variable and ordination axes. See Table 13 for ordination details. This ordination is based on the subset of sites where sediment samples were collected

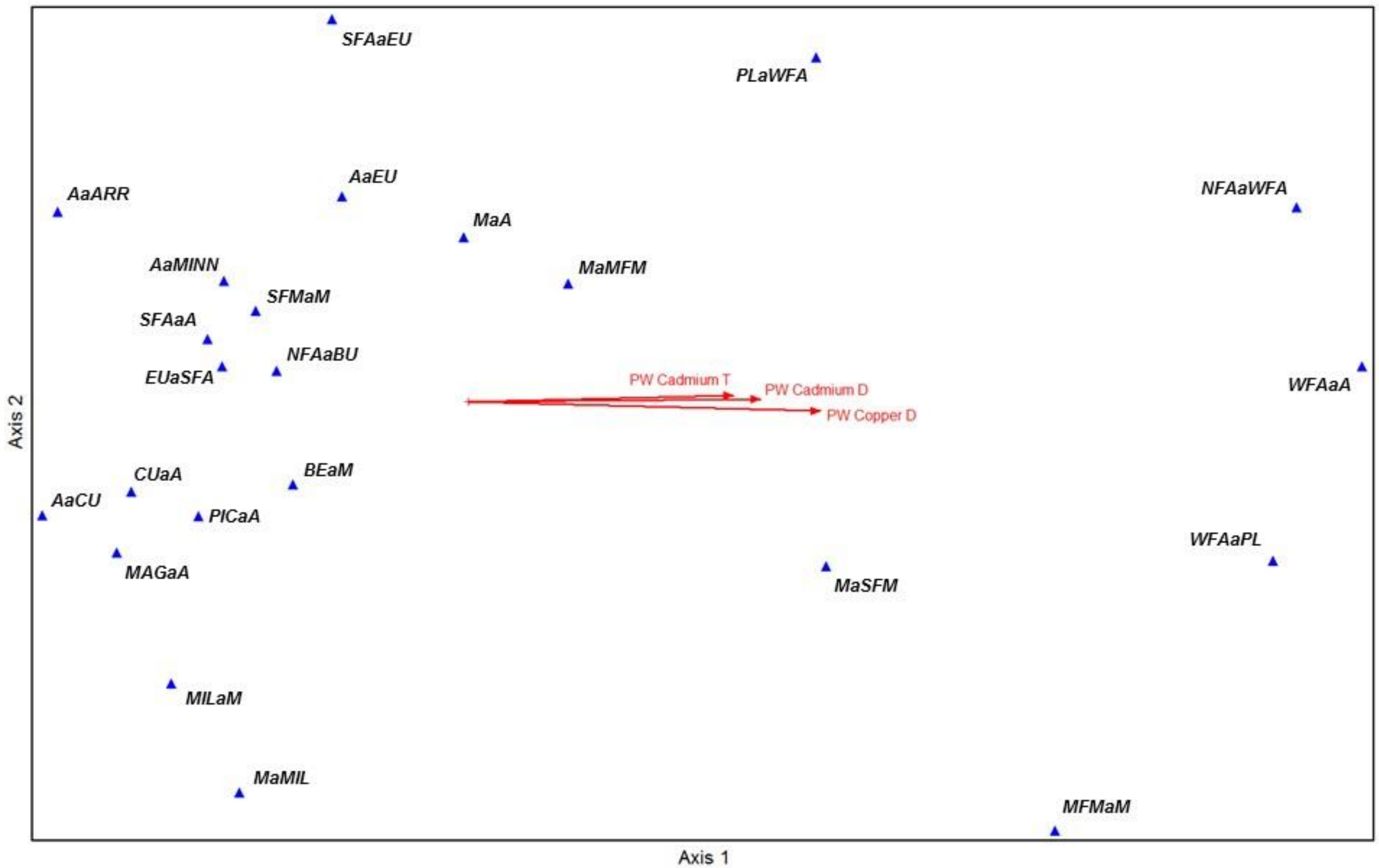

Figure 35: NMS ordination with orthogonal vector lines for metal and mineral concentrations in pore water (r^2 correlations greater than 0.4). The angle and length of the red lines reflect the direction and strength of the relationship between the variable and ordination axes. This ordination is based on the subset of sites where pore water samples were collected. See Table 13 for ordination details

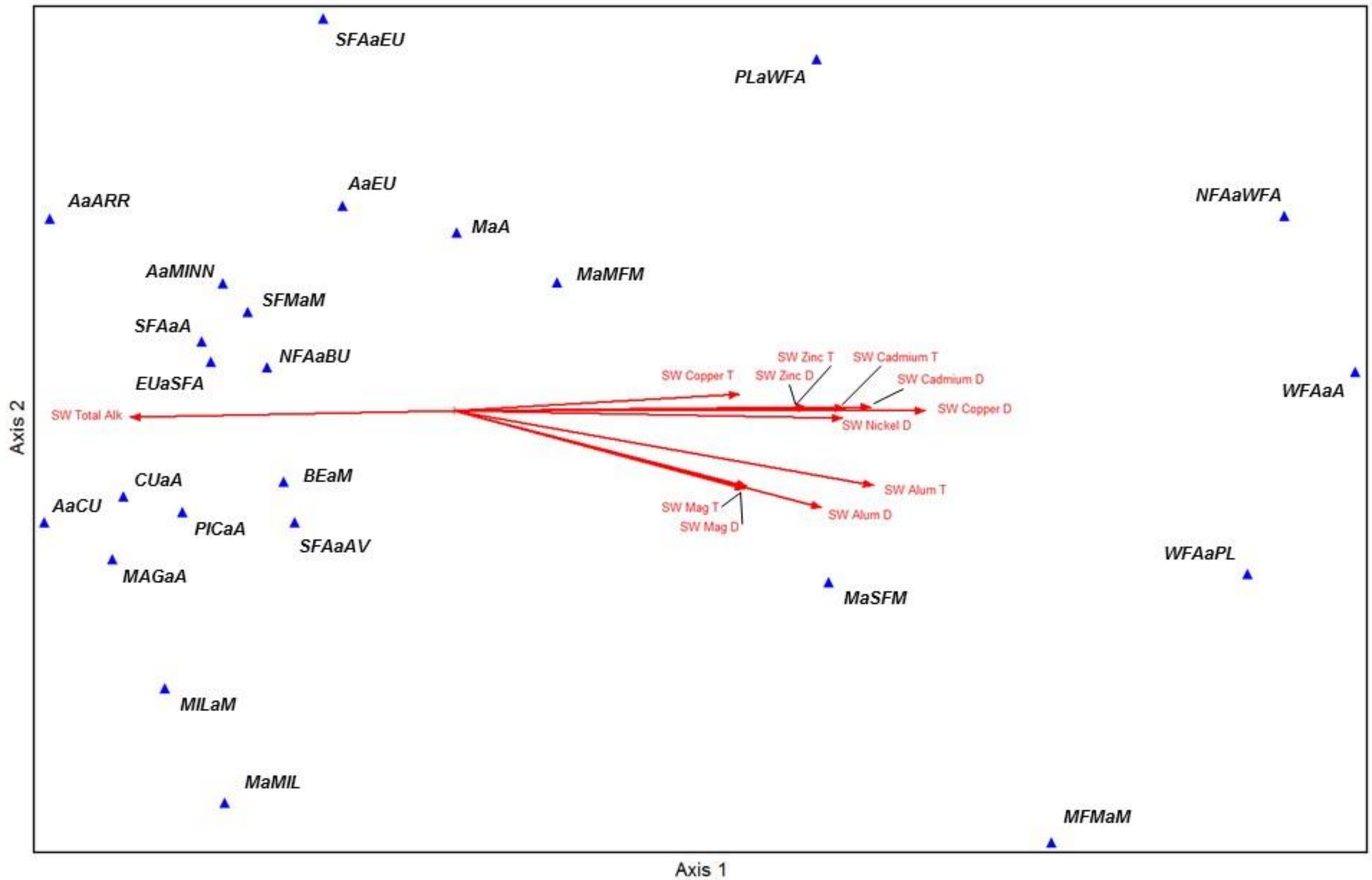

Figure 36: NMS ordination with orthogonal vector lines for metal and mineral concentrations in surface water (r^2 correlations greater than 0.4). The angle and length of the lines reflect the direction and strength of the relationship between the variable and ordination axes. This ordination is based on the subset of sites where surface water samples were collected. See Table 13 for ordination details

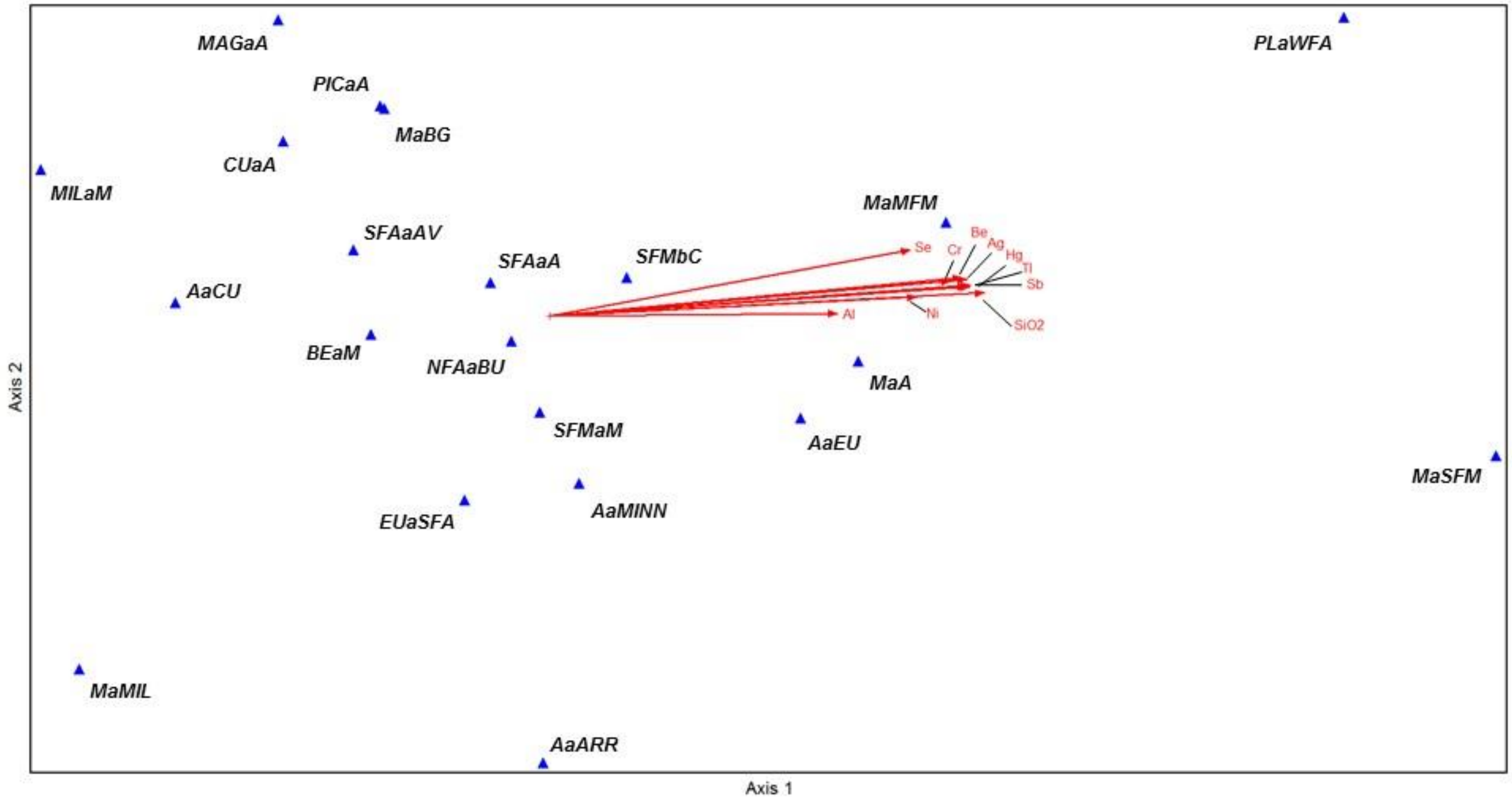

Figure 37: NMS ordination with orthogonal vector lines for metal and mineral concentrations in BMI tissue (r^2 correlations greater than 0.4). The angle and length of the lines reflect the direction and strength of the relationship between the variable and ordination axes. This ordination is based on the subset of sites where BMI tissue samples were collected. See Table 13 for ordination details