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Inter-annual Variability of Benthic Macroinvertebrate Communities in the Bonita Peak Mining District

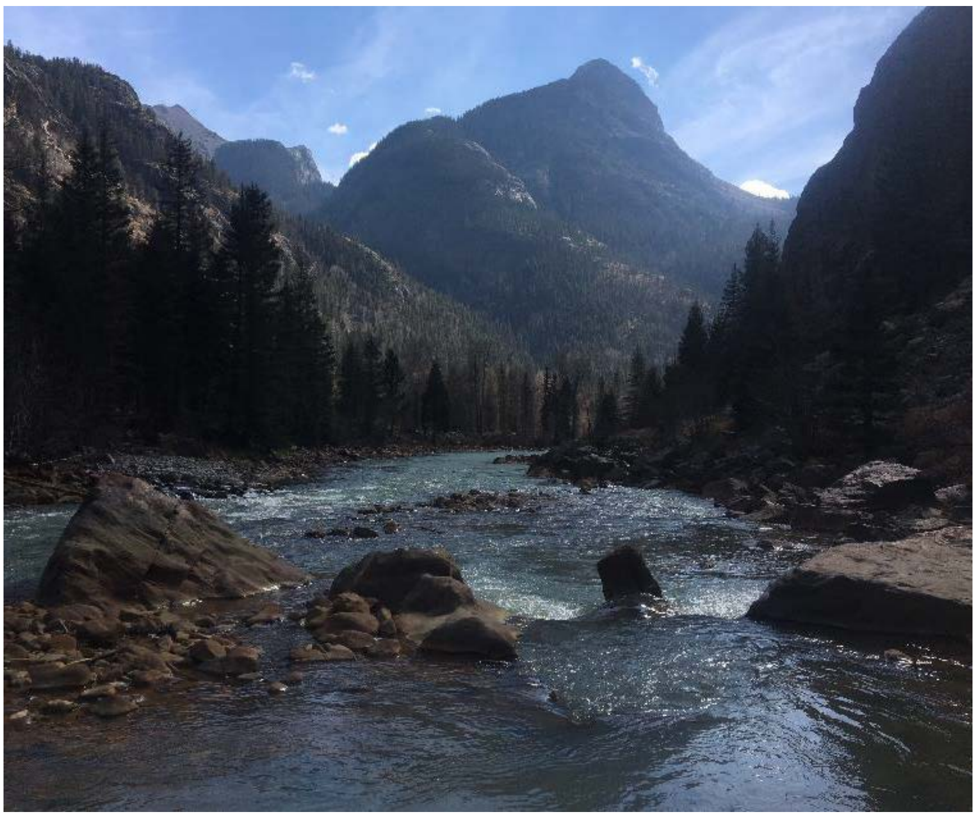

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Table of Contents

1. Introduction **1**

2. Methods **2**

2.1 Monitoring Locations *2*

2.2 Field Survey Methodology *2*
2.2.1 BMI Community Samples 2

2.3 Laboratory Methods *3*
2.3.1 BMI Community Samples 3

2.3 Data Analysis *3*
2.3.1 BMI Metrics 3
2.3.2 Standardizing Sample Size 3
2.3.3 Ecological Data Application System (EDAS) 4
2.3.4 Statistical Analysis 4
3.4.5 EPA Surface Water Quality Data 4

3. Results **4**

3.1 Natural Annual Variability *4*
3.1.1 Context of Natural Annual Variability of 2016-2019 time period 4
3.1.2 Establishment of Natural Annual Variability using the 2016-2019 time period 5
3.1.3 Benthic Sampling in Priority Reaches and Other Locations in 2018-19 7
3.1.4 Potential Influence of Drought on BPMD Benthic Community Structure 8
3.1.5 Potential Influence of Surface Water Quality on BPMD Benthic Community Structure 9

4. Recommendation for BPMD Benthic Monitoring Strategy **10**

4.1 Use of annual variability in BPMD benthic monitoring strategy 10
4.1.1 Strategy focusing on trends in benthic metrics 10
4.1.2 Strategy using NMS ordination simulated trends in benthic community structure 11
4.2 Implications 11

5. Works Cited **12**

6. Tables **16**

7. Figures **27**

8. Maps **59**

List of Tables

Table 1. Benthic monitoring sites and associated sampling dates....17
Table 2. BMI metrics (Pages 18-19)....18
Table 3. Surface water monitoring sites and associated sampling dates that correspond to benthic sample locations....20
Table 4. Climatic and hydrologic conditions from 2015-2020....21
Table 5. Measures of variability across benthic metrics (Pages 22-23)....22
Table 6. NMS ordination: Final stress, percent of variation explained; correlation of common taxa for each ordination group....24
Table 7. Average surface water concentration by year across ten sampling locations....25
Table 8. Distance in ordination space from each sample to reference site centroids....26

List of Figures

Figure 1: Animas River discharge at Durango USGS gage from 1912-2019. 28
Figure 2: Maximum spring runoff of Animas River at Durango USGS gage from 1912-2019. 29
Figure 3: Colorado Multi-metric Index (MMI) – error bars represent annual variability as two standard errors. 30
Figure 4: Taxa richness – error bars represent annual variability as two standard errors. 31
Figure 5: EPT richness – error bars represent annual variability as two standard errors. 32
Figure 6: EPT relative abundance – error bars represent annual variability as two standard errors. 33
Figure 7: Metal Sensitive Family (MSF) richness – error bars represent annual variability as two standard errors. 34
Figure 8: Metal Sensitive Family (MSF) relative abundance – error bars represent annual variability as two standard errors. 35
Figure 9: Shannon Diversity Index (SDI) – error bars represent annual variability as two standard errors. 36
Figure 10: Modified Hilsenhoff Biotic Index (MHBI) – error bars represent annual variability as two standard errors. 37
Figure 11: Chironomidae relative abundance – error bars represent annual variability as two standard errors. 38
Figure 12: Density (#/m^2) – error bars represent annual variability as two standard errors. 39
Figure 13: Colorado Multi-Metric Index (MMI) – 2018-19 supplemental sampling and associated mainstem sites. 40
Figure 14: Taxa richness – 2018-19 supplemental sampling and associated mainstem sites. 41
Figure 15: EPT richness – 2018-19 supplemental sampling and associated mainstem sites. 42
Figure 16: EPT relative abundance – 2018-19 supplemental sampling and associated mainstem sites. 43
Figure 17: Metal Sensitive Family (MSF) richness – 2018-19 supplemental sampling and associated mainstem sites. 44
Figure 18: Metal Sensitive Family (MSF) relative abundance – 2018-19 supplemental sampling and associated mainstem sites. 45
Figure 19: Shannon Diversity Index (SDI) – 2018-19 supplemental sampling and associated mainstem sites. 46
Figure 20: Modified Hilsenhoff Biotic Index (MHBI) – 2018-19 supplemental sampling and associated mainstem sites. 47
Figure 21: Chironomidae relative abundance – 2018-19 supplemental sampling and associated mainstem sites. 48
Figure 22: Density (#/m^2) – 2018-19 supplemental sampling and associated mainstem sites. 49
Figure 23: Measures of variability for each metric averaged across the twelve annual variability assessment sites (2016-19) 50
Figure 24: NMS ordination of ordination group 1 (see table 6) by sample location. 51
Figure 25: NMS ordination of ordination group 1 (see table 6) by reference and non-reference. 52
Figure 26: NMS ordination of ordination group 1 (see table 6) by aquatic life use attainment or impairment based on MMI scores. 53
Figure 27: NMS ordination of ordination group 1 (see table 6) with orthogonal vector lines of richness of metal sensitive families. 54
Figure 28: NMS Ordination of ordination group 2 (see table 6) with orthogonal vector lines for elevation and metal and mineral concentrations in surface water 55
Figure 29: Average surface water concentration for select metals by year across ten sampling locations. ..56
Figure 30: NMS ordination of ordination group 3 (see table 6) by watershed. 57
Figure 31: Hypothetical MMI score from a future sample; 58

Abbreviations and Acronyms

Al	Aluminum
BMI	Benthic Macroinvertebrate
BPMD	Bonita Peak Mining District
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)*
Fe	Iron
Mg	Magnesium
MHBI	Modified Hilsenhoff Biotic Index *(see table 2)*
MMI	Multi-Metric Index *(see table 2)*
MSI	Mountain Studies Institute
MSF	Metal-Sensitive Families - Ephemerellidae, Heptageniidae, and Taeniopterygidae families *(see table 2)*
NMS	Non-metric Multidimensional Scaling Ordination
Pb	Lead
SDI	Shannon-Weaver Diversity Index *(see table 2)*

1. Introduction

Portions of the Upper Animas watershed were designated as the Bonita Peak Mining District (BPMD) Superfund site in 2017 by the Environmental Protection Agency (EPA). EPA and other agencies, partners, and parties are conducting remediation within BPMD to reduce metal contamination of the Animas River and tributaries. Monitoring of benthic macroinvertebrate (BMI) communities across a gradient of metal exposure will be an important component of assessing the effectiveness of remediation efforts in the BPMD.

BMIs are small organisms that have no backbone, can be seen without the aid of magnification, and live all or a part of their life at the bottom of streams, rivers, and lakes. These organisms are diverse and have a wide range of habitat requirements, food preferences, life spans, and tolerances to pollution. BMIs are intimately tied to their habitat, where they are exposed to possible contaminants in sediment and in the water column. Due to these factors, benthic communities are excellent indicators of water quality and the overall condition of aquatic habitat.

Benthic communities can vary substantially from year to year, even in undisturbed waterbodies (Scarsbrook et al. 2000). Long-term, annual monitoring of BMI communities is essential in order to differentiate the direct effects of remediation from natural variability of communities (Chapman 1999; Mazor et al. 2009; Resh et al. 2013). As a part of USGS Professional Paper 1651, which focuses on metal contamination of the Animas River, Anderson (2007) presented a benthic macroinvertebrate monitoring strategy. His recommendations included that, "3 years of pre-remediation data and 3 years of post-remediation data from the same site must be collected to distinguish between the effectiveness of remediation and temporal variation inherent in natural populations." Long-term monitoring has successfully demonstrated improvements in the health of benthic communities following the implementation of remediation projects designed to reduce metal exposure to aquatic life (Clements et a. 2010; Herbst 2018).

To better understand the natural variability of benthic communities in the BPMD, we expanded on work conducted in 2016 and implemented a multiple year monitoring program from 2017-19. These efforts were funded through partnerships with multiple agencies and organizations. In 2016, Mountain Studies Institute (MSI) collected and analyzed data from thirty seven Animas River locations as part of an evaluation of the Animas River for Trout Unlimited and for the EPA's Baseline Ecological Risk Assessment (Roberts 2017; Roberts 2018). The collection, processing, and identification of 2017 and 2018 samples were funded by Colorado Division of Reclamation, Mining, and Safety and by Colorado Department of Public Health and Environment (CDPHE) through the Water Infrastructure Improvements for the Nation (WIIN) Act. Collection, processing, identification, and interpretation of 2019 samples were funded by the EPA through a contract between Mountain Studies Institute (MSI) and TechLaw, Inc. Reporting and interpretation of the inter-annual variability of the 2016-19 dataset described here were funded by CDPHE.

The structure, spatial distribution, and temporal trends of benthic communities in the BPMD have been previously described by Anderson (2000, 2007); Besser and Brumbaugh (2007); Besser, Finger, and Church (20017); Courtney and Clements (2002); EPA (2019a); and Roberts (2015, 2016, 2017a, 2017b). The main objective of this work is to establish the natural annual variability of benthic communities in the BPMD, which will allow researchers in the coming years to be able to more conclusively determine if current and future remediation efforts have successfully improved conditions for aquatic life.

2. Methods

2.1 Monitoring Locations

We implemented a multiple year benthic monitoring program at locations with the following characteristics:

- Distributed across the BPMD within Mineral Creek and the Upper Animas River
- Distributed across a gradient of metal exposure including:
    - Reference sites, with diverse benthic communities and low levels of surface water metals
    - Sites occupied by limited benthic communities and elevated surface water metals
- Located in close downstream proximity to remediation activities
- Located downstream of the district to determine if remediation efforts translate to down-canyon improvement in aquatic life condition
- Located in or near EPA Priority Reaches (EPA 2019b)
- Located at sites where benthic community samples were collected in 2015-16.

We selected twelve locations to sample for three consecutive years from 2017-19: four sites each in the Upper Animas, Mineral Creek, and Animas River Canyon. Four of the twelve locations have lower metal concentrations compared to other BPMD reaches and are considered reference sites (Table 1). We sampled additional locations during this time period in response to special circumstances, such as a mine release, or at the request of agencies and partners. In sum, over the 2017-19 time period, we collected 40 benthic samples from eighteen different locations (Table 1; Maps 1 and 2).

2.2 Field Survey Methodology

2.2.1 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 2007; personal communication). Anderson's method utilized and modified protocols developed by the EPA (Barbour et al. 1999) and CDPHE (CDPHE 2010). 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 (2007) methodology by increasing the amount of habitat sampled per site to 1.15 m^2, which more closely follows the methodology outlined by CDPHE (2010) 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.

2.3 Laboratory Methods

2.3.1 BMI Community Samples

Samples were identified by Scott Roberts (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 Merritt, Cummins, and Berg (2019). 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.

2.3 Data Analysis

2.3.1 BMI Metrics

Several metrics have been developed to assess the composition and health of BMI communities (Table 2). These relatively independent metrics provide multiple lines of evidence of the overall condition of the habitat and water quality of an aquatic system. We focus our analysis on metrics that Roberts (2017a) found to most strongly correlate with metal exposure in the BPMD, which includes total richness; density; Multi-Metric Index (MMI); richness of metal-sensitive families (MSF); and richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT).

2.3.2 Standardizing Sample Size

To eliminate potential bias from differing lab subsample sizes, we employed an algorithm to randomly 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 2.3.3).

2.3.3 Ecological Data Application System (EDAS)

We utilized the Ecological Data Application System (EDAS) developed by Colorado Department of Public Health and the Environment (CDPHE) to calculate all metrics, including the Multi-Metric Index (MMI). MMI scores are based on a fixed count of 300 individuals per sample.

2.3.4 Statistical Analysis

We applied non-metric multi-dimensional scaling ordination (NMS) within PC-ORD software (McCune & Mefford 1999) to assess differences in benthic community structure among sites and years. In addition, we assessed whether surface water metal concentrations drive the variability in community composition among samples. Our NMS analysis was based on Bray-Curtis distance measures of species abundance. To reduce the influence of rare taxa on the ensuing ordination, we limited NMS analysis to species that occurred in at least five percent of samples (Peck 2016).

3.4.5 EPA Surface Water Quality Data

EPA maintains a SCRIBE database containing environmental data that has been collected in recent years from BPMD. For the locations where we present benthic results in this report, we obtained surface water quality data collected from the same location in a comparable time period (i.e., for a benthic sample collected in October 2019 from the Animas River above Cement Creek, we obtained water quality data collected on the nearest adjacent date in which data was available from that site). Water quality data was available from a comparable time period for most benthic samples, but not all (Table 3). For analysis where we include water quality data, we limit the analysis to only include benthic samples that have corresponding water quality data.

3. Results

3.1 Natural Annual Variability

3.1.1 Context of Natural Annual Variability of 2016-2019 time period

The natural annual variability discussed in this report should be interpreted in context of the climatic and hydrological conditions that occurred during the monitoring period. From 2016-2019, aquatic life in the BPMD region experienced a wide range of conditions that included drought, above and below average precipitation, and varied magnitude and timing of Animas River flows (Figures 1 and 2; Table 4). 2016 and 2017 can be characterized by a near average snowpack in the San Juan Mountains and mild drought conditions. 2018 was markedly different. Low winter precipitation, shallow snowpack in the San Juan Mountains, and diminished monsoonal rains lead to substantial drought in the Four Corners area throughout 2018. In the fall of 2018, the Animas River reached the lowest flow for that date ever observed in more than 100-years of records at the Durango USGS gauge (Figure 1). The winter of 2018-19 delivered above average snow accumulations to the San Juan Mountains, a historic avalanche cycle, and subsequent high river flows in 2019.

It is difficult, if not impossible, to exclude all anthropogenic influence when describing the natural annual variability of biological communities. There are several notable anthropogenic influences that could have had an effect on aquatic life within the BPMD region during the 2016-2019 time period. Since October 2015, the EPA has operated the Interim Water Treatment Plant (IWTP) at Gladstone, which treats discharge from the Gold King Mine and thus removes metals that otherwise would have been carried by Cement Creek into the Animas River. In addition, other various mine remediation activities were implemented during the 2016-2019 time period at BPMD locations.

On October 10, 2019, an uncontrolled mine release was reported from the Silver Wing Mine located immediately upstream of our benthic monitoring location, Animas River above Eureka. EPA reported elevated levels of copper, iron, lead, and zinc from samples collected at a location on the Animas River immediately below the Silver Wing Mine. Further downstream, water quality impacts that could be associated with the release were less discernable (EPA 2019c).

3.1.2 Establishment of Natural Annual Variability using the 2016-2019 time period

We illustrate and quantify the natural annual variability of benthic metrics for twelve locations using benthic data collected in sequential years from 2016-2019 in Table 5 and Figures 3-12; 23-28; and 30. Only one of the twelve sites were not sampled annually in the 2016-19 time period, Animas River above Cement Creek; the annual variability we present for this site reflects the 2017-19 time period instead. To visually assess the annual variability among sampled locations, we plotted metrics for each year and each site along with the average for each site across the 2016-19 time period. We depict annual variability as error bars that reflect two standard errors (Figures 3-12). In addition to standard error, we calculated several additional measures of variability: standard deviation; range; coefficient of variation; and relative percent change (Figure 23; Table 5). Across these measures, we found that Shannon Diversity Index (SDI), Modified Hilsenhoff Biotic Index (MHBI), EPT richness, MMI, and Metal Sensitive Family (MSF) richness had lower inter-annual variability, and the relative abundance measures of EPT and MSF had the highest inter-annual variability. This hierarchy of inter-annual variability among metrics should be considered when interpreting future benthic trends in the BPMD with equal consideration of other metric values, such as relevance to metal contamination (Roberts 2017a).

Our data suggest that less impacted reference sites typically have lower inter-annual variability than non-reference sites. This is evident across most metrics and across most measures of variability (Figure 23). One potential explanation for this finding would be if the benthic communities at non-reference sites, that may already have low diversity and abundance, are proven to be more susceptible to extreme climatic conditions such as drought. This conclusion would necessitate observations of benthic communities across a gradient of metal exposure from multiple drought and non-drought years. We intend to explore this topic further in the future.

To further examine the differences in benthic community structure among sites and across years, 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. Samples plotted closer to one another in ordination space have more similar community composition than samples plotted far from one another. We conducted ordination on three different sets of benthic samples (ordination group membership for each sample indicated in Table 1):

> 1) To assess inter-annual variability, we used the group of 47 benthic samples collected from twelve locations across 2016-19;
>
> 2) To assess correlations between surface water quality and community composition, we used the group of benthic samples where corresponding water quality data are available, which includes 42 samples collected from 16 locations from 2016-19;
>
> 3) To assess the relationship of benthic communities to reference centroids, we used all 54 samples discussed in this report.

For all ordinations, we found that a two-dimensional solution provided the optimal ordination. Ordination revealed that although there is inter-annual variability in benthic community structure, most sites were distinct from one another (Figure 24). There is a clear difference in the community composition between reference and non-reference locations; samples from reference sites were distributed on the mid-lower right portion of the plot and non-reference sites were distributed on the left (Figure 25). Similarly, samples in attainment of MMI are largely dissimilar to those that were found to indicate impairment (Figure 26). Almost half of the variability in benthic communities among samples was explained by NMS axis one, while another quarter of the variability was explained by axis two (Table 6). The gradient represented by axis one largely reflects an increase in the abundance of metal sensitive taxa (*Taenionema*, *Serratella*, *Drunella doddsii*, and *Cinygmula*) (Table 6). This is evident by coding the samples in ordination by MSF richness where there is an increase from left to right along axis 1 (Figure 27). Samples from Cascade Creek occupy a unique position in the lower right portion of the plot, perhaps reflecting its topographic position of being at a lower elevation than other reference sites. Inter-annual variability can be interpreted from ordination plots by examining the distances between samples across years from the same sites (see Figure 24). For example, samples across years from the reference site, Picayune, are clustered more tightly in ordination space, and thus are more similar to one another. Conversely, samples from non-reference sites such as the mainstem Mineral Creek sites occur more spread out in ordination space, indicating greater variability from year to year.

3.1.3 Benthic Sampling in Priority Reaches and Other Locations in 2018-19

At the direction of agencies and other partners, and guided by site circumstances, we collected benthic samples in 2018-19 from additional locations within the BPMD beyond the twelve natural annual variability assessment locations discussed thus far. These supplemental locations include sites located within EPA priority reaches on Mineral Creek and the Animas River. From 2017-19, we collected samples from six locations within EPA priority reaches (EPA 2019b) (Maps 1 and 2):

- Priority Reach 1
    - Animas River above Elk Creek
    - Animas River above Cascade Creek
- Priority Reach 2
    - Animas River above Arrastra Gulch
    - Animas River above Cement Creek
- Priority Reach 3
    - South Mineral below Clear Creek
- Priority Reach 4
    - Mineral Creek above Browns Gulch

The Colorado Multi-Metric Index (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 2017). MMI scores from 2019 sampling efforts indicate that benthic communities from all six priority reach sites would be considered in attainment of aquatic life use designation (Figures 3 and 13). Animas River above Cement Creek (priority reach 2) and South Mineral below Clear Creek (priority reach 3) have had MMI scores indicating attainment of aquatic life use designation in all four years of sampling from 2016 to 2019. However, the two sampling locations in priority reach 1 have had inconsistent results, with some years suggesting attainment and other years suggesting impairment. Specifically, MMI scores from Animas River above Elk Creek suggested attainment in 2017 and 2019 but impairment in 2016 and 2018. Animas River above Cascade Creek was in attainment in all years except 2018 (Figures 3). The lower MMI scores in priority reach 1 compared to the upper priority reaches are largely driven by low EPT richness and low abundance of scraper taxa.

NMS ordination is a useful tool to assess how similar or dissimilar benthic community structure within priority reaches is from communities in reference reaches. As illustrated in Figure 28, it is clear that there is variability in the similarity of benthic communities in priority reaches to reference reaches. Benthic community composition has greater similarity between reference sites and priority reaches than between reference sites and sites that are neither reference nor priority reaches.

In addition to priority reaches, we collected samples from other locations in the 2018-19 time period, including Animas River above Minnie Gulch and Cement Creek above Animas (Figures 13-22). Although the benthic community in Cement Creek is low in diversity and density, there is potential for improvement following the numerous mine remediation efforts occurring in the Cement Creek watershed. The Animas River above Minnie Gulch was of interest due to its downstream proximity to remediation efforts underway at Forest Queen Mine, as well as the availability of previously collected benthic data from 2016. However, numerous local issues have made sampling at this location problematic. For example, in 2018-19, beavers constructed a dam immediately upstream of the site, reducing flow through the sampling reach. Then in 2019, substantial avalanche debris filled the valley floor upstream of the site near Eureka, causing the Animas River to braid and meander across the floodplain. While we were able to collect samples at this location in 2016 and 2018, the channel was dry when we visited in 2019. Our monitoring site at the South Fork of Animas River above the Animas was also impacted by avalanche debris during the large 2019 avalanche cycle. When we visited in the fall of 2019, the reach we had sampled in 2016-18 was filled with woody avalanche debris and was dry. Flow in the South Fork of the Animas River had shifted across the valley bottom to a new channel. To accommodate for these changes, we shifted our monitoring reach upstream of the avalanche debris by about 300 meters.

The benthic community at Animas River above Eureka was markedly different in 2019 compared to previous years. NMS ordination depicts the 2019 sample from this site trending noticeably away from samples collected in previous years, and in the opposite direction of samples from reference sites (see point AaEur'19 in top left of plot in Figure 24). This shift in community composition in 2019 is likely in response to reductions in EPT relative abundance (Figure 6), MHBI (Figure 10), and density (Figure 12); and the complete elimination of MSF taxa (Figures 7 and 8) compared to previous years. There were similar reductions in MSF benthic metrics at Mineral Creek sites in 2019, but not nearly as pronounced of a change in community composition as depicted in NMS ordination. As discussed in section 3.1.1, four days before we collected the 2019 benthic community sample from the Animas River above Eureka, an uncontrolled mine release occurred. It is possible that this uncontrolled release had a localized, isolated impact on the benthic community in this reach immediately below the Silver Ledge Mine.

3.1.4 Potential Influence of Drought on BPMD Benthic Community Structure

Due to drought conditions, flows in the Animas River were greatly reduced in 2018 (Figure 1). Reduced flows physically alter aquatic life habitat by decreasing the heterogeneity and area of available wetted habitat (Herbst 2019), but can also alter water quality. In the BPMD, we observed a pronounced increase in the surface water concentration of some metals during the 2018 drought year (Table 7 and Figure 29).

During drought conditions, researchers have documented shifts in community composition and functional feeding groups, including an increased abundance of Chironomidae larvae (Bogan and Lytle 2007; Herbst et al. 2019). At some locations, we observed increased Chironomidae relative abundance and decreased relative abundance of EPT and metal sensitive families in 2018 compared to other years (Figures 6, 8, and 11). This pattern is also evident in NMS ordination as most non-reference samples collected in 2018 trend away from reference sites (Figure 24-25). Interestingly, in NMS ordination, the reference site benthic communities appear to have responded to the 2018 drought year differently than the non-reference site benthic communities; reference sites generally trended negatively along axis 2 while non-reference sites trended negatively along axis 1. Table 8 presents calculated distances in ordination space (depicted in Figure 30) between each benthic sample and the centroid of samples collected from the corresponding reference site within the same watershed (e.g., we calculated the distance from each sample in the Mineral Creek watershed to the centroid of samples collected from 2016-19 at Mill Creek, which is a reference site). On average, samples from 2018 non-reference sites were 40% further from reference centroids than they were in the non-drought years of 2016, 2017, and 2019. These distance measures provide numerical evidence that benthic communities in 2018 at the majority of sites became more dissimilar to reference benthic communities.

Research from the Sierra Nevada mountains demonstrate that the effects of drought on benthic communities are most pronounced after prolonged multi-year drought (Herbst 2019). While 2018 was a historic drought, 2019 brought above average snowfall and above average river levels, effectively curtailing the 2018 drought period. Benthic communities at some sites in the BPMD seem to reflect this pattern, with recovery occurring in 2019 as Chironomidae abundance returned to levels more similar to pre-drought observations. NMS also depicts this pattern as many samples trended back toward reference conditions from 2018 to 2019 (Figures 24-25).

To understand the relationship between the BPMD benthic communities and drought, more data are needed across a broader gradient of climatic conditions.

3.1.5 Potential Influence of Surface Water Quality on BPMD Benthic Community Structure

Environmental variables, such as elevation and surface water metal concentrations, can be added to ordination plots as orthogonal vector lines to visualize correlations between these variables and benthic community composition. 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.

The surface water concentration of several metals and minerals had strong correlations ($>=0.4\ r^2$) with NMS ordination axis 1: total and dissolved aluminum, iron, and magnesium; dissolved lead, and sulfate. Elevation had a strong correlation with axis 2. This suggests that these factors may be directly or indirectly responsible for the differences in benthic communities we observed (Figure 28). Orthogonal vector lines indicate that higher concentrations of these metals and minerals were more closely associated with the non-reference benthic communities that are depicted on the left-hand side of the ordination plot.

Previous examinations of benthic community distribution have focused on the portion of the BPMD upstream of Silverton. One such study found additional metals (copper, cadmium, nickel, and zinc) that had strong correlations with NMS ordination of benthic community structure (Roberts 2017a). The fewer metals with strong correlations presented here could reflect the broader geographic extent and inclusion of lower elevation Animas River Canyon sites in this report, which were not included in the previous work.

4. Recommendation for BPMD Benthic Monitoring Strategy

4.1 Use of annual variability in BPMD benthic monitoring strategy

We propose the following two procedures when evaluating results from future benthic samples from the BPMD sites (based on work by Anderson 2007, Herbst et al. 2018, and Roberts 2018).

4.1.1 Strategy focusing on trends in benthic metrics

Here, we use MMI as an example, but this procedure applies to any BMI metric.

1. Plot the average MMI observed in samples from fall 2016-2019.
   (note: use a reference time frame that reflects climatic variability and minimizes anthropogenic influence such as a uncontrolled mine release)

2. Add error bars that reflect two standard errors of the fall 2016-2019 MMI scores above and below the plotted average. This approximates inter-annual variability.

3. Plot MMI from the newly collected sample of interest.

4. Assessment (see hypothetical example in Figure 31, demonstrating a scenario in which an increase in MMI at Mineral Creek above the Animas River is beyond the natural variability documented from 2016-2019).

   a. Is the new data point within error bars?
      i. If yes, then the new sample is within the estimated window of natural variability and any change observed may not be attributed to anthropogenic factors with any certainty.
      ii. If no, then the new sample is beyond the estimated window of natural variability for this site; proceed to step b.

   b. If samples from reference sites were collected concurrently, are the new samples from reference sites within the reference site error bars?
      i. If no, and the direction of change observed in the reference site(s) is the same as the direction of change observed at Mineral Creek above the Animas River, then the change observed in the new sample from Mineral Creek above the Animas River likely occurred watershed-wide.

ii. If yes, then it is possible that the change observed in the new sample from Mineral Creek above the Animas River could be the result of anthropogenic factors rather than a watershed-wide phenomenon.

4.1.2 Strategy using NMS ordination simulated trends in benthic community structure

One way to assess how dissimilar the benthic community at a non-reference site is from a reference benthic community is to calculate the distance of the site in NMS ordination space from a reference centroid. Trends in benthic community structure over time could be evaluated by assessing whether the community at a particular site is trending toward or away from the reference centroid (i.e., whether the quantifiable distance from the reference centroid is increasing or decreasing) (For example, see table 8).

4.2 Implications

The inter-annual variability established in this report can be used to better detect potential changes in benthic communities that are attributable to remediation. The inter-annual variability of benthic communities in the BPMD presented here reflect the wide range of climatic and hydrologic conditions that occurred during the 2016-19 monitoring period. The 2018 drought corresponded to low water levels and reduced benthic diversity at most sites. Researchers assessing BPMD benthic community trends in the future may choose to interpret new observations within the wide inter-annual variability captured in the 2016-19 time period, or they may choose to limit their dataset to samples collected from years with similar climatic and hydrologic conditions. This later approach could allow for a narrower estimate of inter-annual variability, as opposed to the wide-ranging conditions (e.g., drought and non-drought) that occurred during 2016-19.

We found evidence that inter-annual variability of benthic communities in the BPMD may be larger in mineralized streams than in non-mineralized streams (Figure 23). A greater period of record beyond the four years assessed in this study would help confirm this conclusion. If habitat conditions and benthic community structure of mineralized streams are more heavily influenced by differing climatic scenarios than non-mineralized streams, then a greater frequency of benthic sample collection may be necessary in the future to differentiate the direct effects of remediation from natural variability of these communities.

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6. Tables

Table 1. Benthic monitoring sites and associated sampling dates

Stream Name	Site Name	ID	Lat	Long	2016	2017	2018	2019
Upper Animas River								
*Picayune Gulch	Above Animas River	PIC	37.91253	-107.56168	10/5/2016 [1,2,3]	10/17/2017 [1,3]	10/16/2018 [1,3]	10/11/2019 [1,3]
Animas River	Above Eureka	AaEur	37.89849	-107.55928	10/11/2016 [1,2,3]	10/17/2017 [1,2,3]	10/16/2018 [1,2,3]	10/14/2019 [1,2,3]
SF Animas River	Above Animas River	SFAaA	37.88231	-107.58458	10/6/2016 [1,2,3]	10/17/2017 [1,2,3]	10/16/2018 [1,2,3]	10/11/2019 [1,2,3]
Animas River	Above Minnie Gulch	AaMinnie	37.86334	-107.57155	10/11/2016 [2,3]		10/22/2018 [2,3]	
Animas River	Above Arrastra Cr	AaArrastra	37.82762	-107.62431	10/2/2016 [2,3]			10/14/2019 [2,3]
Animas River	Above Cement Creek	AaCEM	37.81197	-107.65867		10/14/2017 [1,2,3]	10/16/2018 [1,2,3]	10/18/2019 [1,2,3]
Cement Creek								
Cement Creek	Above Animas River	CEMaA	37.81739	-107.66197				10/24/2019 [2,3]
Mineral Creek								
*Mill Cr	Above Mineral Cr	Mill	37.87268	-107.73540	10/17/2016 [1,2,3]	10/16/2017 [1,3]	10/21/2018 [1,2,3]	10/17/2019 [1,2,3]
Mineral Cr	Above Browns Gulch	MINaBrG	37.85640	-107.72626	10/17/2016 [2,3]			10/17/2019 [2,3]
Mineral Cr	Above SF Mineral Cr	MINaSF	37.81980	-107.71783	10/13/2016 [1,2,3]	10/16/2017 [1,2,3]	10/21/2018 [1,2,3]	10/17/2019 [1,2,3]
SF Mineral Cr	Below Clear Cr	SFMIN	37.80470	-107.76956	10/13/2016 [1,2,3]	10/16/2017 [1,2,3]	10/21/2018 [1,2,3]	10/17/2019 [1,2,3]
Mineral Cr	Above Animas River	MINaA	37.80272	-107.67260	10/12/2016 [1,2,3]	10/14/2017 [1,2,3]	10/16/2018 [1,2,3]	10/18/2019 [1,2,3]
Animas River Canyon								
Animas River	Above Elk Creek	AaELK	37.72237	-107.65479	10/20/2016 [1,3]	10/20/2017 [1,3]	10/19/2018 [1,2,3]	10/16/2019 [1,2,3]
*Elk Creek	Above Animas River	ELK	37.72195	-107.65367	10/20/2016 [1,3]	10/20/2017 [1,3]	10/19/2018 [1,2,3]	10/16/2019 [1,2,3]
Animas River	Above Cascade Creek	AaCAS	37.59979	-107.77104	10/19/2016 [1,3]	10/20/2017 [1,3]	10/19/2018 [1,2,3]	10/16/2019 [1,2,3]
*Cascade Cr	Above Animas River	CAS	37.60179	-107.77901	10/19/2016 [1,3]	10/20/2017 [1,3]	10/19/2018 [1,2,3]	10/16/2019 [1,2,3]

*Note: * indicates reference sites.*
Superscript numbers at the end of each date indicates NMS ordination group (see table 6)

Table 2. BMI metrics (Pages 18-19)

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).

BMI Metric	Metric Description, Justification, and Source	
Functional Feeding Groups - *Relative abundance of scraper taxa*	Proportion of BMI community composed of scraper taxa	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 2012).
Multi-metric Index (MMI)	MMI is a bioassessment tool developed by Colorado Water Quality Control Division and the Environmental Protection Agency (CDPHE 2017). 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 (2017) 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 2017 for more details.	
Modified Hilsenhoff Biotic Index (MHBI)	MHBI is an index of the overall tolerance/sensitivity 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 modified index value ranges from 0 (more tolerant) to 10 (more sensitive). MHBI is the inverse of the traditional HBI so that high MHBI scores reflect a community that has a high proportion of sensitive taxa.	
Shannon-Weaver Diversity Index (SWDI)	SWDI is a measure of the diversity and evenness of a community (Shannon 1948).	

Table 3. Surface water monitoring sites and associated sampling dates that correspond to benthic sample locations.

Location		2016	2017	2018	2019
Upper Animas River and Cement Creek					
*Picayune Gulch	Above Animas River	9/29/2016	*none*	*none*	*none*
Animas River	Above Eureka	9/29/2016	9/27/2017	9/25/2018	9/24/2019
SF Animas River	Above Animas River	9/27/2016	9/28/2017	9/26/2018	9/26/2019
Animas River	Above Minnie Gulch	9/29/2016	9/28/2017	9/26/2018	9/25/2019
Animas River	Above Arrastra Cr	9/27/2016	9/26/2017	9/25/2018	9/24/2019
Animas River	Abv Cement Creek	9/27/2016	9/26/2017	9/25/2018	9/24/2019
Cement Creek					
Cement Creek	Abv Animas River	9/27/2016	9/26/2017	9/25/2018	10/14/2019
Mineral Creek					
*Mill Cr	Above Mineral Cr	9/30/2016	*none*	9/27/2018	9/24/2019
Mineral Cr	Above Browns Gulch	9/29/2016	9/27/2017	9/26/2018	9/25/2019
Mineral Cr	Above SF Mineral Cr	9/27/2016	9/27/2017	9/25/2018	9/24/2019
*SF Mineral Cr	Below Clear Cr	9/28/2016	9/27/2017	9/26/2018	9/24/2019
Mineral Cr	Above Animas River	9/27/2016	9/26/2017	9/25/2018	9/24/2019
Animas River Canyon					
Animas River	Abv Cascade Creek	*none*	*none*	9/25/2018	9/25/2019
Animas River	Abv Elk Creek	*none*	*none*	9/27/2018	9/24/2019
*Cascade Cr	Abv Animas River	*none*	*none*	9/25/2018	9/25/2019
*Elk Creek	Abv Animas River	*none*	*none*	9/27/2018	9/24/2019

*Note: * indicates reference sites.*

Table 4. Climatic and hydrologic conditions from 2015-2020

	Palmer Drought Severity Index - September (CO Basin)	SWE % of April 1 Median (Animas River Basin)	SWE April 1 (Red Mountain Pass)	SWE % of April 1 Median (Red Mountain Pass)
2015	1.48	53	17.3	76
2016	-0.68	77	20	88
2017	-1.84	120	26.6	117
2018	-8	47	14.1	62
2019	-1.14	171	33	145
2020	-4.95	104	23.6	104

Table 5. Measures of variability across benthic metrics (Pages 22-23)

Stream Name	Site Name	MMI					Taxa Richness					EPT Richness					EPT Relative Abundance				
		St Dev	Range	CV	RPD	2SE	St Dev	Range	CV	RPD	2SE	St Dev	Range	CV	RPD	2SE	St Dev	Range	CV	RPD	2SE
Upper Animas River and Cement Creek																					
Picayune Gulch	Above Animas River	6.1	14.0	8.6	19.8	6.1	2.4	5.0	8.5	18.0	2.4	0.8	2.0	4.5	11.1	0.8	10.3	22.1	11.8	25.4	10.3
Animas River	Above Eureka	5.7	12.1	12.1	25.8	5.7	3.3	8.0	24.9	60.4	3.3	1.5	3.0	16.2	32.4	1.5	19.6	41.7	22.7	48.3	19.6
SF Animas River	Above Animas River	5.9	14.3	10.3	25.0	5.9	2.2	5.0	10.8	25.0	2.2	0.5	1.0	4.4	8.9	0.5	18.0	40.3	22.4	50.3	18.0
Animas River	Abv Cement Creek	4.5	8.3	7.9	14.5	5.2	0.6	1.0	2.5	4.3	0.7	2.0	4.0	15.4	30.8	2.3	7.0	13.9	8.2	16.3	8.0
Mineral Creek																					
Mill Cr	Above Mineral Cr	5.2	12.4	7.8	18.7	5.2	3.9	8.0	15.3	31.1	3.9	2.9	7.0	20.2	49.1	2.9	12.6	26.4	16.3	34.2	12.6
Mineral Cr	Above SF Mineral Cr	5.1	11.1	29.3	63.9	5.1	2.9	7.0	34.0	82.4	2.9	1.0	2.0	28.6	57.1	1.0	9.3	19.0	40.3	82.8	9.3
SF Mineral Cr	Below Clear Cr	1.9	4.0	2.4	5.1	1.9	1.0	2.0	3.5	7.2	1.0	1.3	3.0	6.9	16.4	1.3	4.6	10.6	5.0	11.7	4.6
Mineral Cr	Above Animas River	11	24.4	36.6	77.9	11.5	3.2	7.0	36.6	80.0	3.2	2.1	4.0	48.5	94.1	2.1	11.0	23.6	13.3	28.5	11.0
Animas River Canyon																					
Elk Creek	Abv Animas River	3	6.4	4.2	8.9	3.0	2.9	6.0	9.7	20.2	2.9	1.0	2.0	5.0	10.4	1.0	21.8	46.0	26.9	56.8	21.8
Animas River	Abv Elk Creek	13	31.2	27.5	64.8	13.3	3.3	7.0	19.2	40.6	3.3	3.8	8.0	38.3	80.0	3.8	23.8	48.3	30.3	61.6	23.8
Cascade Cr	Abv Animas River	5.8	11.2	8.0	15.5	5.8	2.7	6.0	7.3	16.2	2.7	2.5	6.0	13.6	32.4	2.5	7.8	17.8	9.9	22.7	7.8
Animas River	Abv Cascade Creek	12	24.8	19.5	40.5	12.0	2.8	6.0	12.3	26.1	2.8	2.1	5.0	16.8	40.8	2.1	21.7	50.1	34.7	80.1	21.7

Note: StDev = standard deviation; Range = max-min; cv = coefficient of variation; RPD = relative percent difference; 2SE = two standard errors.

Stream Name	Site Name	MHBI					Shannon Diversity					MSF Richness					MSF Relative Abundance				
		St Dev	Range	CV	RPD	2SE	St Dev	Range	CV	RPD	2SE	St Dev	Range	CV	RPD	2SE	St Dev	Range	CV	RPD	2SE
Upper Animas River and Cement Creek																					
Picayune Gulch	Above Animas River	0.4	1.0	5.7	12.7	0.4	0.6	1.1	18.6	37.2	0.6	0.8	2.0	16.3	40.0	0.8	17.2	39.8	31.9	74.1	17.2
Animas River	Above Eureka	0.6	1.3	7.9	16.9	0.6	0.7	1.5	28.0	60.6	0.7	1.0	2.0	66.7	133	1.0	8.8	18.9	68.9	149	8.8
SF Animas River	Above Animas River	0.6	1.3	7.9	17.0	0.6	0.2	0.5	8.8	20.4	0.2	0.6	1.0	23.1	40.0	0.6	14.3	31.0	80.8	176	14.3
Animas River	Abv Cement Creek	0.3	0.7	4.5	8.9	0.4	0.7	1.4	25.0	49.7	0.8	0.0	0.0	0.0	0.0	0.0	25.8	49.2	45.9	87.6	29.7
Mineral Creek																					
Mill Cr	Above Mineral Cr	1	2.1	13.5	28.6	1.0	0.3	0.6	8.8	18.4	0.3	0.5	1.0	9.5	19.0	0.5	7.4	14.2	11.4	21.7	7.4
Mineral Cr	Above SF Mineral Cr	0.4	0.9	7.2	15.9	0.4	0.4	0.8	22.5	51.5	0.4	0.0	0.0	0.0	0.0	0.0	1.7	3.9	51.0	121	1.7
SF Mineral Cr	Below Clear Cr	0.5	1.1	6.0	14.2	0.5	0.3	1	8.0	19.1	0.3	1.7	4.0	38.5	88.9	1.7	13.6	31.8	37.0	86.5	13.6
Mineral Cr	Above Animas River	0.4	0.8	4.7	10.3	0.4	0.5	1.1	33.2	68.9	0.5	1.2	2.0	115	200	1.2	5.8	12.2	148	314	5.8
Animas River Canyon																					
Elk Creek	Abv Animas River	0.8	1.7	10.6	22.8	0.8	0.5	1.3	17.4	42.2	0.5	0.5	1.0	9.5	19.0	0.5	20.4	48.7	33.7	80.7	20.4
Animas River	Abv Elk Creek	0.8	1.7	10.0	21.5	0.8	0.3	0.6	11.7	27.2	0.3	0.5	1.0	22.2	44.4	0.5	4.1	8.8	46.8	101	4.1
Cascade Cr	Abv Animas River	1.1	2.5	14.4	32.4	1.1	0.3	0.8	8.0	19.2	0.3	0.5	1.0	7.4	14.8	0.5	11.1	25.0	38.8	87.9	11.1
Animas River	Abv Cascade Creek	0.8	1.6	11.0	22	0.8	0.2	0.5	7.9	17.8	0.2	0.8	2.0	27.2	66.7	0.8	20.1	44.9	52.2	116	20.1

Note: StDev = standard deviation; Range = max-min; cv = coefficient of variation; RPD = relative percent difference; 2SE = two standard errors.

Table 6. NMS ordination: Final stress, percent of variation explained; correlation of common taxa for each ordination group.

Ordination Group #	Ordination Group Description	# of sites	Final stress	% of variation		Correlation coefficients of common taxa (occurring in greater than ¼ of samples) to each axis with Pearson's r > 0.40		corresponding figure #s
				Axis 1	Axis 2	Axis 1	Axis 2	
1	Inter-annual variability benthic dataset (2016-19)	47	16.9	46%	26%	Negative Correlation: *n/a* Positive Correlation: *Capnia* (0.40) *Ameletus* (0.44) *Megarcys* (0.49) *Cinygmula* (0.50) *Drunella doddsii* (0.50) *Serratella* (0.54) *Taenionema* (0.63)	Negative Correlation: *Dicranota* (-0.43) *Arctopsyche* (-0.49) *Epeorus* (-0.53) *Baetis* (-0.57) Positive Correlation: *Suwallia* (0.42) *Rhyacophila vofixa* (0.48) *Rhyacophila hyalinata* (0.49) *Zapada* (0.61)	24-27
2	Group of benthic samples where corresponding water quality data are available (2016-19)	42	17.6	49%	21%	Negative Correlation: *n/a* Positive Correlation: *Pagastia* (0.41) *Capnia* (0.44) *Cinygmula* (0.47) *Ameletus* (0.47) *Drunella doddsii* (0.47) *Serratella* (0.48) *Taenionema* (0.56)	Negative Correlation: *Dicranota* (-0.45) *Arctopsyche* (-0.41) *Epeorus* (-0.50) *Baetis* (-0.53) Positive Correlation: *Suwallia* (0.40) *Rhyacophila vofixa* (0.47) *Rhyacophila hyalinata* (0.48) *Zapada* (0.57)	28
3	All benthic samples discussed in this report	54	17.13	49%	23%	Negative Correlation: *n/a* Positive Correlation: *Megarcys* (0.40) *Capnia* (0.41) *Ameletus* (0.42) *Cinygmula* (0.45) *Drunella doddsii* (0.48) *Serratella* (0.49) *Taenionema* (0.62)	Negative Correlation: *Dicranota* (-0.43) *Arctopsyche* (-0.43) *Epeorus* (-0.51) *Baetis* (-0.57) Positive Correlation: *Suwallia* (0.42) *Rhyacophila vofixa* (0.46) *Rhyacophila hyalinata* (0.50) *Zapada* (0.60)	30

Table 7. Average surface water concentration by year across ten sampling locations.

	Average concentration (ug/L)			
	2016	2017	2018	2019
Aluminum D	796	1,172	1,657	1,342
Aluminum T	1,557	1,923	2,154	1,844
Cadmium D	1.6	1.5	2.1	1.2
Cadmium T	1.8	1.5	2.1	1.4
Calcium D	56,650	71,090	94,360	76,560
Calcium T	59,500	72,450	94,660	77,270
Chloride T	1,010	5,660	4,500	3,400
Copper D	19.5	9.9	13.6	7.6
Copper T	26.9	17.8	23.9	11.9
Fluoride T	630	830	1,090	670
Hardness D	158,400	197,600	260,300	211,200
Iron D	1,218	1,762	2,516	1,584
Iron T	1,873	2,431	3,442	2,275
Lead D	2.2	1.9	2.3	1.4
Lead T	4.4	4.5	3.7	3.1
Magnesium D	4,072	4,915	5,974	4,876
Magnesium T	4,226	5,055	5,920	4,988
Manganese D	1,146	1,218	1,567	1,102
Manganese T	1,214	1,235	1,563	1,110
Nickel D	2.8	1.4	4.8	1.3
Nitrate/Nitrite as N T	260	6,000	4,600	1,700
Sulfate as SO4 T	161,980	203,550	265,320	200,830
Total Alkalinity T	20,490	21,350	20,880	20,680
Zinc D	513	497	751	457
Zinc T	527	493	768	465

Note: Red shading indicates that average concentration was highest in 2018. Averages were calculated for ten surface water sampling locations where surface water analytical results were available for all four years, and were locations where benthic samples have been collected: AaEur, SFAaA, AaMinnie, AaArrastra, AaCEM, CEMaA, MINaBrG, MINaSF, SFMIN, and MINaA (see table 1 for site codes). T=total; D=dissolved.

Table 8. Distance in ordination space from each sample to reference site centroids.

Site ID	Stream Name	Site Name	Corresponding Reference Centroid	2016	2017	2018	2019	Average distance of non-drought years ('16, '17, '19) to reference centroid	% difference in distance from reference centroid between the 2018 drought year and non-drought years*
Upper Animas River and Cement Creek									
AaEur	Animas River	Above Eureka	Picayune	0.86	1.14	1.42	1.88	1.30	9.26
SFAaA	SF Animas River	Above Animas River		1.04	1.49	1.64	1.52	1.35	21.76
AaMinnie	Animas River	Above Minnie Gulch		0.63		1.14		0.63	81.21
AaArrastra	Animas River	Above Arrastra Cr		1.10			0.66	0.88	
AaCEM	Animas River	Above Cement Creek			0.43	1.00	0.46	0.45	122.87
CEMaA	Cement Creek	Above Animas River					3.11	3.11	
Mineral Creek									
MINaBrG	Mineral Cr	Above Browns Gulch	Mill Cr	0.74			0.66	0.70	
MINaSF	Mineral Cr	Above SF Mineral Cr		2.60	2.73	3.32	2.77	2.70	22.93
SFMIN	SF Mineral Cr	Below Clear Cr		1.16	1.20	0.66	0.64	1.00	-33.78
MINaA	Mineral Cr	Above Animas River		1.64	1.95	2.57	2.03	1.87	37.62
Animas River Canyon - Upper									
AaELK	Animas River	Above Elk Creek	Elk Cr	1.28	1.06	1.82	1.43	1.26	44.78
Animas River Canyon - Lower									
AaCAS	Animas River	Above Cascade Creek	Cascade Cr	0.74	0.78	1.18	0.84	0.79	49.82

Note: Red shading indicates instances where the distance in ordination space to reference site centroids was greatest in 2018.
*For example, the distance from the 2018 AaCEM sample to the reference centroid (1.00) was 122.87% further than the average distance from the 2016, 2017, and 2019 AaCEM samples to the reference centroid (0.45).

7. Figures

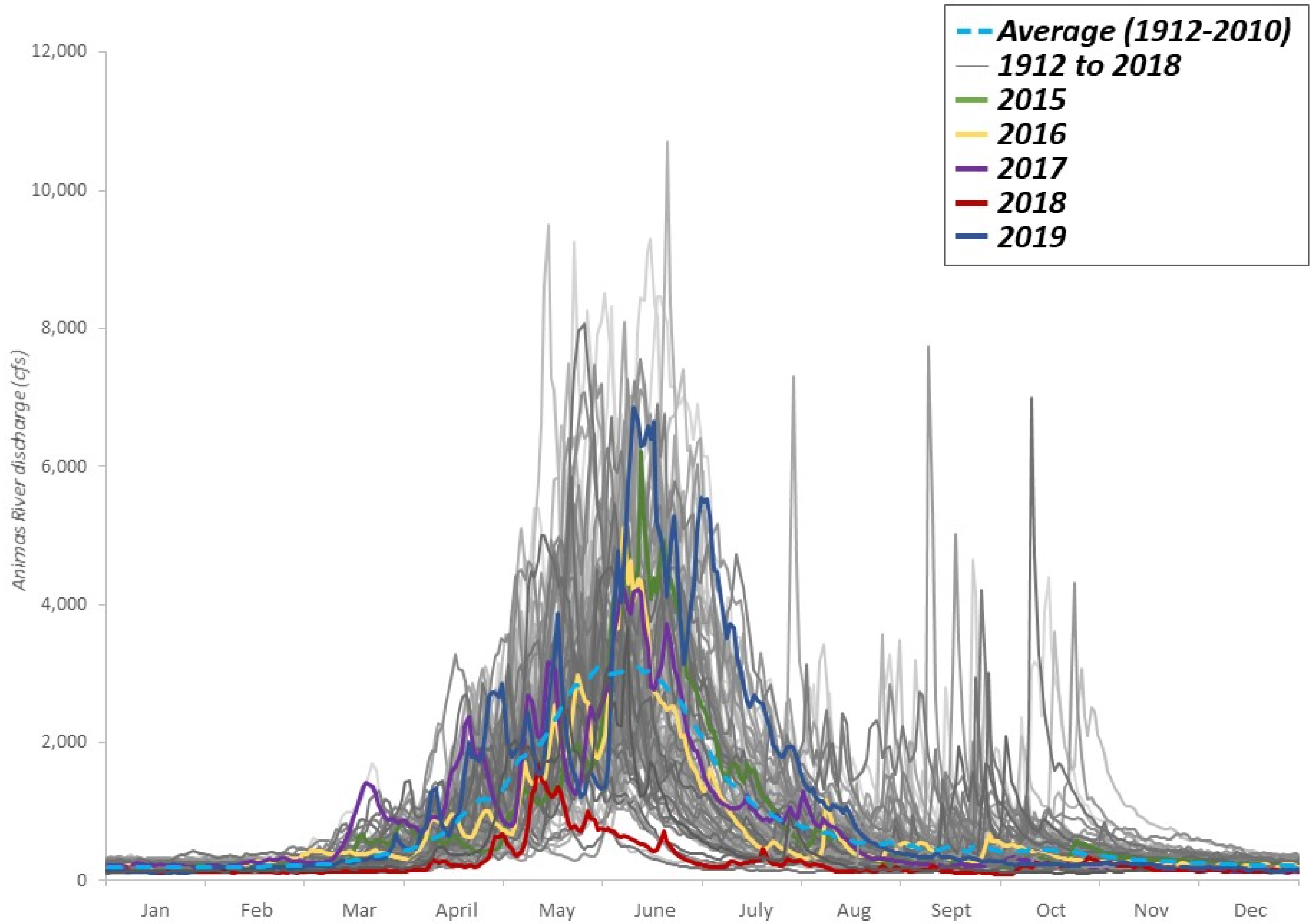


Figure 1: Animas River discharge at Durango USGS gage from 1912-2019.

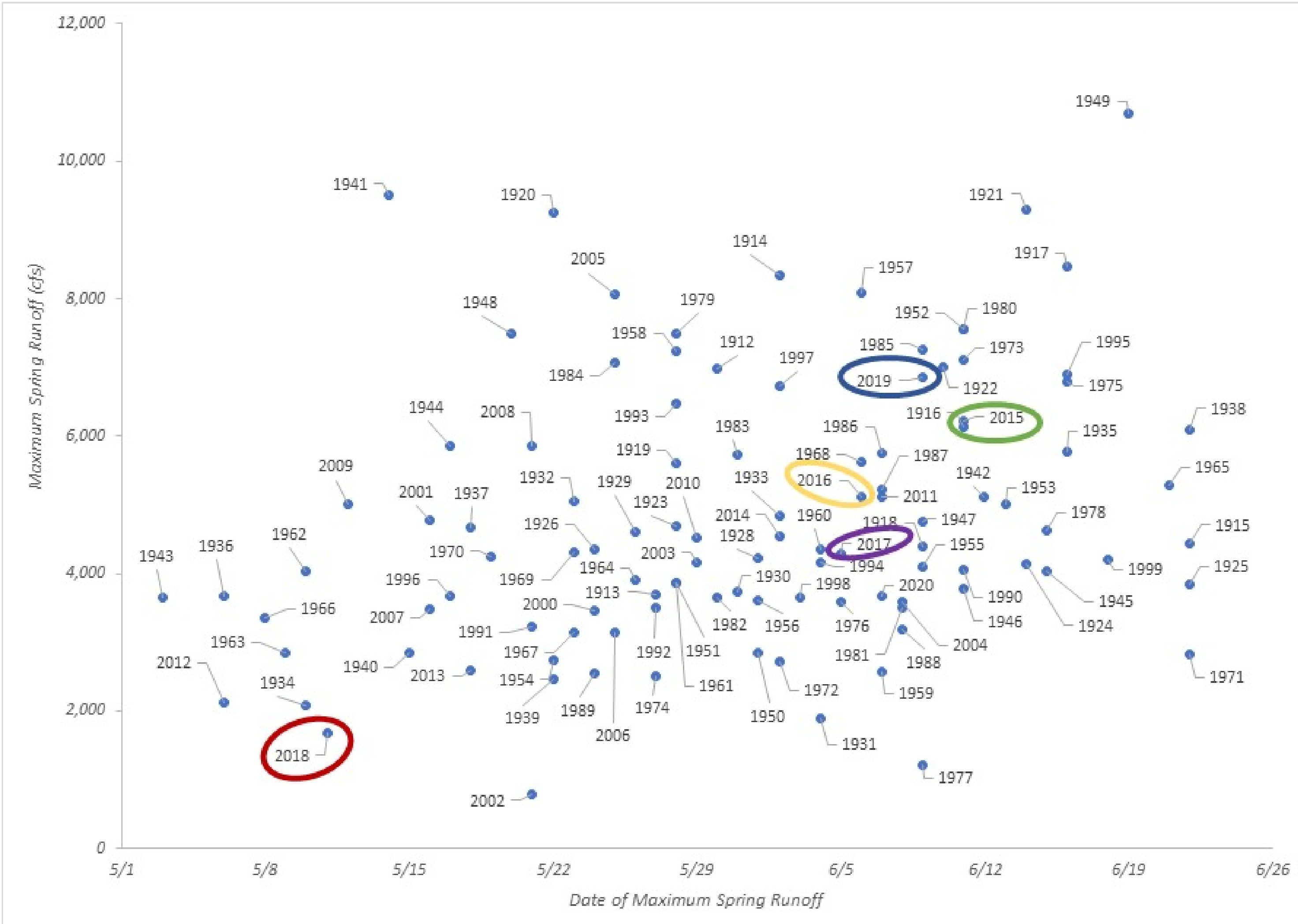


Figure 2: Maximum spring runoff of Animas River at Durango USGS gage from 1912-2019.

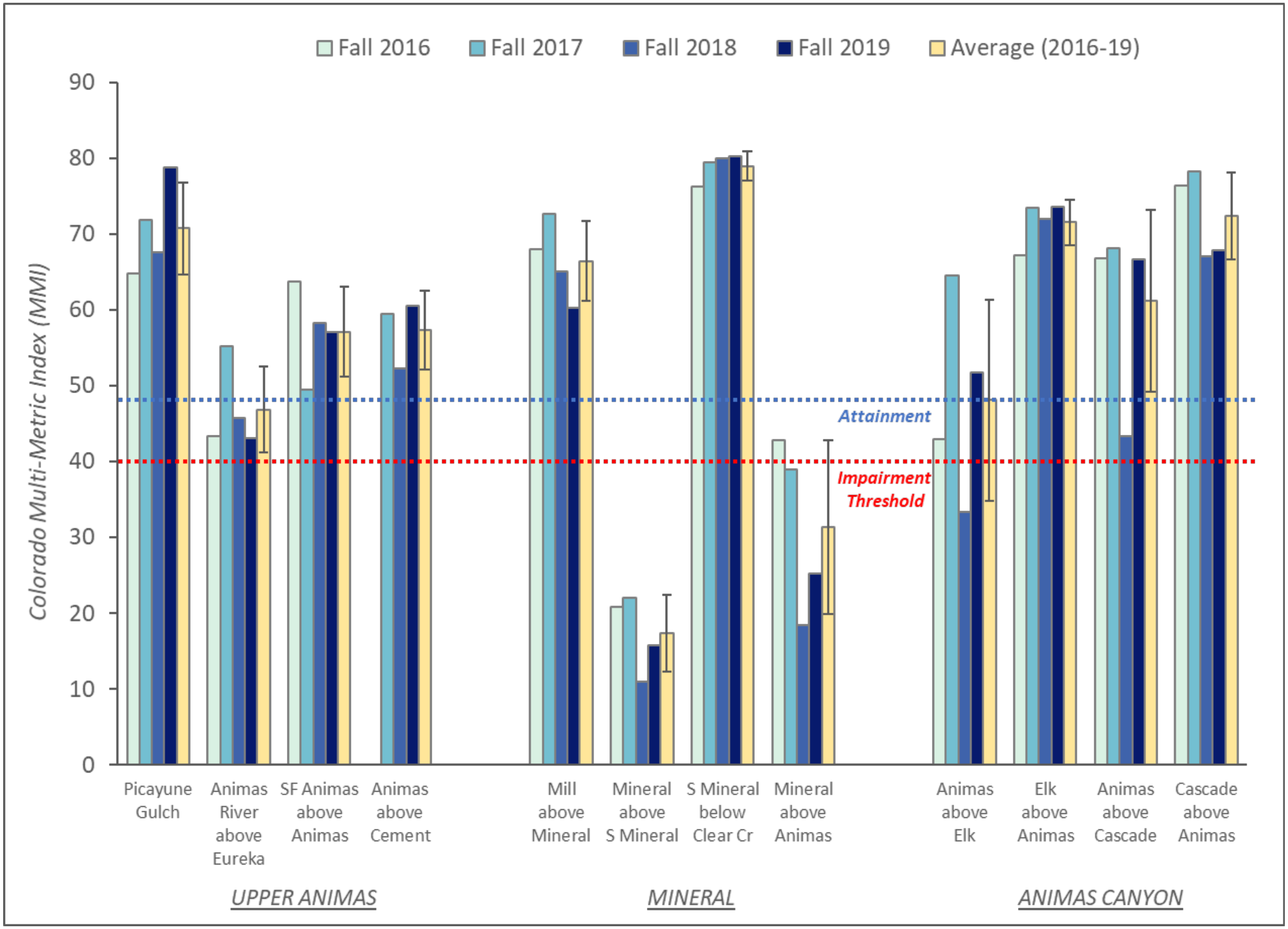


Figure 3: Colorado Multi-metric Index (MMI) – error bars represent annual variability as two standard errors.

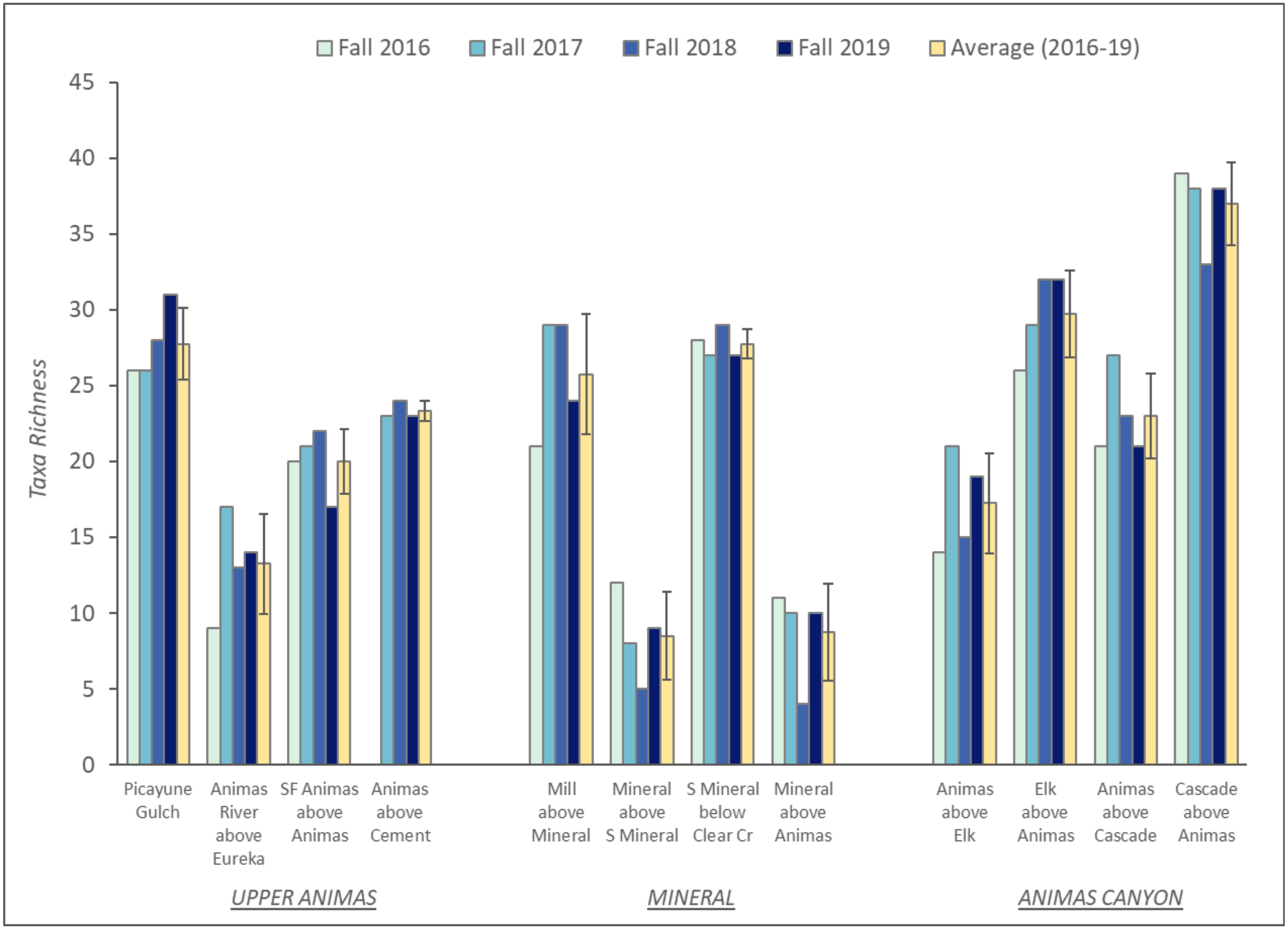


Figure 4: Taxa richness – error bars represent annual variability as two standard errors.

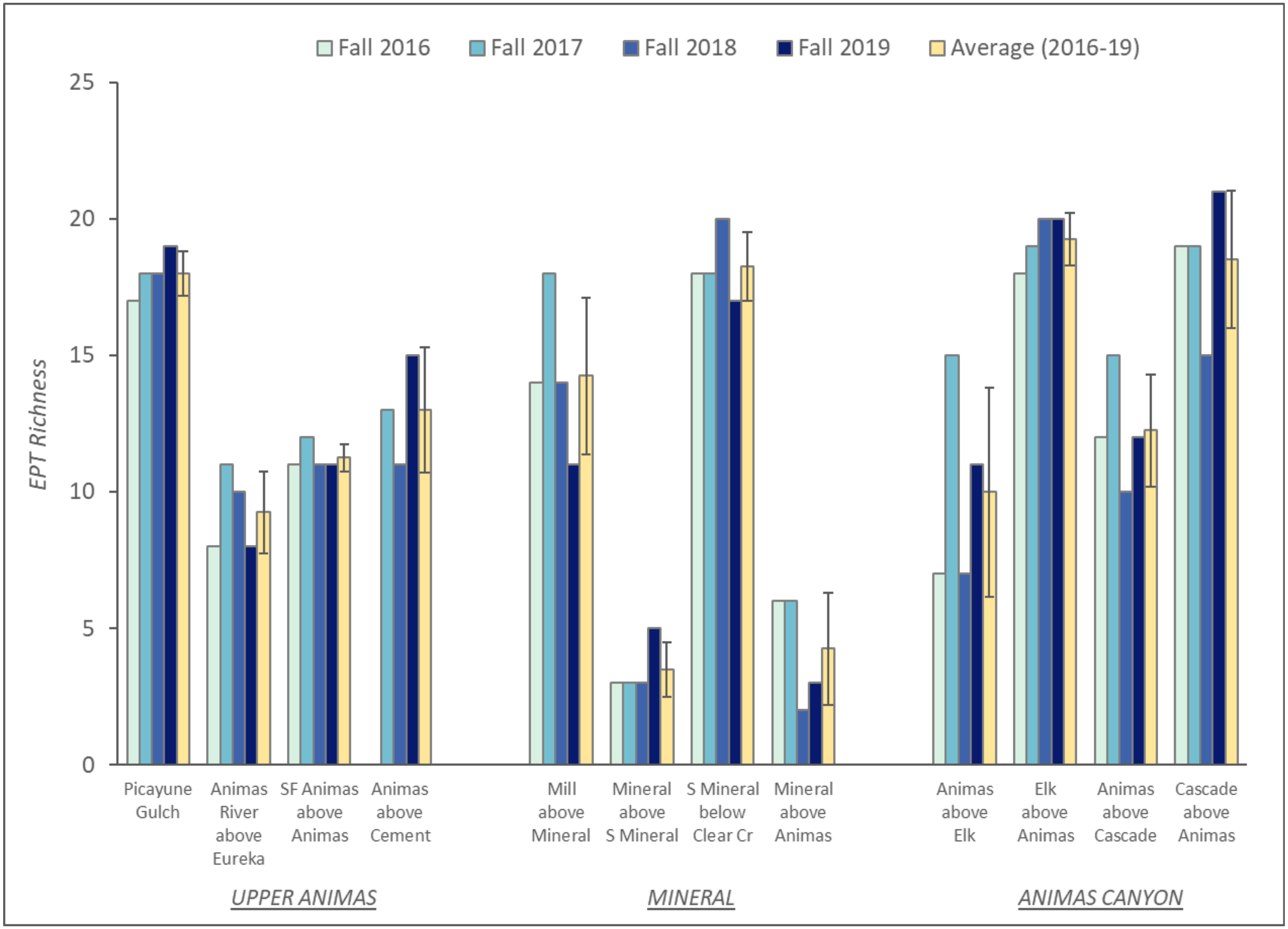


Figure 5: EPT richness – error bars represent annual variability as two standard errors.

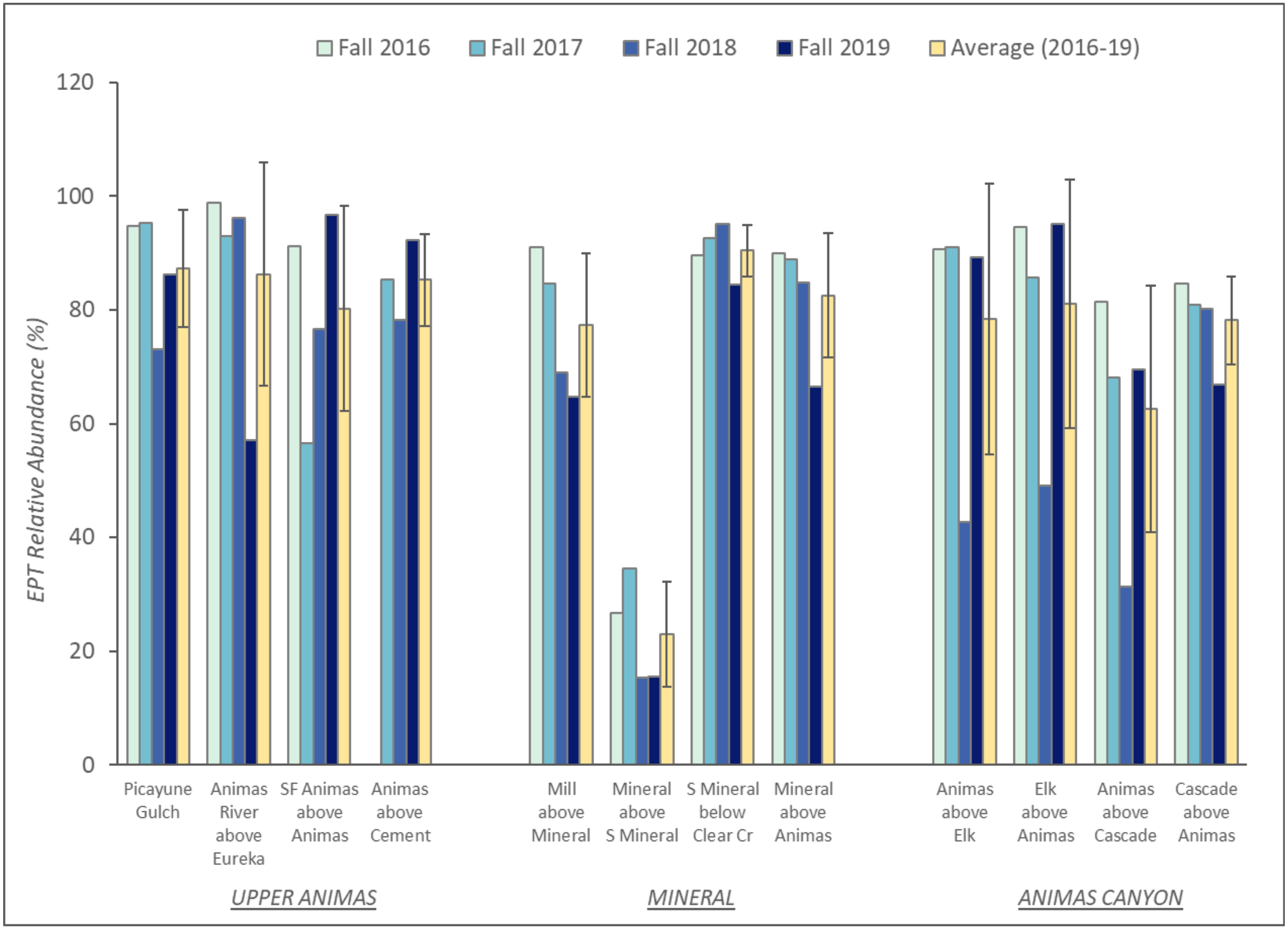


Figure 6: EPT relative abundance – error bars represent annual variability as two standard errors.

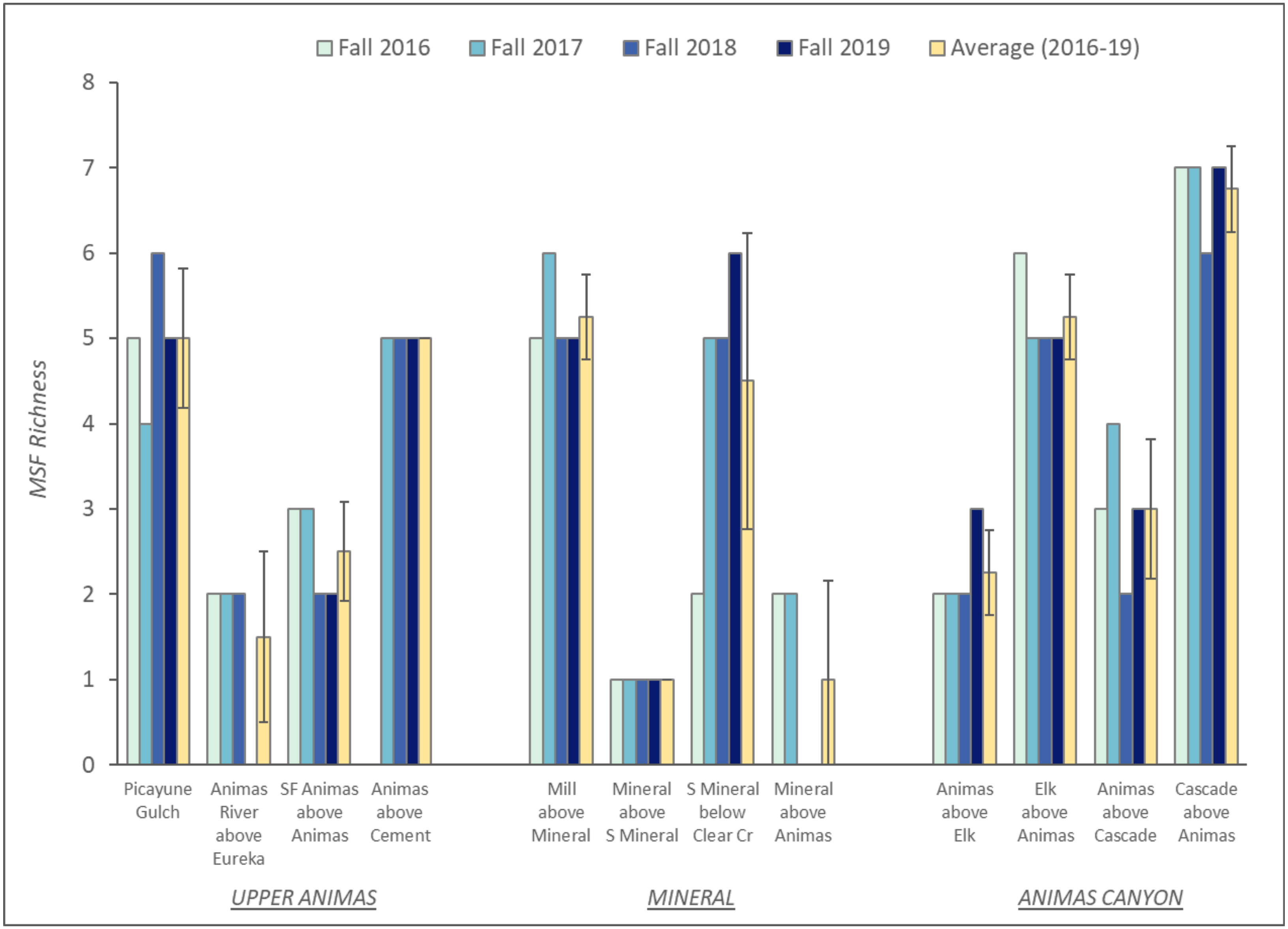


Figure 7: Metal Sensitive Family (MSF) richness – error bars represent annual variability as two standard errors.

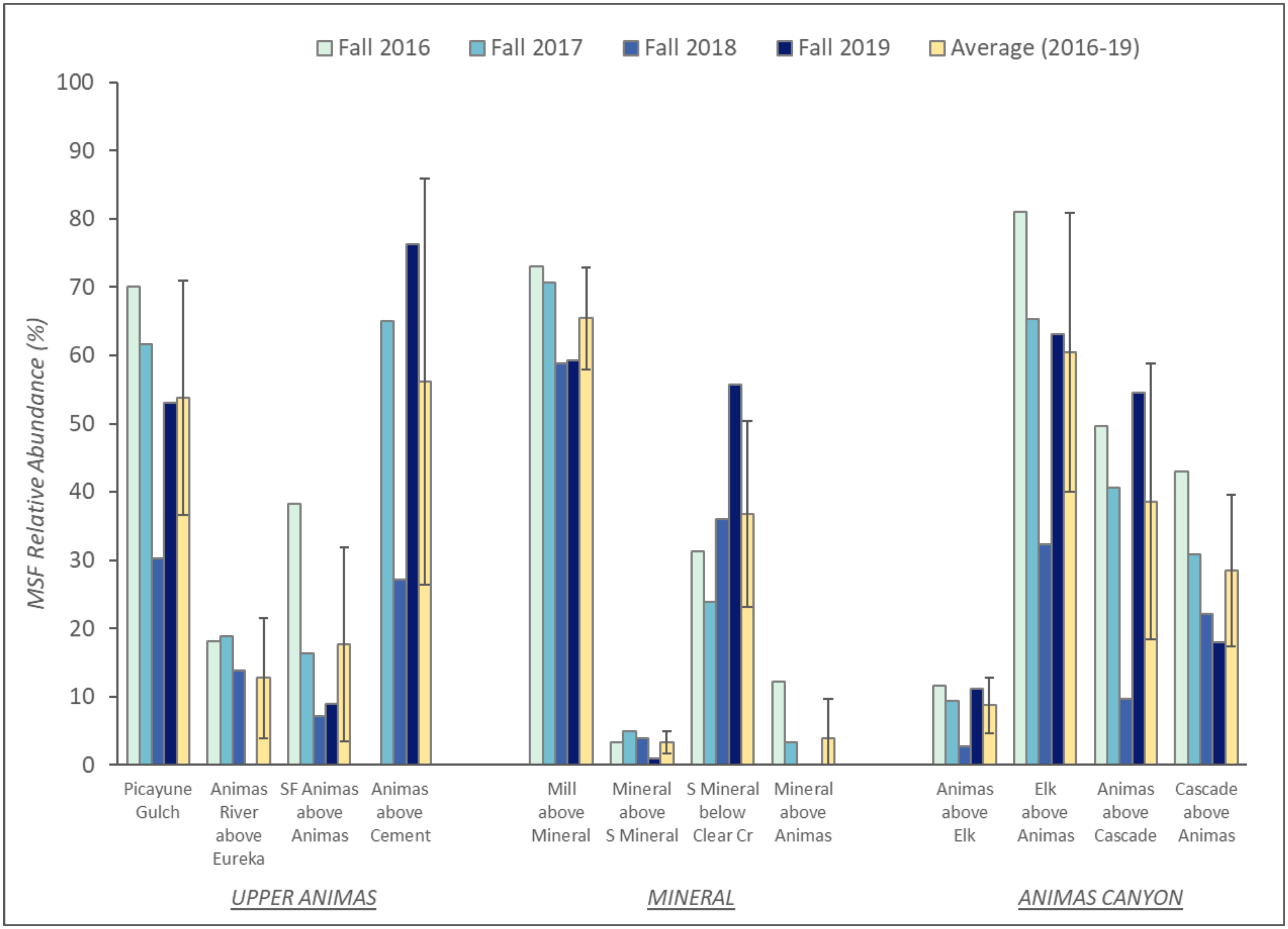


Figure 8: Metal Sensitive Family (MSF) relative abundance – error bars represent annual variability as two standard errors.

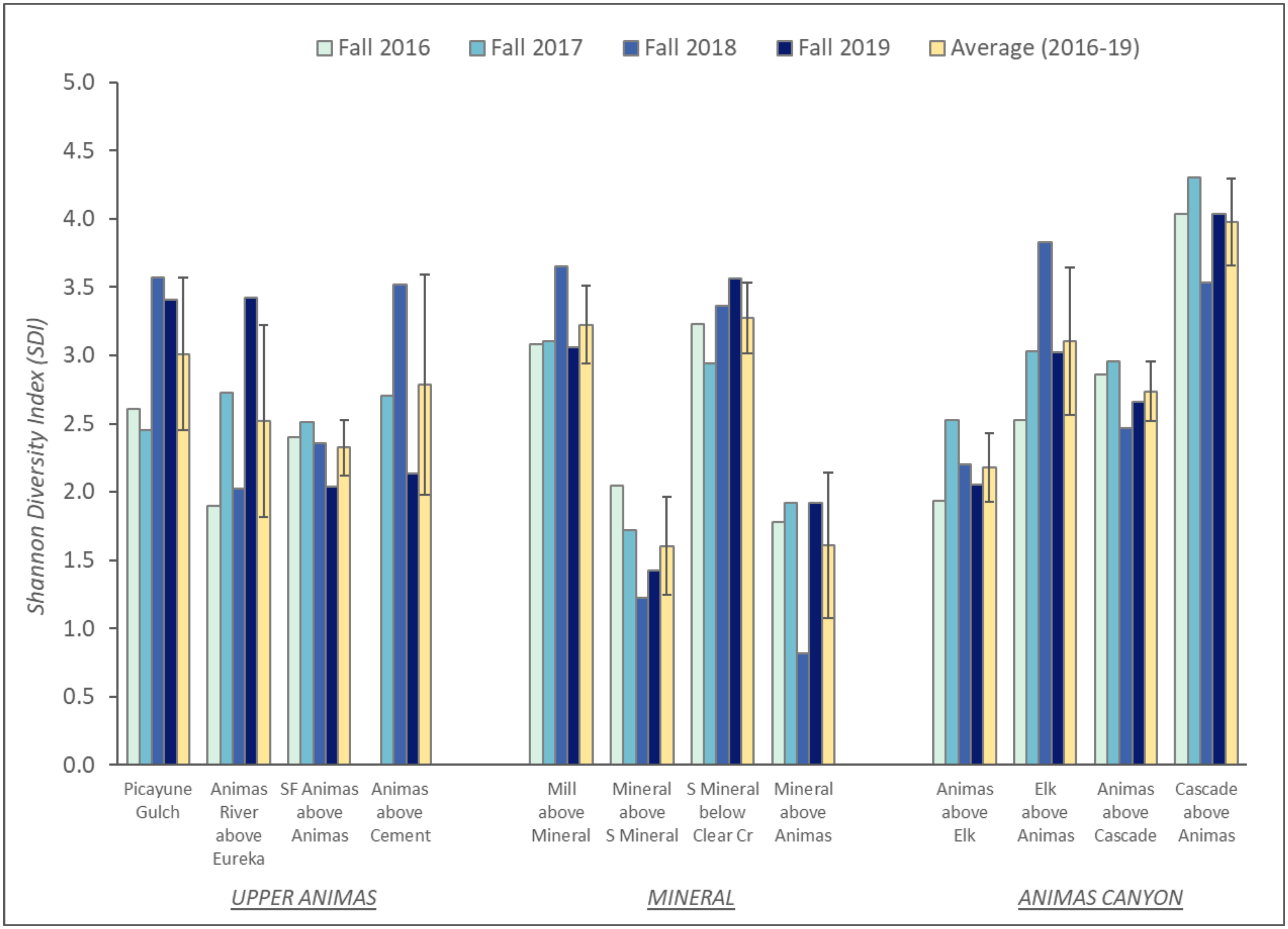


Figure 9: Shannon Diversity Index (SDI) – error bars represent annual variability as two standard errors.

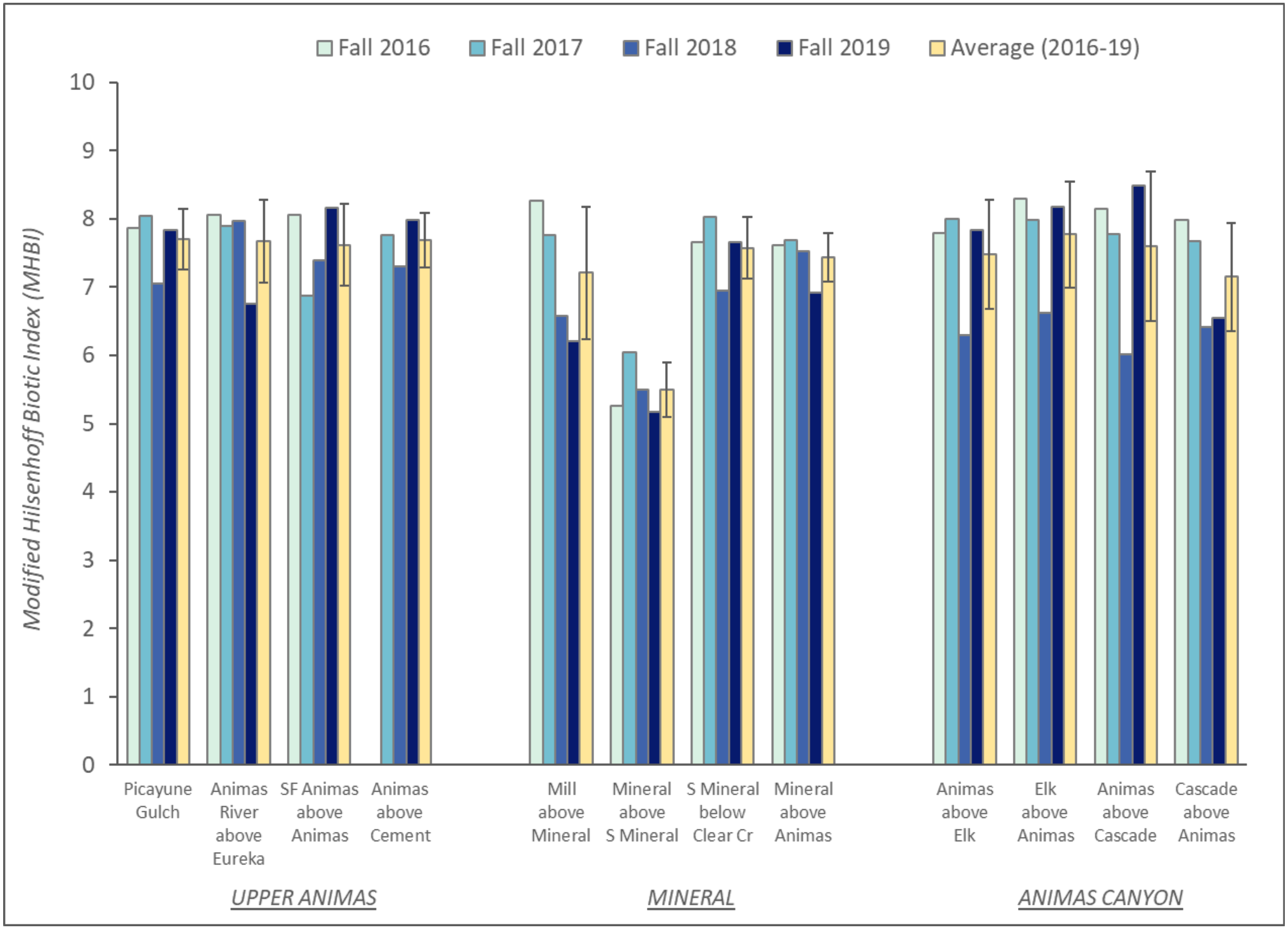


Figure 10: Modified Hilsenhoff Biotic Index (MHBI) – error bars represent annual variability as two standard errors.

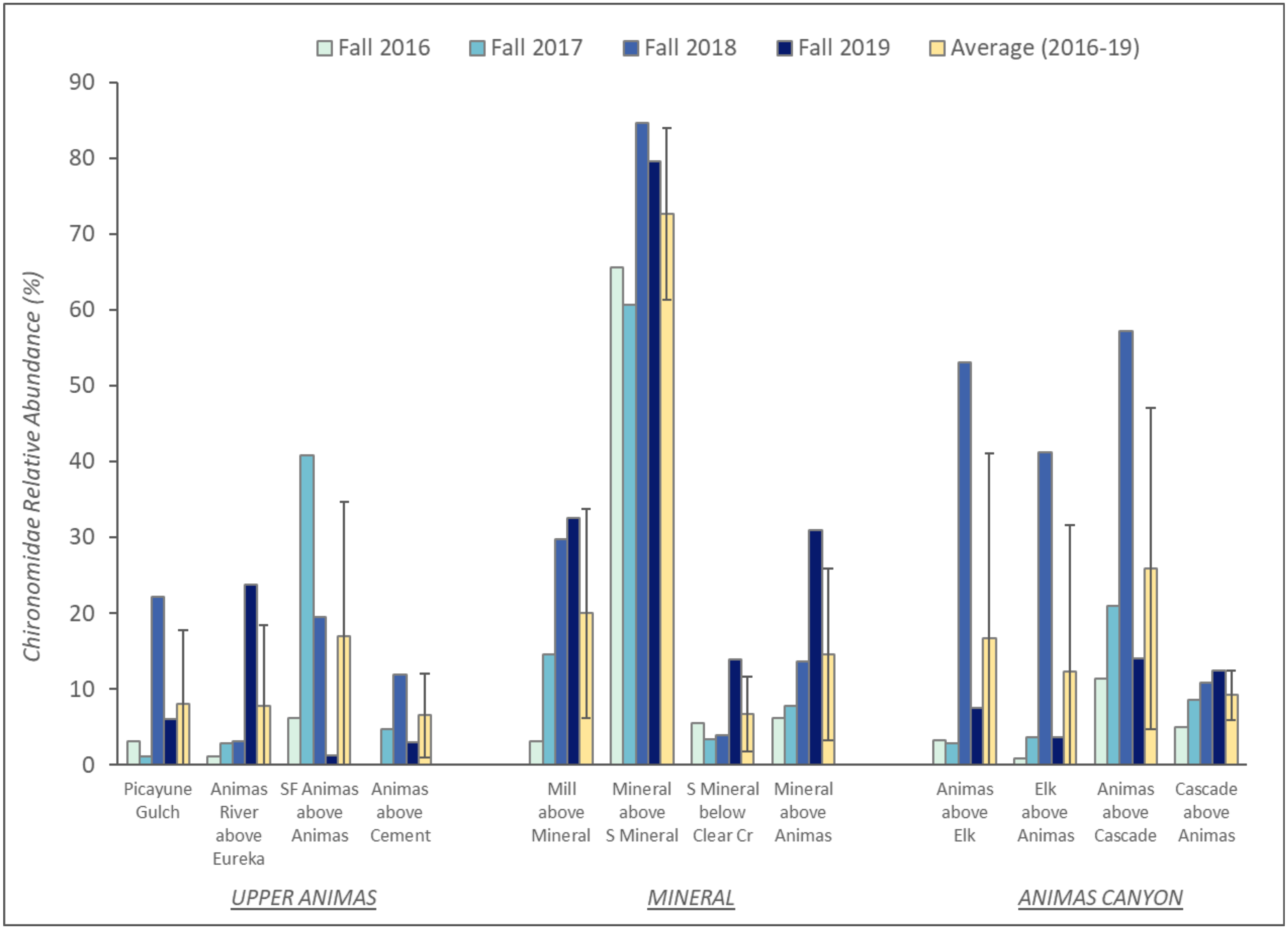


Figure 11: Chironomidae relative abundance – error bars represent annual variability as two standard errors.

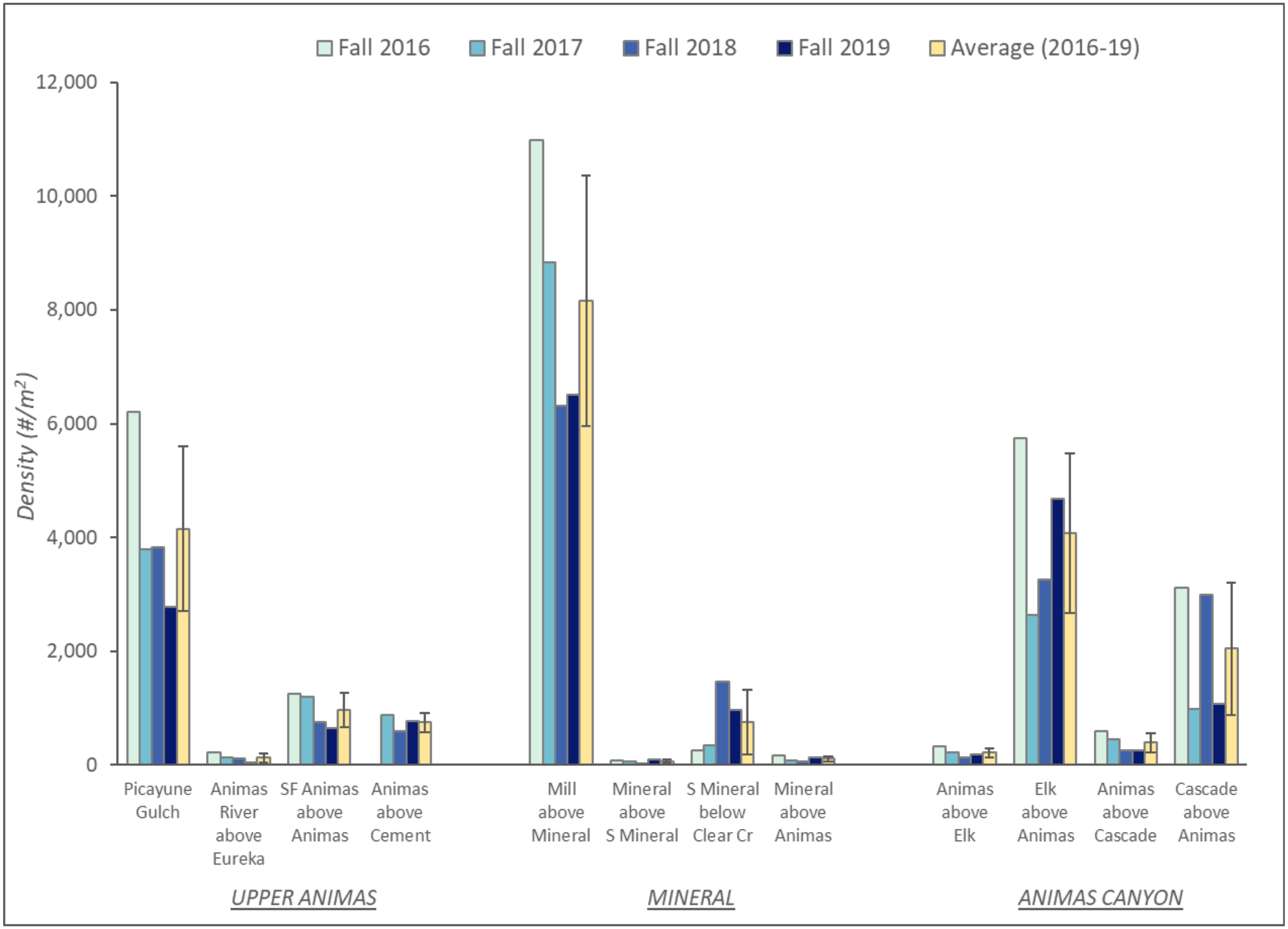


Figure 12: Density (#/m²) – error bars represent annual variability as two standard errors.

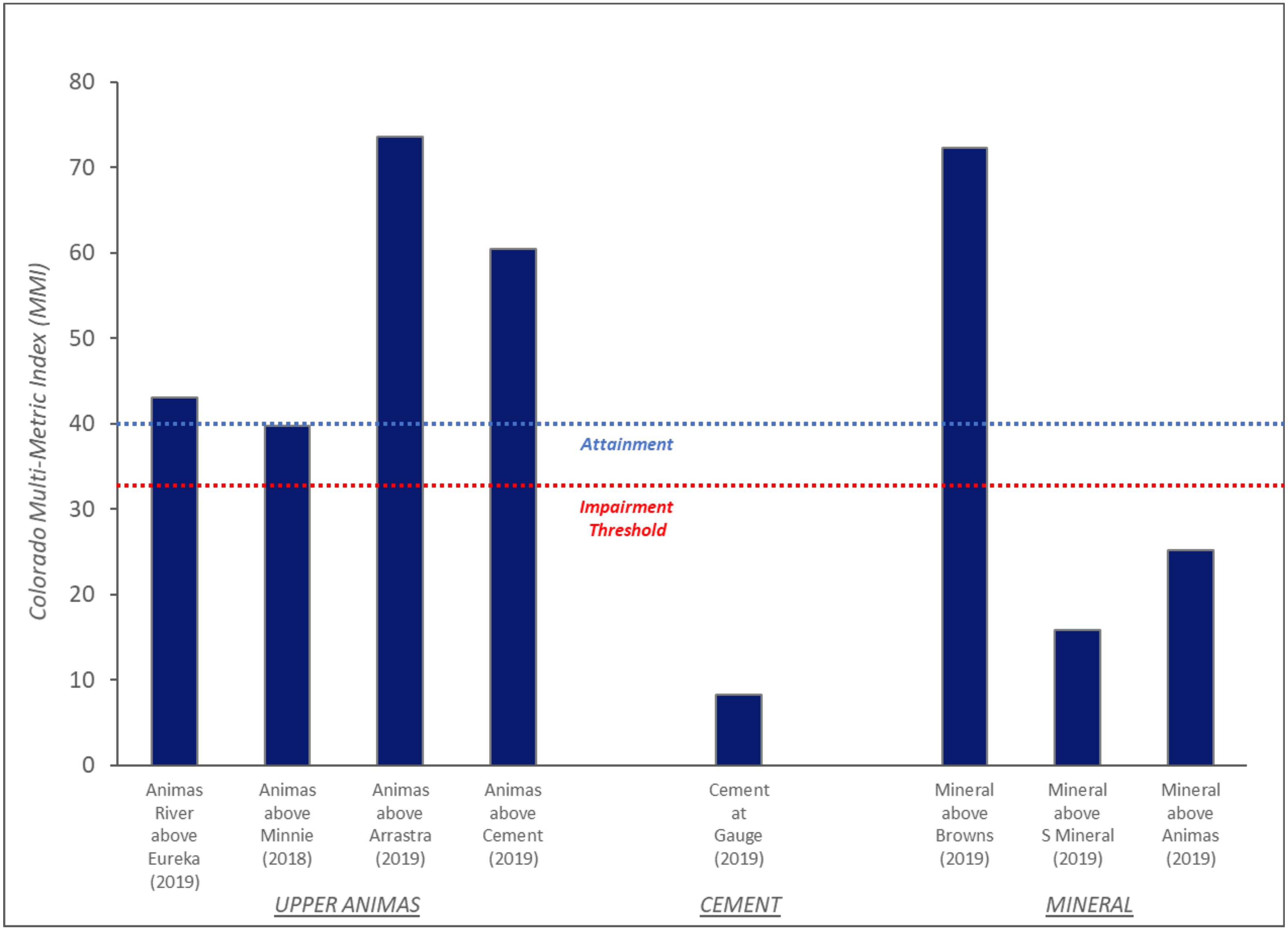


Figure 13: Colorado Multi-Metric Index (MMI) – 2018-19 supplemental sampling and associated mainstem sites.

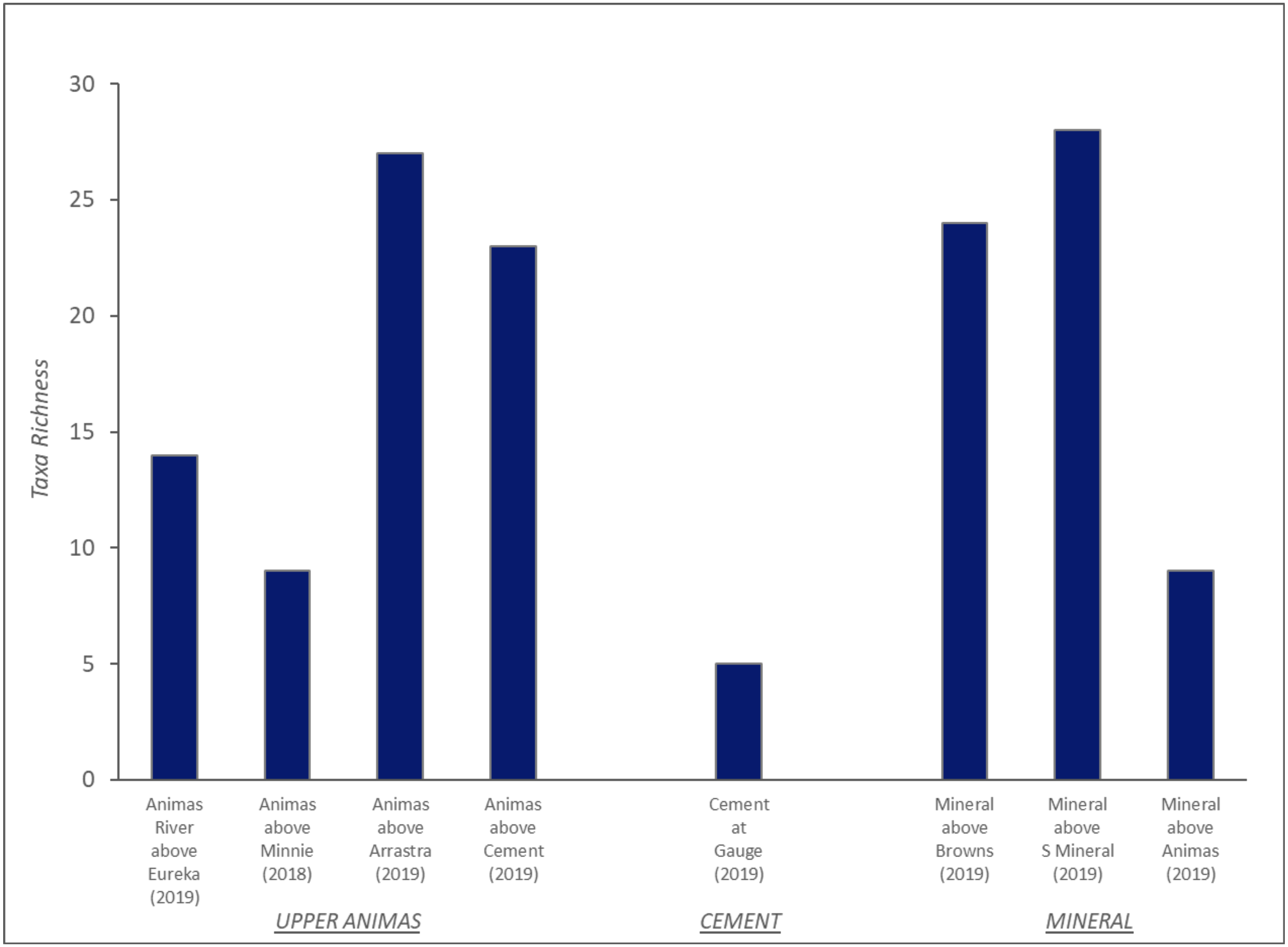


Figure 14: Taxa richness – 2018-19 supplemental sampling and associated mainstem sites.

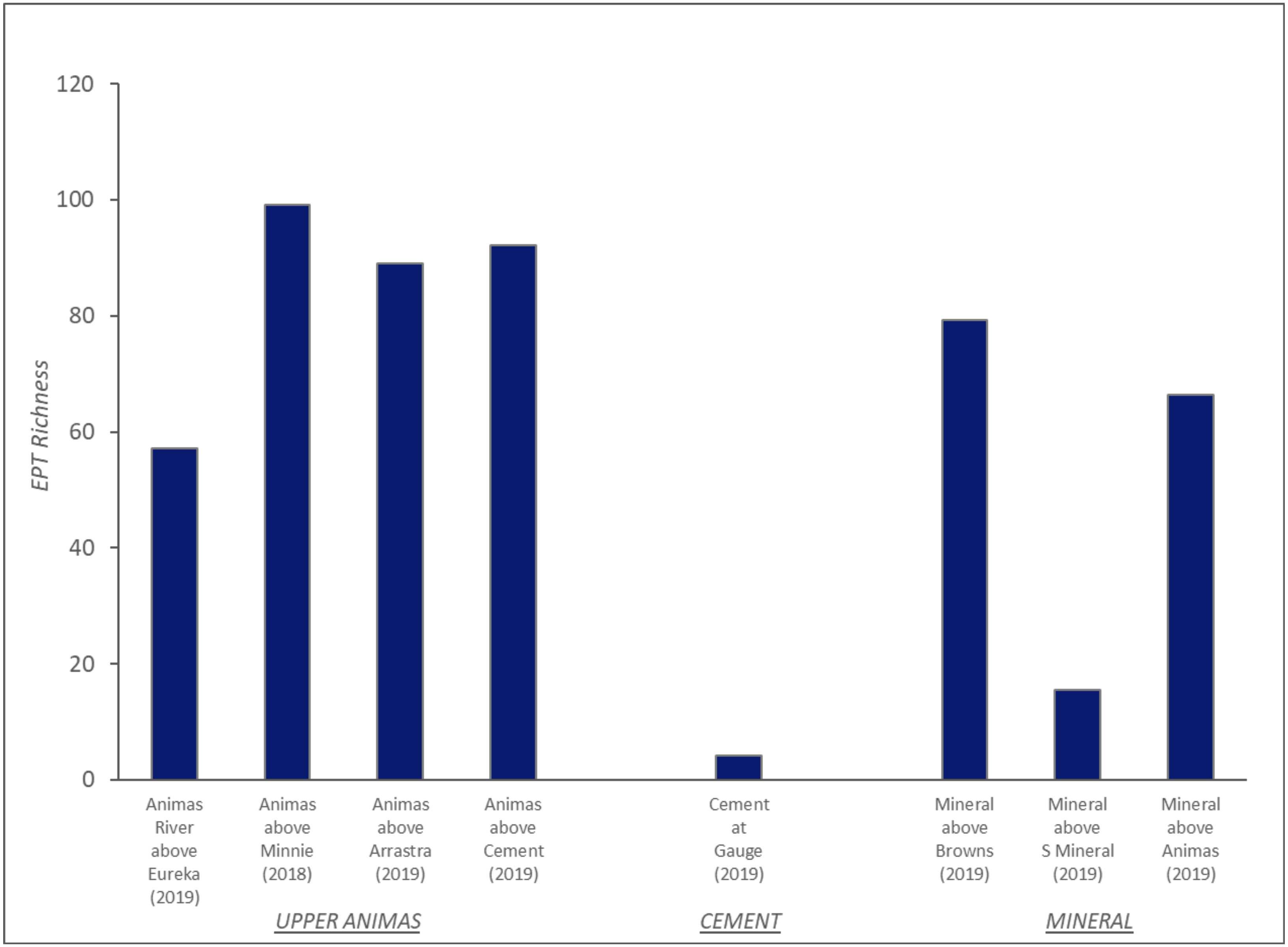


Figure 15: EPT richness – 2018-19 supplemental sampling and associated mainstem sites.

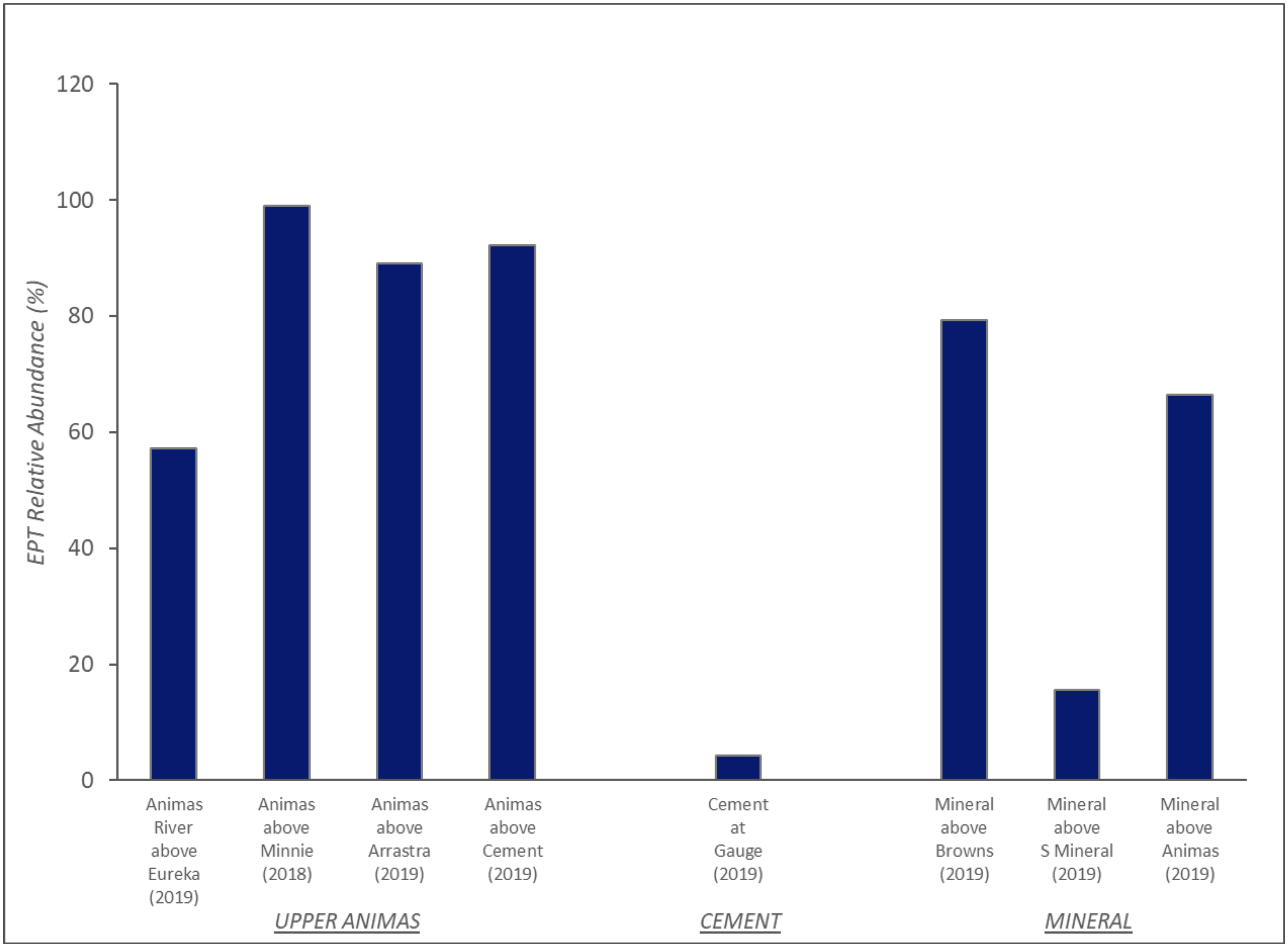


Figure 16: EPT relative abundance – 2018-19 supplemental sampling and associated mainstem sites.

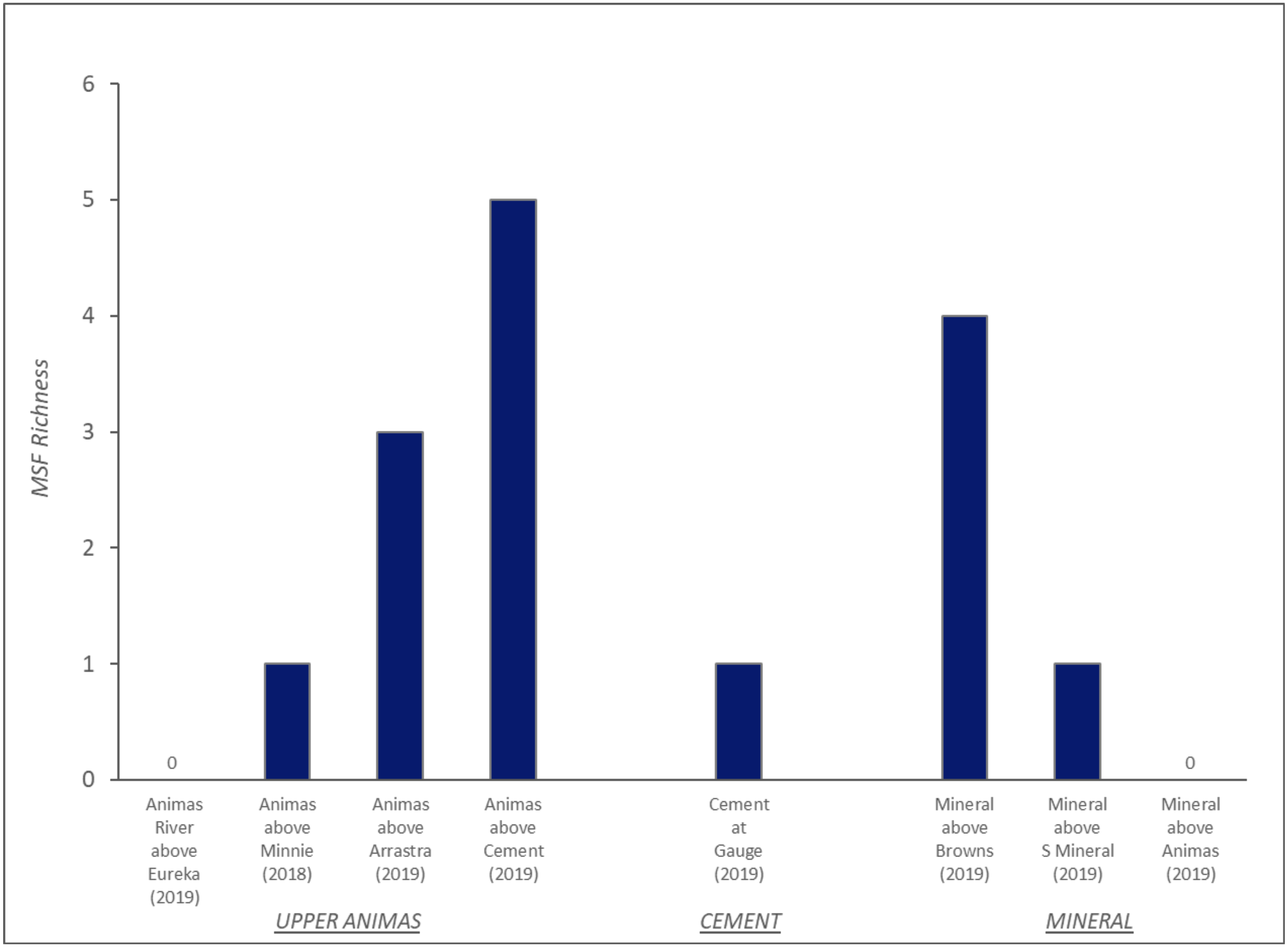


Figure 17: Metal Sensitive Family (MSF) richness – 2018-19 supplemental sampling and associated mainstem sites.

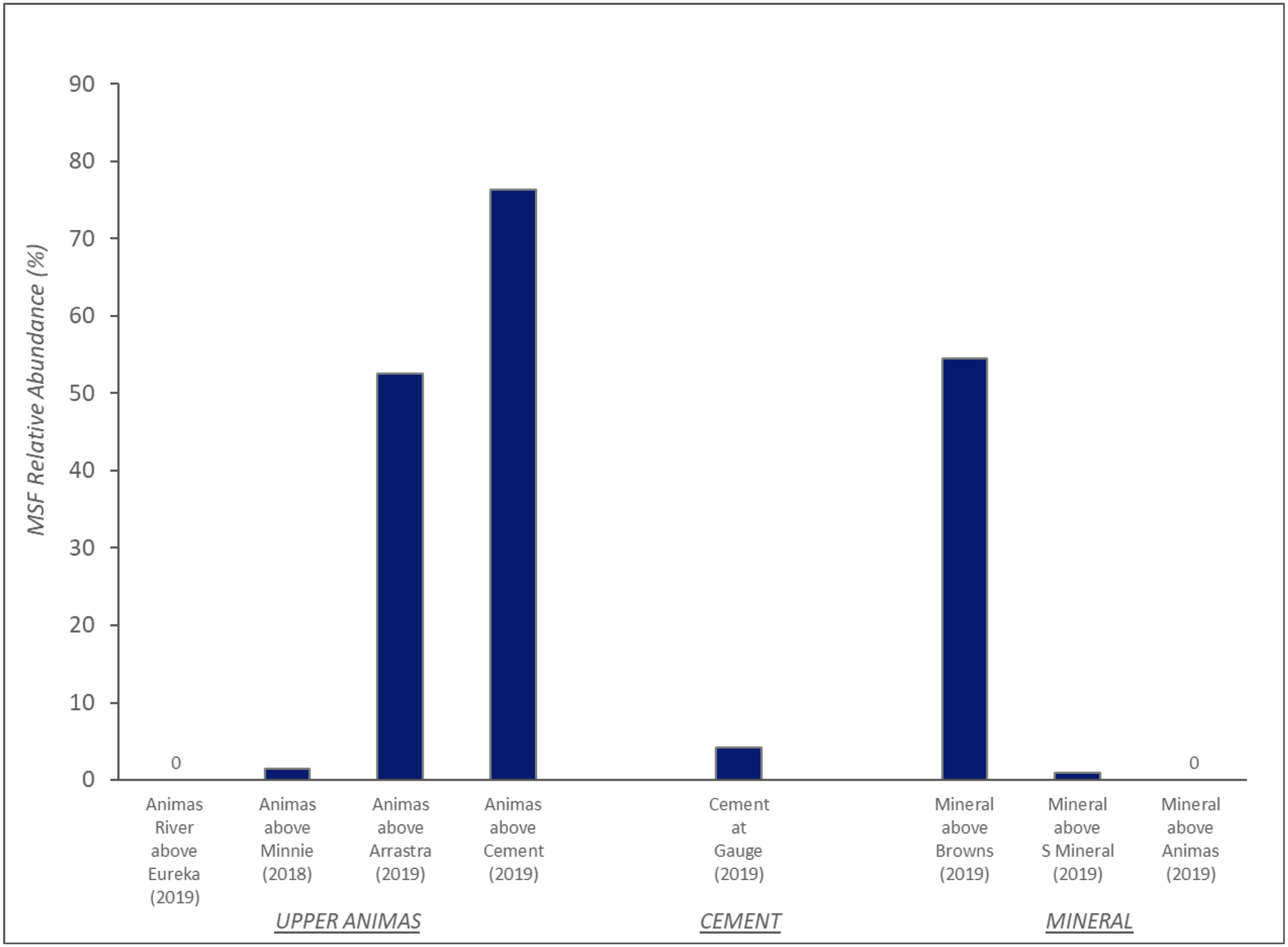


Figure 18: Metal Sensitive Family (MSF) relative abundance – 2018-19 supplemental sampling and associated mainstem sites.

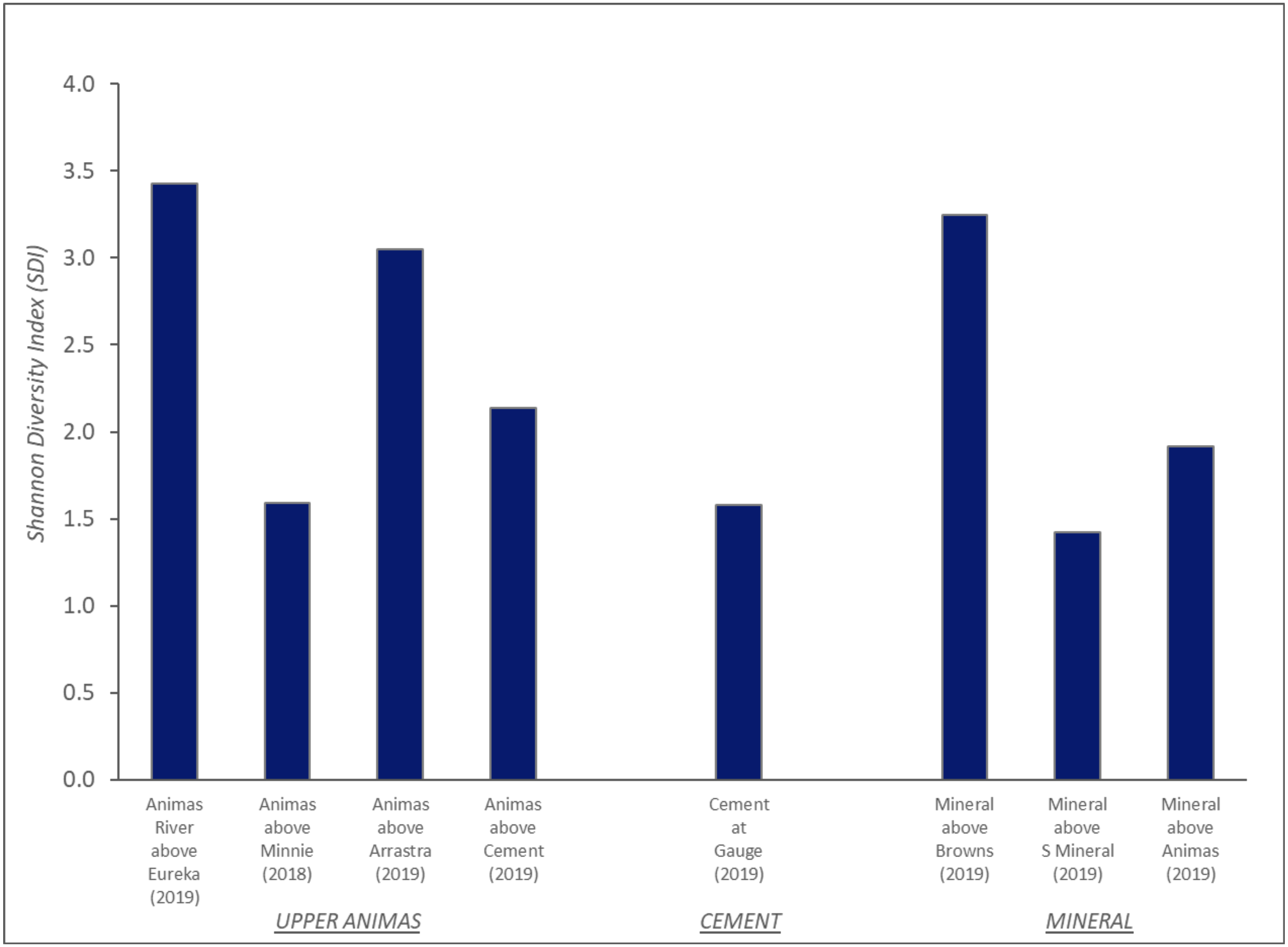


Figure 19: Shannon Diversity Index (SDI) – 2018-19 supplemental sampling and associated mainstem sites.

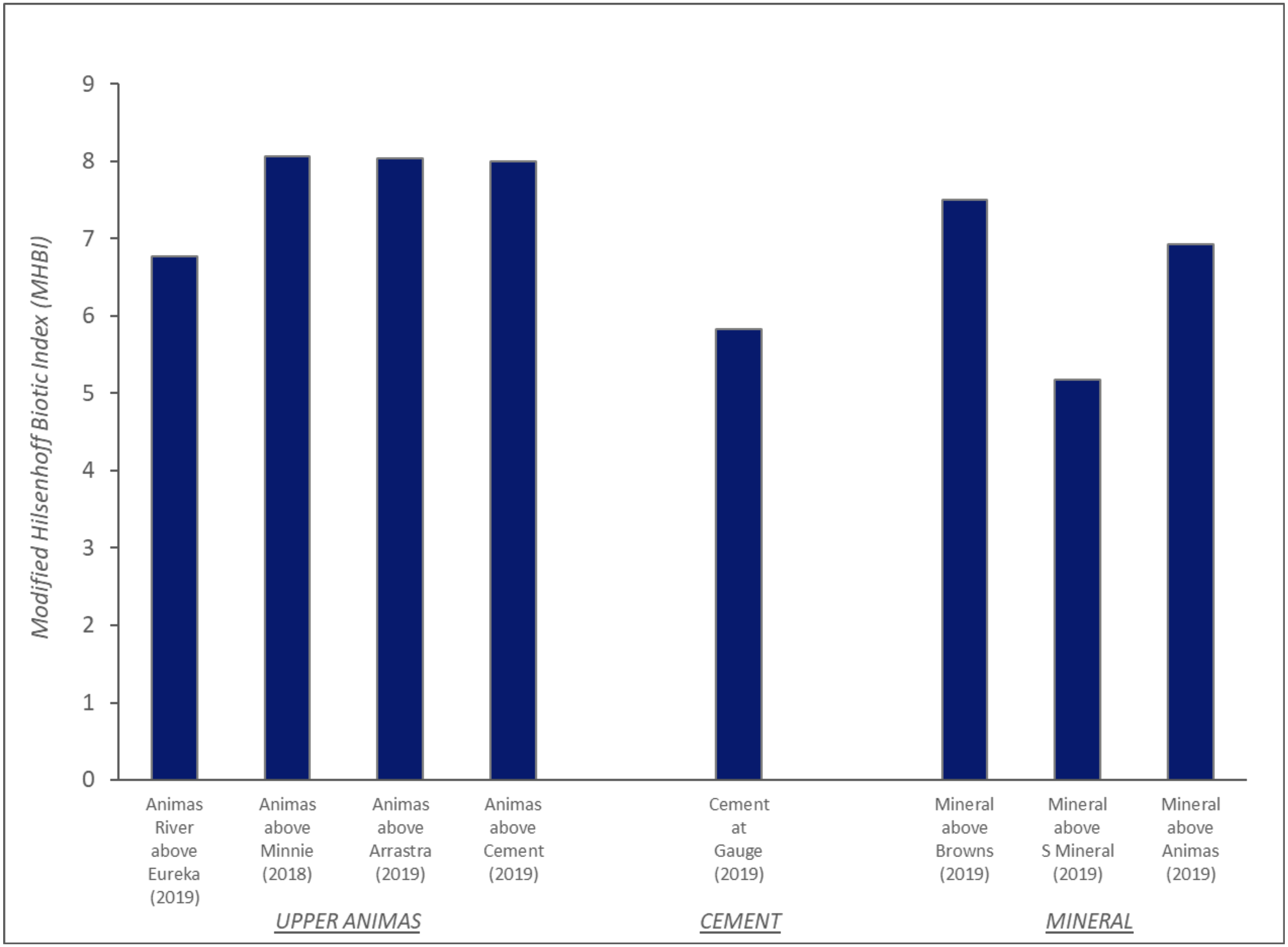


Figure 20: Modified Hilsenhoff Biotic Index (MHBI) – 2018-19 supplemental sampling and associated mainstem sites.

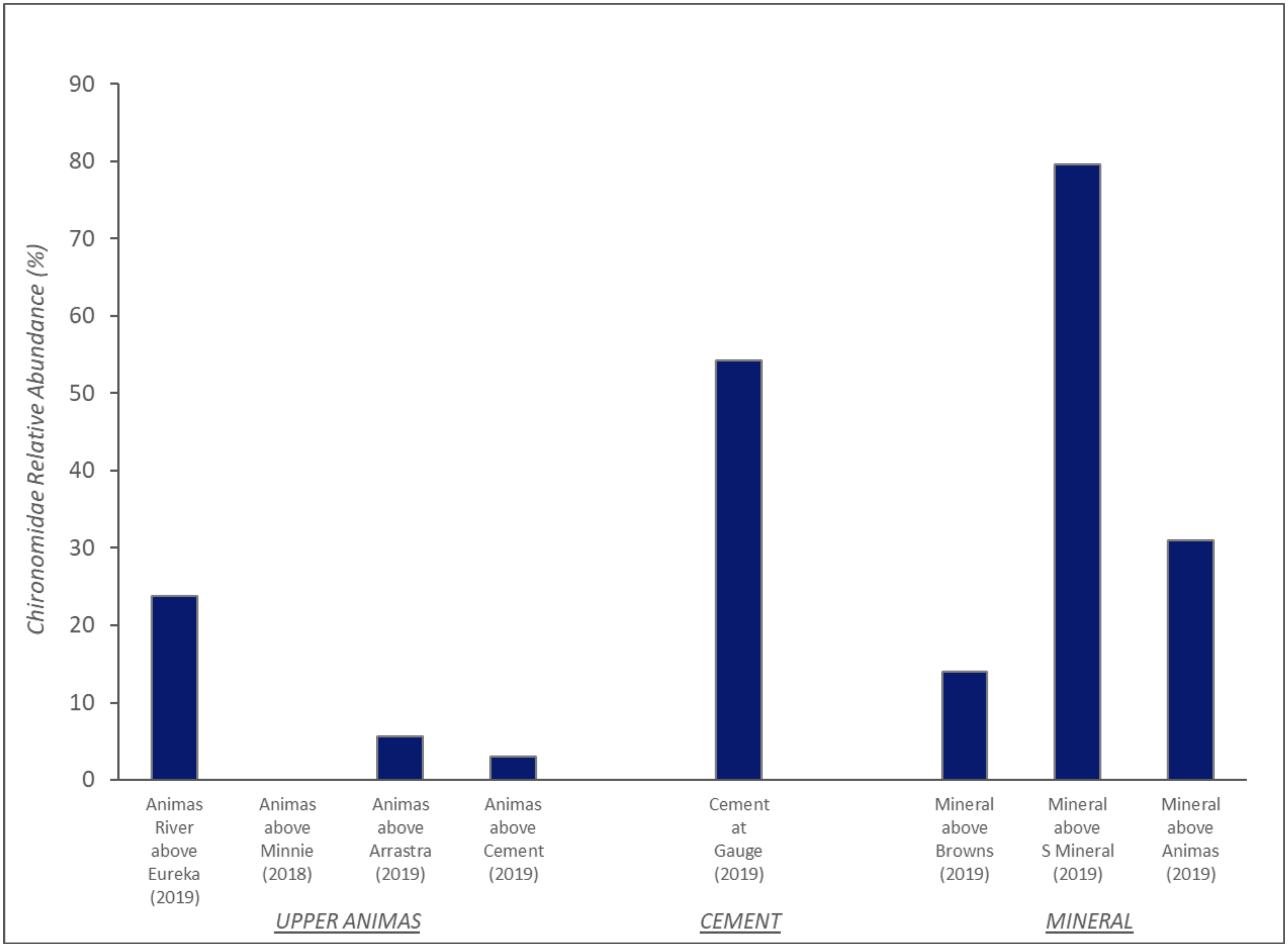


Figure 21: Chironomidae relative abundance – 2018-19 supplemental sampling and associated mainstem sites.

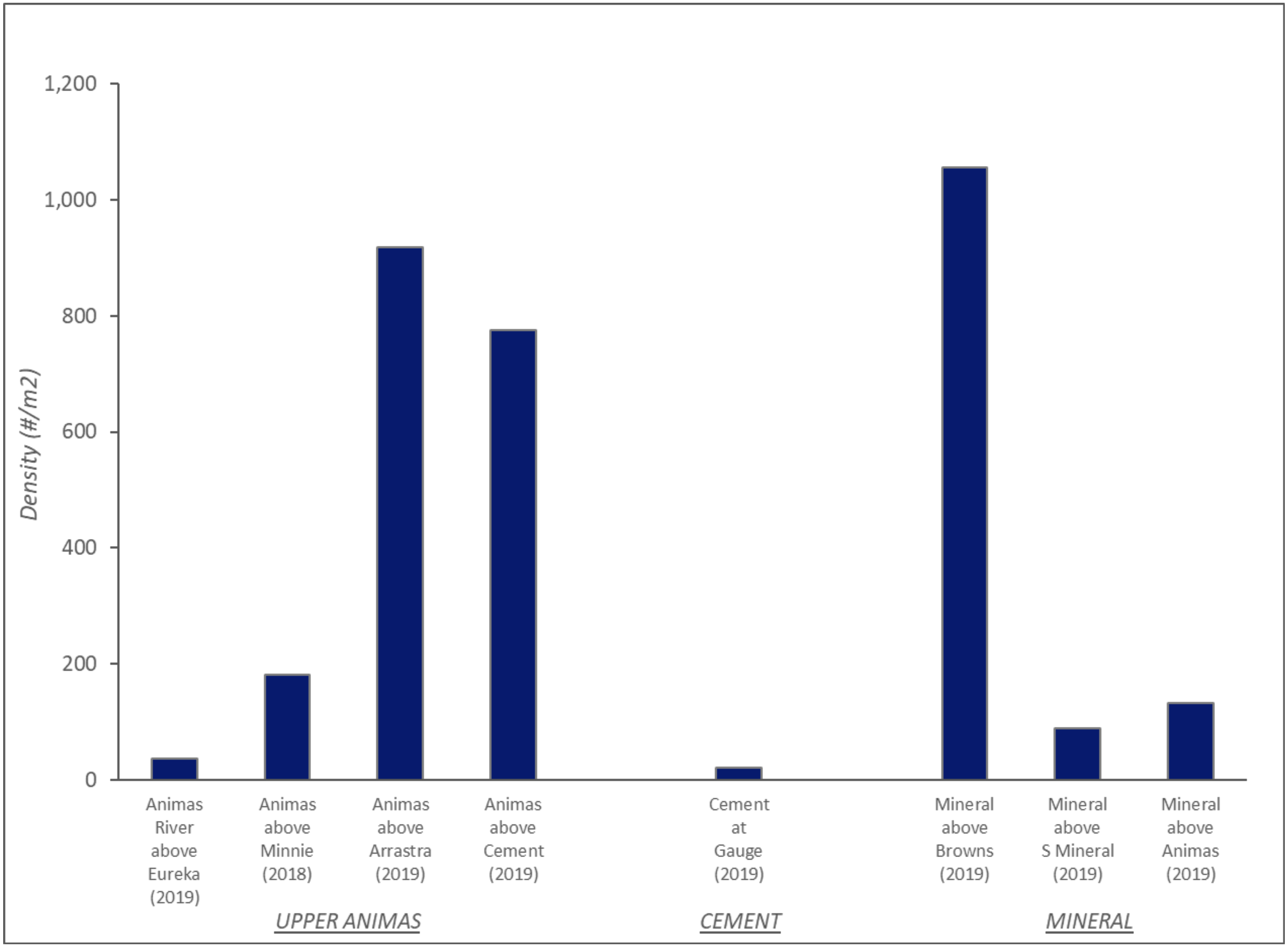


Figure 22: Density (#/m²) – 2018-19 supplemental sampling and associated mainstem sites.

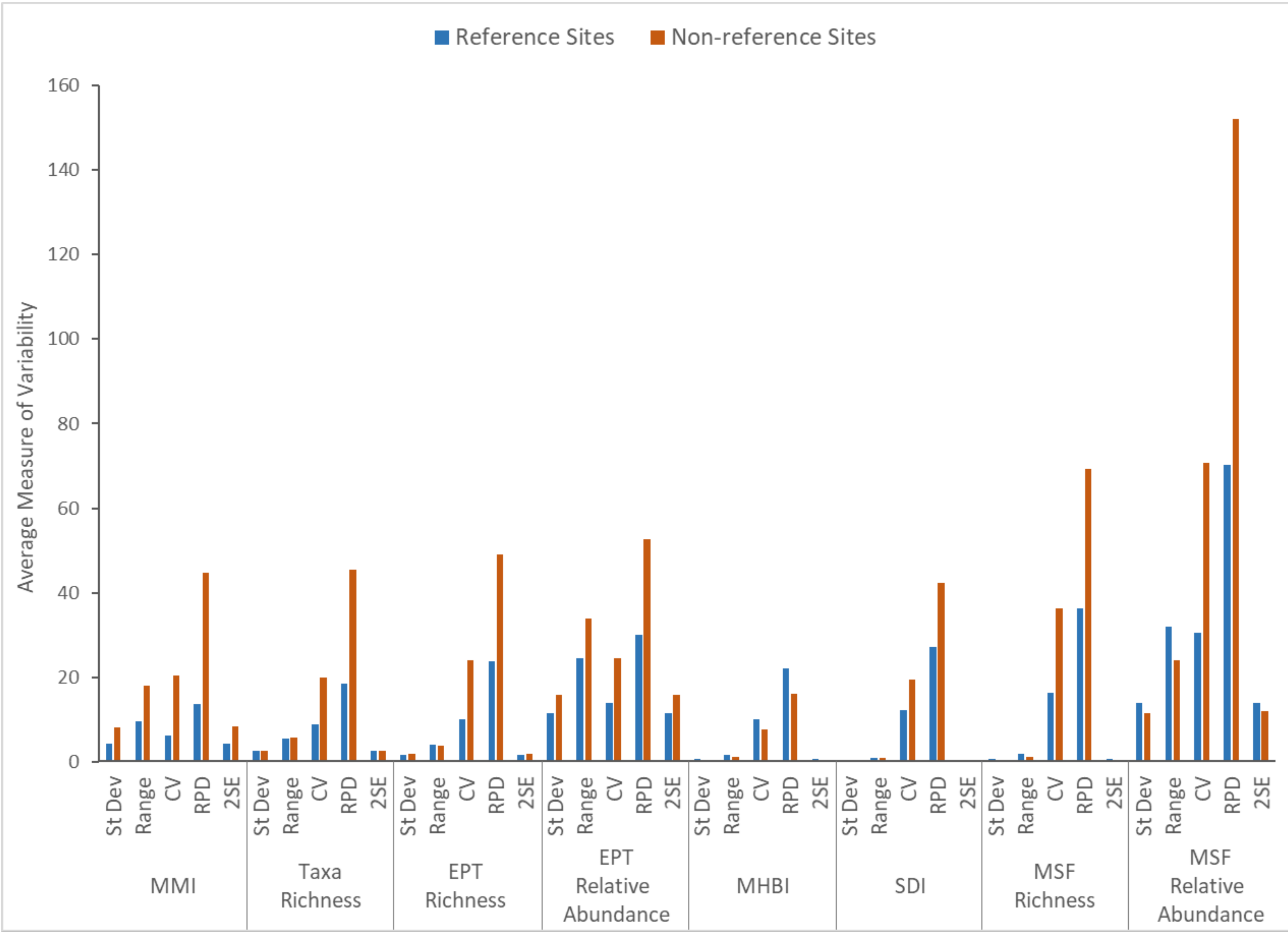


Figure 23: Measures of variability for each metric averaged across the twelve annual variability assessment sites (2016-19). ***StDev = standard deviation; Range = max-min; cv = coefficient of variation; RPD = relative percent difference; 2SE = two standard errors.***

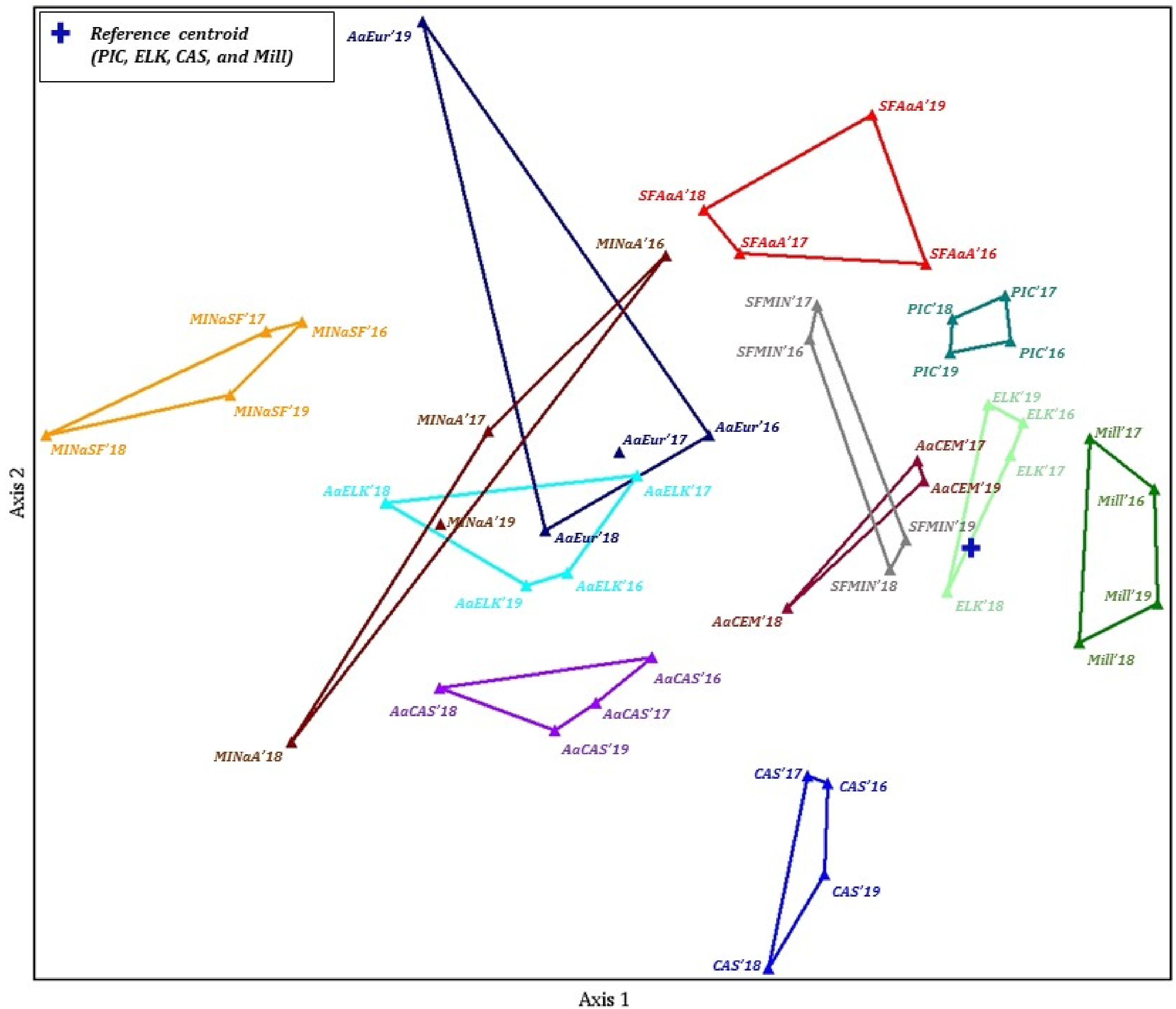


Figure 24: NMS ordination of ordination group 1 (see table 6) by sample location.

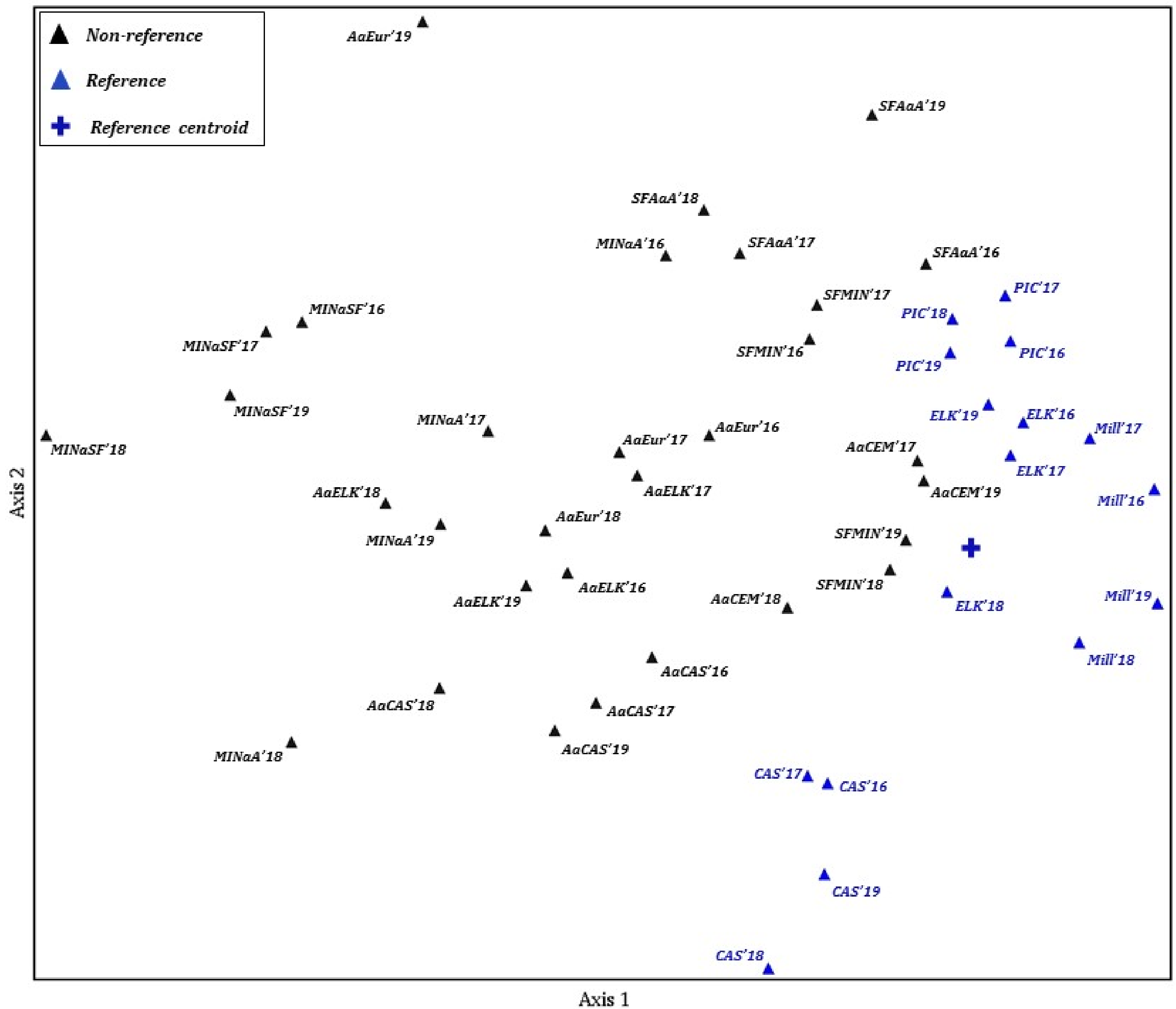


Figure 25: NMS ordination of ordination group 1 (see table 6) by reference and non-reference.

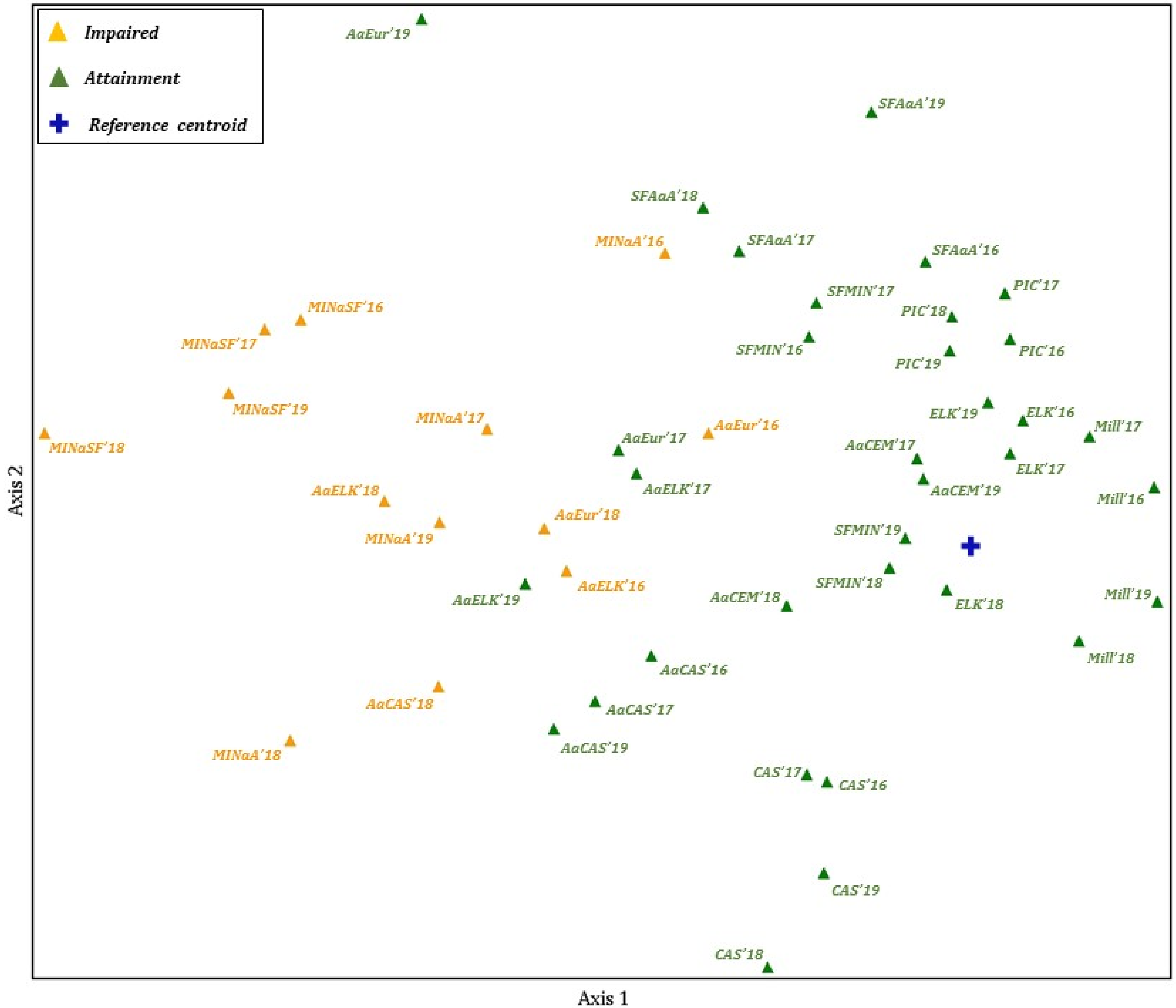


Figure 26: NMS ordination of ordination group 1 (see table 6) by aquatic life use attainment or impairment based on MMI scores. The assessment of attainment is for illustration only since it is based on a theoretical assumption that all sites are designated as having a class one aquatic life use designation.

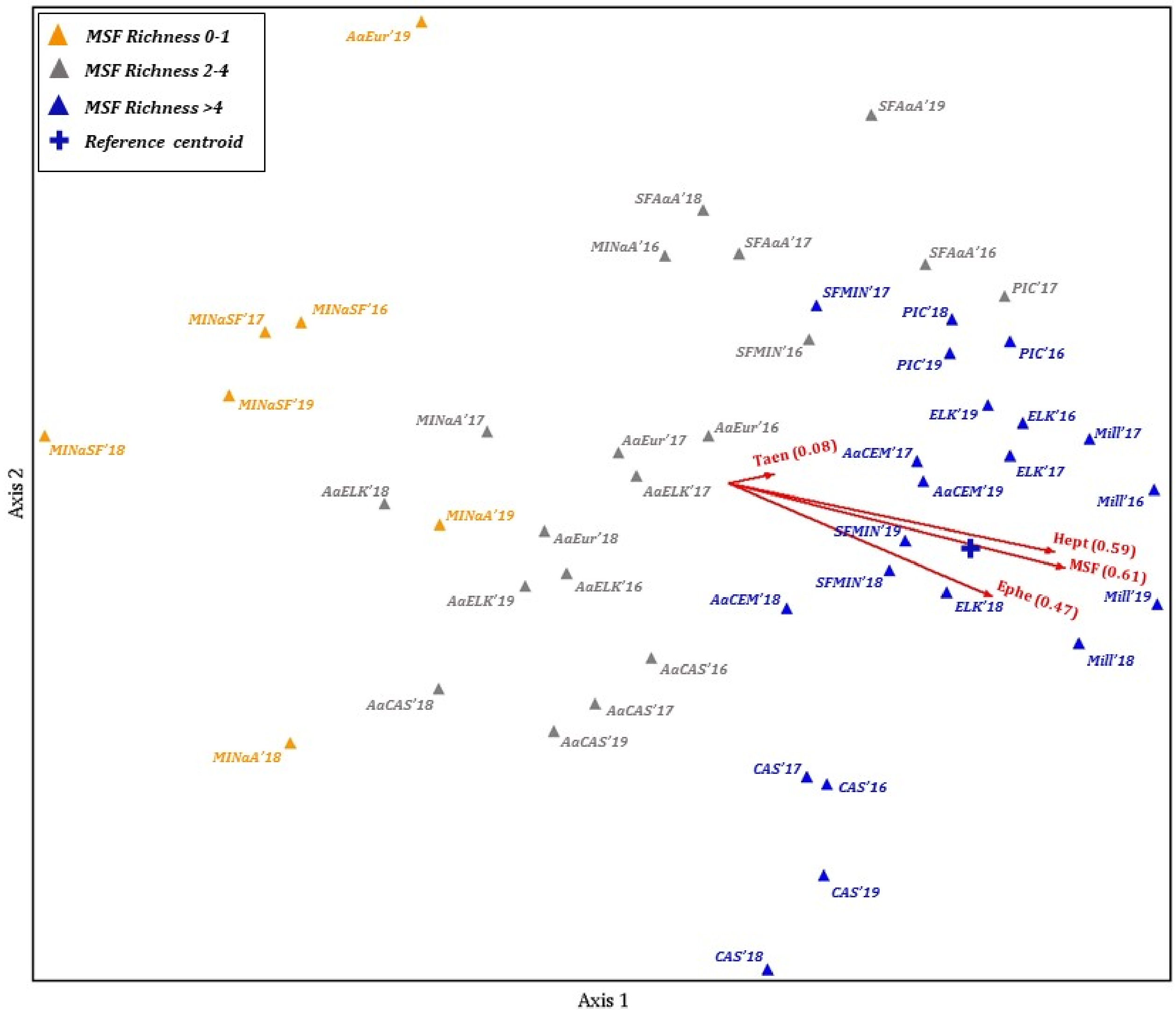


Figure 27: NMS ordination of ordination group 1 (see table 6) with orthogonal vector lines of richness of metal sensitive families. The angle and length of the lines reflect the direction and strength of the relationship between the variable and ordination axes. Taen=richness of Taeniopterygidae; Hept=richness of Heptageniidae; MSF=richness of all three metal sensitive families combined; Ephe=richness of Ephemerellidae. Number in parenthesis are r^2 correlations with Axis 1.

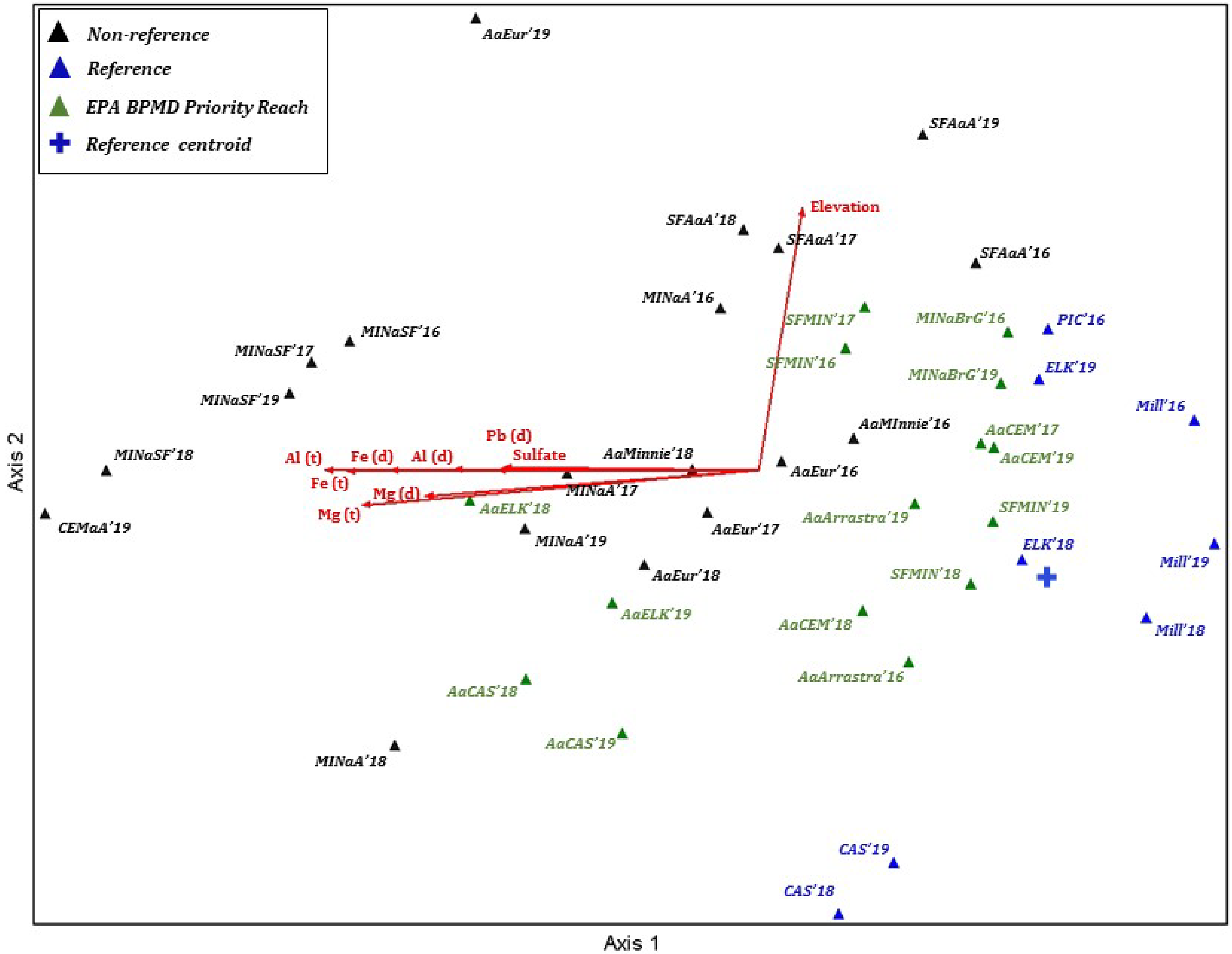


Figure 28: NMS Ordination of ordination group 2 (see table 6) with orthogonal vector lines for elevation and metal and mineral concentrations in surface water (r^2 correlations greater than 0.4). The angle and length of the lines reflect the direction and strength of the relationship between the variable and ordination axes. This ordination is based on the subset of sites where corresponding surface water data was available. Samples grouped by reference, non-reference, and EPA BPMD priority reaches. Al=aluminum; Fe=iron; Mg=magnesium; Pb=lead; (t)=total; (d)=dissolved

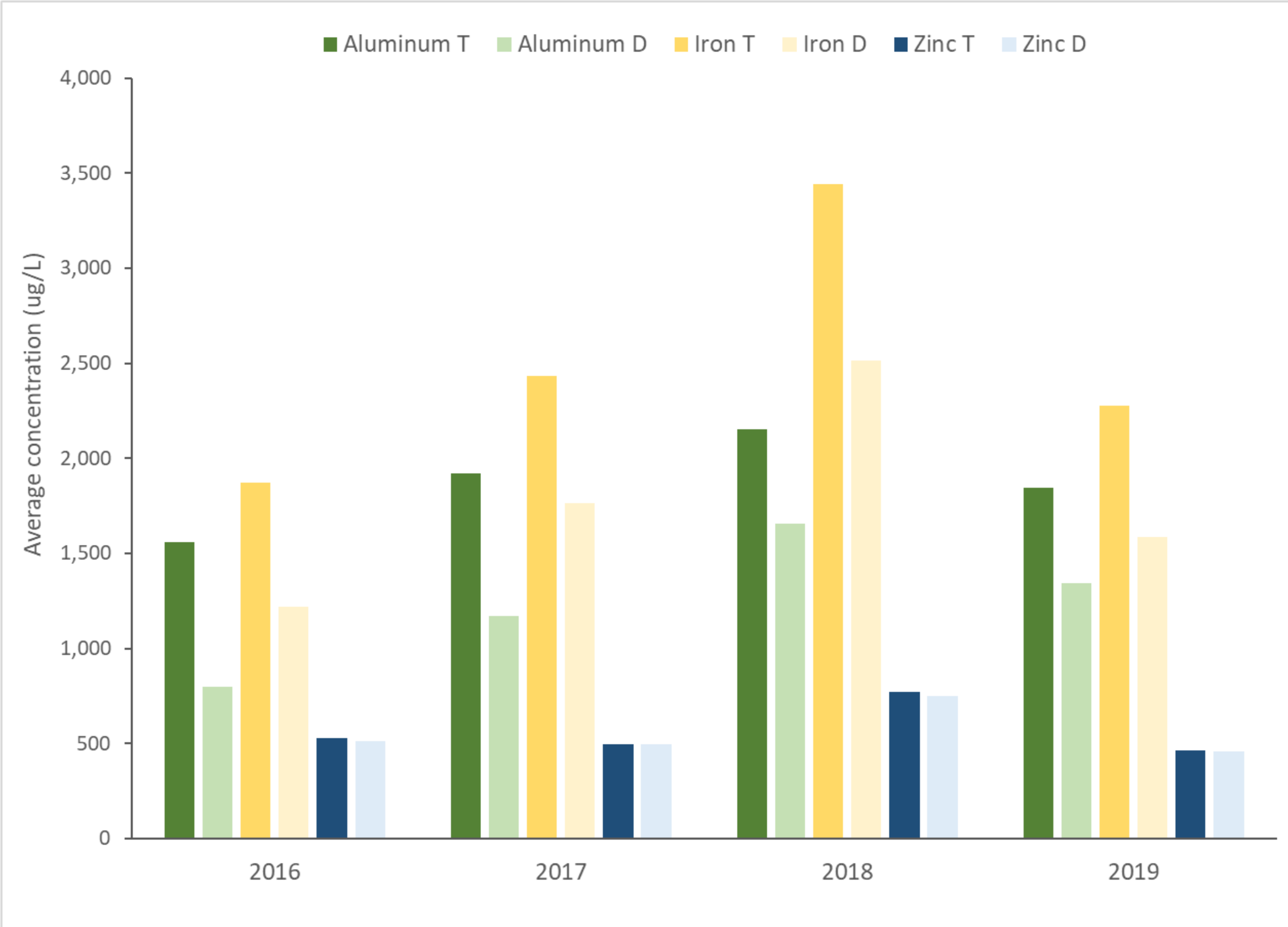


Figure 29: Average surface water concentration for select metals by year across ten sampling locations.

Note: Averages were calculated for ten surface water sampling locations where surface water analytical results were available for all four years, and were locations where benthic samples have been collected: AaEur, SFAaA, AaMinnie, AaArrastra, AaCEM, CEMaA, MINaBrG, MINaSF, SFMIN, and MINaA (see table 1 for site codes).

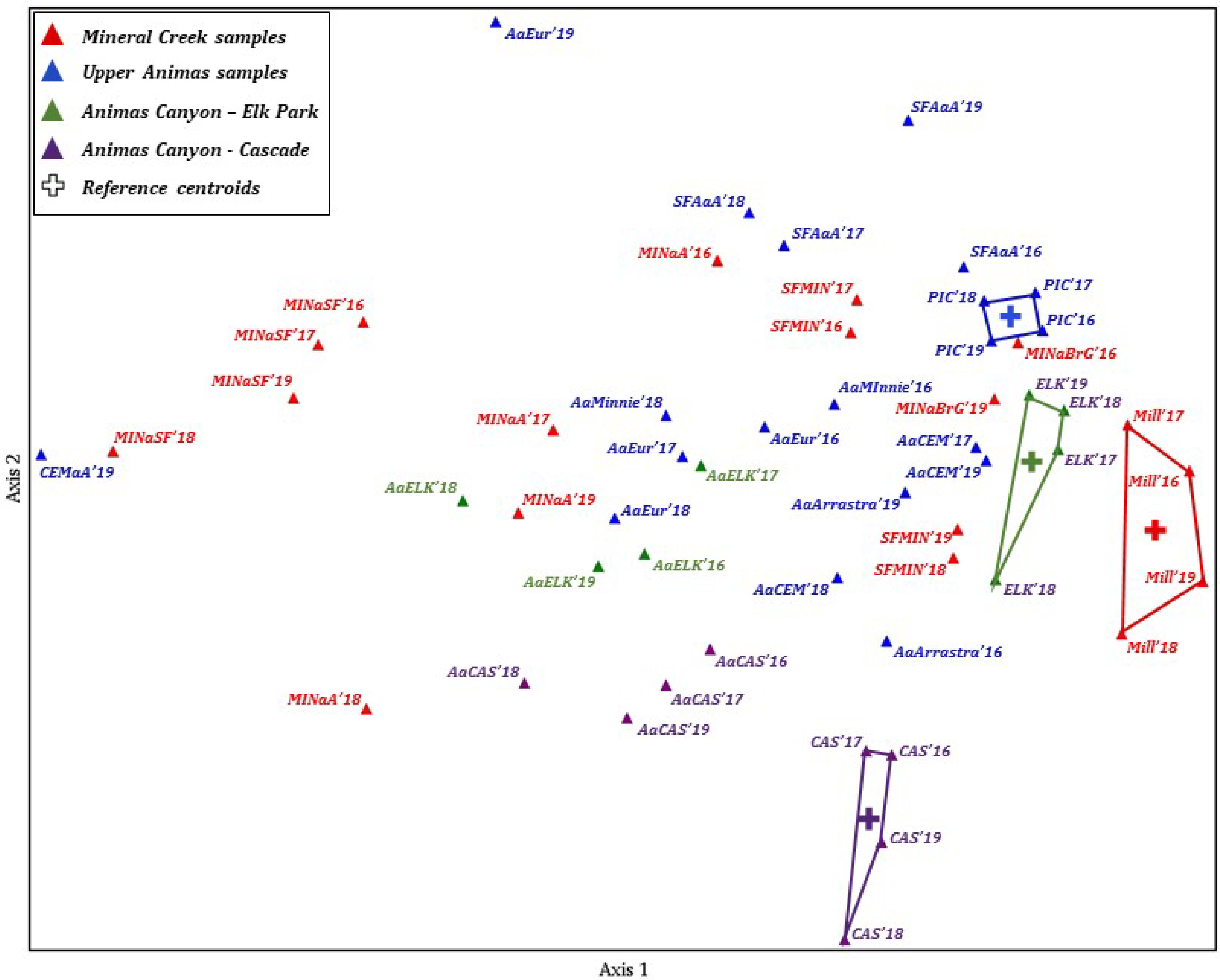


Figure 30: NMS ordination of ordination group 3 (see table 6) by watershed.

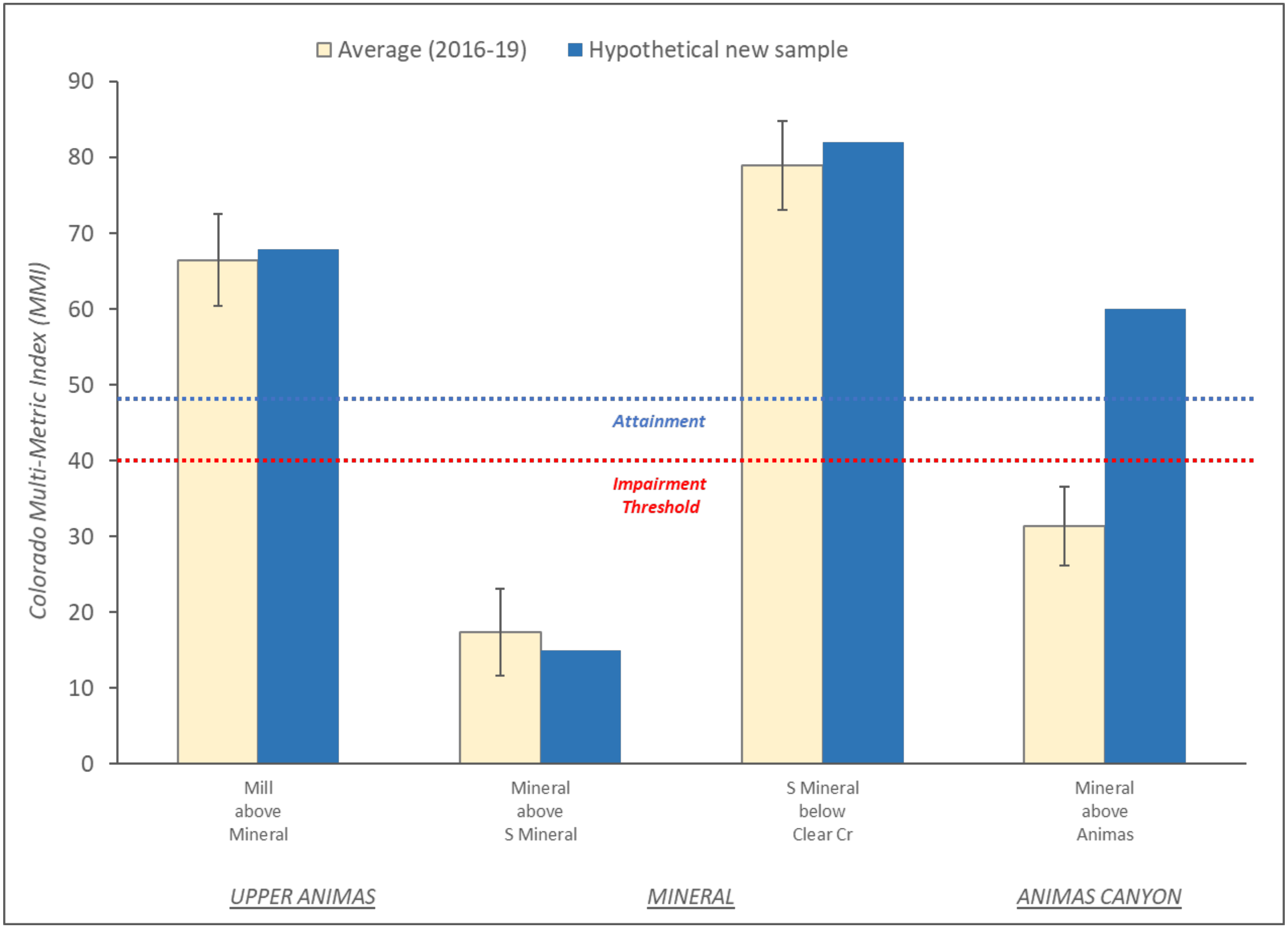
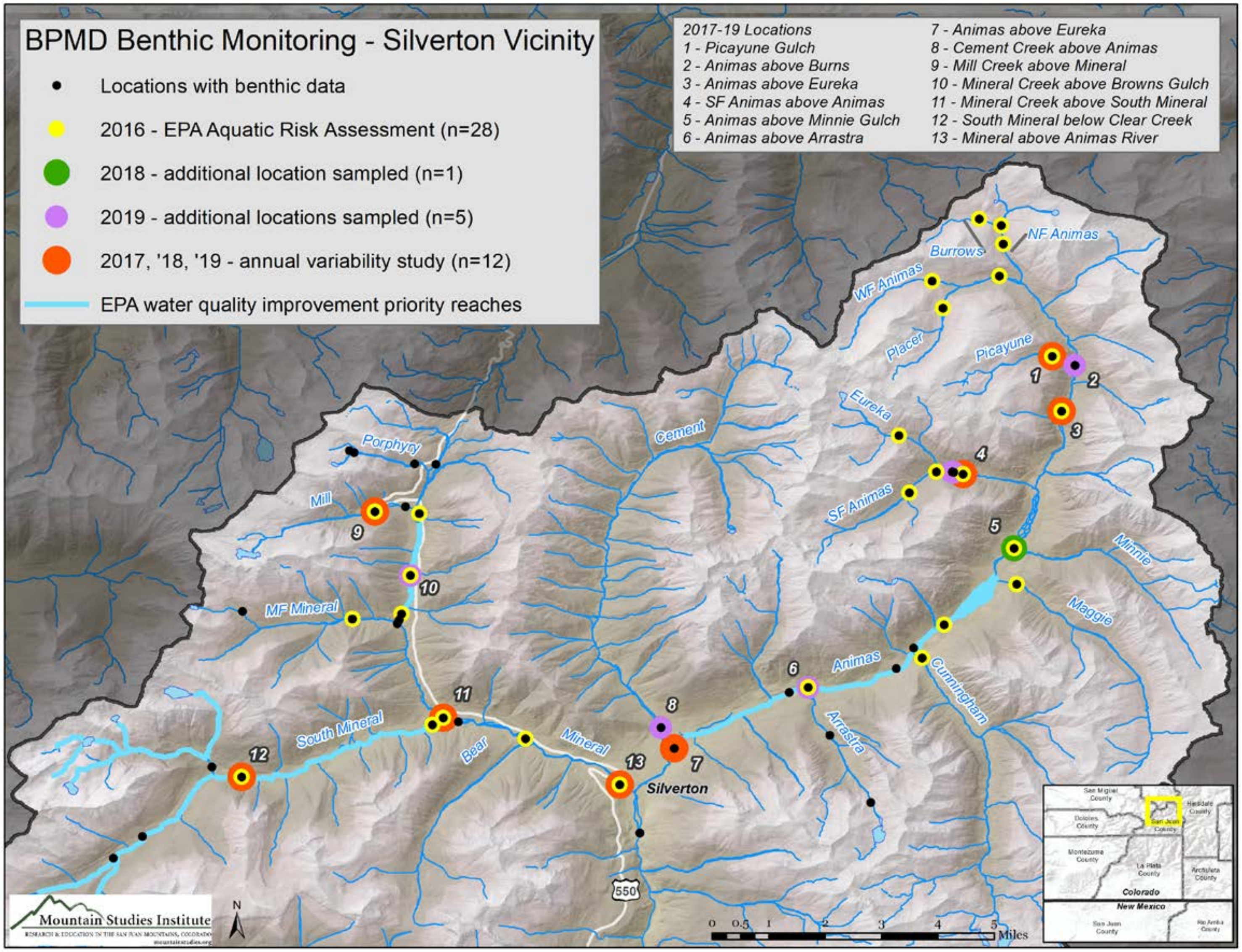
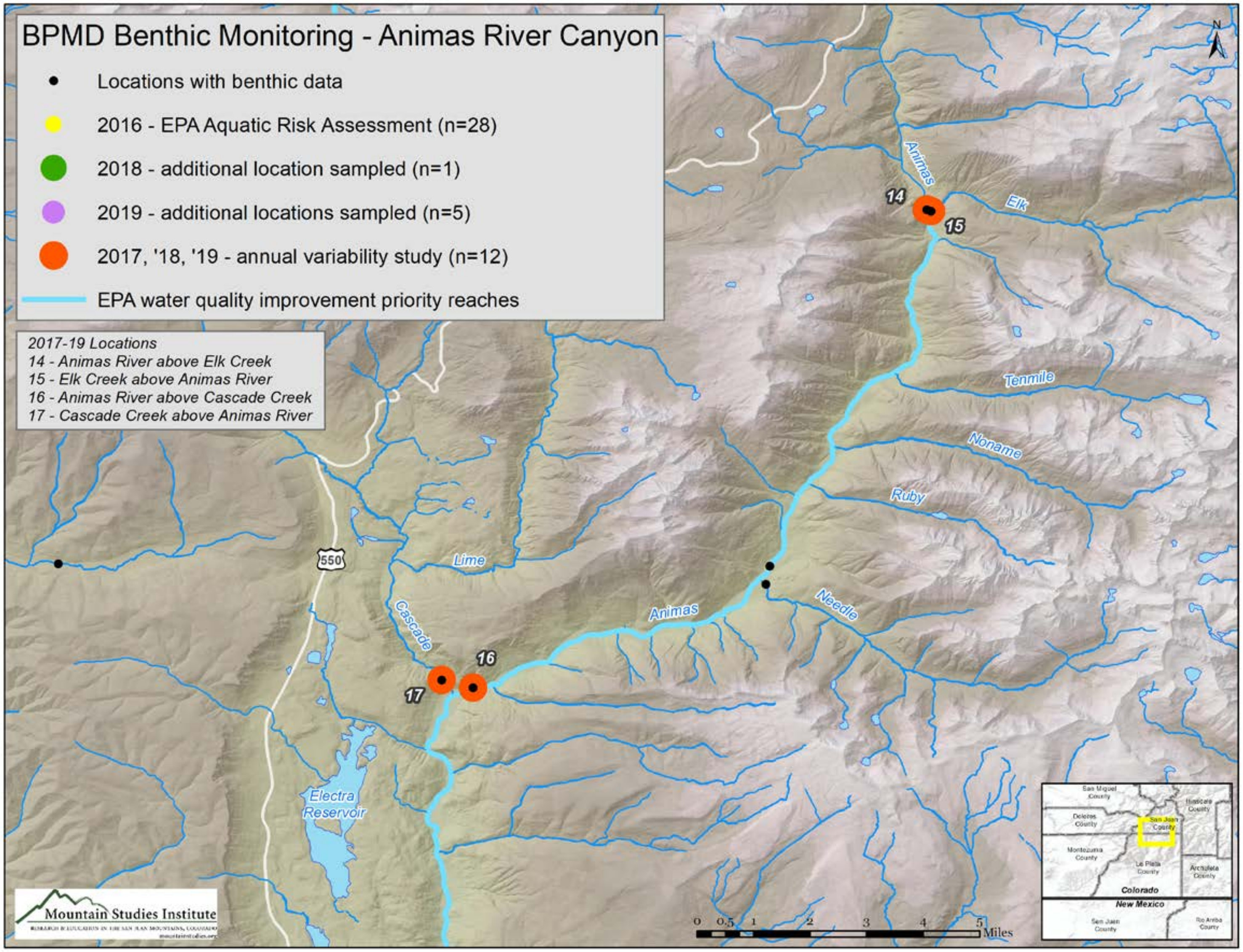


Figure 31: Hypothetical MMI score from a future sample; an example of incorporating natural annual variability into trend assessment.

8. Maps



Map 1: BPMD Benthic Monitoring Locations in Silverton Vicinity.



Map 2: BPMD Benthic Monitoring Locations in the Animas River Canyon.