Mountain Studies Institute
SAN JUAN MOUNTAINS, COLORADO

Animas River
2017 Benthic Macroinvertebrate Assessment

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La Plata County
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Abbreviations and Acronyms

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 4)*
GKMR	Gold King Mine release
MHBI	Modified Hilsenhoff Biotic Index *(see table 4)*
MSF	Metal-Sensitive Families - Ephemerellidae, Heptageniidae, and Taeniopterygidae families *(see table 4)*
MMI	Multi-Metric Index *(see table 4)*
MSI	Mountain Studies Institute
SDI	Shannon-Weaver Diversity Index *(see table 4)*

Macroinvertebrate Latin names used in this report

Arctopsyche	Genus - net-spinner caddis
Baetis	Genus - blue winged olive mayflies
Brachycentrus	Genus - American grannom / log cabin cade caddis
Chironomidae	Family - midges
Drunella	Genus – Ephemerellidae mayfly
Ephemerella	Genus - pale morning dun (PMD) mayflies
Ephemerellidae	Family – spiny crawler mayflies
Ephemeroptera	Order - mayflies
Heptageniidae	Family – flathead mayflies
Hydropsyche	Genus - net-spinner caddis
Plecoptera	Order - stoneflies
Taeniopterygidae	Family – winter stoneflies
Taenionema	Genus – willowfly stonefly
Trichoptera	Order - caddisflies

1. Executive Summary

The benthic macroinvertebrate community (BMI) of the Animas River is a valuable indicator of water quality and the overall health of aquatic life in the river. Over the past twenty years, Animas River benthic community monitoring has occurred infrequently and at irregular intervals, making it difficult to evaluate long-term trends in BMI community health. We expanded on recent sampling efforts to establish a robust baseline condition that will enable future monitoring efforts to more effectively track changes in Animas River benthic communities. We have included several key monitoring program components: three years of consecutive BMI data (2014-2016) from several Animas River sites; relatively undisturbed reference sites; and an estimation of the natural inter-annual variability of benthic communities in the Animas River watershed. Incorporating these components into our proposed monitoring strategy will allow for greater interpretation of Animas River BMI samples collected in the future. Specifically, we will be able to more conclusively determine whether observed changes in benthic community health are due to region-wide fluctuations such as climate, or whether they can be directly attributed to anthropogenic actions, such as mine remediation or land use change.

There are a number of potential threats to Animas River benthic communities including elevated metal concentrations, excessive nutrients, contaminants, and low flows and warm temperatures during drought years. Colorado Department of Public Health and the Environment (CDPHE) developed a Multi-Metric Index (MMI) to assess the extent to which benthic 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). The MMI was previously applied on the Animas River and results indicated that several sites from Silverton to Durango were in impairment of their designated aquatic life use (Roberts 2016). In 2017, CDPHE refined the MMI to improve model performance. We used the newly refined version of MMI to assess aquatic life use attainment for benthic samples collected from 2014-2016. Results from the newly refined version of MMI indicate much less impairment among Animas River benthic communities than was indicated by the prior version. Four Animas River sites that previously had at least one 2014-2016 sample indicating impairment had no samples indicating impairment according to the new version of MMI. Results do raise concern for the health of benthic communities at several sites: the Animas River above Elk Creek was impaired in all three years from 2014-2016; samples collected from the Animas River above Cascade Creek in 2014 and from the Animas River at 32nd Street in 2015 suggest impairment; and samples from the Animas River above Lightner Creek and the Animas River at Purple Cliffs had MMI scores that were very close to impairment.

Much attention has focused on potential impacts to Animas River aquatic life from the 2015 Gold King Mine release (GKMR). Researchers found no initial impacts to Animas River aquatic life in the immediate months following the release (Roberts 2016; White 2016). However, there were persisting concerns about whether sub-lethal impacts had occurred that could affect subsequent generations of insects. We compared fall 2014 benthic communities, collected the year prior to the GKMR, to fall 2016 benthic communities, collected one full year after the event when most insects would have completed at least one life cycle since exposure to the release. We found that BMI data from before and after the GKMR do not demonstrate a negative impact to BMI survival, diversity, abundance, or community health indices. These findings should be interpreted within the historical context of metal contamination within the Animas watershed. BMI communities in the Animas River have long been stressed by long-term exposure to mine-related impacts and other stressors (Anderson 2007b; EPA 2015; Smith 1976). BMI species that are most sensitive to metal contamination are either absent or occur at a low diversity in large portions of the Animas River, which reduces the ability to detect an impact from the GKMR. It is possible that we could have seen a greater impact to aquatic life from the GKMR in parts of the Animas River if the resident aquatic life had not already been impacted by long-term exposure to metals. Our finding that Animas River BMI community composition and structure does not appear to have changed one year after the release suggests that the GKMR did not cause substantial sub-lethal impacts, such as reduced reproduction success.

We compiled and assessed trends in Animas River benthic data collected over the past 20 years, focusing our evaluation on two questions: 1) Are recent observations of BMI communities (2014-2017) significantly different from historical observations (1997-2010)?; 2) Have there been any detectable downstream changes in BMI communities following the 2003-2004 closure of the mine wastewater treatment plant operated on Cement Creek?

We found that reach-wide BMI metrics in recent years (2014-17) were consistent with historical observations (1997-2010). One exception is that benthic community diversity has declined in recent years at a site on the Animas River above Elk Creek. A number of mine remediation activities have occurred in the Upper Animas watershed including a water treatment plant that was operated from 1978-2004 to remove metals from water draining the American Tunnel and at times, all of Cement Creek (ARSG 2017). Following the closure of the treatment plant, there was a potentially related decline in the water quality of Cement Creek and trout abundance in the Animas River canyon downstream of Silverton (ARSG 2017; White 2010; White 2016). We evaluated whether any downstream changes in Animas River benthic communities occurred following the closure of the treatment plant by comparing benthic samples collected from 1997-2004 to samples collected from 2005-2015. We found evidence that benthic community health may have declined at sites in closest downstream proximity to the treatment plant. The effect was most pronounced at the Animas River above Elk Creek, where there was a substantial decrease in benthic community diversity following the closure of the treatment plant. Given the elevated metal concentrations that occur in the Animas River above Elk Creek (EPA 2015), insects known to be especially sensitive to elevated metals are of particular interest at this site. We found that insects in the metal-sensitive family Ephemerellidae were present in the Animas River above Elk Creek while the treatment plant was in operation, but disappeared from all samples collected after the treatment plant was closed. Roberts (2017) reported that Ephemerellidae may be the most sensitive of the metal-sensitive families and are often absent from sites with levels of metals that are still tolerated by Heptageniidae and Taeniopterygidae. Monitoring the status of Ephemerellidae at the Animas River above Elk Creek could serve as a useful indicator of the downstream effectiveness of continued mine remediation in the Bonita Peak Mining District.

2. Introduction

The benthic macroinvertebrate community (BMI) of the Animas River is a valuable indicator of water quality and the overall health of aquatic life in the river. There is some concern that the BMI community in the Animas River may be exposed to unfavorable conditions such as elevated metal concentrations, contaminants, excessive nutrients, and low flows and warm temperatures during drought years. Over the years, anglers have anecdotally reported shifts in the composition and abundance of larvae and adult BMIs in the Animas River. Monitoring of Animas River BMI communities has occurred infrequently and at irregular intervals over the past couple of decades, making it difficult to evaluate long-term trends in BMI community health. Most of the monitoring and research has focused on the impacts to BMI communities from the legacy of historic mining in the upper portion of the Animas watershed (e.g., Anderson 2000; Anderson 2007a; Courtney and Clements 2002; etc.). Anderson (2010) documented a decline in benthic community health from 2004 to 2010 in Cement Creek and at a site in the Animas River immediately below Silverton. The Environmental Protection Agency (EPA 2015) evaluated the potential risks to the environment from metal contamination of water and sediments in the Animas watershed and concluded that BMI communities were impaired in the Animas River from Silverton to Baker's Bridge. Researchers have documented impacted BMI communities downstream of Baker's Bridge as well. Anderson (2007b) found a decline in BMI diversity during 2003 and 2005 in the Animas River from Baker's Bridge to Durango and attributed the decline to multiple stressors including metal concentrations, habitat degradation, sediment, and excessive nutrients. At sites along the Durango reach of the Animas River, Roberts (2015) documented an increase from previous years in the relative abundance of the caddisfly, *Brachycentrus*, which appeared to be closely tied to the prevalence of an aquatic moss (*Fontinalis*) (Glime 2015). Results from BMI samples collected in 2014 and 2015 indicated that several sites along the Animas River downstream of Baker's Bridge may not be in attainment of an aquatic life use designation (Roberts 2016). In August 2015, a large volume of impounded metal contaminated water was released into the Animas River from the Gold King Mine near Silverton, Colorado. Although researchers found no evidence of initial impacts to aquatic life in the Animas River from the Gold King Mine release (GKMR) (MSI 2016; White 2016), members of the community continue to express concern about potential sub-lethal or longer-term impacts to BMIs.

Through a partnership with Trout Unlimited, Southwest Water Conservation District, La Plata County, the City of Durango, and Colorado Parks and Wildlife, we designed a study to address these concerns through the following objectives:

1) Expand upon recent monitoring initiated by Trout Unlimited (2014) and the EPA (2014-15) by adding a 3rd year of data collection so that three consecutive years of BMI data (2014-2016) from several Animas River sites can serve as a robust baseline condition.

2) Develop a monitoring strategy that incorporates the annual variability of Animas River benthic communities, enabling future monitoring efforts to more effectively track changes in benthic communities.

3) Assess whether the GKMR caused any measurable impacts to benthic communities in the Animas River one year after the release.

4) Evaluate aquatic life use attainment of Animas River benthic communities using the recently updated Colorado Multi-Metric Index (MMI).

5) Analyze temporal trends in Animas River benthic communities over the past twenty years.

3. Methods

3.1 Monitoring Locations

We collected BMI community composition data from nine sites along a roughly 40-mile stretch of the Animas River from Elk Park to Durango (Table 1 and Map 1). Seven sites were located on the Animas River. Two sites were located on tributaries (Elk Cr and Cascade Cr), which will serve as relatively undisturbed reference locations. Including reference sites in monitoring programs allows us to differentiate changes in BMI communities specific to the Animas River from region-wide fluctuations due to larger scale influences, such as climate.

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 2016). 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.

At each site we collected five samples at equal intervals along a 100-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 five samples into a single sample container representing 0.575 m^2 of habitat at each site.

We consistently used the same sampling methodology in 2014-16 across all sites.

3.2.2 Physical Habitat Evaluation

In 2016, we evaluated physical habitat along ten cross-sectional transects equidistantly located within the 100-meter reach delineated for BMI community sampling. At five points along each of the ten 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 50 substrates at each site. We estimated embeddedness as the percent surface area of cobble sized substrate (i.e., substrate with intermediate axis longer than 64 mm in length) that was covered by fine sediment or biofilm mats.

At each site we photographed the perspective at the bottom (0m) of the reach. 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 collected in 2014 were identified by Timberline Aquatics in Fort Collins, Colorado (www.timberlineaquatics.com). Samples collected in 2015 and 2016 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.

3.4 Data Analysis

3.4.1 Data Preparation

BMI samples have been collected at irregular intervals over the past couple of decades from each of the nine sites included in this report (Tables 2 and 3). Historical datasets that have been identified by different taxonomists are often problematic to analyze. For example, different taxonomists may identify organisms to varying taxonomic levels (e.g., order, family, sub-family, genus), which can result in ambiguous taxonomic identifications. For this study, we implemented a method of resolving ambiguous taxonomic identifications described by Cuffney and others (2007). For example, if some specimens were identified to species (e.g., *Eukiefferiella brehmi*), but other specimens were only identified to genus (e.g., *Eukiefferiella*), we used the coarser level of identification (e.g., genus instead of species) so that samples were comparable.

To address the inconsistent taxonomic-level identifications found in the historical data, we prepared two separate data sets for analysis. The first data set includes samples collected from 2014 to 2017, which were identified to a higher-resolution taxonomic level. Analyzing these samples separately from the historical data is advantageous for several reasons: 1) the higher-resolution Chironomidae identifications are required for MMI assessment; 2) the higher-resolution identifications allow for a more detailed assessment of community composition; and 3) Samples from 2014-2017 were collected using a protocol that targets a consistent amount of habitat area (approximately 0.575 m^2) across all sites, which makes it possible to calculate BMI abundance. The second data set includes all samples from 1997 to 2017. To ensure samples were comparable across years, it was necessary to use coarser level identifications to match the historical data. Although using coarser-level identifications allows for analysis of trends across a nearly twenty-year period, it does prevent the use of this data for legitimate assessment using the MMI.

3.4.2 Standardizing Sample Size

To eliminate potential bias from differing sample sizes, we employed an algorithm to subsample all samples to a fixed count of 300 individuals. All metrics discussed in this report are based on the 300 count subsampled data.

3.4.3 Ecological Data Application System (EDAS)

We utilized the Ecological Data Application System (EDAS) developed by CDPHE to calculate the MMI.

3.4.4 BMI Metrics

Several metrics have been developed to assess the composition and health of BMI communities (Table 4). These relatively independent metrics provide multiple lines of evidence pertaining to the overall water quality and habitat conditions of an aquatic system.

3.4.5 Statistical Analysis

Using JMP statistical software (JMP 2013), we conducted paired t-tests to determine whether changes in BMI metrics were consistent across all Animas River sites. To evaluate the relationship between BMI metrics and measures of drought, we calculated Pearson correlation coefficients.

4. Results

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

4.1 Physical Habitat

The most notable differences in physical habitat among sites were related to vegetative cover, embeddedness, and the proportion of substrate in the fines, pebble, and boulder size classes (Table 5). We found that the Durango stretch of the Animas River had the highest vegetative cover of the surveyed sites. Interestingly, while A32nd and APC had high vegetative cover, AaLC, which is located between A32nd and APC, had much lower vegetative cover. CASaA also had abundant algae, but had no moss. There was a gradient of embeddedness across sites. ELKaA, CASaA, and AJR had very little embeddedness. AaElk had very high embeddedness associated with metal precipitates and biofilm mats. A32nd and APC also had relatively high embeddedness due to the accumulation of fines associated with the abundant moss that occurs at these sites. Boulder-sized substrate was most prevalent at the sites in the Animas River canyon (AaElk, AaCas, ELKaA, and CASaA) as well as at site A32nd. The abundant boulders within the A32nd reach are likely associated with Pleistocene glacial moraines (Johnson and Gillam 1995). We found that the tributary reference sites (ELKaA and CaC) had a higher proportion of pebble-sized substrate than Animas River sites.

4.2 BMI Community Composition

4.2.1 Spatial overview

Benthic community composition and structure differed among sites (Figures 1-18). EPT (Ephemeroptera, Plecoptera, and Trichoptera) richness, and MMI scores were generally highest at the two reference sites (ELKaA and CaC) (Figures 1 & 6). The benthic community at AaElk has been clearly impacted by elevated metal concentrations reflected by low richness of metal-sensitive families, and MMI scores that suggest impairment (Figures 1 & 7). Higher EPT richness and MMI scores at AaCas suggest that the health of the benthic community improved from AaElk to AaCas, and generally persisted at this level of health through ABB and AJR. Sites along the Durango stretch of the Animas River (A32nd, AaL, and APC) had lower EPT richness and lower MMI scores than sites immediately upstream (ABB, and AJR).

4.2.2 Gold King Mine release – potential impacts

Much attention has focused on potential impacts to Animas River aquatic life from the 2015 GKMR. Researchers found no initial impacts to Animas River aquatic life in the immediate months following the release (Roberts 2016; White 2016). However, there were persisting concerns about whether sub-lethal impacts had occurred that could affect subsequent generations of insects. If sub-lethal impacts did occur and were not detected during 2015 surveys, it is logical to expect that they should have been evident in 2016, when most insects would have completed at least one life cycle since the GKMR.

To assess the potential for sub-lethal impacts associated with the GKMR, we compared fall 2016 benthic communities, collected one full year after the GKMR, to fall 2014 benthic communities, collected the year prior to the release (Figures 1-9). We evaluated Animas River BMI communities that were exposed to the GKMR as well as BMI communities from reference tributaries that were not exposed to the release. The use of reference sites is particularly important to BMI studies because of the temporally dynamic nature of BMI populations. Examining changes in BMI community health at a single site could result in misleading conclusions. For example, from 2014 to 2016, taxa richness declined at AaLC (Figure 2). Since AaLC was exposed to the release, one may inappropriately conclude that the decline was solely attributable to the GKMR. However, taxa richness declined at a similar magnitude from 2014 to 2016 at Elk Creek, which was not exposed to the GKMR. Because this decline in taxa richness occurred at a site that was exposed to the release as well as a site that was not exposed, this decline cannot be solely attributed to the GKMR. There were several similar instances where changes in BMI community composition occurred from 2014 to 2016, but the changes were not attributable to the GKMR because analogous changes to BMI community composition also occurred at reference sites. There was no decline in the richness of BMI families known to be sensitive to elevated metals (Heptageniidae, Ephemerellidae, and Taeniopterygidae) between fall 2014 and fall 2016 at any site affected by the GKMR (Figure 7).

In order to determine whether any changes in BMI populations from fall 2014 to fall 2016 were consistent across all sites that were exposed to the GKMR, we used a statistical technique called a paired t-test. We found no statistically significant difference in any BMI metric between fall 2014 and fall 2016, indicating that the GKMR did not consistently alter BMI community health or structure at surveyed sites in the Animas River one year after the release. We present statistical test results comparing BMI metrics between 2014 and 2015, and between 2014 and 2016 in Table 6.

A note on spring samples:

It would be beneficial to assess potential impacts to BMI communities from the GKMR using observations from samples collected in the spring in addition to the observations from the fall samples discussed above. However, we only have one sample collected in the spring to represent the BMI community immediately prior to the GKMR. Furthermore, we have no concurrent spring samples from a reference site to serve as a comparison. The lack of data limits our ability to conclusively assess potential impacts to spring BMI communities from the GKMR. We will evaluate recent spring samples in context of historical data in Section 4.3.5.

4.2.3 Colorado 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 2017). In 2017, CDHPE developed a new version of the MMI model to improve indicators, refine stream classifications, and add new benthic samples collected from reference and test sites throughout Colorado (CDPHE 2017). It was anticipated that the new 2017 version of the MMI could produce different scores than the 2010 version of the MMI. The 2010 version of MMI was previously applied on the Animas River and results indicated that several sites from Silverton to Durango were in impairment of their designated aquatic life use (Roberts 2016).

We used the newly refined version of MMI to assess aquatic life use attainment for benthic samples collected from 2014-2016 (Figure 1), including many of the samples previously reported by Roberts (2016). We found some agreement between the 2010 and 2017 versions of MMI, but profound differences (Table 7). Results from both MMI versions suggested that AaElk was impaired. However, samples from other sites flipped from being considered impaired by the 2010 MMI version, to being considered in attainment by the 2017 MMI version. Most noteworthy, perhaps, is that five of the nine sites (ELKaA, ABB, AJR, AaLC, and APC) had at least one sample that indicating impairment according to the 2010 MMI version, but had no samples that indicated impairment according to the 2017 MMI version. Results do raise concern for the health of benthic communities at several sites: AaElk was impaired in all three years from 2014-2016; samples collected at AaCas in 2014 and at A32nd in 2015 suggest impairment; and samples from AaLC and APC had MMI scores that were very close to impairment. We did not assess attainment of spring samples as they were collected outside of the approved MMI sampling period (CDPHE 2017). We did not assess attainment of historical samples (1997-2010) due to incomplete Chironomidae taxonomic resolution.

4.2.4 Inter-annual variability

Benthic communities can vary substantially from year to year, even in undisturbed waterbodies (Scarsbrook et al. 2000). Without an understanding of the natural inter-annual variability of benthic populations, it is difficult to conclusively determine whether observed changes in benthic community health can be directly attributed to anthropogenic actions such as mine remediation or land use change (Anderson 2007a; Chapman 1999; Mazor et al. 2009; Resh et al. 2013). One of the objectives of this study was to estimate inter-annual variability of Animas River benthic communities so that future monitoring efforts will be able to more effectively track changes in benthic communities. We evaluated the variability of BMI metrics from samples collected in three sequential years (fall, 2014-2016) from seven sites on the mainstem of the Animas River. We also evaluated the variability of BMI metrics from two Animas River tributaries where human disturbance is relatively absent. In Table 7, we include several measures of variability: range; coefficient of variation; relative percent change; and standard errors. We found that Shannon Diversity Index (SDI), Metal Sensitive Family (MSF) richness, EPT richness, and MMI had lower inter-annual variability than total taxa richness, Modified Hilsenhoff Biotic Index (MHBI), or MSF relative abundance. We propose the following procedure when evaluating results from future Animas River BMI monitoring (based on previous work by Anderson (2007a)). Here, we use EPT richness as an example, but this procedure applies to any BMI metric.

1. Plot the average EPT richness observed in samples from fall 2014-2016.

2. Add error bars that reflect two standard errors of the fall 2014-2016 data above and below the plotted average. This represents inter-annual variability.

3. Plot taxa richness from the new sample.

4. Assessment (see hypothetical example in Figure 19, demonstrating a scenario in which an increase in EPT richness at AaElk is beyond the natural variability documented from 2014-2016).

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

    b. Are the new samples from reference sites within reference site error bars?
        i. If no, then the change likely occurred watershed-wide;
        ii. If yes, then it is possible that the change could be the result of anthropogenic factors occurring locally at the site or further upstream.

4.2.5 Historical data – trends in BMI data from 1997-2017

We assessed whether there have been any shifts in Animas River BMI community composition or structure in the past twenty years by focusing on the following questions:

- Are recent observations of BMI communities (2014-2017) significantly different from historical observations (1997-2010)?

- Have there been any detectable downstream changes in BMI communities following the 2004 closure of the mine wastewater treatment plant operated at Gladstone along Cement Creek?

- Are trends in Animas River benthic communities related to drought, snowpack, or Animas River water levels?

There has been substantial year-to-year variability of BMI metrics (Figures 20--33). As such, it is helpful to examine BMI trends over time in context of this variability, so for each subset of years of interest (e.g., recent years, 2014-2016), we plotted average BMI metrics with error bars that represent the year-to-year variability that occurred during the corresponding subset of years (Figures 34-61).

Recent years (2014-2017) compared to historical (1997-2010)

We found that reach-wide BMI metrics in recent years (2014-2017) were not significantly different from historical observations (1997-2010) (Table 9). However, we found evidence of some changes in BMI community composition at a handful of sites. At site AaElk, benthic community health appears to have declined in recent years. MMI, SDI, EPT richness, and MSF richness all were substantially lower at AaElk in recent years as compared to historical data (Figures 34, 36, 39, & 40). Other sites exhibited signs of improved benthic community health in recent years; ABB had greater BMI diversity and at AaLC, there were more taxa known to be sensitive to metal contamination (MSF richness). Data from samples collected in the fall from A32nd suggest overall benthic community health may have declined, reflected by lower MMI scores, but the number of metal-sensitive taxa increased in the same time period. Observations of spring benthic communities at AJR and A32nd in recent years (2015-2017) were consistent with historical observations from the spring (1997-2007) (Figures 41-47).

Gladstone treatment plant operation (1997-2004) compared to post-operation (2005-2015)

A number of mine remediation activities occurred in the Upper Animas watershed during the 1990s and early 2000s, including the installation of bulkheads and the operation of a mine wastewater treatment plant. The treatment plant was operated from 1978 to 2004 to remove metals from water draining the American Tunnel and at times, all of Cement Creek (ARSG 2017). In the fall of 2014, there was an increase in the volume of water draining the Red and Bonita and perhaps other mines in the Gladstone area (ARSG 2017). Following the closure of the treatment plant, there was a potentially related decline in water quality in Cement Creek (ARSG 2017). Concurrently, diversity of the benthic community in Cement Creek and an Animas River site immediately downstream of Silverton decreased (Anderson 2010) and further downstream in the Animas River canyon, the density of brook trout and rainbow trout also declined (White 2010; White 2016).

We evaluated whether any downstream changes in Animas River benthic communities occurred following the closure of the treatment plant by comparing benthic samples collected from 1997-2004 to samples collected from 2005-2015 (Figures 48-61). To eliminate any influence from the new water treatment plant that began operating at Gladstone in late 2015, we did not include data from fall 2016. We used the statistical technique, paired t-test, to determine whether any reach-wide (AaElk to APC) changes in BMI communities occurred consistently at all sites downstream of the treatment plant. Although most reach-wide metrics were not significantly different during plant operation (fall 1997-2004) and after plant operation (fall 2005-2015), we found that MMI was consistently lower at all sites after the treatment plant closed. At reference sites ELKaA and CASaA, which would have been unaffected by the operation of the treatment plant, MMI scores increased, suggesting that the observed reduction in MMI at Animas River sites was likely due to an upstream event rather than a watershed-wide phenomenon. Taxa richness was consistently lower at most sites after the treatment plant closed, but since taxa richness also decreased at reference site CASaA, the decrease observed at Animas River sites cannot be solely attributed to an upstream influence, such as the treatment plant.

Examining changes in BMI metrics at a site-by-site level revealed additional trends. We found evidence that benthic community health may have declined at sites in closest downstream proximity to the treatment plant: AaElk, AaCas, ABB, and AJR. There was a decrease in fall EPT richness at AaCas after the treatment plant closed, but all other metrics at this site did not decline substantially. A reduction of fall MMI and EPT richness at ABB suggests deteriorating BMI community health, but our confidence in interpreting trends from ABB is limited due to only having one sample from the period when the treatment plant was operational. We found reduced SDI and EPT richness in spring samples collected at AJR after the treatment plant closed, but we did not observe a similar decline in BMI metrics from samples collected in the fall. Multiple lines of evidence do conclusively suggest that a decline in benthic community health occurred at AaElk following the closure of the treatment plant (reductions in EPT richness, MMI, and SDI). Given the elevated metal concentrations that occur at AaElk (EPA 2015), insects known to be especially sensitive to elevated metals are of particular interest at this site. We found that after the closure of the treatment plant, there was a reduction in MSF richness (Figure 54) as well as an elimination of Ephemerellidae, which is a metal-sensitive family of mayflies. Ephemerellidae (genus *Drunella*) was observed at AaElk in 1996 (not included in this study, but documented by Anderson 2007a), 1997, and 2004, but has not been collected in samples from 2014, 2015, or 2016 (Figure 62). Caution should be used when assessing the presence or absence of a single taxon that occurs at low abundances. However, the absence of Ephemerellidae in every sample since 2004 is definitive. Roberts (2017) reported that Ephemerellidae may be the most sensitive of the metal-sensitive families and is often absent from sites with levels of metals that are still tolerated by Heptageniidae and Taeniopterygidae. Monitoring the status of Ephemerellidae at AaElk could serve as a useful indicator of the downstream effectiveness of continued mine remediation in the Bonita Peak Mining District.

Drought, snowpack, and river levels

We assessed whether trends in BMI metrics were related to measures of drought, snowpack, and river levels. For each year that a benthic sample was collected, we obtained the Palmer Drought Severity Index, the April 1st snow water equivalency as compared to average, and the Animas River baseflow and peak spring runoff as compared to average. Palmer Drought Severity Index and snow water equivalency represented basin-wide conditions. We obtained Animas River levels from the USGS gauge in Durango. Correlation coefficients suggest that BMI metrics are related to drought, snowpack, and river levels, but the relationship is not consistent across all sites (Tables 10 and 11). We found evidence that drought conditions may negatively affect Animas River benthic communities. For example, samples from years characterized by the Palmer Drought Severity Index as 'extreme drought' corresponded with lower benthic community diversity at AaCas, AJR, A32nd, and AaLC. In years with low baseflow, EPT and taxa richness were reduced at AaCas. Conversely, in years with lower than average April 1st snowpack, there was a higher BMI diversity at CASaA and AaLC. The volume of peak spring runoff did not strongly correlate with many BMI metrics. The conclusiveness of statistical results is highly dependent on sample size and the range of conditions represented. Currently, we only have one year of benthic data from an extreme drought year (2003), and one year of data from an extremely wet year (1997) (Figure 65). The patterns we present here are largely driven by these two years. A greater number of samples collected across a wider array of conditions (i.e., collected across a spectrum of drought and non-drought years) would allow for much stronger inference. This analysis should be revisited when additional years of BMI data have been obtained.

Other notable trends: Trout Food Availability

Most BMI metrics reflect the overall health of a community by incorporating measures of diversity and/or the proportion of the community made up of taxa known to be sensitive to contaminants. Rader (1997) developed a Trout Food Availability (TFA) index that represents the relative availability of benthic macroinvertebrates as food source for salmonids. Rather than being based on the sensitivity of taxa to contaminants, TFA is based on morphological, habit, and life cycle traits that are more likely to expose an individual to trout. Given the importance of the Animas River as a recreational fishery, we evaluated whether TFA at sites along the river have changed over time. Similar to other BMI metrics, we found no evidence that TFA was reduced in response to the GKMR (Table 6), but changes in TFA have occurred at several sites. Across the 1997-2017 time period, it appears that TFA increased at AaElk and AJR, and declined at AaLC (Figures 24 and 31). At AaElk the increase in TFA was due to a decrease in the abundance of the stonefly *Taenionema,* which are relatively unavailable to trout, and an increase in abundance of the caddisfly *Arctopsyche*, which are more available to trout (Rader 1997). The general increase in TFA at AJR in the fall and spring was attributable to an increase in the abundance of the mayfly *Baetis*, which is highly available to trout. Conversely, the decline in TFA at AaLC reflected a decrease in the abundance of *Baetis*. As evident by the relatively stable TFA at several Animas River sites (ABB, A32nd, APC), reach-wide changes in TFA do not appear to have occurred over the 1997-2017 time period.

Other notable trends: Ephemerella (pale morning dun)

There have been anecdotal reports from anglers in recent years that there may have been shifts in Animas River populations of the mayfly, *Ephemerella* (Pale Morning Dun). We found that *Ephemerella* mayflies were absent from most of the Animas River BMI samples collected in the fall of 1997-2016 (Figures 66 and 67). When present in fall samples, *Ephemerella* occurred at very low abundances, comprising less than 2% of the benthic community. Interestingly, *Ephemerella* only occurred in one fall sample across all seven Animas River sites from 1997-2015, but occurred at four of the seven sites in 2016. A similar trend occurred in samples collected in the spring; there was a greater abundance of *Epheremella* at A32nd during 2015-2017 than during 1997-2005 (Figure 68). These data suggest that *Ephemerella* in the Animas River may have increased in abundance in recent years.

Other notable trends: The Durango stretch of the Animas River

We found much greater year-to-year variability in BMI density at sites along the Durango stretch of the Animas River as compared to sites located further upstream (Figure 8). This may be related to the higher prevalence of aquatic moss found at these lower elevation sites. BMI density can be strongly tied to the amount of algae or moss that is available for use as habitat structure. The increase in BMI density that occurred at A32nd, AaLC, and APC in 2015 was largely driven by an increase in the relative abundance of Chironomidae midges, which are known to inhabit particularly thick moss (Glime 2015). *Brachycentrus* caddis flies also densely inhabit moss. During the 2014-2016 period at A32nd, the relative abundance of *Brachycentrus* decreased while the relative abundance of Chironomidae increased. It is possible that this shift could be driven by fluctuations in moss abundance, which in turn could be dependent on nutrient availability, water temperatures, and late summer water levels. Further monitoring that includes nutrient sampling and a method of tracking moss and algae abundance over time would be useful for understanding this phenomenon.

5. Conclusions, Research Recommendations, and Further Questions

Over the past twenty years, Animas River benthic community monitoring has occurred infrequently and at irregular intervals, making it difficult to evaluate long-term trends in BMI community health. We expanded on recent sampling efforts funded by EPA and TU to establish a robust baseline condition that will enable future monitoring efforts to more effectively track changes in Animas River benthic communities. We have included several key monitoring program components: three years of consecutive BMI data (2014-2016) from several Animas River sites; relatively undisturbed reference sites; and an estimation of the natural inter-annual variability of benthic communities in the Animas River watershed. These monitoring components will allow for greater interpretation of Animas River BMI samples collected in the future. Specifically, we will be able to more conclusively determine whether observed changes in benthic community health are due to region-wide fluctuations such as climate, or whether they can be directly attributed to anthropogenic actions, such as mine remediation or land use change. We developed a monitoring strategy that uses BMI data from fall 2014-2016 as a baseline condition and incorporates the inter-annual variability of benthic communities in the Animas River and reference tributaries.

There are a number of potential threats to Animas River benthic communities including elevated metal concentrations, contaminants, excessive nutrients, and low flows and warm temperatures during drought years. CDPHE developed the MMI to assess the extent to which benthic 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). The MMI was previously applied on the Animas River and results indicated that several sites from Silverton to Durango were in impairment of their designated aquatic life use (Roberts 2016). In 2017, CDPHE refined the MMI to improve model performance. We used the newly refined version of MMI to assess aquatic life use attainment for benthic samples collected from 2014-2016. Results from the newly refined version of MMI indicate much less impairment among Animas River benthic communities than was indicated by the prior version. Four Animas River sites (ABB, AJR, AaLC, and APC) that previously had at least one 2014-2016 sample indicating impairment had no samples indicating impairment according to the new version of MMI. Results do raise concern for the health of benthic communities at several sites: AaElk was impaired in all three years from 2014-2016; samples collected at AaCas in 2014 and at A32nd in 2015 suggest impairment; and samples from AaLC and APC had MMI scores that were very close to impairment.

Much attention has focused on potential impacts to Animas River aquatic life from the 2015 GKMR. Researchers found no initial impacts to Animas River aquatic life in the immediate months following the release (Roberts 2016; White 2016). However, there were persisting concerns about whether sub-lethal impacts had occurred that could affect subsequent generations of insects. We compared fall 2014 benthic communities, collected the year prior to the GKMR, to fall 2016 benthic communities, collected one full year after the event when most insects would have completed at least one life cycle since exposure to the release. We found that BMI data from before and after the GKMR do not demonstrate a negative impact to BMI survival, diversity, abundance, or community health indices. These findings should be interpreted within the historical context of metal contamination within the Animas watershed. BMI communities in the Animas River have long been stressed by long-term exposure to mine-related impacts and other stressors (Anderson 2007b; EPA 2015; Smith 1976). BMI species that are most sensitive to metal contamination are either absent or occur at a low diversity in large portions of the Animas River, which reduces the ability to detect an impact from the GKMR. It is possible that we could have seen a greater impact to aquatic life from the GKMR in parts of the Animas River if the resident aquatic life had not already been impacted by long-term exposure to metals. Our finding that Animas River BMI community composition and structure does not appear to have changed one year after the release suggests that the GKMR did not cause substantial sub-lethal impacts, such as reduced reproduction success.

We compiled and assessed trends in Animas River benthic data collected over the past 20 years, focusing our evaluation on two questions: 1) Are recent observations of BMI communities (2014-2017) significantly different from historical observations (1997-2010)?; 2) Have there been any detectable downstream changes in BMI communities following the 2003-2004 closure of the mine wastewater treatment plant operated on Cement Creek?

We found that reach-wide BMI metrics in recent years (2014-17) were consistent with historical observations (1997-2010). One exception is that benthic community diversity has declined at AaElk in recent years. A number of mine remediation activities have occurred in the Upper Animas watershed including a water treatment plant that was operated from 1978-2004 to remove metals from water draining the American Tunnel and at times, all of Cement Creek (ARSG 2017). Following the closure of the treatment plant, there was a potentially related decline in the water quality of Cement Creek and trout abundance in the Animas River canyon downstream of Silverton (ARSG 2017; White 2010; White 2016). We evaluated whether any downstream changes in Animas River benthic communities occurred following the closure of the treatment plant by comparing benthic samples collected from 1997-2004 to samples collected from 2005-2015. We found evidence that benthic community health may have declined at sites in closest downstream proximity to the treatment plant. The effect was most pronounced at AaElk, where there was a substantial decrease in benthic community diversity following the closure of the treatment plant. Given the elevated metal concentrations that occur at AaElk (EPA 2015), insects known to be especially sensitive to elevated metals are of particular interest at this site. We found that mayflies in the metal-sensitive family Ephemerellidae were present at AaElk while the treatment plant was in operation, but disappeared from all samples collected after the treatment plant was closed. Roberts (2017) reported that Ephemerellidae may be the most sensitive of the metal-sensitive families and is often absent from sites with levels of metals that are still tolerated by Heptageniidae and Taeniopterygidae. Monitoring the status of Ephemerellidae at AaElk could serve as a useful indicator of the downstream effectiveness of continued mine remediation in the Bonita Peak Mining District.

This report is focused on seven Animas River sites and two reference tributary sites. Interpretation of long-term benthic community trends could be improved by incorporating additional BMI data. For further historical analysis, it would be useful to include data from Mineral Creek, Cement Creek, and sites A68 and A72 on the Animas River.

In Summary:

- This work establishes three consecutive years of BMI data (2014-2016) from several mainstem Animas River sites and reference tributaries to serve as a robust baseline condition.

- We propose a monitoring strategy that incorporates the inter-annual variability of Animas River benthic communities, enabling future monitoring efforts to more effectively track changes.

- We found no evidence of any change in Animas River BMI community composition and structure one year after the GKMR, indicating that the event does not appear to have caused sub-lethal impacts, such as reduced reproduction success.

- The newly refined MMI indicates much less impairment among Animas River benthic communities than was indicated by the prior version. However, AaElk was impaired in all three years assessed (2014-2016); samples collected at AaCas in 2014 and at A32nd in 2015 suggest impairment; and samples from AaLC and APC were very close to impairment.

- Recent observations of BMI communities (2014-2017) were consistent with historical observations (1997-2010) at most sites. One exception is that benthic community diversity has declined at AaElk in recent years.

- We attempted to evaluate the relationship between BMI metrics and measures of drought. Although we found evidence that drought conditions may negatively affect Animas River benthic communities, the conclusiveness of our findings was severely limited by our small sample size (a maximum of 4-7 years for each site). Of the years in which BMI samples have been collected, sampling only occurred during one "extreme drought" (2003) year and only one "extremely wet" (1997) year. Additional samples collected across a wider array of drought conditions would allow for much stronger inference. This analysis should be revisited when additional years of BMI data have been obtained.

- After the closure of the Gladstone water treatment plant in 2004, benthic community health may have declined at sites in closest downstream proximity to the plant. The effect was most pronounced at AaElk, where there was a substantial decrease in benthic community diversity. We found that insects in the metal-sensitive family Ephemerellidae were present at AaElk while the treatment plant was in operation, but disappeared from all samples collected after the treatment plant was closed. Monitoring the status of Ephemerellidae at AaElk could serve as a useful indicator of the downstream effectiveness of continued mine remediation in the Bonita Peak Mining District.

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7. 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. 2010. 2010 Benthic macroinvertebrate data analysis, for the Animas River Stakeholders Group.

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

Animas River Stakeholders Group (ARSG). 2017. Cement Creek Historical Timeline. http://animasriverstakeholdersgroup.org/blog/index.php/2015/10/23/gold-king-timeline/ Accessed 11/28/2017.

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). 2016. Benthic macroinvertebrate sampling - standard operating procedure. Water Quality Control Division.

CDPHE. 2017. Aquatic life use attainment, methodology to determine use attainment for rivers and streams. Policy Statement 10-1.

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.

Cuffney, T., M. Bilger, and A. Haigler. 2007. Ambiguous taxa: effects on the characterization and interpretation of invertebrate assemblages. Journal of the North American Benthological Society, 26(2):286-307.

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

Glime, J. 2015. Aquatic Insects: Bryophyte Habitats and Fauna. Chapt. 11-3. *In*: J. Glime, Bryophyte Ecology, Volume 2: Bryological Interaction.

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

Johnson, M., and M. Gillam. 1995. Composition and construction of late Pleistocene end moraines, Durango, Colorado. GSA Bulletin, 107(10): 1241-1253.

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.

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.

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

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 report, Gold King Mine release monitoring. Mountain Studies Institute.

Roberts, S. 2017. Bonita Peak Mining District, 2016 benthic macroinvertebrate assessment. Mountain Studies Institute.

Scarsbrook M, I. Boothroyd, and J. Quinn. 2000. New Zealand's national river water quality network: long-term trends in macroinvertebrate communities. New Zealand Journal of Marine and Freshwater Research, 34: 289–302.

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.

White, J. 2010. 2010 Animas River report. Colorado Parks and Wildlife (CPW), Durango, CO.

White, J. 2016. 2015 Fishery inventory report for the San Juan and Upper Dolores River Basins. Colorado Parks and Wildlife (CPW), Durango, CO.

8. Tables

Table 1. Monitoring sites, associated recent sampling date (2014-2017), and funding source.

Site ID	Stream Name	Site Name	Lat	Long	Dates Collected					
					Fall			Spring		
					2014	2015	2016	2015	2016	2017
Animas River										
AaElk	Animas River	Above Elk Creek	37.722367	-107.654795	10/16/14**	10/15/15***	10/20/16^			
AaCas	Animas River	Above Cascade Creek	37.597757	-107.775842	10/16/14**	10/15/15***	10/19/16^			
ABB	Animas River	Baker's Bridge	37.453774	-107.801439	9/26/14**	9/21/15***	9/27/16^			
AJR	Animas River	James Ranch	37.417822	-107.814819	9/26/14**	9/21/15***	9/30/16^		3/18/2016^	3/27/2017^
A32nd	Animas River	32nd Street	37.297274	-107.870280	9/26/14*	9/22/15***	9/27/16^	3/18/15^	3/18/2016^	3/27/2017^
AaLC	Animas River	Above Lightner Creek	37.268929	-107.886295	9/26/14*	9/20/15***	9/27/16^			
APC	Animas River	Purple Cliffs	37.221889	-107.862038	9/26/14*	9/22/15***	9/30/16^			
Reference Sites										
ELKaA	Elk Creek	Above Animas River	37.721954	-107.653666	10/16/14**	10/15/15***	10/20/16^			
CASaA	Cascade Creek	Above Animas River	37.598120	-107.776258	10/16/14**	10/15/15***	10/19/16^			

*Note: *collection and analysis funded by 2014 TU study; **collection and analysis funded by 2014 EPA Baseline Ecological Risk Assessment; ***collection and analysis funded by 2015 EPA Gold King response; ^collection and analysis funded by partnership between TU, SWCD, La Plata County, City of Durango, Colorado Parks and Wildlife, and MSI.*

Table 2. Fall sampling dates from 1997 to 2016.

ID	Stream Name	Site Name	Fall Dates Collected							
			1997	2003	2004	2005	2010	2014	2015	2016
Animas River										
AaElk	Animas River	Above Elk Creek	10/03/97	-	10/01/04	-		10/16/14	10/15/15	10/20/16
AaCas	Animas River	Above Cascade Creek	10/03/97		10/01/04		10/15/10	10/16/14	10/15/15	10/19/16
ABB	Animas River	Baker's Bridge	-	-	10/13/04	-	-	09/26/14	09/21/15	09/27/16
AJR	Animas River	James Ranch	09/19/97	09/15/03	10/01/04	10/10/05	-	09/26/14	09/21/15	09/30/16
A32nd	Animas River	32nd Street	09/19/97	09/15/03	10/01/04	11/04/05	-	09/26/14	09/22/15	09/27/16
AaLC	Animas River	Above Lightner Creek	09/16/97	09/15/03	10/01/04	11/04/05	-	09/26/14	09/20/15	09/27/16
APC	Animas River	Purple Cliffs	09/15/97	-	10/01/04	-	-	09/26/14	09/22/15	09/30/16
Reference Sites										
ELKaA	Elk Creek	Above Animas River	10/03/97	-	10/01/04	-	-	10/16/14	10/15/15	10/20/16
CASaA	Cascade Creek	Above Animas River	10/03/97	-	10/01/04	-	10/15/10	10/16/14	10/15/15	10/19/16

Note: Additional historical data were not included in this report. Data from 1992 were excluded as they only exist for one site. Data from 1996 were excluded because they were collected using a different style sampling net.

Table 3. Spring sampling dates from 1997 to 2017.

ID	Stream Name	Site Name	Spring Dates Collected								
			1997	2003	2004	2005	2006	2007	2015	2016	2017
Animas River											
AaElk	Animas River	Above Elk Creek	-	-	-	-	-	-	-	-	-
AaCas	Animas River	Above Cascade Creek	-	-	-	-	-	-	-	-	-
ABB	Animas River	Baker's Bridge	-	-	-	-	-	-	-	-	-
AJR	Animas River	James Ranch	4/17/1997	3/15/2003	3/15/2004	2/28/2005	-	-	-	3/18/2016	3/27/2017
A32nd	Animas River	32nd Street	-	3/15/2003	3/15/2004	2/28/2005	3/13/2006	2/3/2007	3/18/15	3/18/2016	3/27/2017
AaLC	Animas River	Above Lightner Creek	-	-	-	-	-	-	-	-	-
APC	Animas River	Purple Cliffs	-	-	-	-	-	-	-	-	-
Reference Sites											
ELKaA	Elk Creek	Above Animas River	-	-	-	-	-	-	-	-	-
CASaA	Cascade Creek	Above Animas River	-	-	-	-	-	-	-	-	-

Table 4. BMI metrics.

BMI Metric	Metric Description	Justification and Source
Metal Sensitive Families (MSF)	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 4 (cont.)

BMI Metric	Metric Description, Justification, and Source	
Functional Feeding Groups -	Proportion of BMI community composed of each group	Functional Feeding Groups include collector-filterers (cf), collector-gatherers (cg), omnivores (o), predators (p), scrapers (sc), and shredders (sh).
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 (SDI)	SDI is a measure of the diversity and evenness of a community (Shannon 1948).	
Trout Food Availability (TFA)	Rader (1997) developed an index representing the relative availability of benthic macroinvertebrates as food for salmonids based on several BMI traits: propensity to drift during aquatic or adult stage, flow exposure, mobility, drag, emergence behavior, ovipositing behavior, diel activity, size, and abundance.	

Table 5. Physical habitat results – substrate size, embeddedness, and vegetative cover.

Site			Substate Size (mm)						Substrate Class Size (%)						Embeddedness	Proportion of substrates with vegetative cover (%)	
ID	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
Animas River																	
AaElk	Animas River	Above Elk Creek	169.12	119.35	90	165	237.5	310	12.00	0.00	0.00	8.00	56.00	24.00	37.50	0.00	0.00
AaCas	Animas River	Above Cascade	253.80	170.56	113	225	357.5	478	0.00	0.00	0.00	6.00	46.00	48.00	6.74	4.00	0.00
ABB	Animas River	Bakers Bridge	133.70	55.12	96.3	120	160	220	0.00	0.00	0.00	6.00	92.00	2.00	5.32	6.00	0.00
AJR	Animas River	James Ranch	137.58	45.41	90	110	135	166	0.00	0.00	0.00	2.00	94.00	4.00	2.70	4.00	0.00
A32nd	Animas River	32nd St	254.71	229.42	115	230	345	441	8.00	0.00	0.00	4.00	42.00	46.00	21.43	28.00	74.00
AaLC	Animas River	Above Lightner	153.21	126.12	70	125	216.3	273	10.00	0.00	4.00	8.00	62.00	16.00	6.04	6.00	6.00
APC	Animas River	Purple Cliffs	147.90	53.66	100	140	210	286	0.00	0.00	0.00	4.00	96.00	0.00	16.15	48.00	98.00
Reference Sites																	
ELKaA	Elk Creek	Above Animas River	200.80	135.75	90	195	282.5	410	0.00	0.00	0.00	14.00	56.00	30.00	1.00	0.00	0.00
CASaA	Cascade Creek	Above Animas River	238.20	204.44	92.5	165	337.5	518	0.00	0.00	2.00	14.00	44.00	40.00	2.73	40.00	0.00

Table 6. Statistical test (paired t-test) results comparing BMI metrics before (2014) and after (2015 and 2016) the Gold King Mine release.

Metric	Fall 2014 Mean	Fall 2015 Mean	Fall 2016 Mean	2014 compared to 2015		2014 compared to 2016	
				degrees of freedom	p value	degrees of freedom	p value
MMI	49.06	50.50	52.64	6	0.6534	6	0.2981
Taxa Richness	18.29	20.29	19.71	8	0.4097	8	0.4914
SDI	1.75	1.85	1.92	6	0.5446	6	0.1272
MHBI	6.34	6.07	6.31	6	0.6110	6	0.8819
TFA	63.48	58.59	61.96	6	0.0988	6	0.4185
EPT Rich	7.43	7.57	7.86	6	0.8688	6	0.5338
MSF Rich	1.43	2.00	2.00	6	0.2308	6	0.1030
MSF Relative Abundance	3.79	8.63	10.79	6	0.3442	6	0.1832
Density	2414	7745	5080	8	0.0769	8	0.1876

Note: Results indicate no statistically significant differences at p<0.05; see table 4 for an explanation of BMI metrics.

Table 7. MMI scores and attainment using the 2010 and 2017 versions of MMI.

ID	Stream Name	Site Name	MMI Scores					
			2010 Version			2017 Version		
			2014	2015	2016	2014	2015	2016
Animas River								
AaElk	Animas River	Above Elk Creek	30.7	40.7	-	37.9	42.4	43.0
AaCas	Animas River	Above Cascade Creek	52	47.1	-	47.8	55.8	63.8
ABB	Animas River	Baker's Bridge	51.8	60.2	-	52.2	61.4	58.2
AJR	Animas River	James Ranch	61.2	46.7	-	55.8	52.6	63.5
A32nd	Animas River	32nd Street	30.8	20	-	47.4	41.8	48.3
AaLC	Animas River	Above Lightner Creek	48.5	47.3	-	57.3	46.1	46.0
APC	Animas River	Purple Cliffs	46.4	50.7	-	45.0	53.4	45.7
Reference Sites								
ELKaA	Elk Creek	Above Animas River	65.2	49.5	-	78.4	59.2	71.9
CASaA	Cascade Creek	Above Animas River	68.9	90.7	-	64.7	80.8	76.4

Note: Gold highlight indicates sites that are impaired according to MMI scores. For 2016 samples, we only calculated scores using the 2017 version of MMI. These scores are derived from samples collected in the fall. We did not assess attainment of spring samples as they were collected outside of the approved MMI sampling period.

Table 8. Measures of inter-annual variability of BMI metrics from samples collected in the fall of 2014, 2015, and 2016.

ID	Stream Name	Site Name	MMI				Taxa Richness				EPT Richness				MHBI				Shannon Diversity				MSF Richness				MSF Relative Abundance			
			Range	CV	RPD	2SE	Range	CV	RPD	2SE	Range	CV	RPD	2SE	Range	CV	RPD	2SE	Range	CV	RPD	2SE	Range	CV	RPD	2SE	Range	CV	RPD	2SE
Animas River																														
AaElk	Animas River	Above Elk Creek	5.1	6.8	12.4	3.2	8.0	27.6	54.5	4.7	2.0	15.1	26.1	1.3	0.5	3.2	6.1	0.3	0.3	13.5	26.6	0.2	0.0	0.0	0.0	0.0	6.3	38.6	76.7	3.6
AaCas	Animas River	Above Cascade Creek	16.0	14.3	28.7	9.2	3.0	7.8	15.3	1.8	2.0	11.9	20.7	1.3	1.9	13.3	24.6	1.2	0.3	7.4	14.4	0.2	0.0	0.0	0.0	0.0	34.1	52.0	92.0	22.3
ABB	Animas River	Baker's Bridge	9.2	8.2	16.1	5.4	12.0	26.0	50.0	7.2	3.0	15.8	31.0	1.8	0.9	7.6	14.5	0.5	0.6	15.9	29.9	0.4	3.0	57.3	112.5	1.8	7.4	77.1	153.2	4.3
AJR	Animas River	James Ranch	10.9	9.8	19.0	6.5	3.0	7.5	14.8	1.8	3.0	15.8	31.0	1.8	0.9	8.7	17.1	0.6	0.4	12.6	25.3	0.3	1.0	21.7	37.5	0.7	3.3	81.0	157.9	2.0
A32nd	Animas River	32nd Street	6.5	7.7	14.2	4.1	5.0	14.7	27.8	3.1	2.0	21.7	37.5	1.3	1.6	15.7	31.3	0.9	0.6	17.0	34.1	0.4	1.0	43.3	75.0	0.7	2.0	68.9	128.6	1.2
AaLC	Animas River	Above Lightner Creek	11.3	13.0	22.7	7.5	8.0	18.4	35.3	4.8	3.0	24.1	47.4	1.8	2.2	19.5	37.4	1.3	0.3	8.0	15.7	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
APC	Animas River	Purple Cliffs	8.4	9.7	17.5	5.4	3.0	9.2	18.0	1.8	2.0	20.0	40.0	1.2	0.7	6.6	13.2	0.4	0.1	4.4	7.8	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Reference Sites																														
ELKaA	Elk Creek	Above Animas River	19.2	14.0	27.5	11.3	10.0	22.2	44.1	5.8	10.0	30.2	60.0	5.8	0.1	0.5	0.9	0.04	0.1	3.8	7.1	0.1	3.0	43.3	75.0	2.0	0.7	0.4	0.8	0.4
CASaA	Cascade Creek	Above Animas River	16.1	11.3	21.8	9.6	15.0	23.2	46.4	8.7	2.0	6.3	10.9	1.3	1.5	10.6	21.0	0.9	0.5	9.9	19.6	0.3	0.0	0.0	0.0	0.0	10.0	15.7	30.6	5.9

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

Table 9. Statistical test (paired t-test) results comparing fall BMI metrics observed in recent years (2014-2016) with historical observations (1997-2010), and comparing fall BMI metrics during (1997-2004) and after (2005-2015) operation of the Gladstone water treatment plant.

Metric	1997-2010 compared to 2014-2016				1997-2004 compared to 2005-2015			
	Fall 1997-2010 Mean	Fall 2014-2016 Mean	degrees of freedom	p value	Fall 1997-2004 Mean	Fall 2005-2015 Mean	degrees of freedom	p value
MMI	49.68	47.05	6	0.1575	50.08	46.33	6	0.0158
Taxa Richness	13.31	13.10	6	0.7441	13.81	12.55	6	0.0454
SDI	1.62	1.64	6	0.9252	1.67	1.54	6	0.2783
MHBI	6.42	6.23	6	0.5764	6.45	6.35	6	0.7026
TFA	61.24	62.48	6	0.7325	59.83	63.69	6	0.2316
EPT Rich	7.51	7.10	6	0.5317	7.62	6.90	6	0.2769
MSF Rich	1.42	1.62	6	0.4774	1.43	1.43	6	1.0000
MSF Relative Abundance	11.77	7.72	6	0.3238	12.04	6.38	6	0.1939

Note: Gold highlight indicates a statistically significant difference at p<0.05; see table 4 for an explanation of BMI metrics.

Table 10. Summary of correlation results between BMI metrics and measures of drought, snowpack, and Animas River water level.

			Diversity			Proportion of Sensitive Species			MMI	TFA
			SDI	Total richness	EPT rich	MHBI	MSF rich	MSF relative abundance		
When…	*…Baseflow…*	*…was below average…*	-	↓ AaCas	↓ AaCas	-	-	-	-	↑ AaCas
	…Peak Spring Runoff…	*…was below average…*	-	-	-	-	-	-	-	↑ CASaA
	…snow pack…	*…was below average…*	↑ CASaA; AaLC	-	-	-	-	-	↓ A32nd	
	…Drought…	*…was severe…*	↓ AJR; A32nd	↓ AaCas; AJR; A32nd; AaLC	↓ AaCAS; AJR; A32nd; AaLC	-	↓ AJR	↓ AJR		↑ AaCas

Note: Arrow indicates the direction of strong correlations (>=0.75 Pearson correlation coefficient). For example, "when baseflow was below average, total richness declined at AaCas"; "-" indicates an instance where no correlation coefficients were greater than or equal to 0.75. Only sites with at least 6 years of data are included. see table 4 for an explanation of BMI metrics.

Table 11. Pearson correlation coefficients for BMI metrics and measures of drought, snowpack, and Animas River water level.

		Peak Spring Runoff	Baseflow - % of average	Palmer Hydrological Drought Index	Palmer Drought Severity Index	April 1 SWE - % of average
CASaA	Total Richness	0.26	0.25	0.28	0.28	-0.70
	MMI	0.31	-0.27	-0.31	-0.31	-0.24
	TFA	-0.77	-0.41	-0.37	-0.37	0.72
	SDI	0.53	0.31	0.24	0.24	-0.95
	EPT Richness	0.53	0.58	0.70	0.70	-0.12
	MHBI	-0.07	0.32	0.27	0.27	0.25
	MSF Richness	0.67	0.27	0.20	0.20	0.10
	MSF Relative Abundance	0.23	-0.24	-0.27	-0.27	0.12
AaCas	Total Richness	0.45	0.90	0.88	0.88	0.17
	MMI	0.31	0.11	0.12	0.12	-0.73
	TFA	-0.66	-0.83	-0.81	-0.81	0.61
	SDI	0.10	0.53	0.48	0.48	0.03
	EPT Richness	0.44	0.89	0.94	0.94	-0.10
	MHBI	0.13	0.01	-0.03	-0.03	0.22
	MSF Richness	0.67	0.27	0.20	0.20	0.10
	MSF Relative Abundance	0.65	0.39	0.38	0.38	-0.72
AJR	Total Richness	0.26	0.68	0.80	0.88	0.47
	MMI	-0.42	-0.15	-0.09	0.00	0.17
	TFA	0.33	0.02	0.42	0.31	0.19
	SDI	0.25	0.67	0.56	0.76	0.18
	EPT Richness	0.26	0.57	0.74	0.84	0.37
	MHBI	0.38	0.32	0.69	0.67	0.30
	MSF Richness	0.15	0.63	0.58	0.91	-0.25
	MSF Relative Abundance	0.18	0.72	0.54	0.82	-0.30
A32nd	Total Richness	-0.10	0.64	0.33	0.76	-0.39
	MMI	0.28	0.61	0.49	0.37	0.77
	TFA	-0.19	-0.22	0.27	0.19	0.24
	SDI	0.10	0.51	0.53	0.80	-0.01
	EPT Richness	0.41	0.61	0.77	0.83	0.43
	MHBI	0.12	0.20	0.26	0.54	-0.44
	MSF Richness	0.14	0.39	0.19	0.54	-0.40
	MSF Relative Abundance	-0.07	0.07	0.06	0.29	-0.19
AaLC	Total Richness	-0.05	0.55	0.43	0.81	-0.28
	MMI	-0.15	0.47	0.15	0.60	-0.40
	TFA	0.27	-0.06	0.10	-0.34	0.64
	SDI	-0.23	0.43	0.14	0.63	-0.77
	EPT Richness	0.10	0.58	0.41	0.78	-0.37
	MHBI	0.53	0.45	0.38	0.31	-0.14
	MSF Richness	-0.10	-0.27	-0.19	0.00	-0.40
	MSF Relative Abundance	-0.19	-0.24	-0.18	0.00	-0.17

Note: Pearson correlation coefficients; Gold-highlight indicates positive correlations >= 0.75; Green-highlight indicates negative correlations <-0.75. Drought indices are expressed from 1 = extreme drought to 3 = extremely moist. A positive correlation between total richness and Palmer Hydrological Drought Index indicates that higher richness occurs in wetter years. Only sites with at least 6 years of data are included.

9. Figures

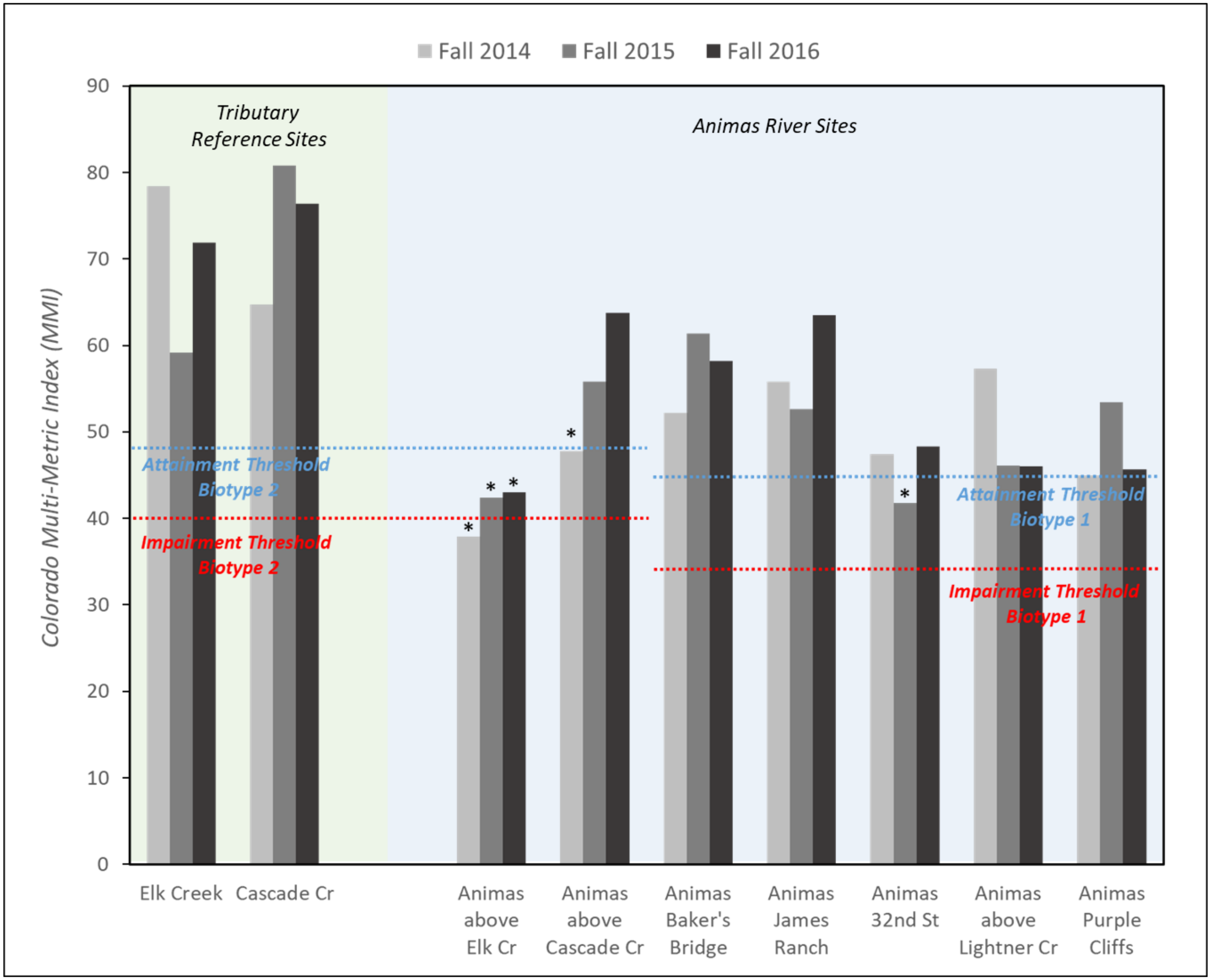


Figure 1: Colorado Multi-Metric Index (MMI) – Fall 2014-2016

*Note: * indicates impairment according to MMI; see table 4 for an explanation of BMI metrics.*

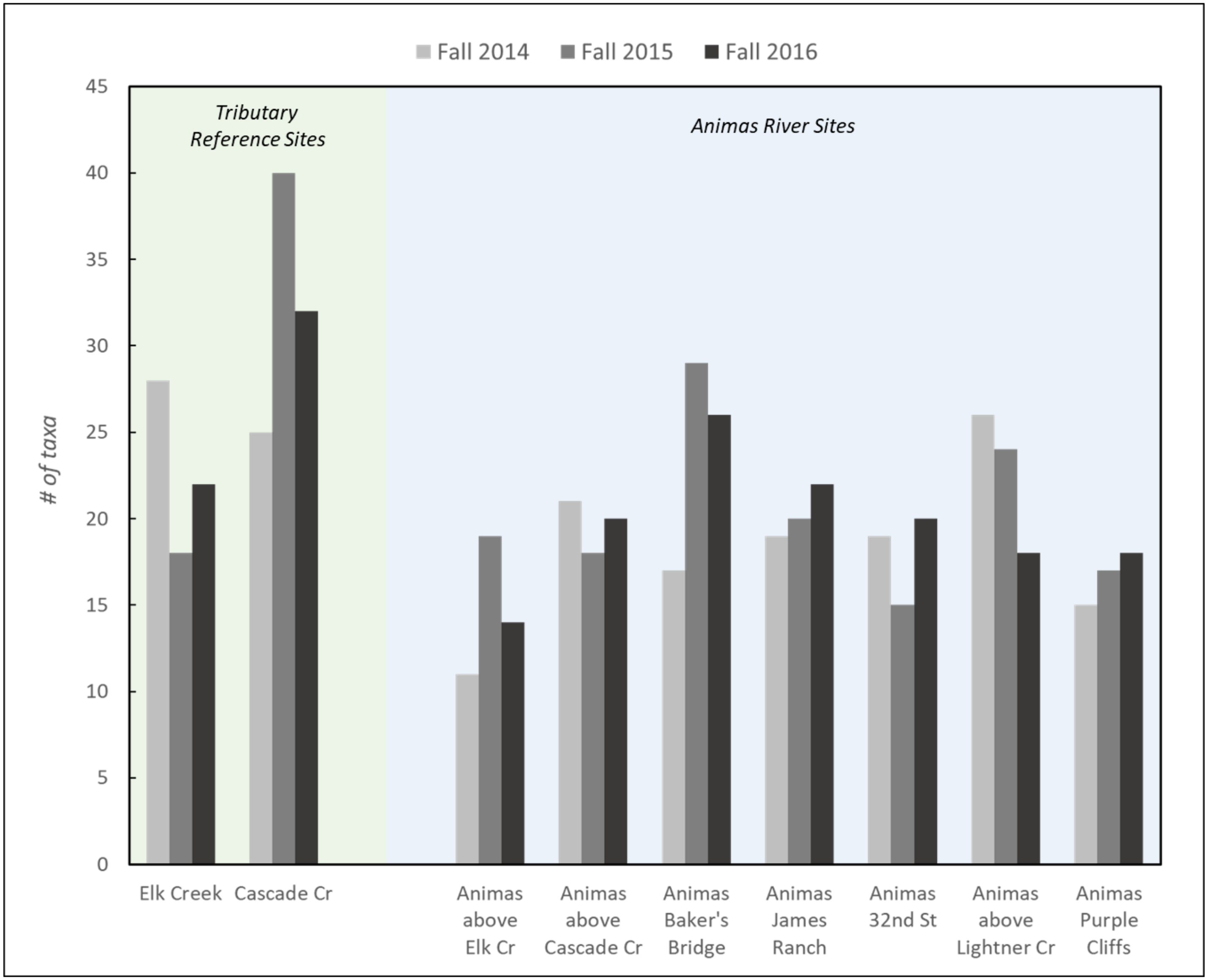


Figure 2: Taxa richness – Fall 2014-2016

Note: see table 4 for an explanation of BMI metrics.

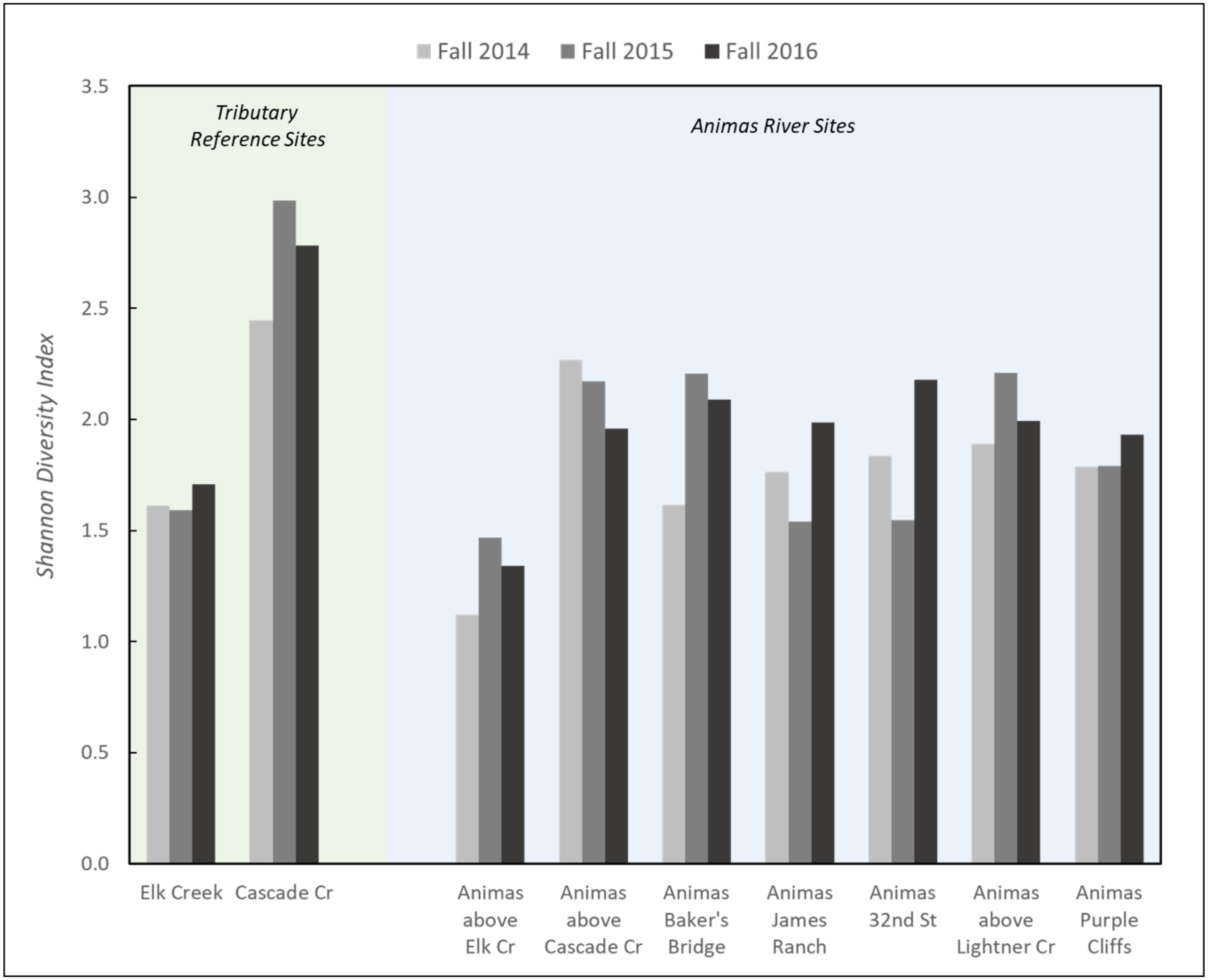


Figure 3: Shannon Diversity Index (SDI) – Fall 2014-2016

Note: see table 4 for an explanation of BMI metrics.

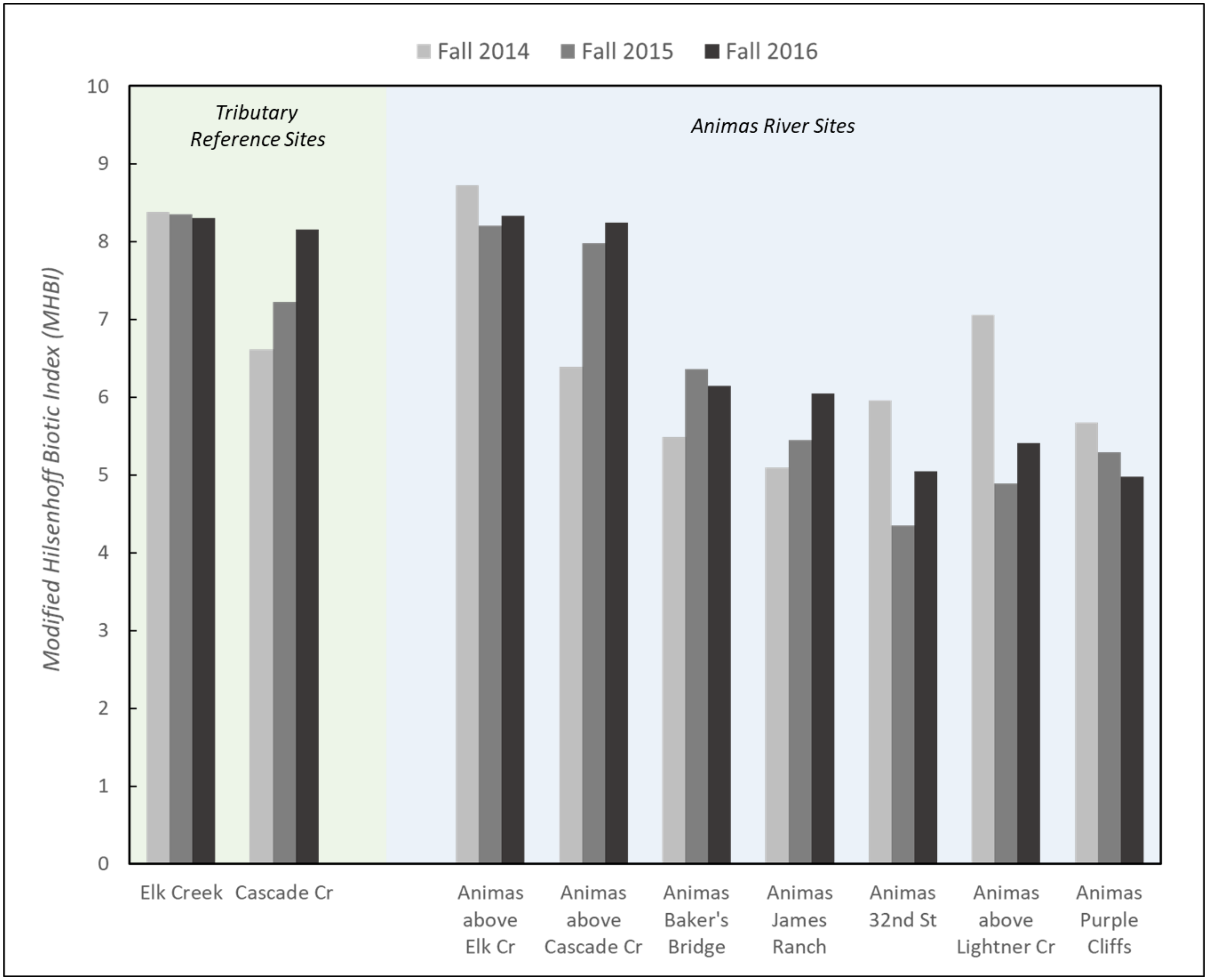


Figure 4: Modified Hilsenhoff Biotic Index (MHBI) – Fall 2014-2016

Note: see table 4 for an explanation of BMI metrics.

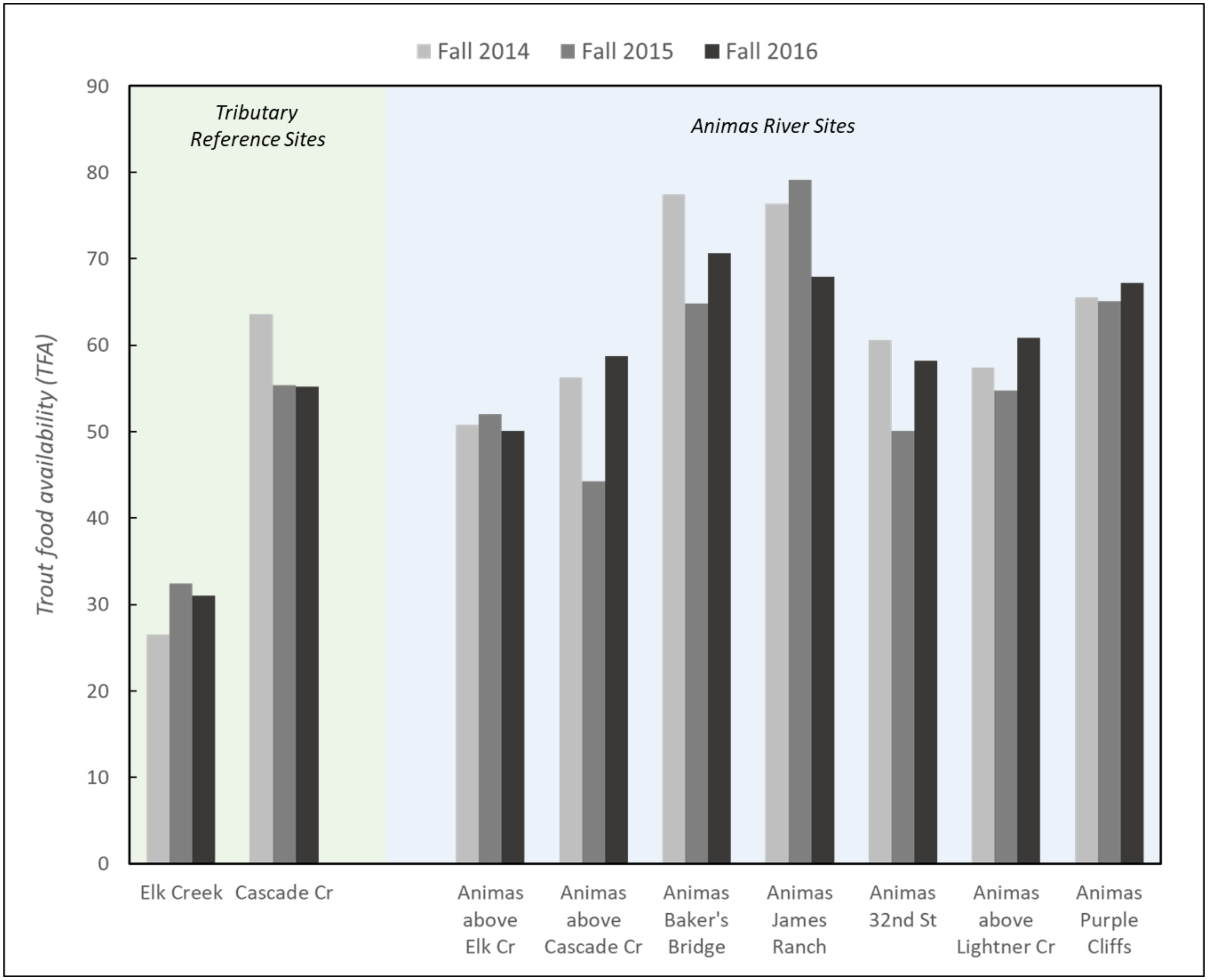


Figure 5: Trout Food Availability (TFA) – Fall 2014-2016

Note: see table 4 for an explanation of BMI metrics.

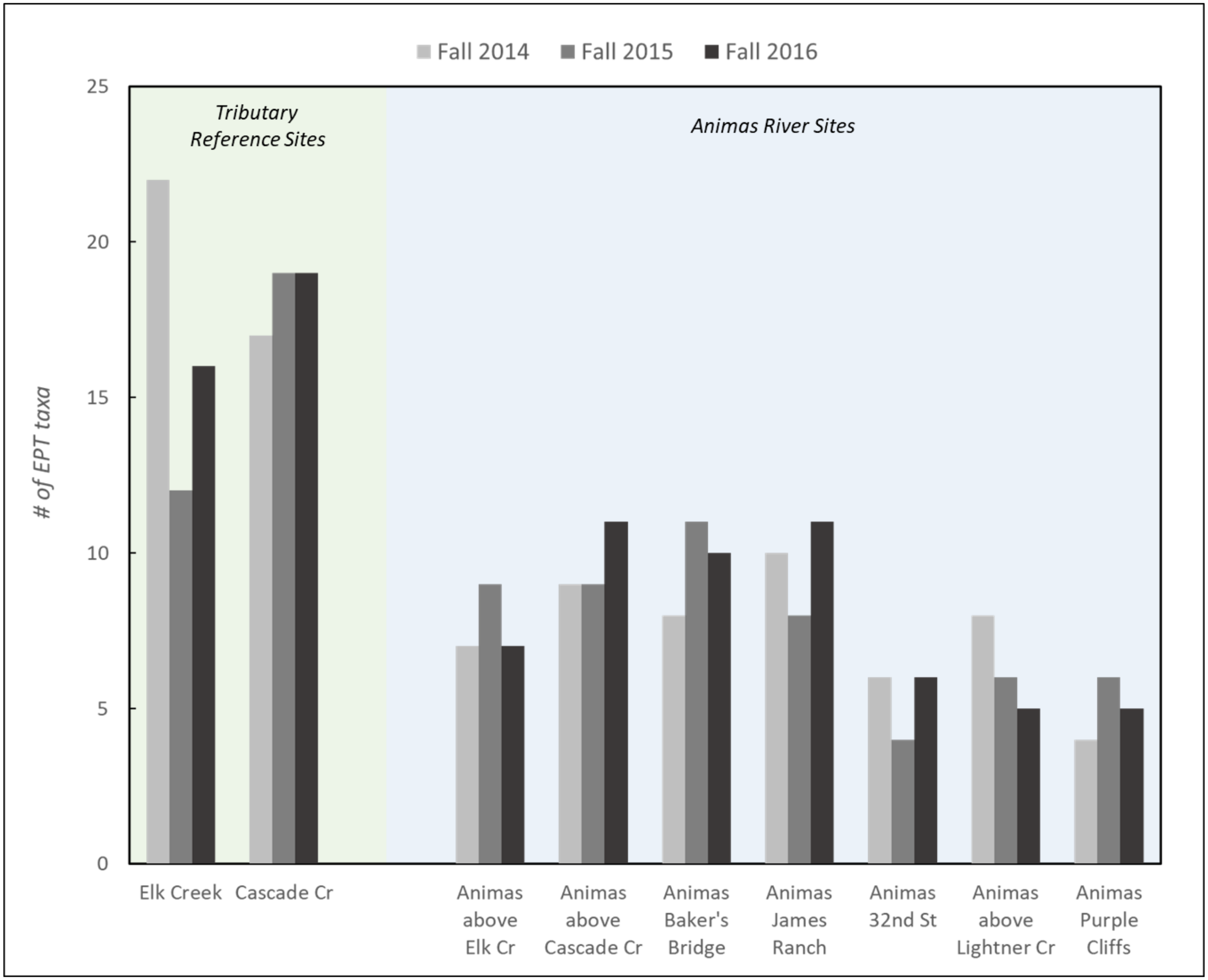


Figure 6: EPT taxa richness – Fall 2014-2016

Note: see table 4 for an explanation of BMI metrics.

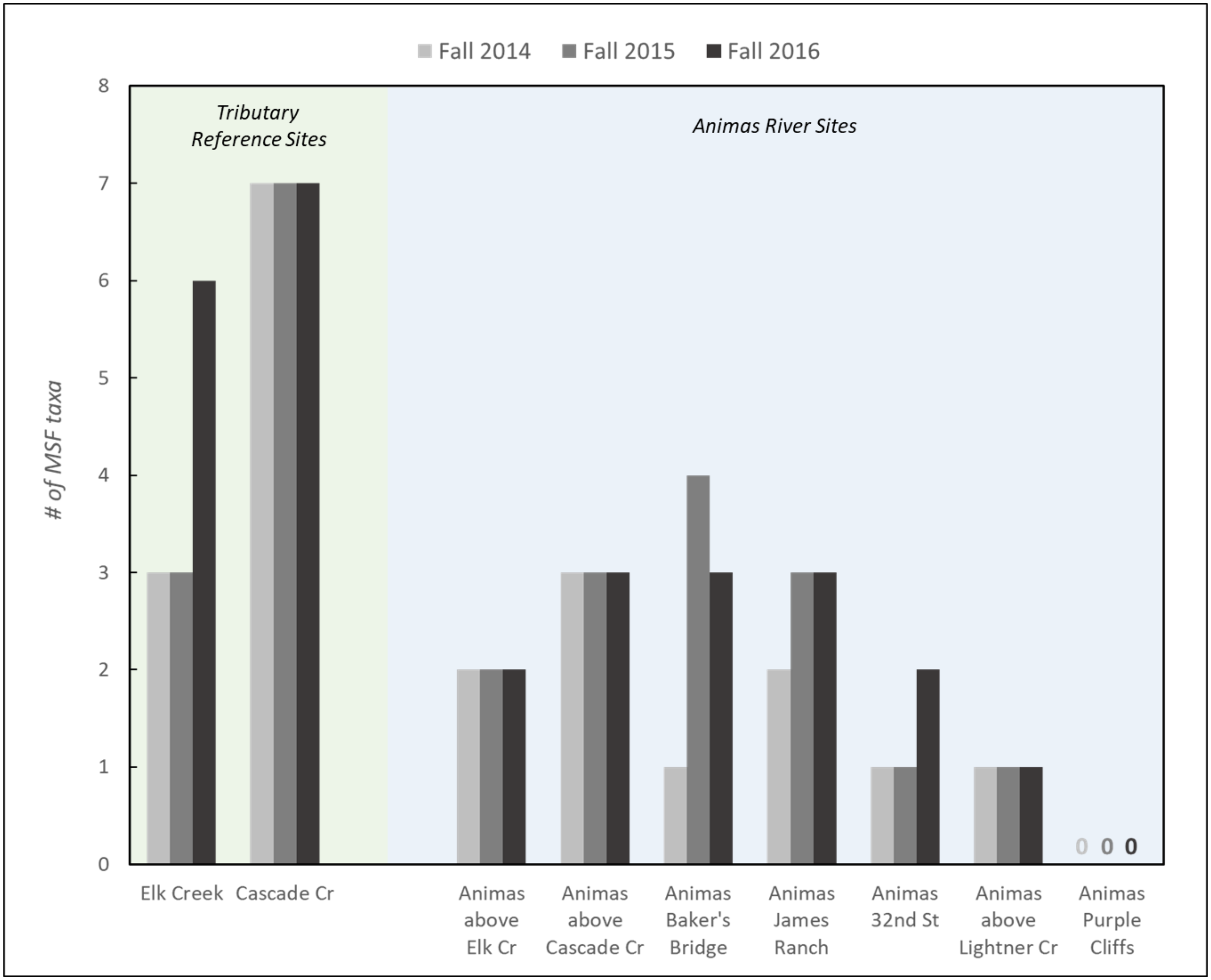


Figure 7: Metal-sensitive family (MSF) taxa richness – Fall 2014-2016

Note: 0 indicates that a sample was collected, but no MSF taxa were present; see table 4 for an explanation of BMI metrics.

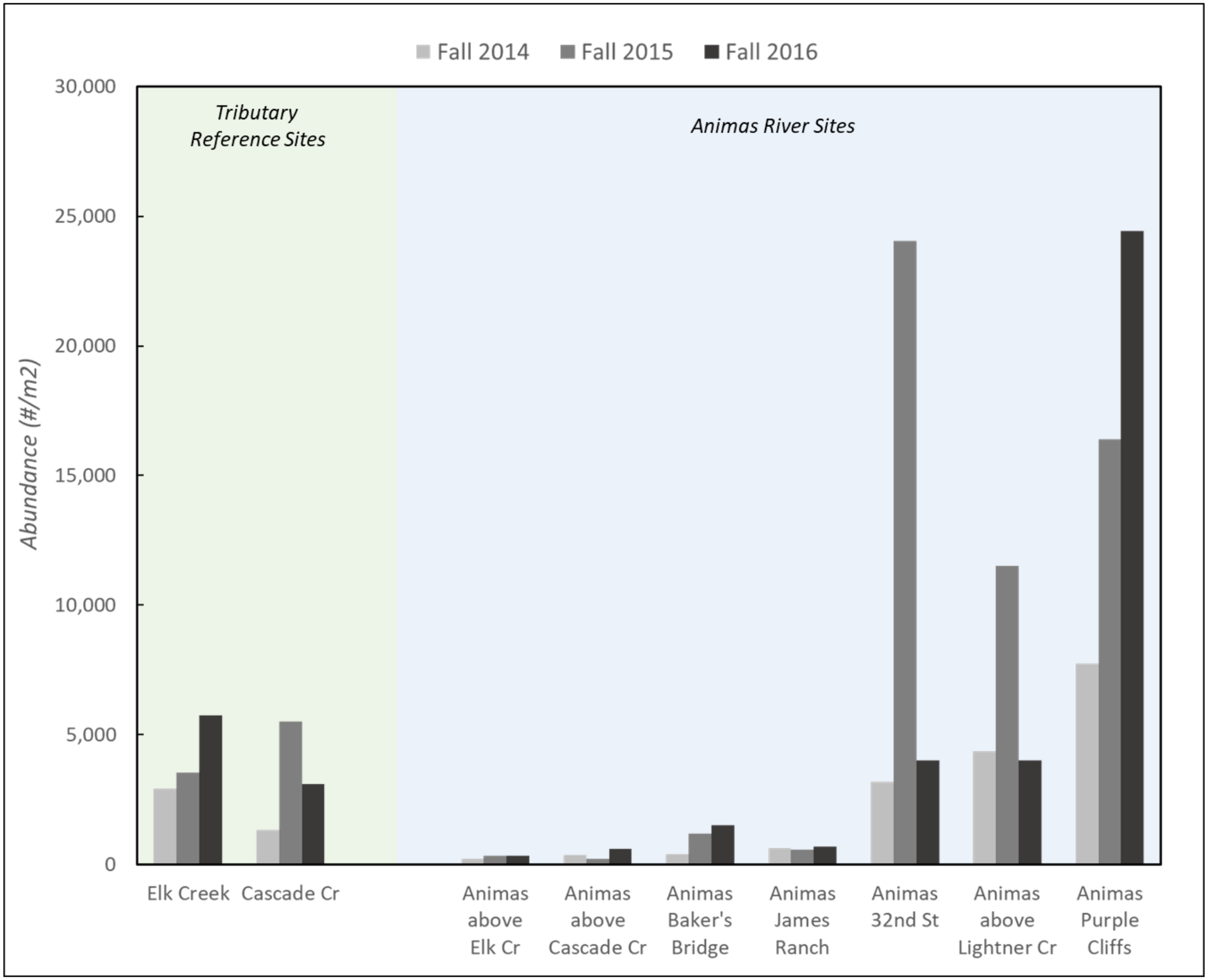


Figure 8: BMI abundance (#/m²)– Fall 2014-2016

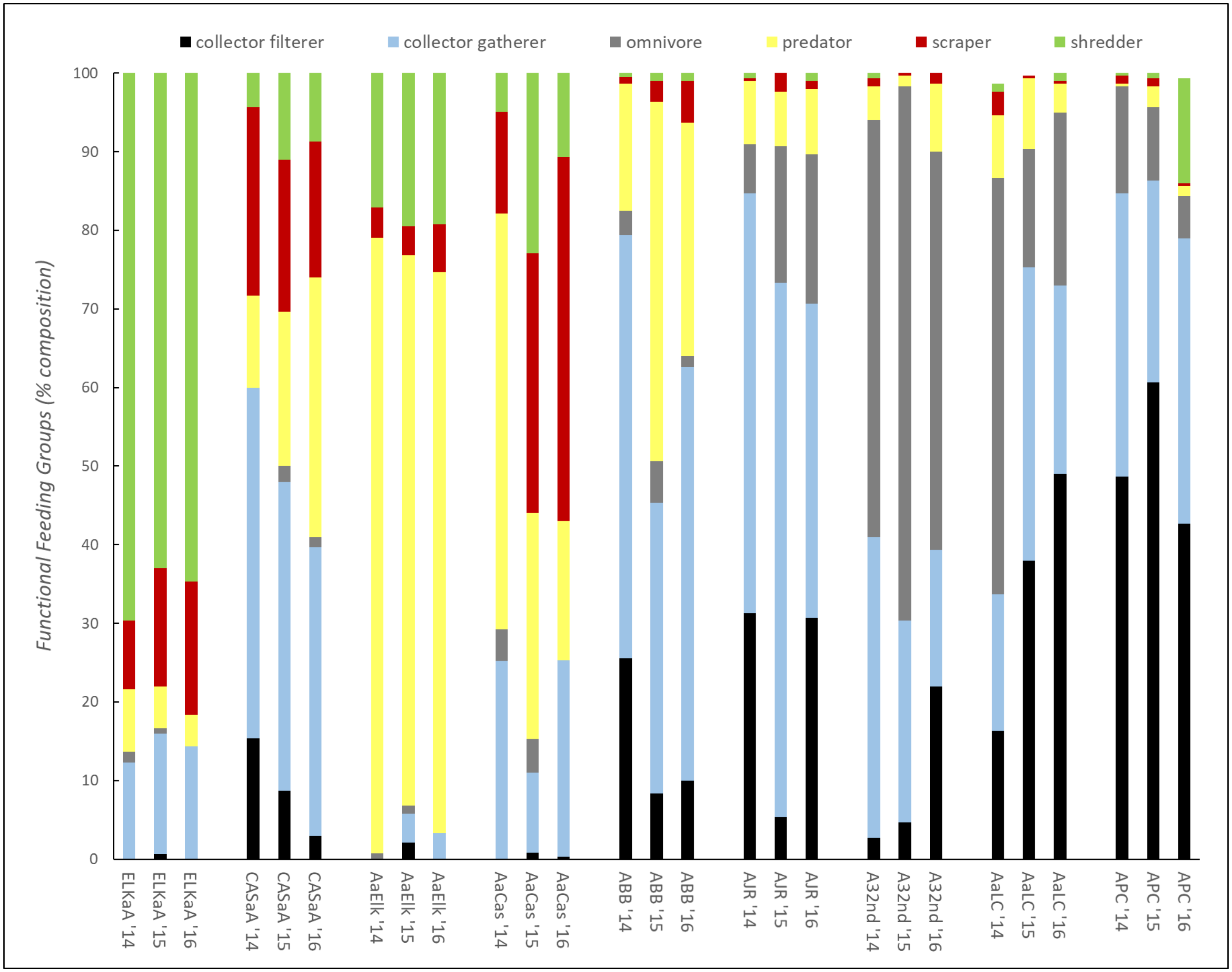


Figure 9: Relative abundance of functional feeding groups – Fall 2014-2016

Note: see table 4 for an explanation of BMI metrics.

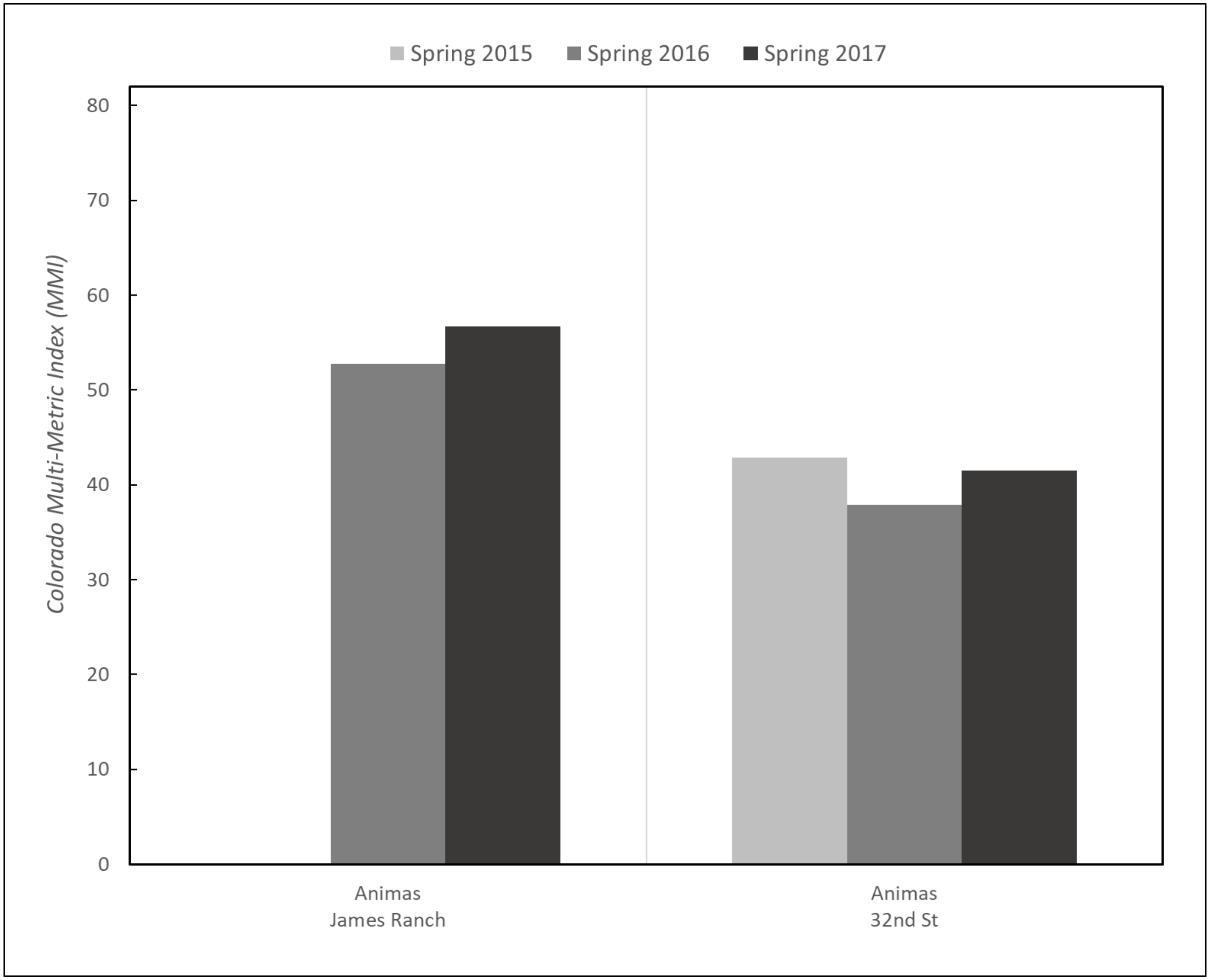


Figure 10: Colorado Multi-Metric Index (MMI) – Spring 2015-2017

Note: James Ranch was not sampled in the spring of 2015; We did not assess attainment of spring samples as they were collected outside of the approved MMI sampling period (CDPHE 2017); see table 4 for an explanation of BMI metrics.

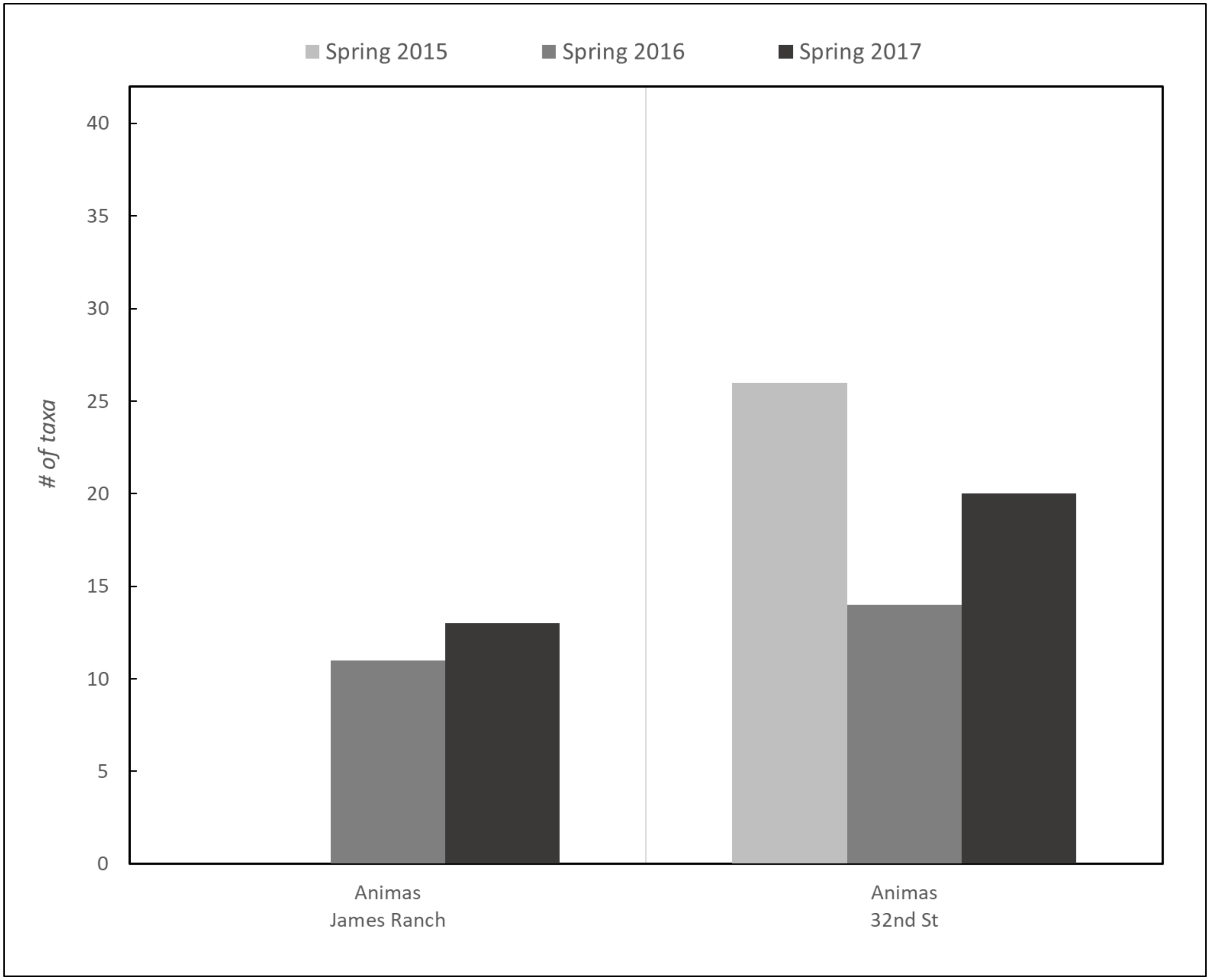


Figure 11: Taxa richness – Spring 2015-2017

Note: James Ranch was not sampled in the spring of 2015; see table 4 for an explanation of BMI metrics.

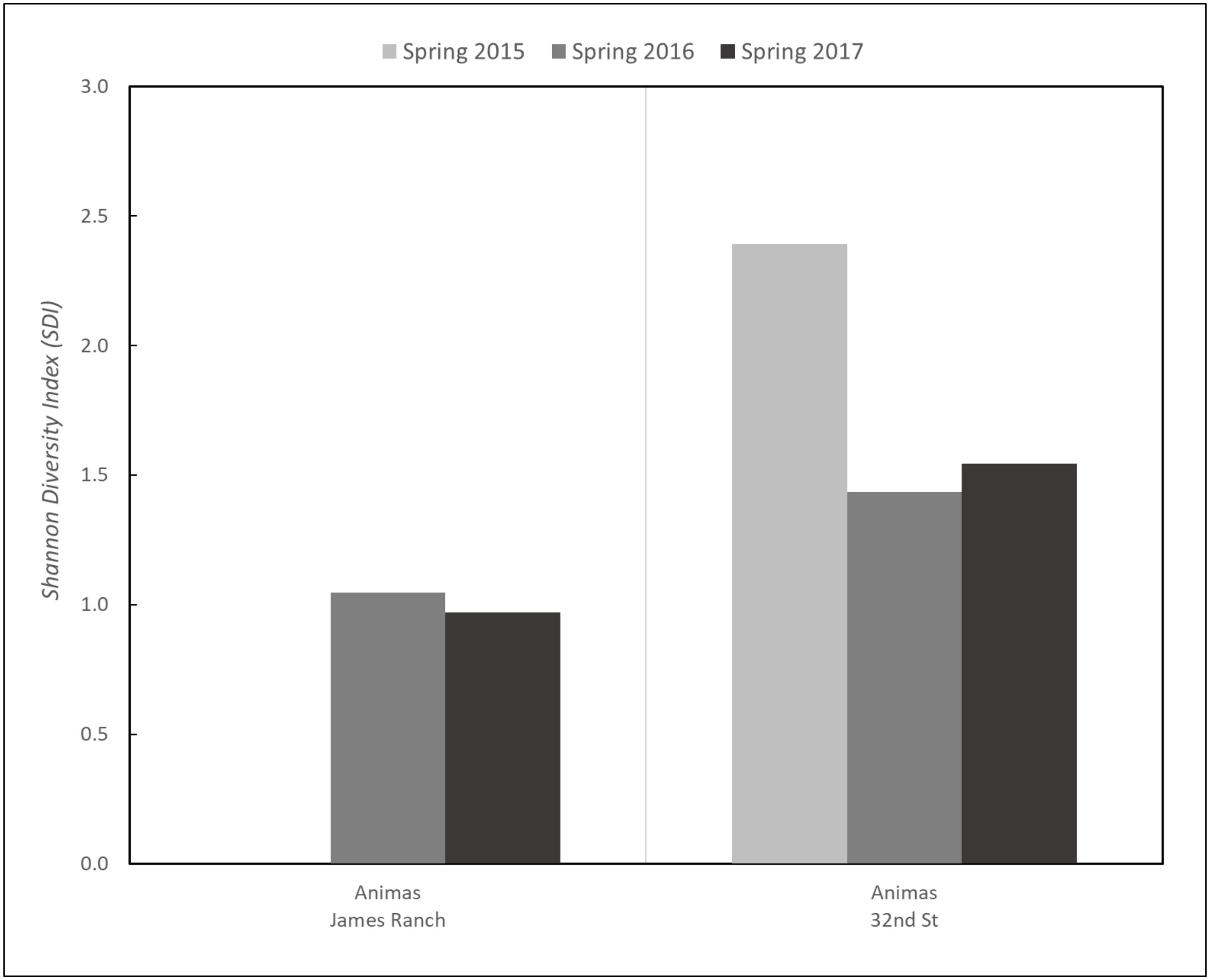


Figure 12: Shannon Diversity Index (SDI) – Spring 2015-2017

Note: James Ranch was not sampled in the spring of 2015; see table 4 for an explanation of BMI metrics.

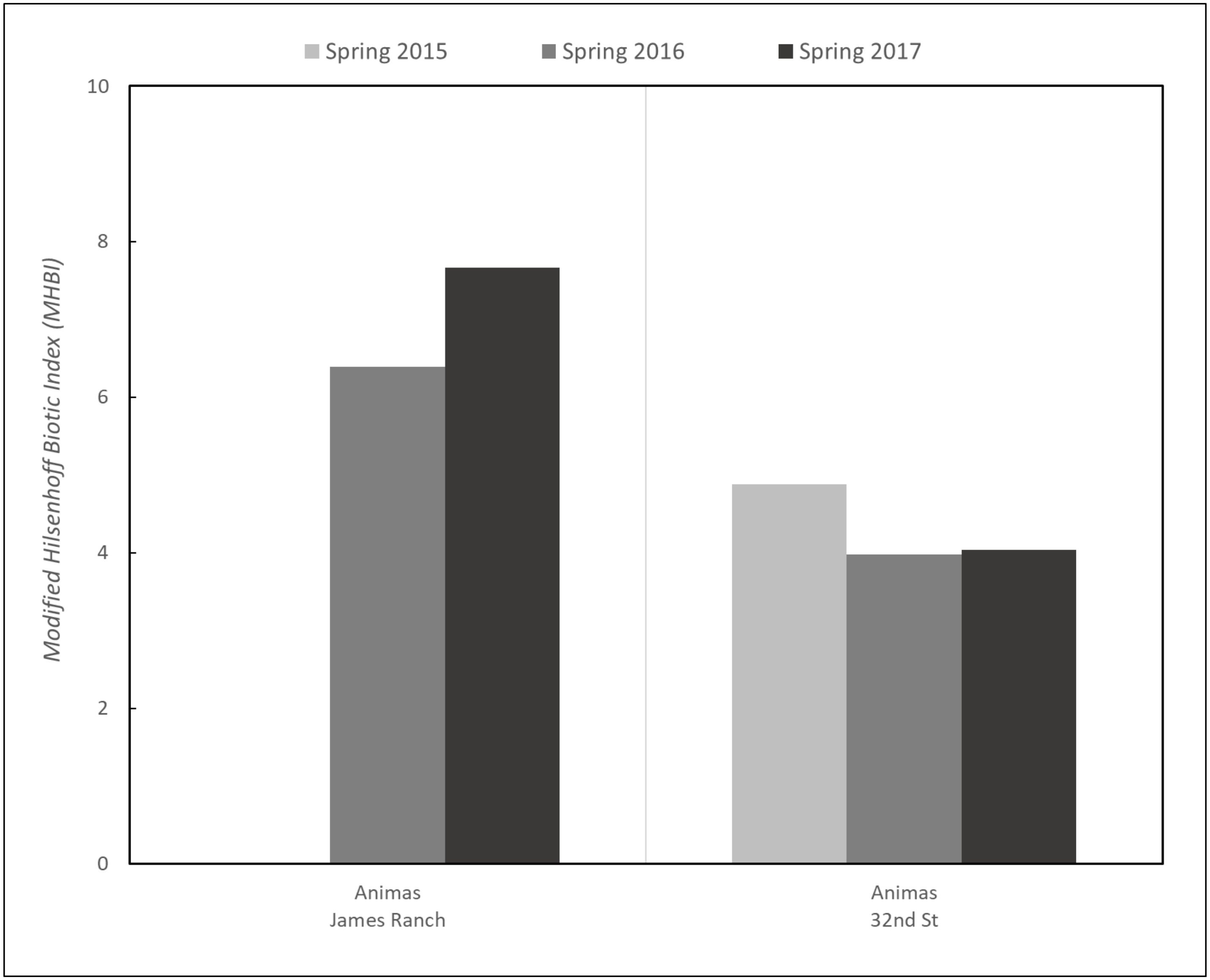


Figure 13: Modified Hilsenhoff Biotic Index (MHBI) – Spring 2015-2017

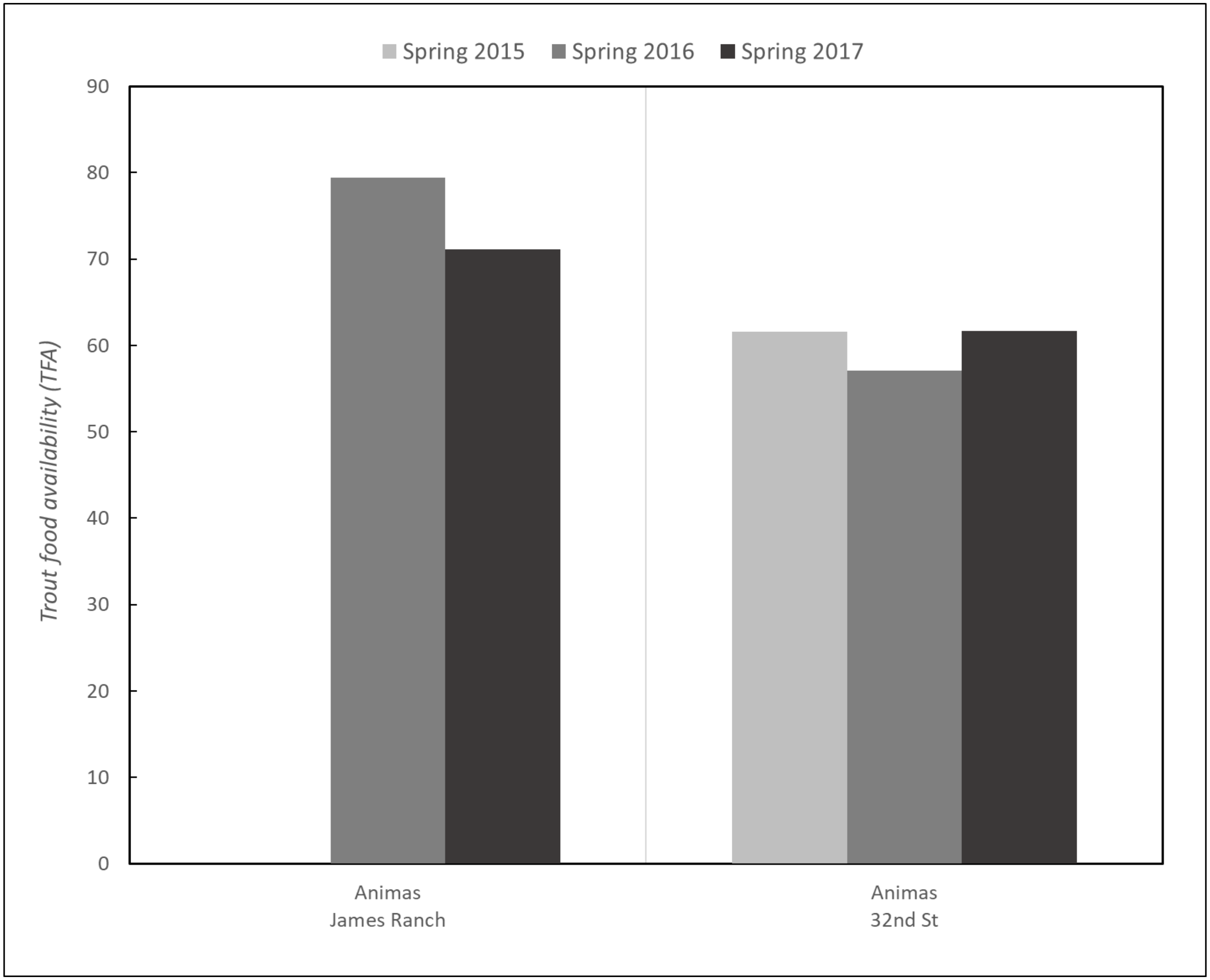


Figure 14: Trout Food Availability (TFA) – Spring 2015-2017

Note: James Ranch was not sampled in the spring of 2015; see table 4 for an explanation of BMI metrics.

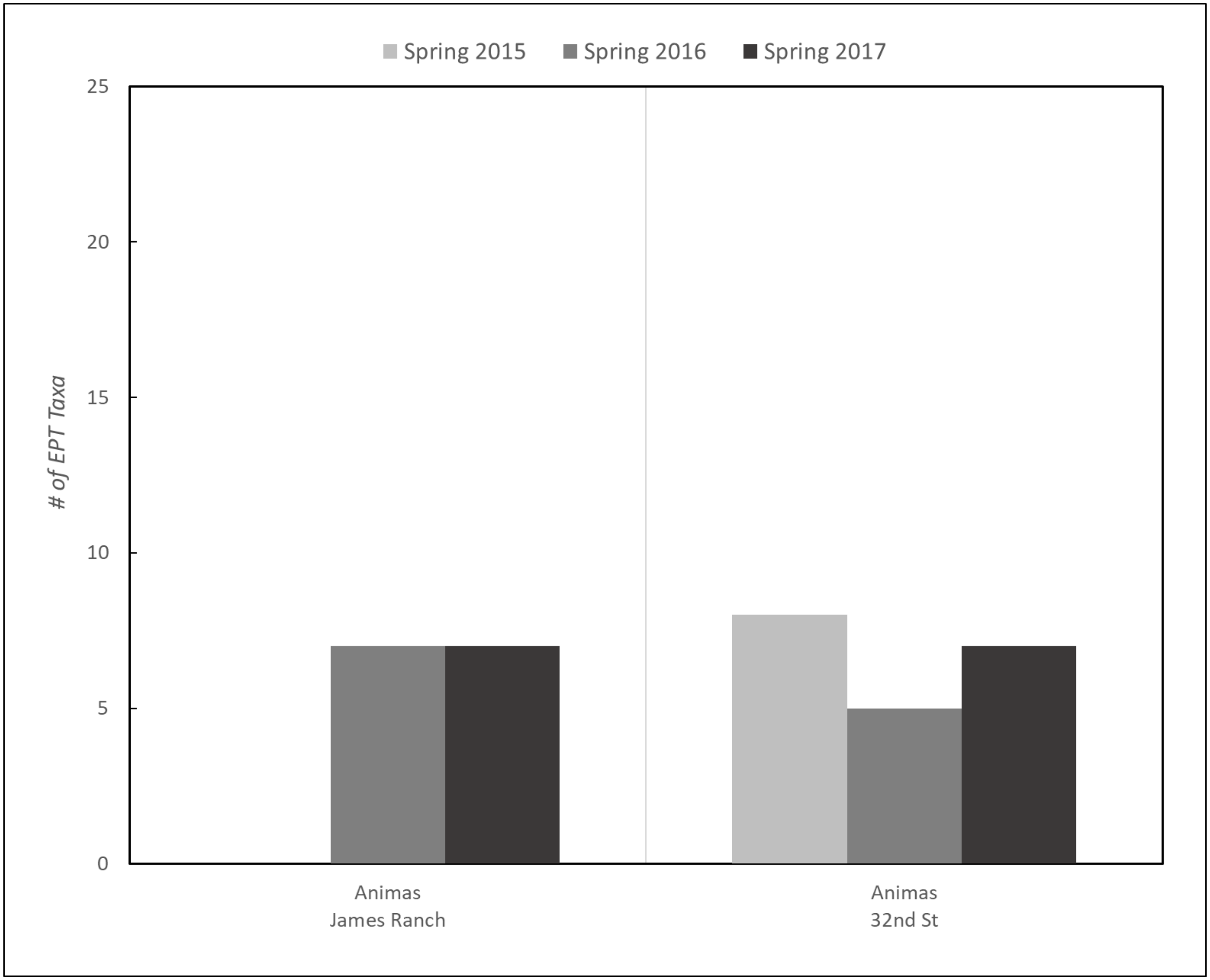


Figure 15: EPT richness – Spring 2015-2017

Note: James Ranch was not sampled in the spring of 2015; see table 4 for an explanation of BMI metrics.

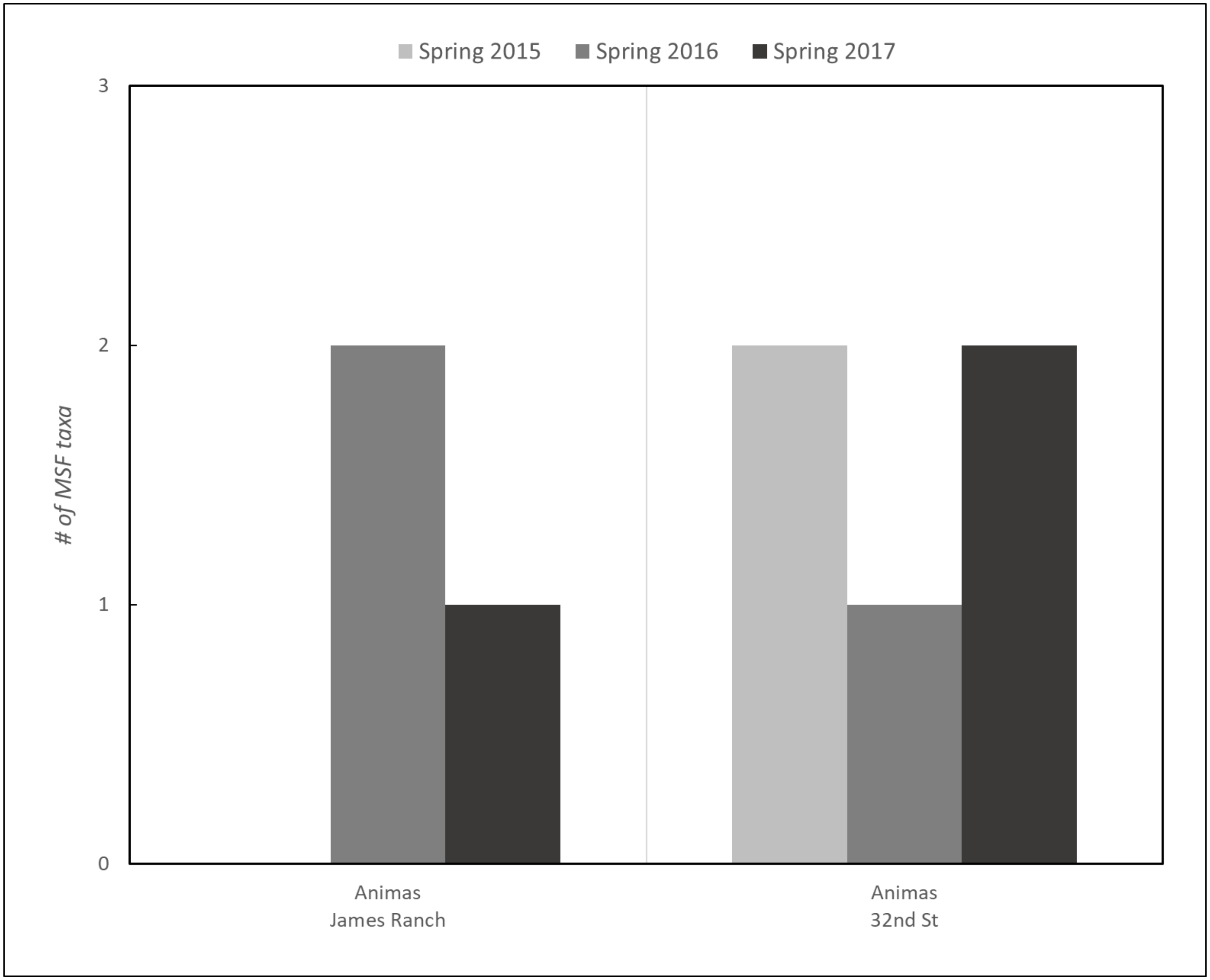


Figure 16: Metal-sensitive family (MSF) taxa richness – Spring 2015-2017

Note: James Ranch was not sampled in the spring of 2015; see table 4 for an explanation of BMI metrics.

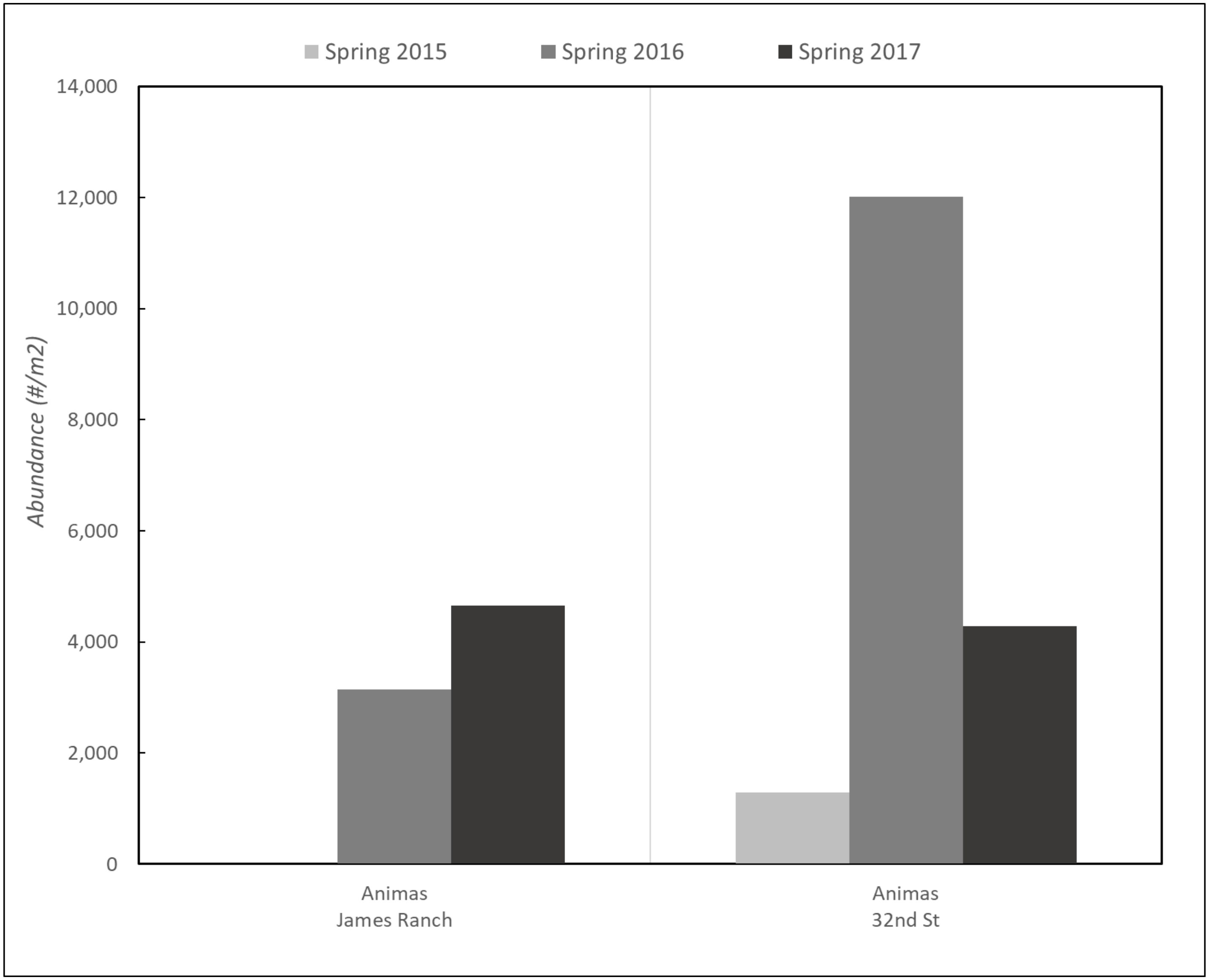


Figure 17: BMI abundance (#/m^2) – Spring 2015-2017

Note: James Ranch was not sampled in the spring of 2015; see table 4 for an explanation of BMI metrics.

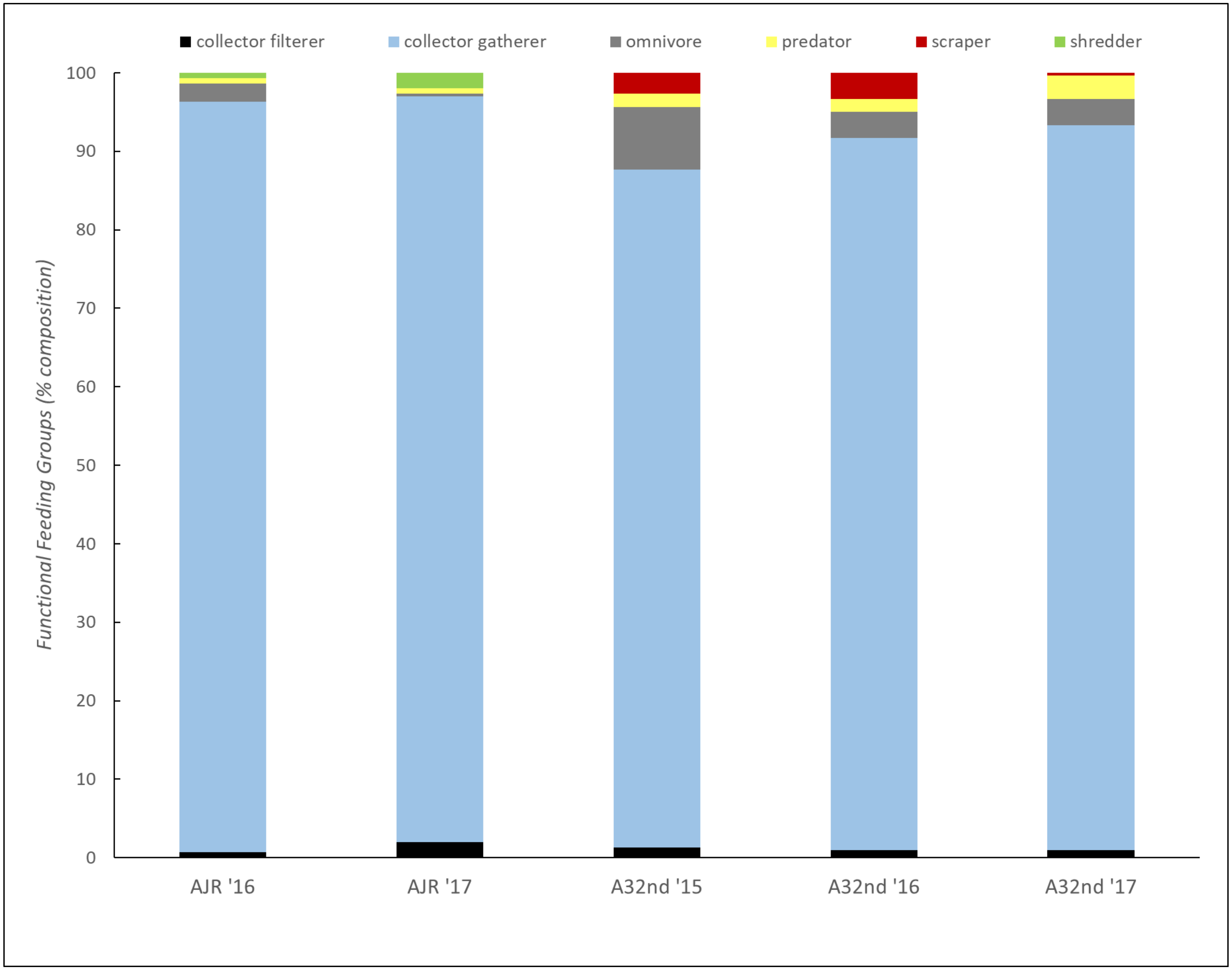


Figure 18: Relative abundance of functional feeding groups – Spring 2015-2017

Note: James Ranch was not sampled in the spring of 2015; see table 4 for an explanation of BMI metrics.

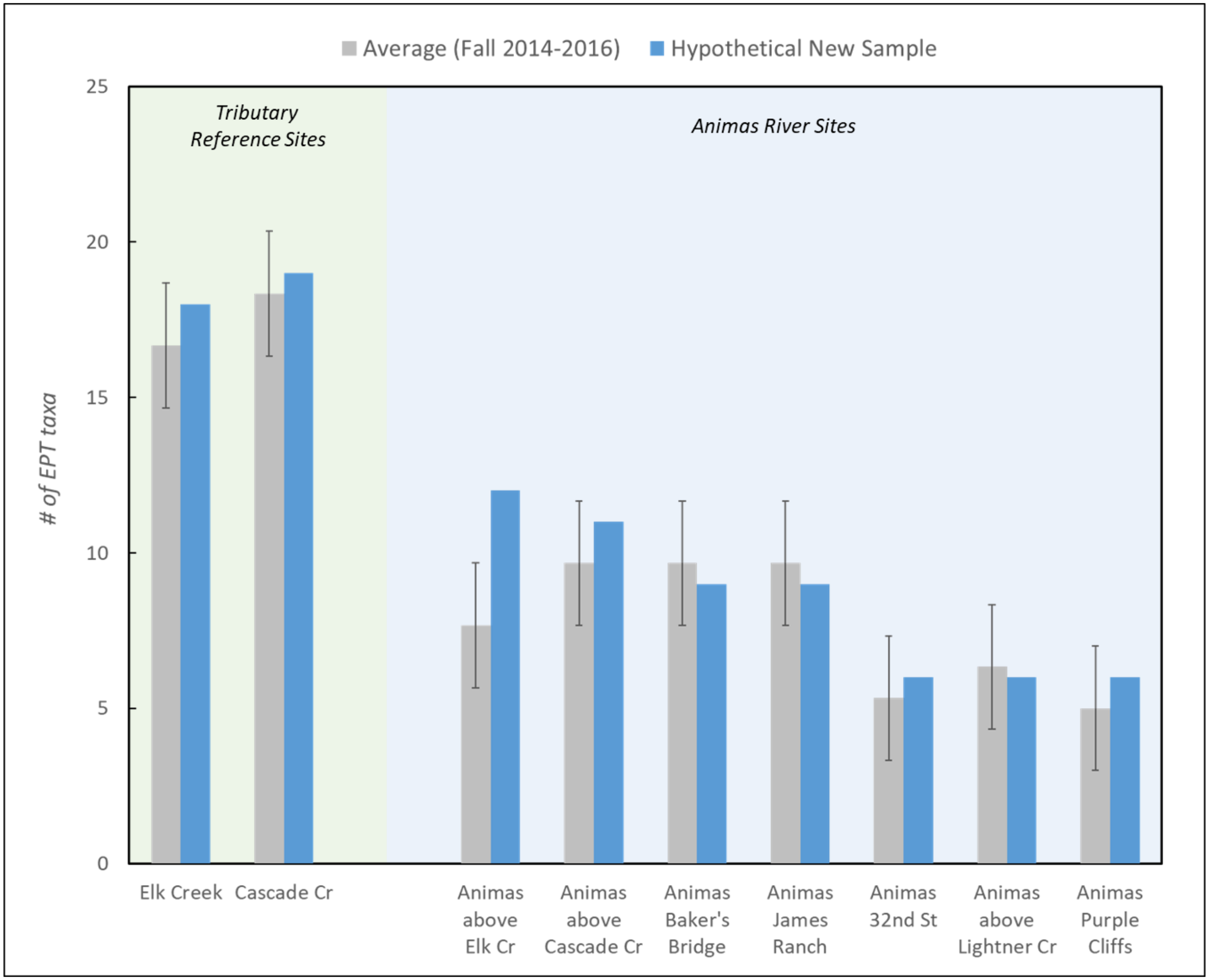


Figure 19: Hypothetical example of how a new set of data could be assessed in context of 2014-2016 data.
Note: This example demonstrates a scenario in which an increase in EPT richness at "Animas above Elk Cr" is beyond the natural variability documented from 2014-2016. Error bars are two standard errors.

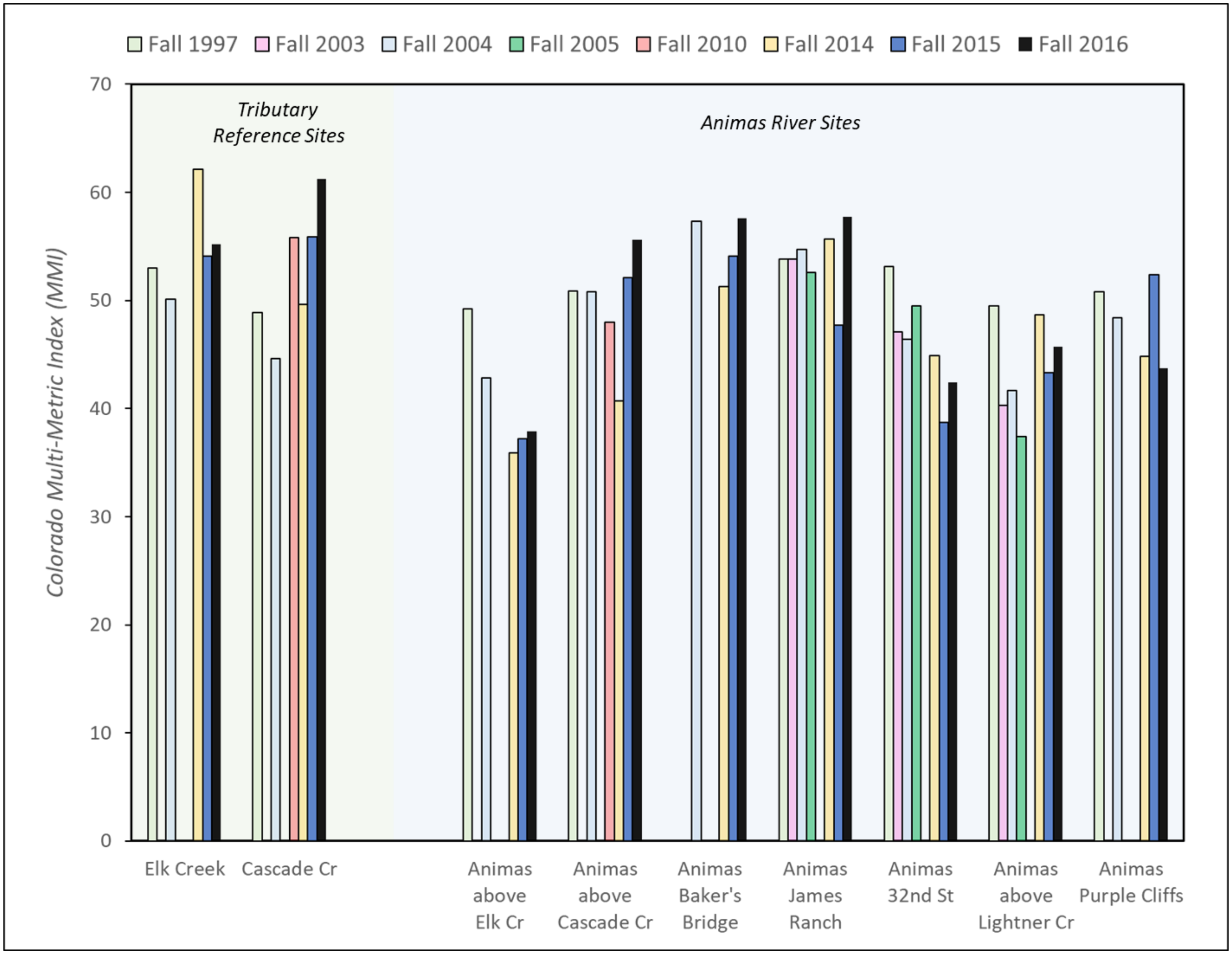


Figure 20: Colorado Multi-Metric Index (MMI) – Fall 1997-2016

Note: We did not assess attainment due to incomplete Chironomidae taxonomic resolution in the historical data; See table 4 for an explanation of BMI metrics.

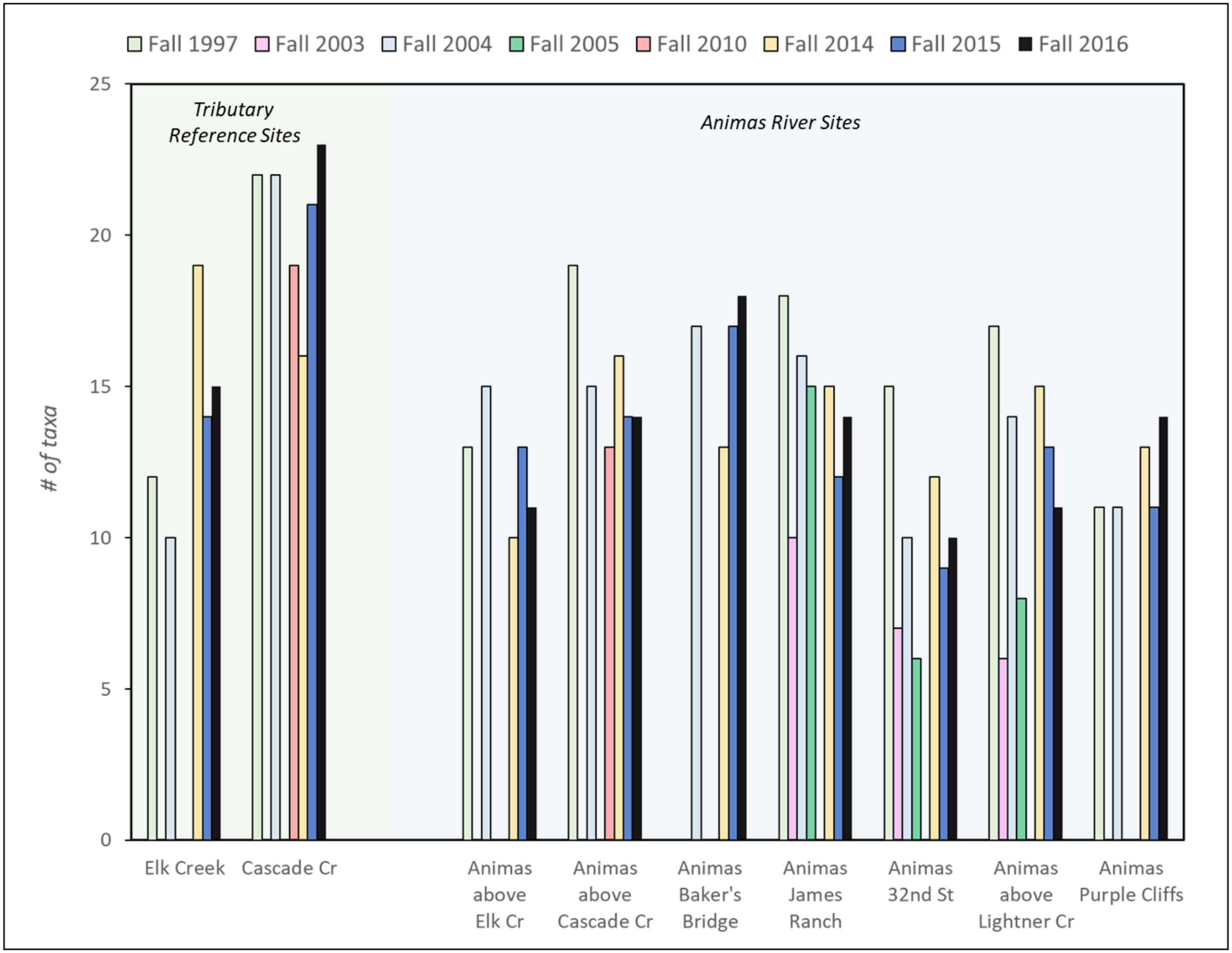


Figure 21: Taxa richness – Fall 1997-2016

Note: see table 4 for an explanation of BMI metrics.

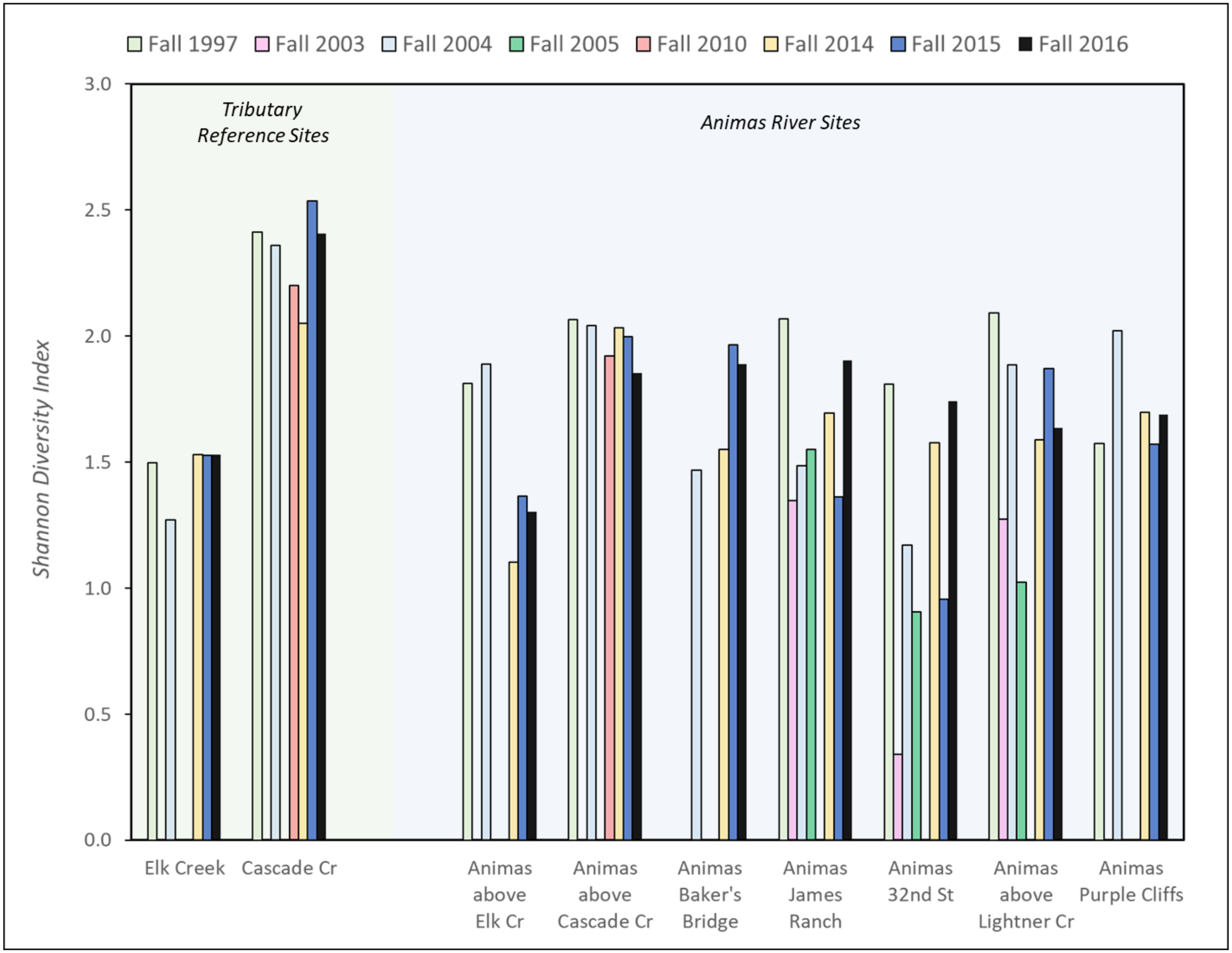


Figure 22: Shannon Diversity Index (SDI) – Fall 1997-2016
Note: see table 4 for an explanation of BMI metrics.

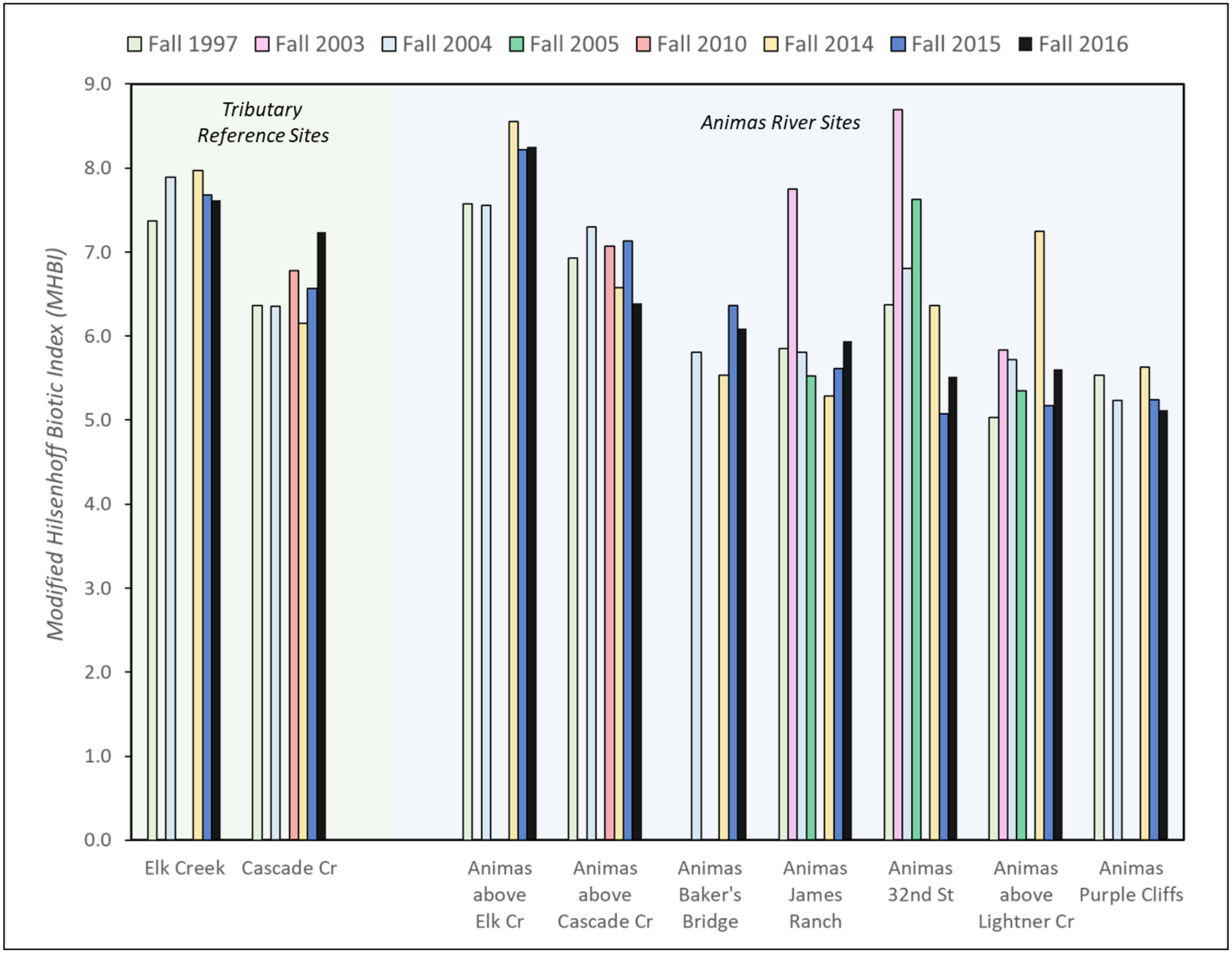


Figure 23: Modified Hilsenhoff Biotic Index (MHBI) – Fall 1997-2016

Note: see table 4 for an explanation of BMI metrics.

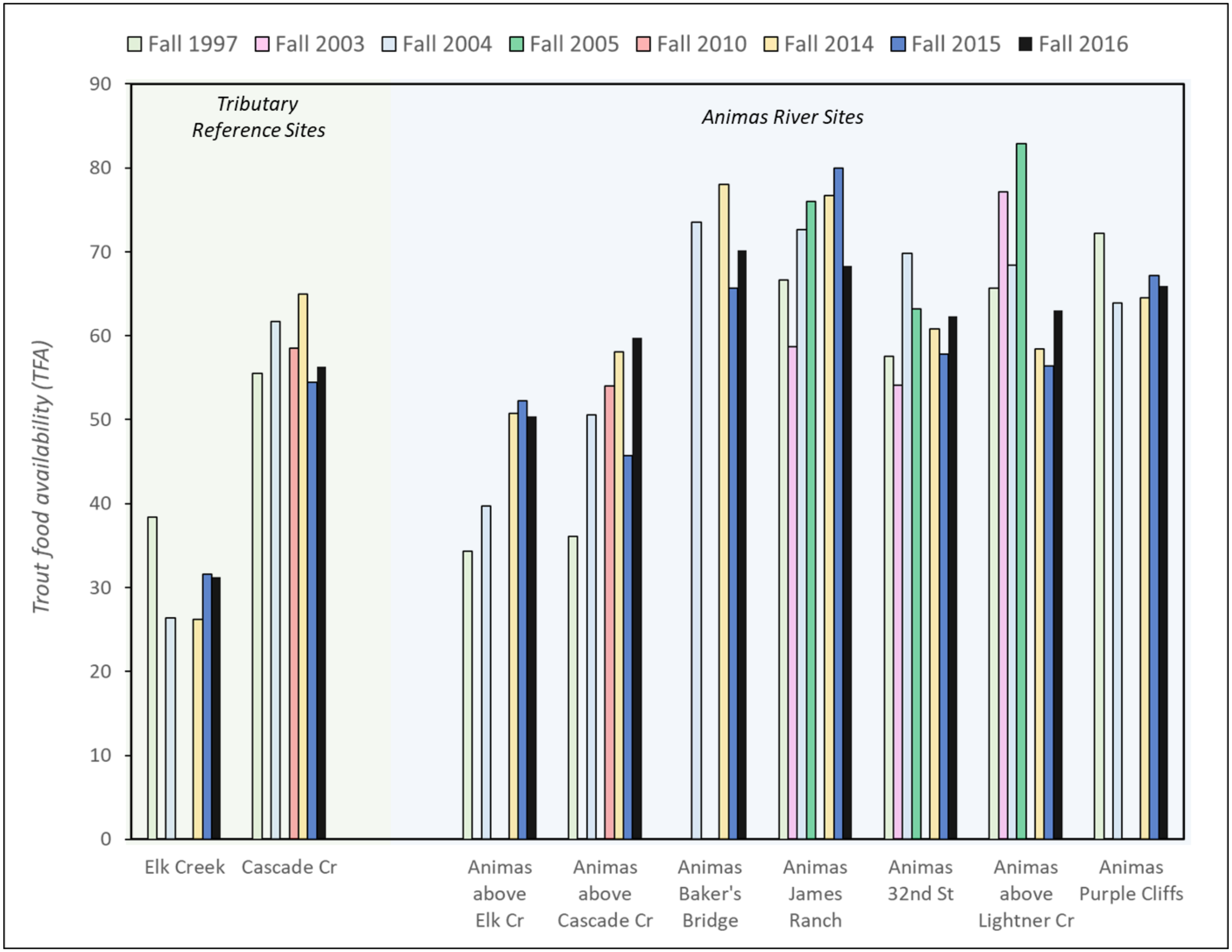


Figure 24: Trout Food Availability (TFA) – Fall 1997-2016

Note: see table 4 for an explanation of BMI metrics.

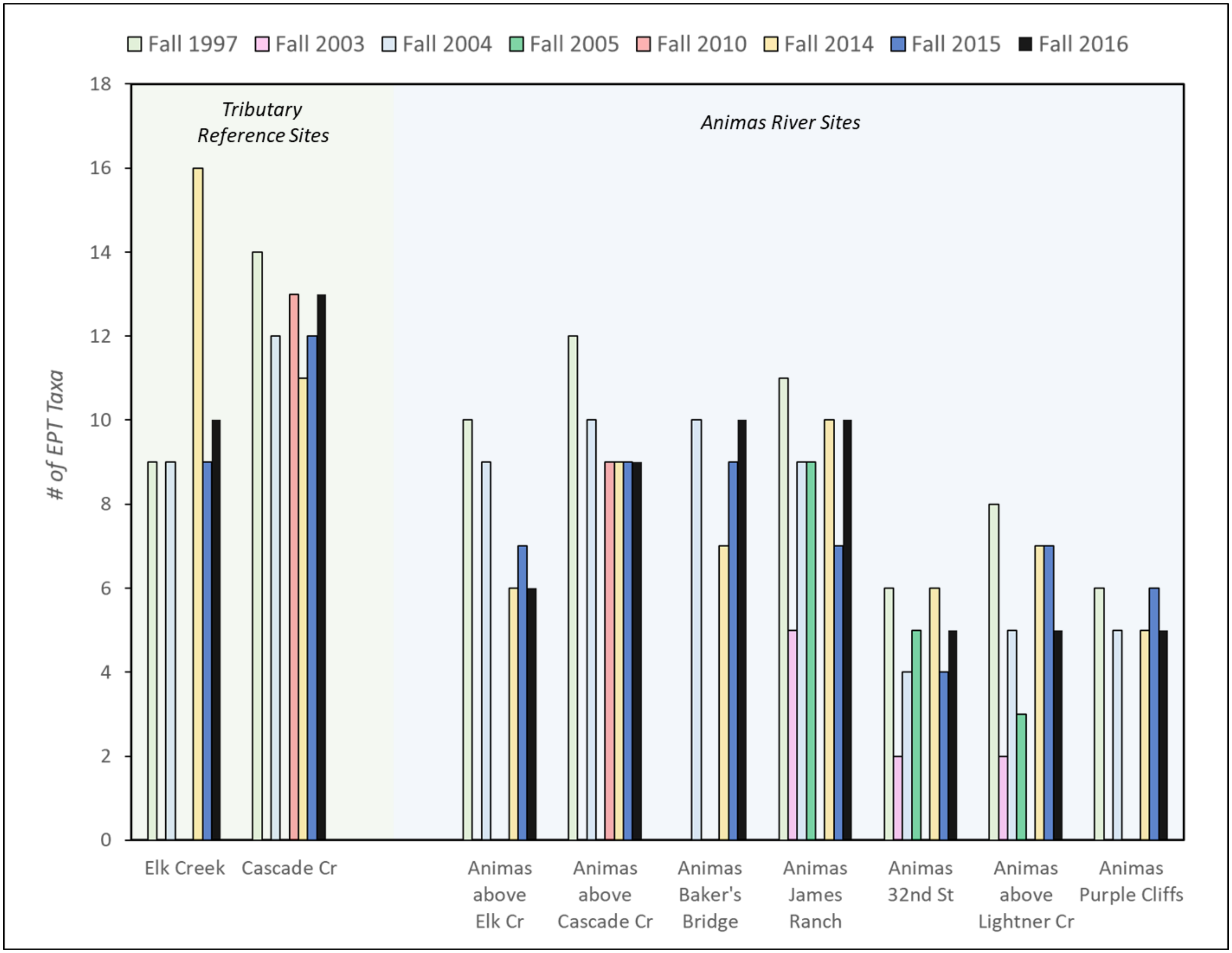


Figure 25: EPT taxa richness – Fall 1997-2016

Note: see table 4 for an explanation of BMI metrics.

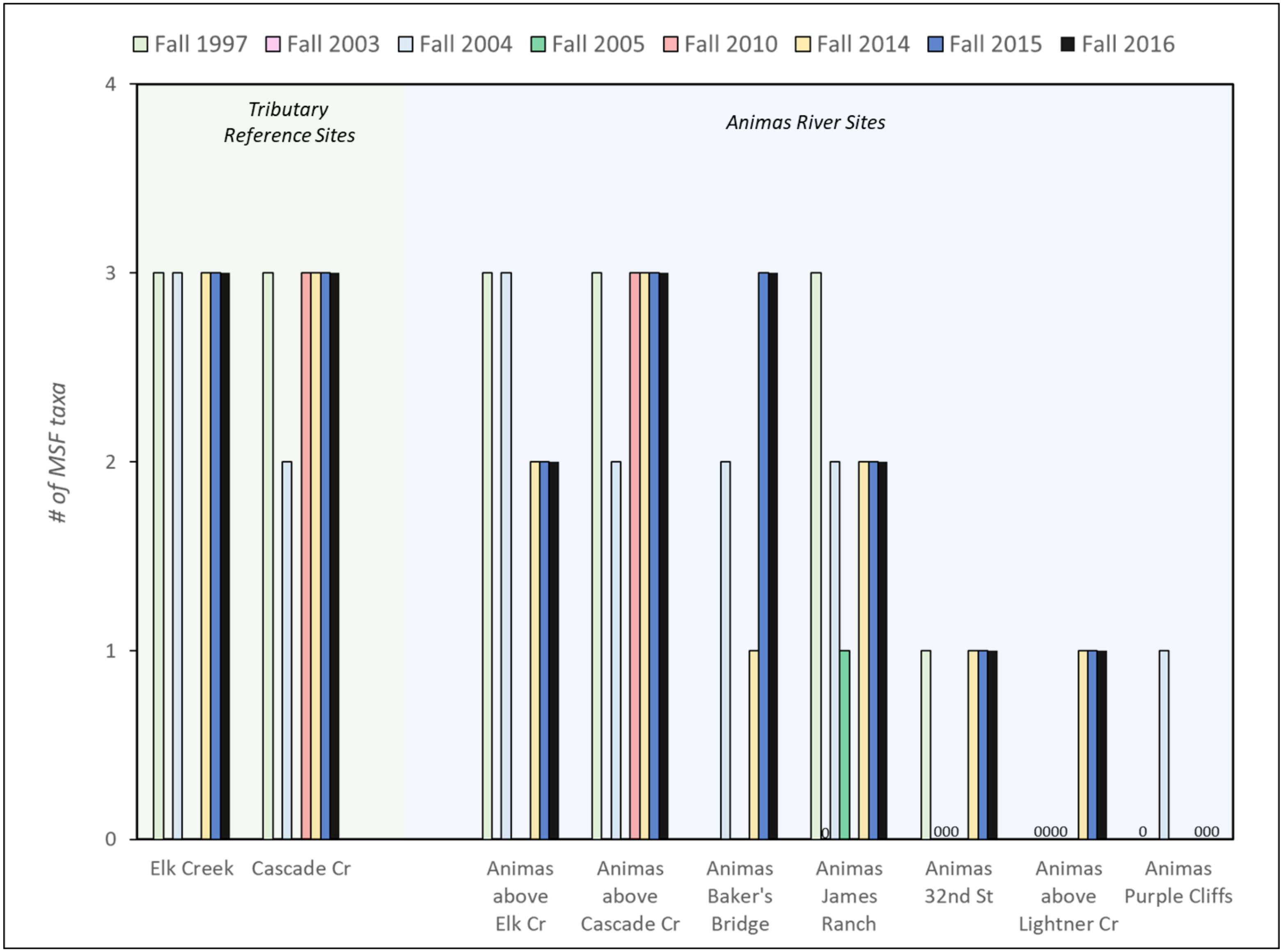


Figure 26: Metal-sensitive family (MSF) taxa richness – Fall 1997-2016

Note: 0 indicates that a sample was collected, but no MSF taxa were present; see table 4 for an explanation of BMI metrics.

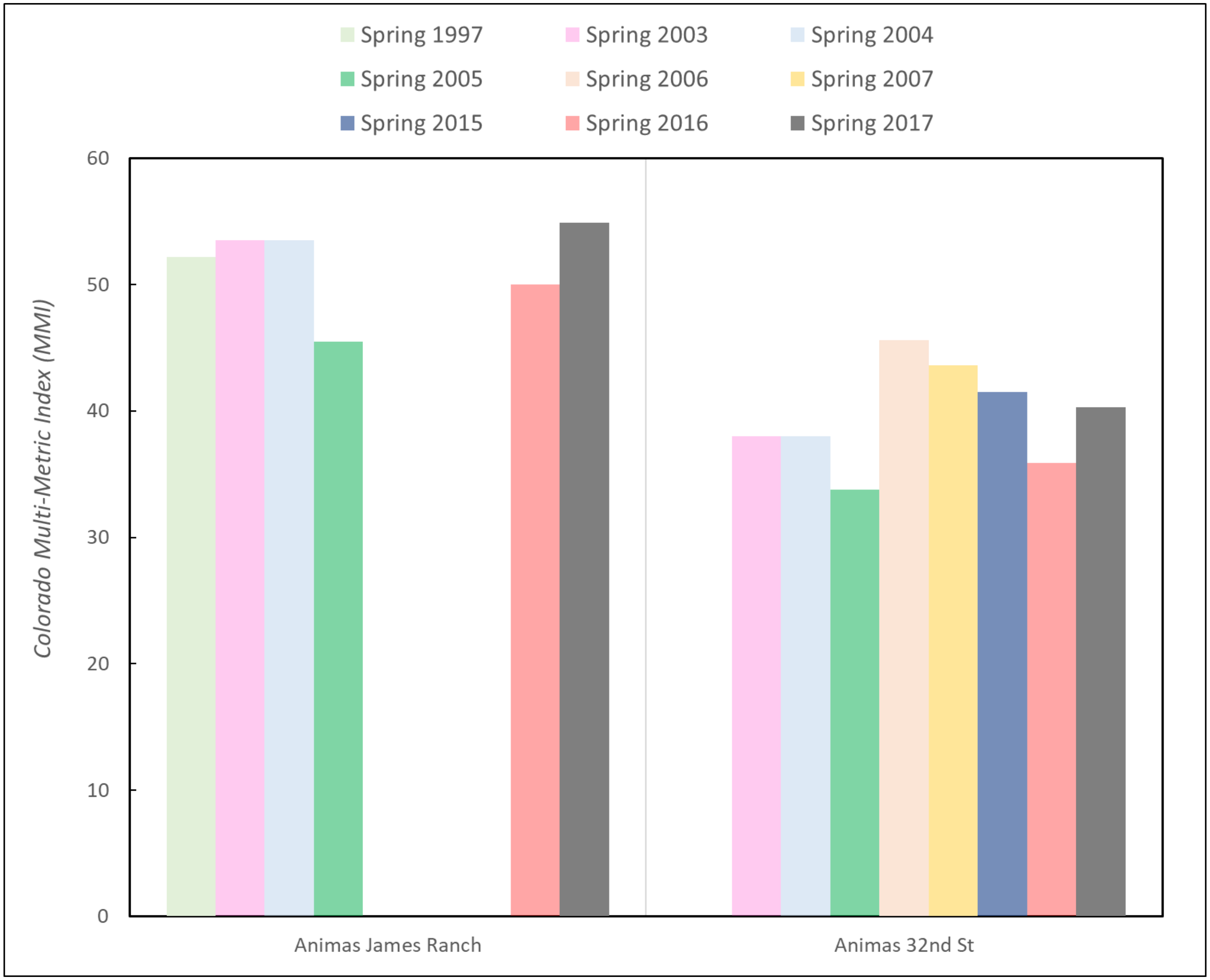


Figure 27: Colorado Multi-Metric Index (MMI) – Spring 1997-2017

Note: We did not assess attainment due to incomplete Chironomidae taxonomic resolution in the historical data; See table 4 for an explanation of BMI metrics.

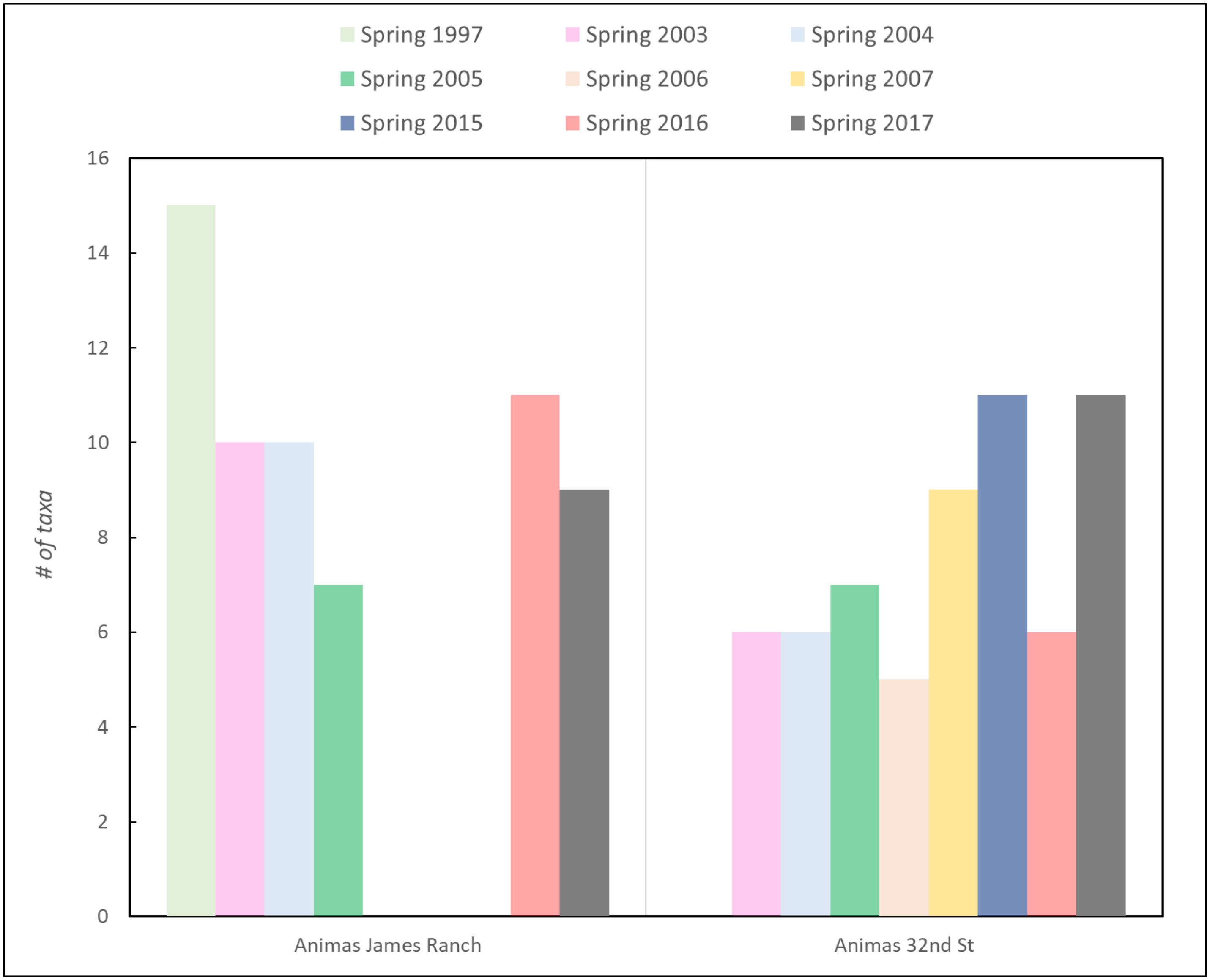


Figure 28: Taxa richness – Spring 1997-2017
Note: see table 4 for an explanation of BMI metrics.

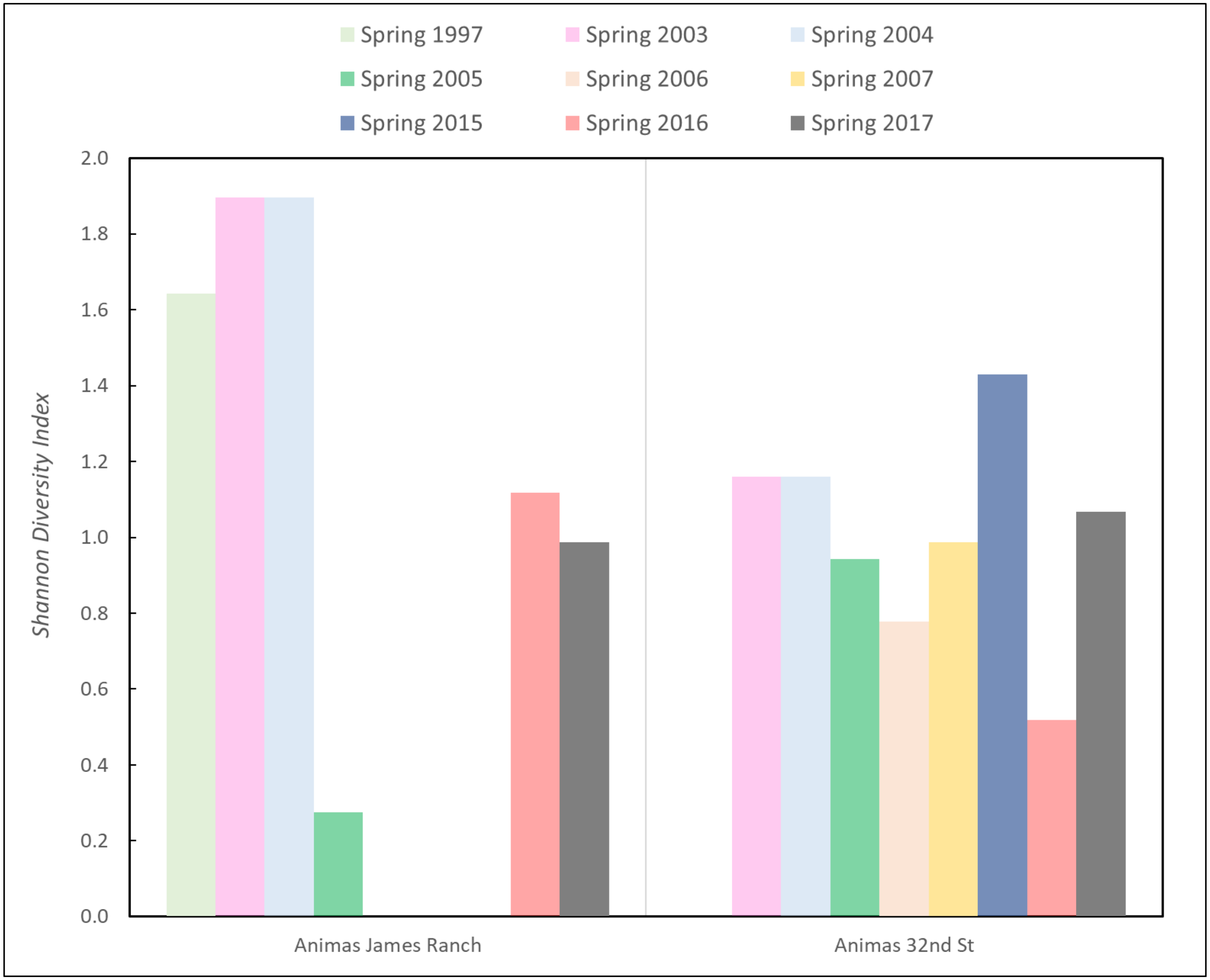


Figure 29: Shannon Diversity Index (SDI) – Spring 1997-2017

Note: see table 4 for an explanation of BMI metrics.

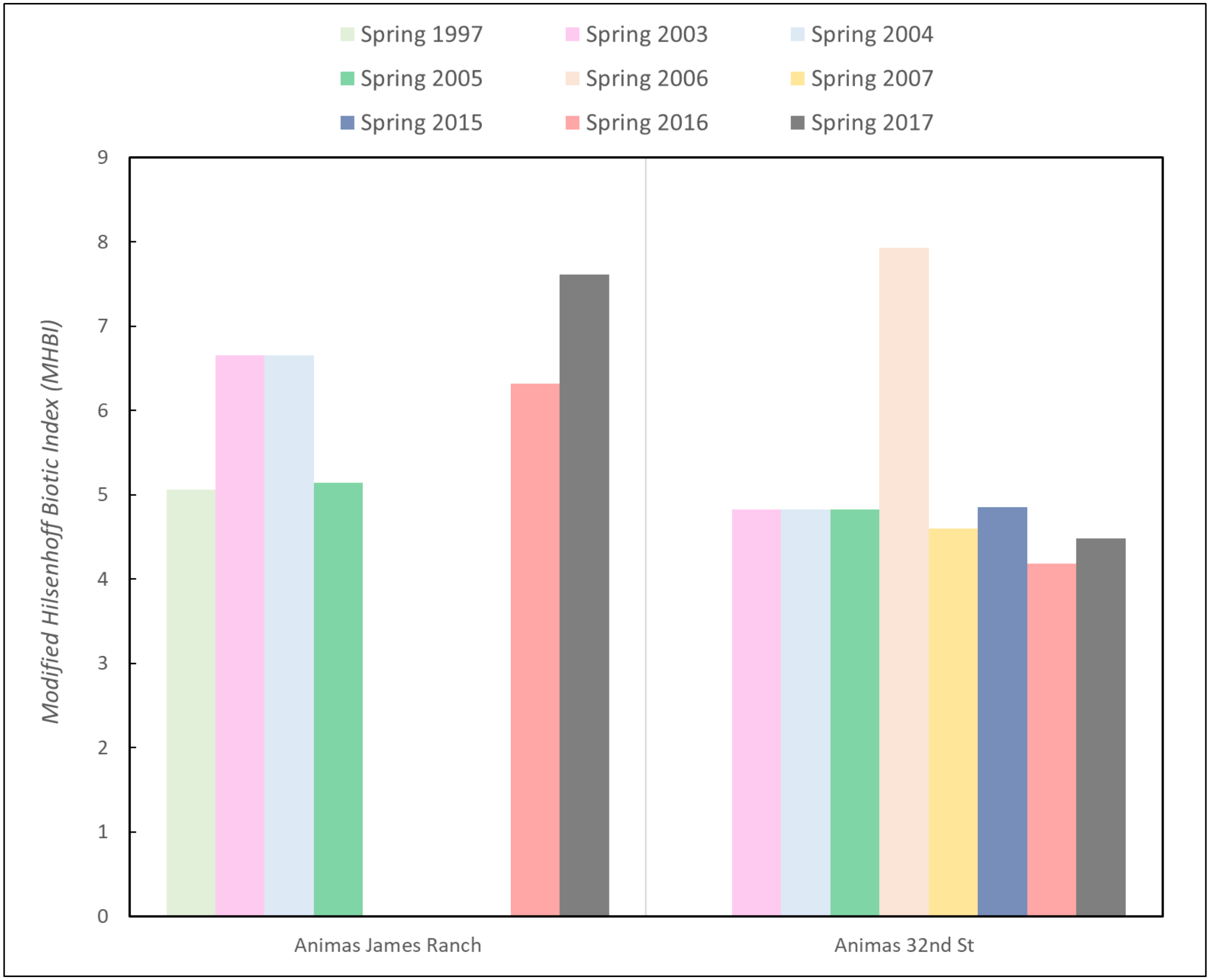


Figure 30: Modified Hilsenhoff Biotic Index (MHBI) – Spring 1997-2017

Note: see table 4 for an explanation of BMI metrics.

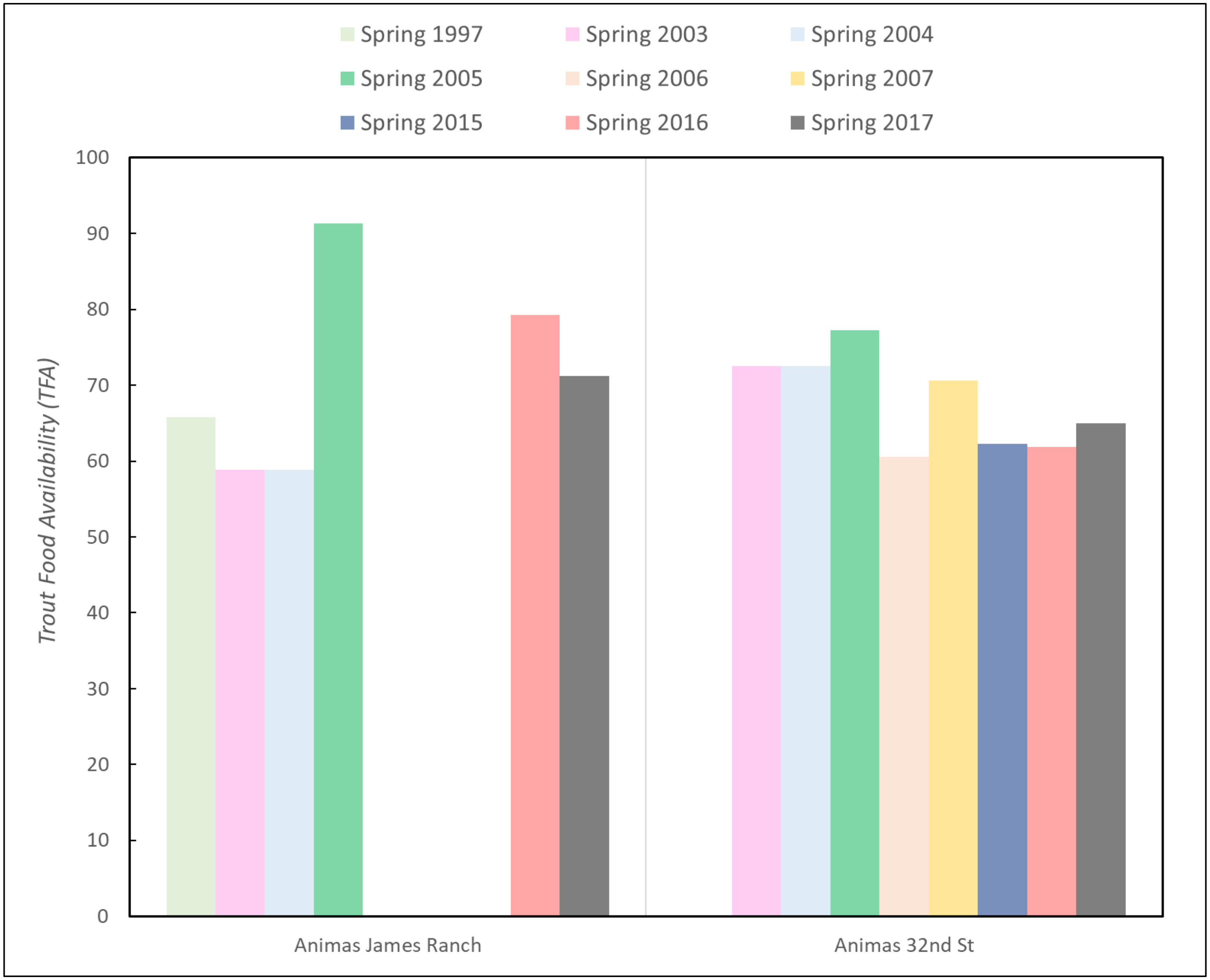


Figure 31: Trout Food Availability (TFA) – Spring 1997-2017

Note: see table 4 for an explanation of BMI metrics.

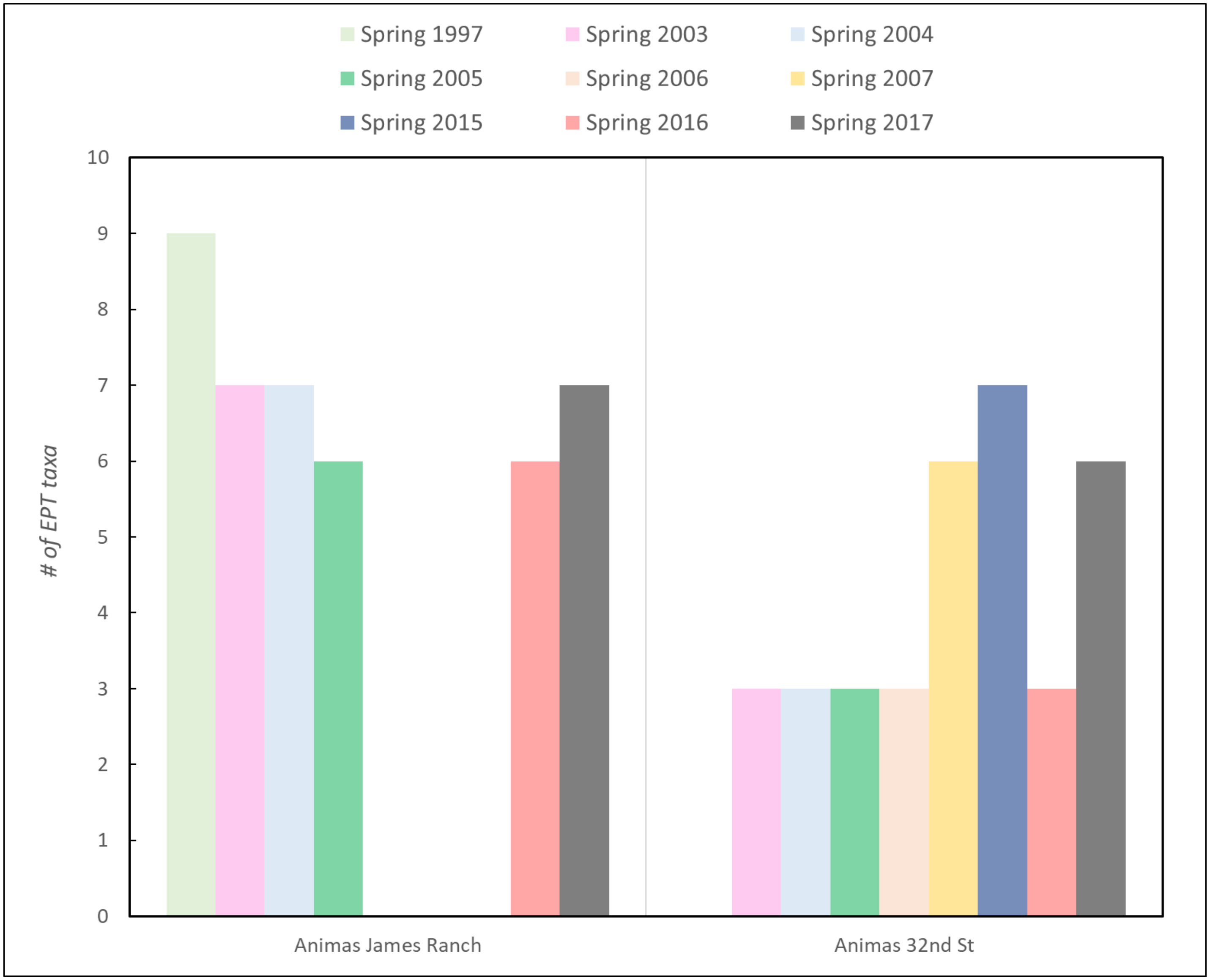


Figure 32: EPT taxa richness – Spring 1997-2017
Note: see table 4 for an explanation of BMI metrics.

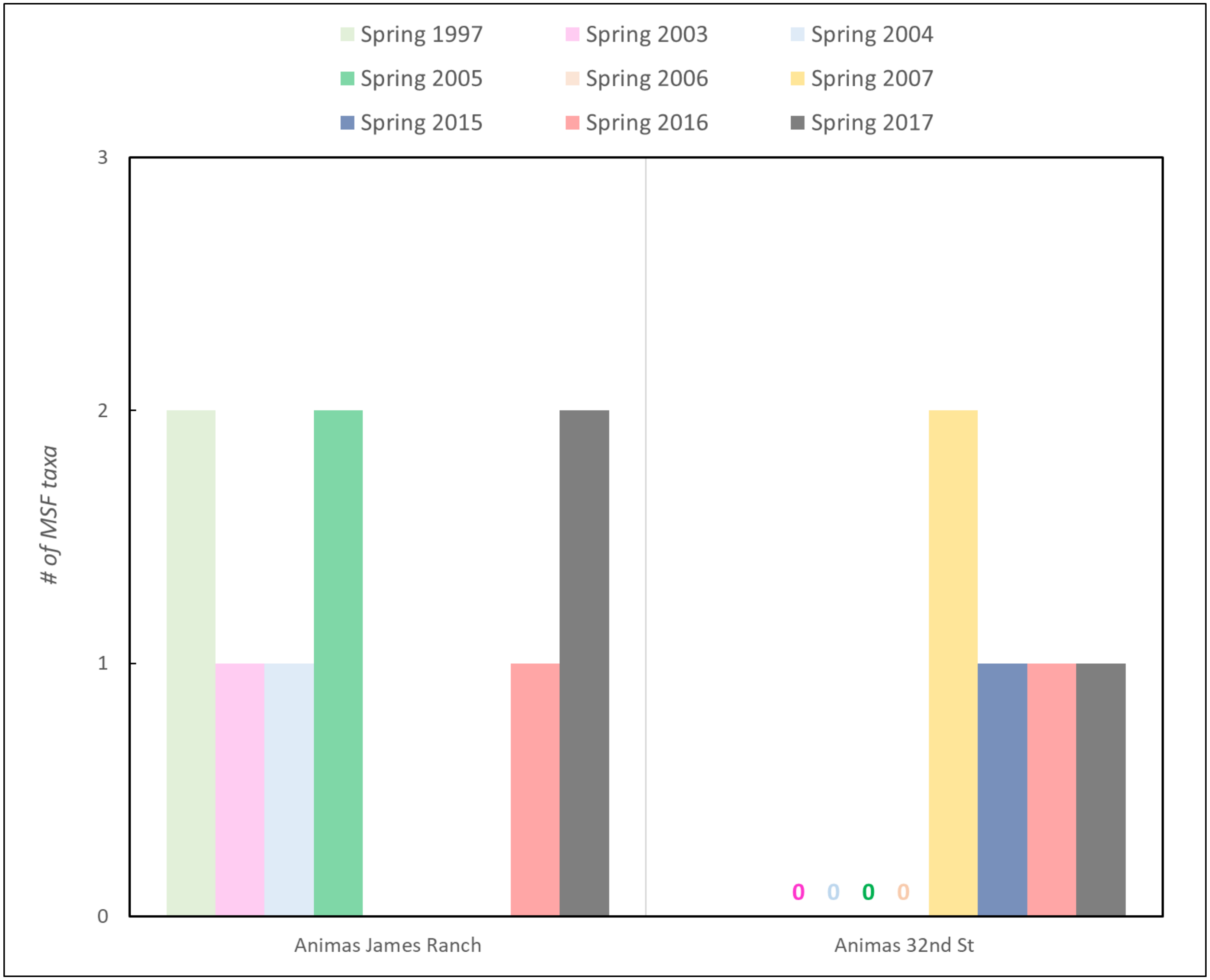


Figure 33: Metal-sensitive family (MSF) taxa richness – Spring 1997-2017

Note: 0 indicates that a sample was collected, but no MSF taxa were present; see table 4 for an explanation of BMI metrics.

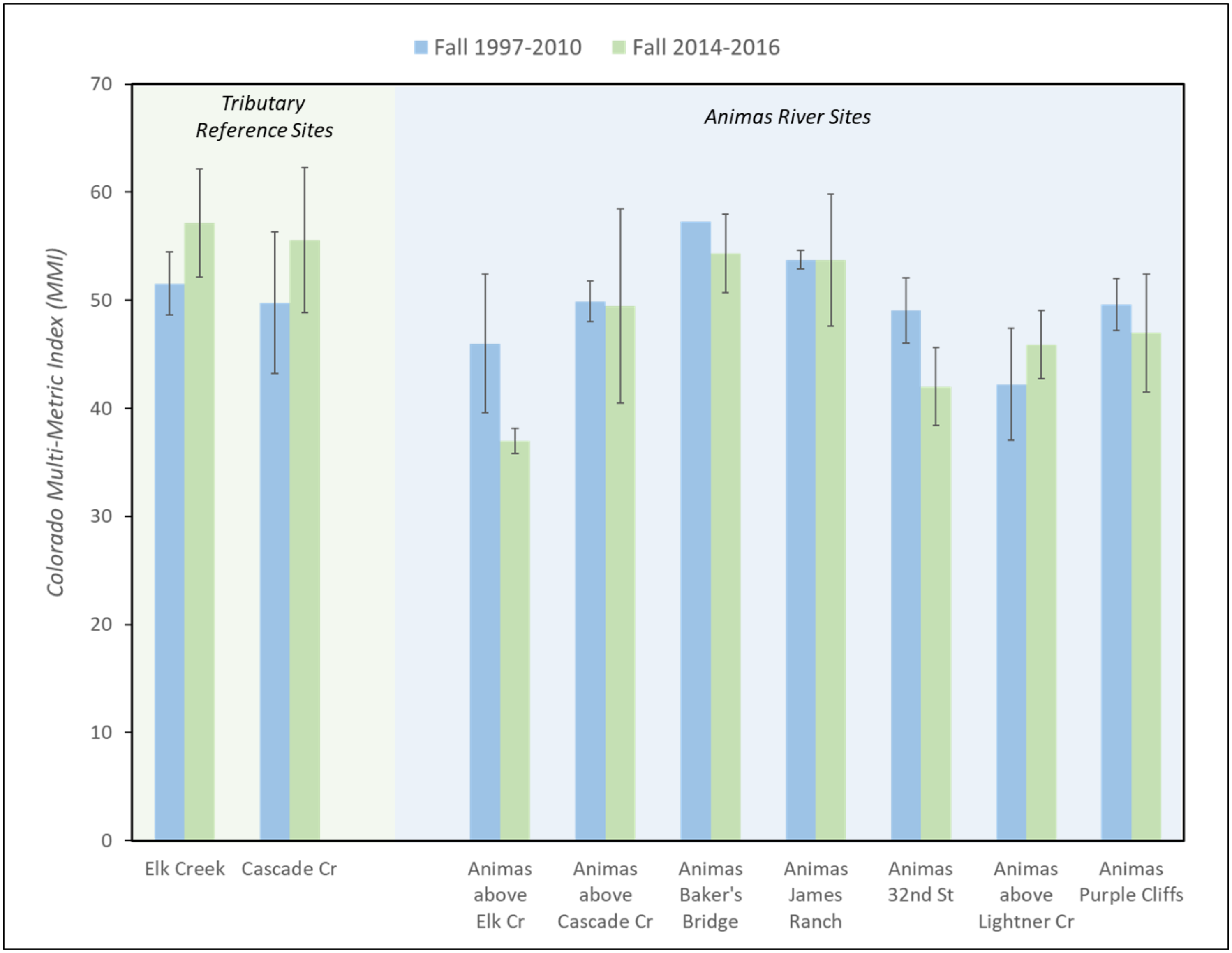


Figure 34: Colorado Multi-Metric Index (MMI) – Fall 1997-2010 compared to Fall 2014-2016

Note: See table 4 for an explanation of BMI metrics.

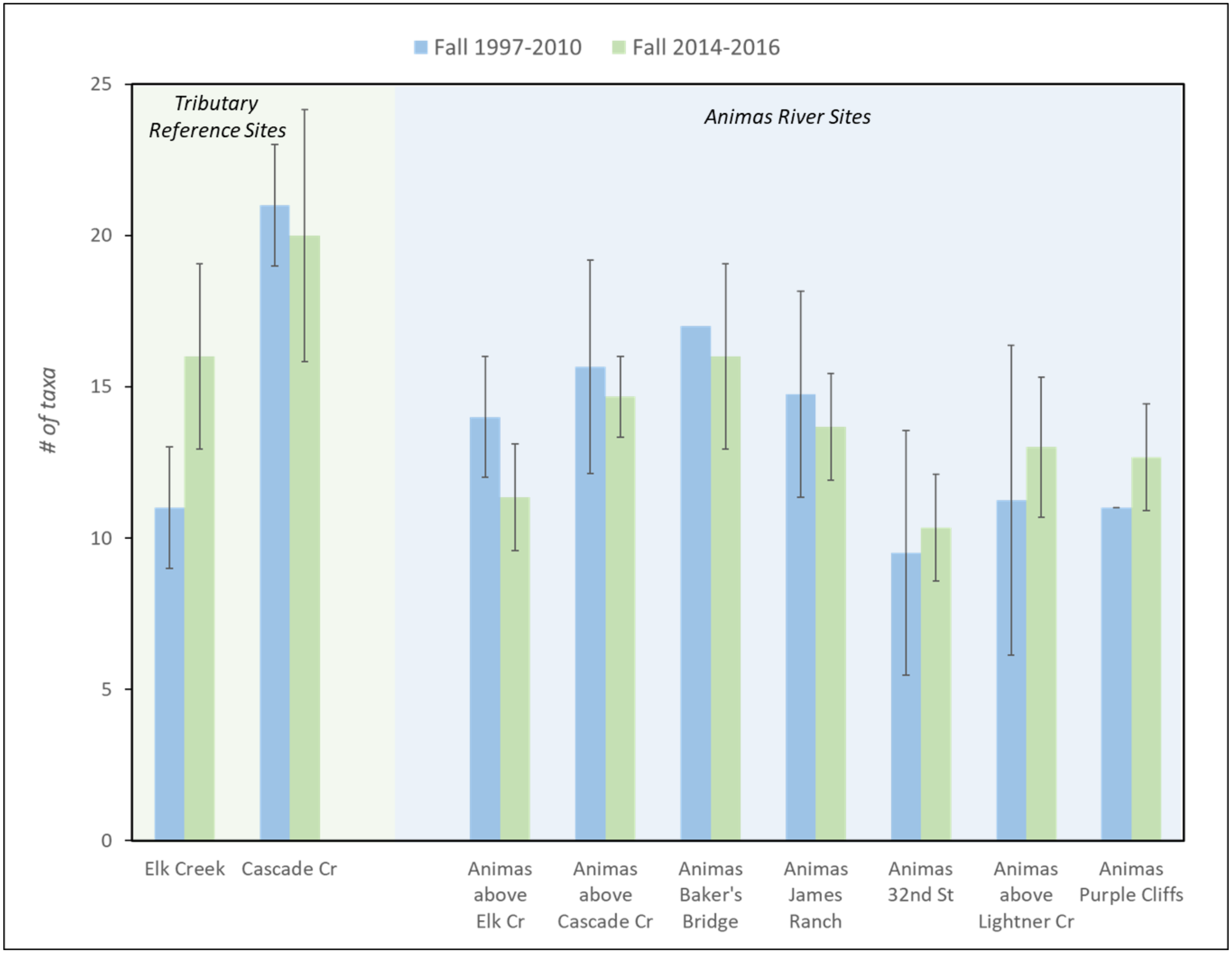


Figure 35: Taxa richness – Fall 1997-2010 compared to Fall 2014-2016

Note: see table 4 for an explanation of BMI metrics.

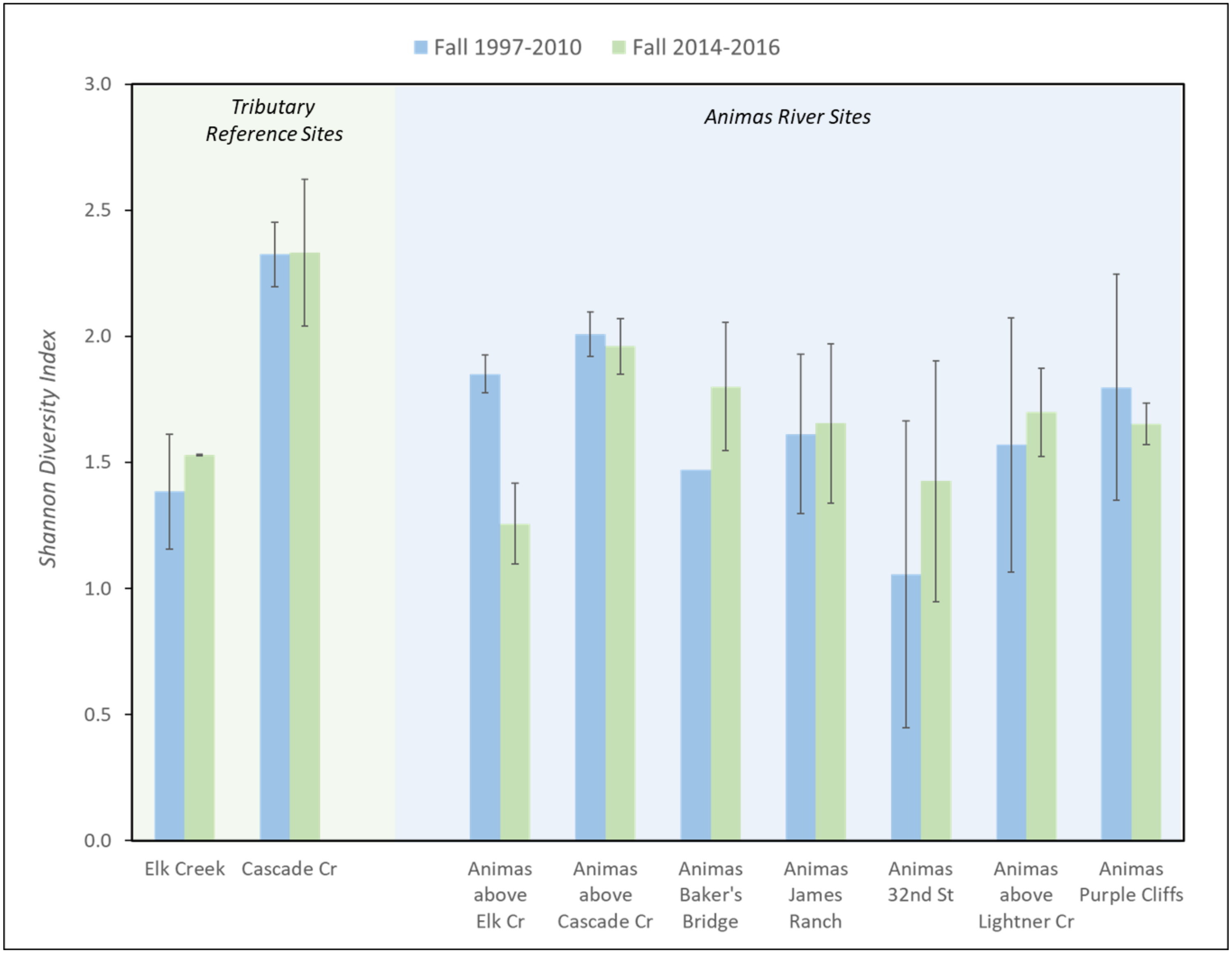


Figure 36: Shannon Diversity Index (SDI) – Fall 1997-2010 compared to Fall 2014-2016

Note: see table 4 for an explanation of BMI metrics.

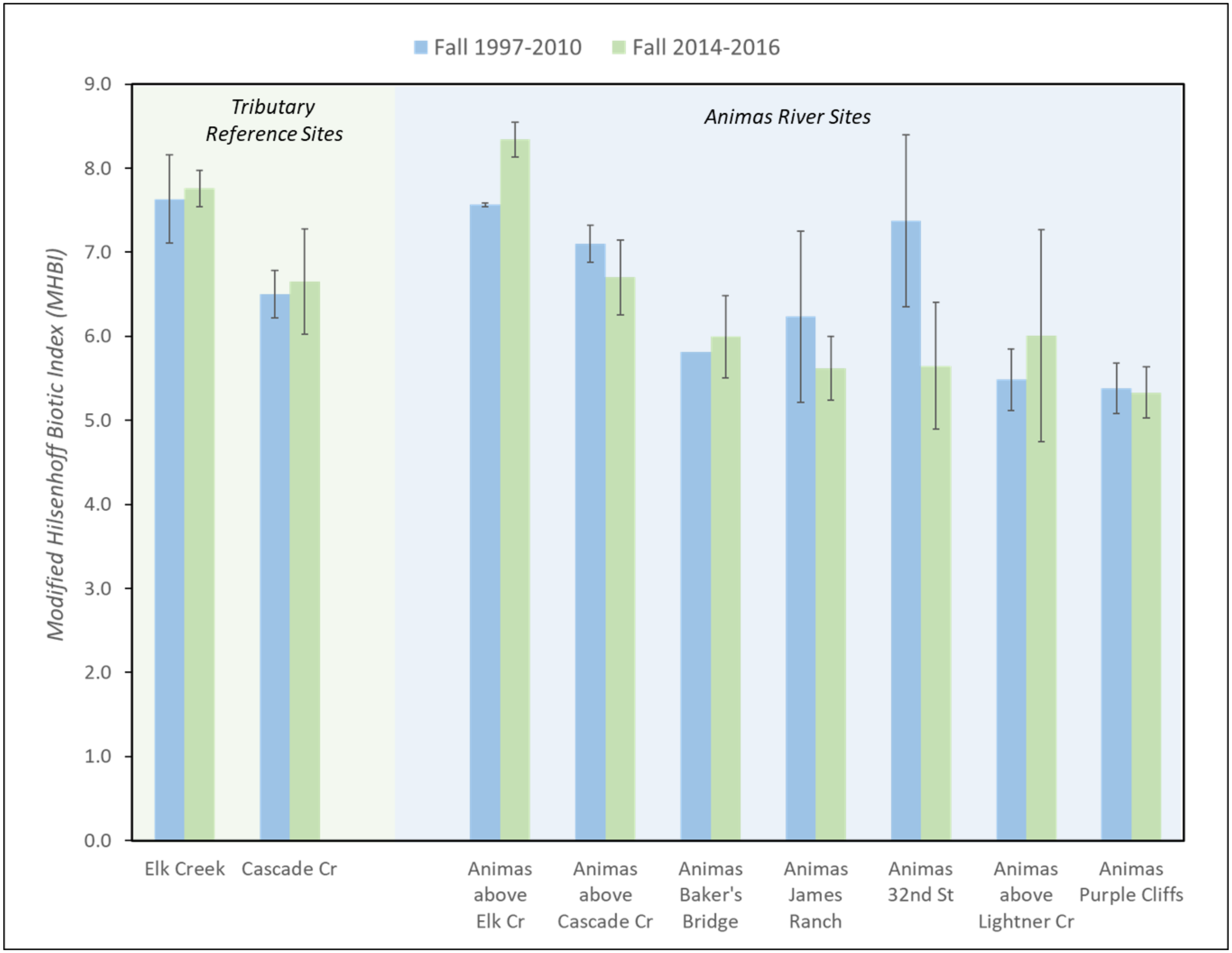


Figure 37: Modified Hilsenhoff Biotic Index (MHBI) – Fall 1997-2010 compared to Fall 2014-2016

Note: see table 4 for an explanation of BMI metrics.

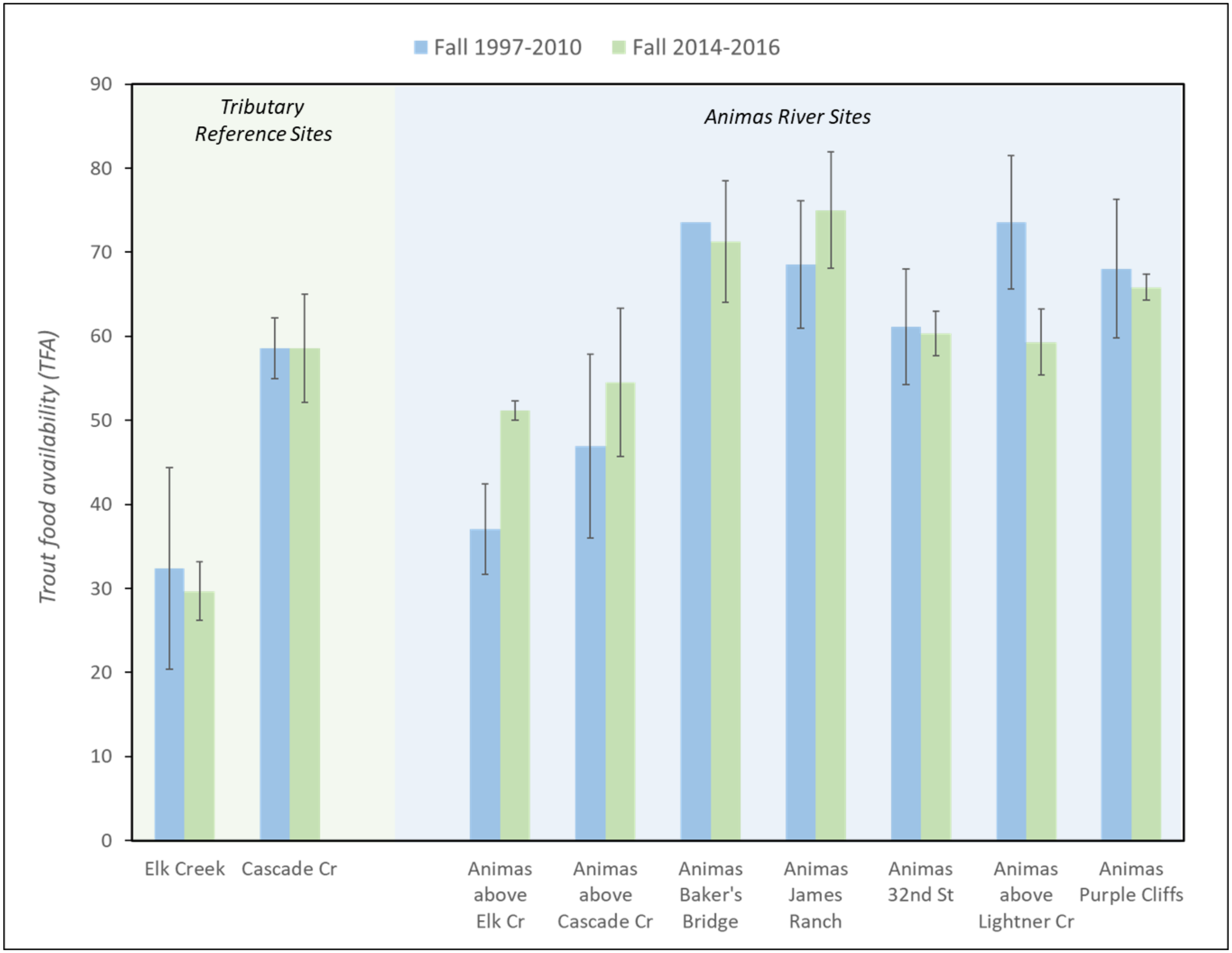


Figure 38: Trout Food Availability (TFA) – Fall 1997-2010 compared to Fall 2014-2016

Note: see table 4 for an explanation of BMI metrics.

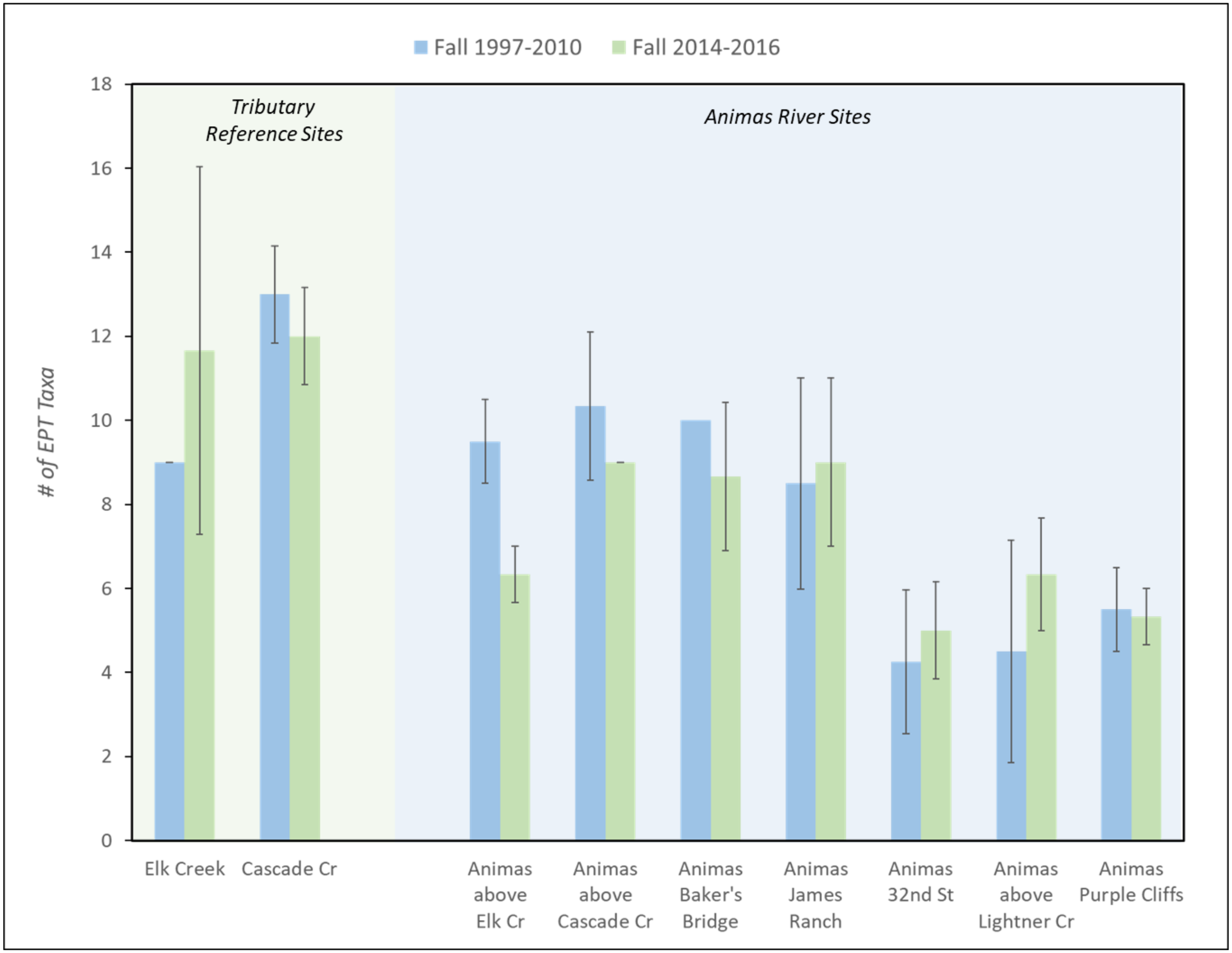


Figure 39: EPT taxa richness – Fall 1997-2010 compared to Fall 2014-2016
Note: see table 4 for an explanation of BMI metrics.

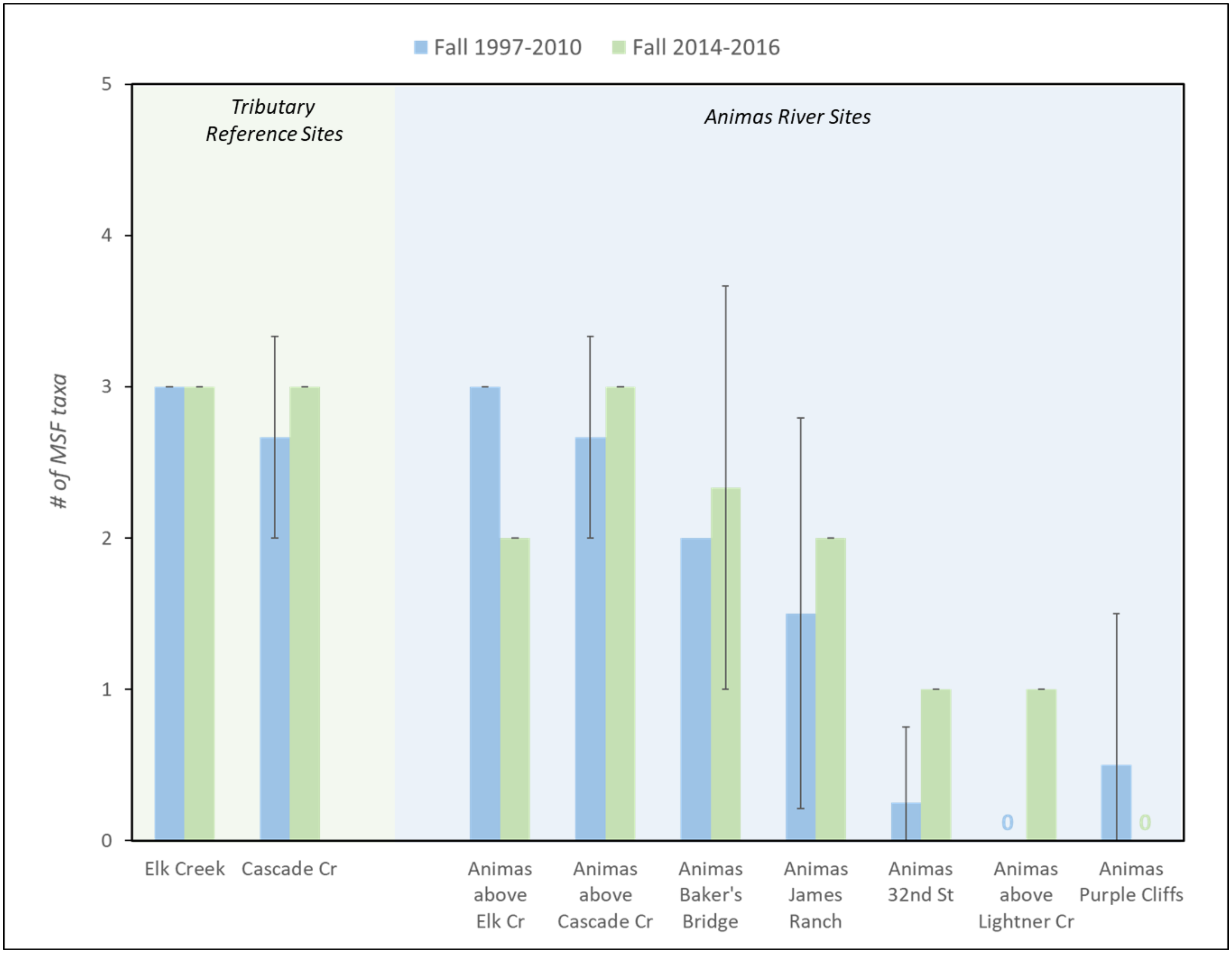


Figure 40: Metal-sensitive family (MSF) taxa richness – Fall 1997-2010 compared to Fall 2014-2016

Note: 0 indicates that a sample was collected, but no MSF taxa were present; see table 4 for an explanation of BMI metrics.

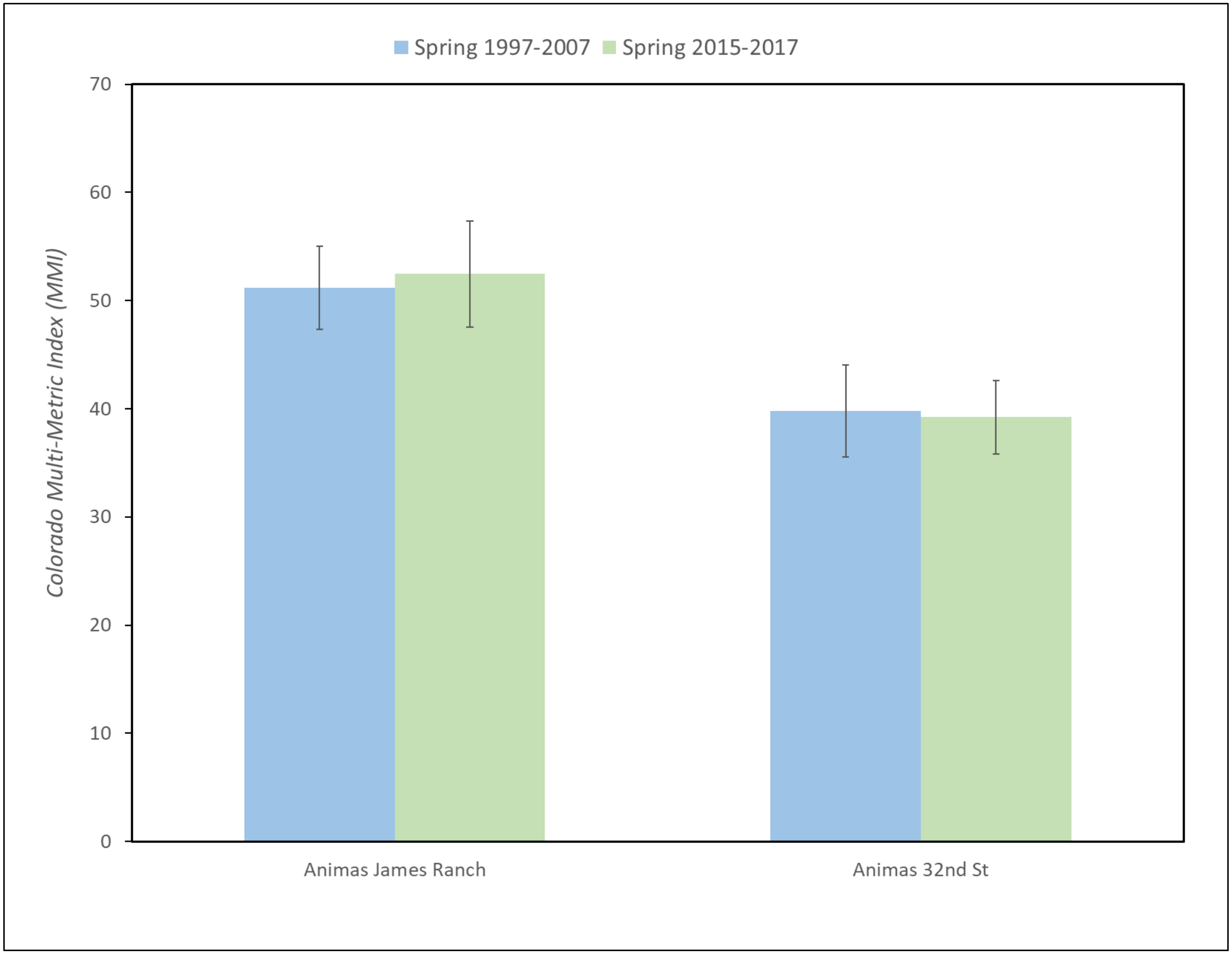


Figure 41: Colorado Multi-Metric Index (MMI) – Spring 1997-2007 compared to Spring 2015-2017
Note: See table 4 for an explanation of BMI metrics.

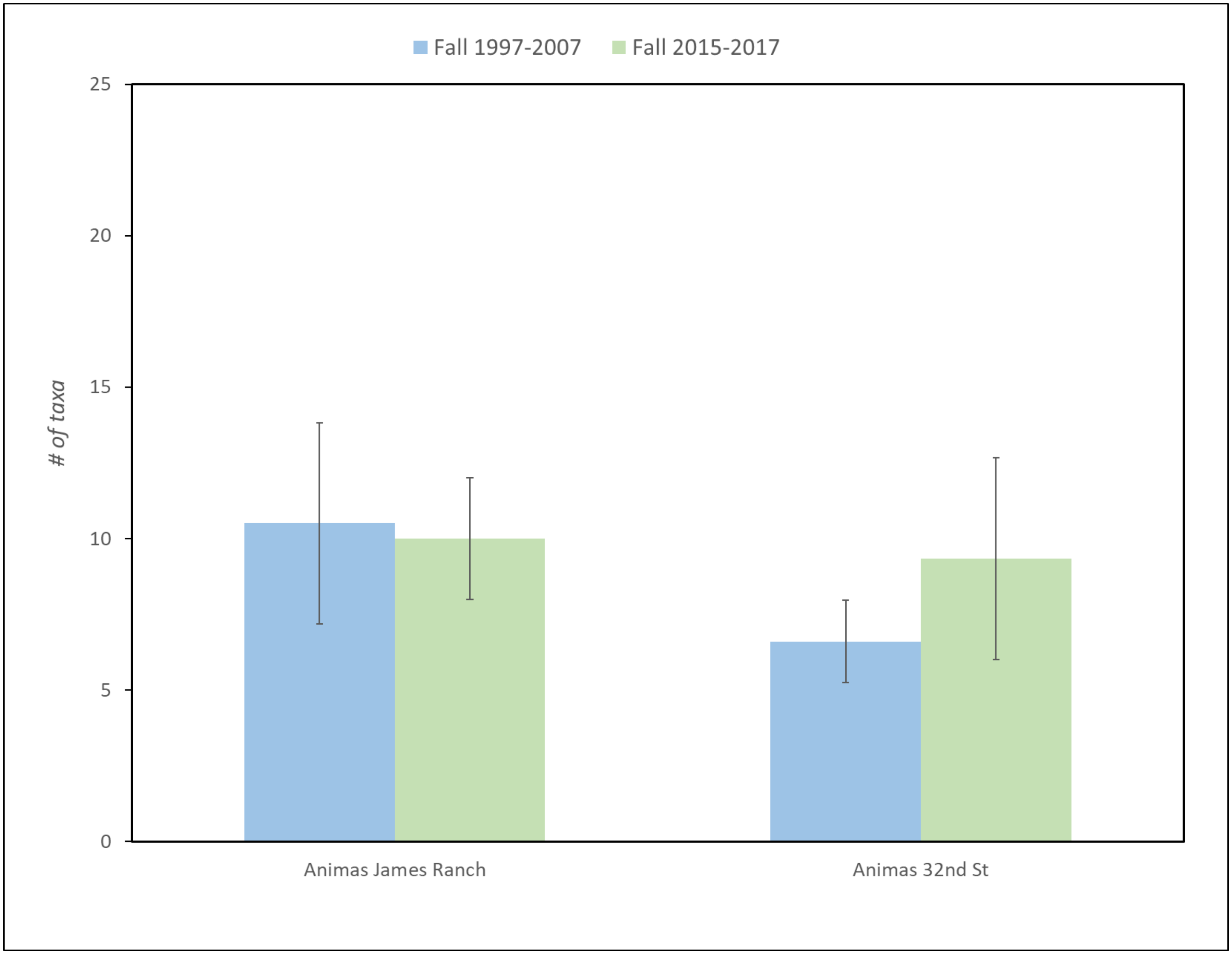


Figure 42: Taxa richness – Spring 1997-2007 compared to Spring 2015-2017

Note: see table 4 for an explanation of BMI metrics.

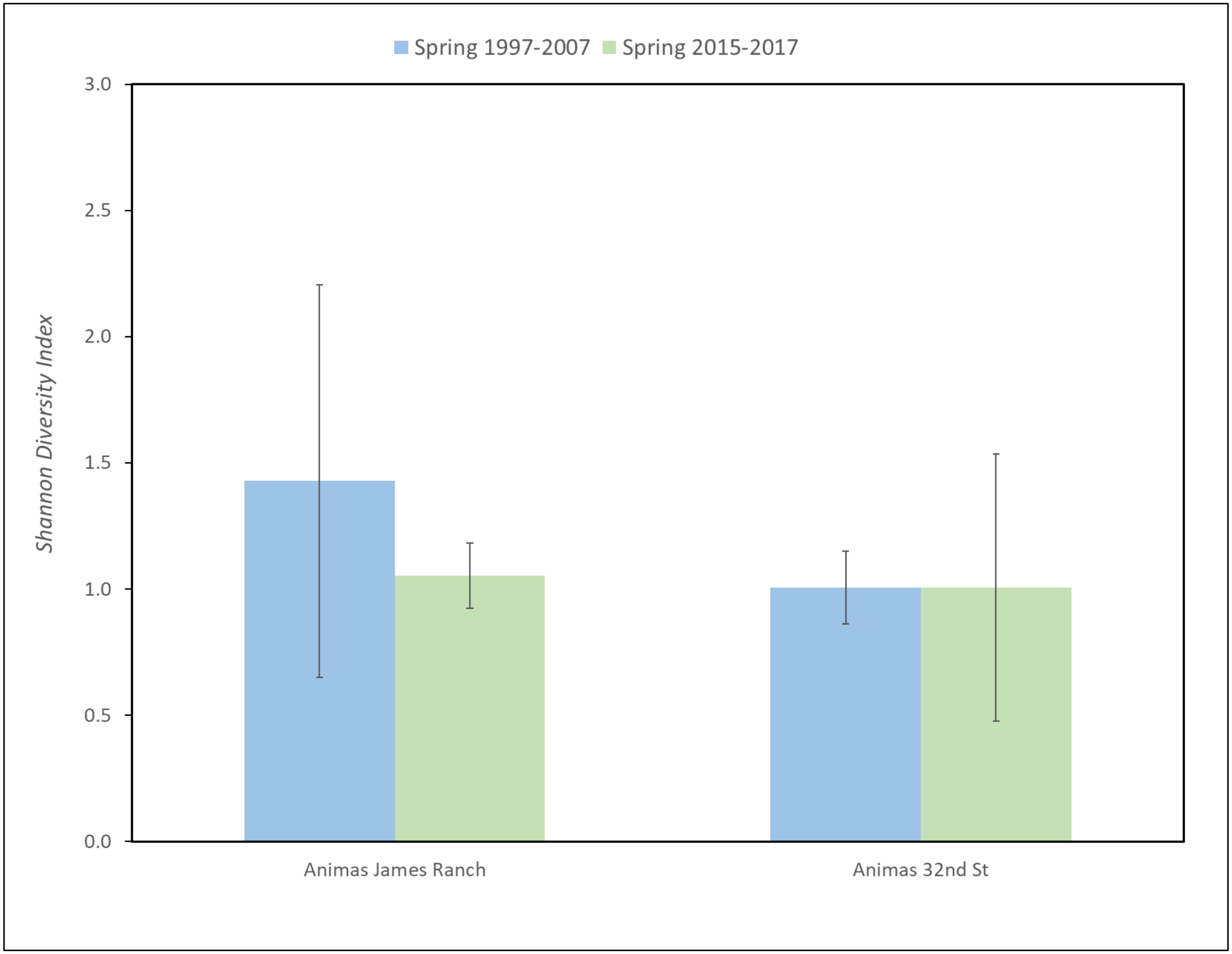


Figure 43: Shannon Diversity Index (SDI) – Spring 1997-2007 compared to Spring 2015-2017
Note: see table 4 for an explanation of BMI metrics.

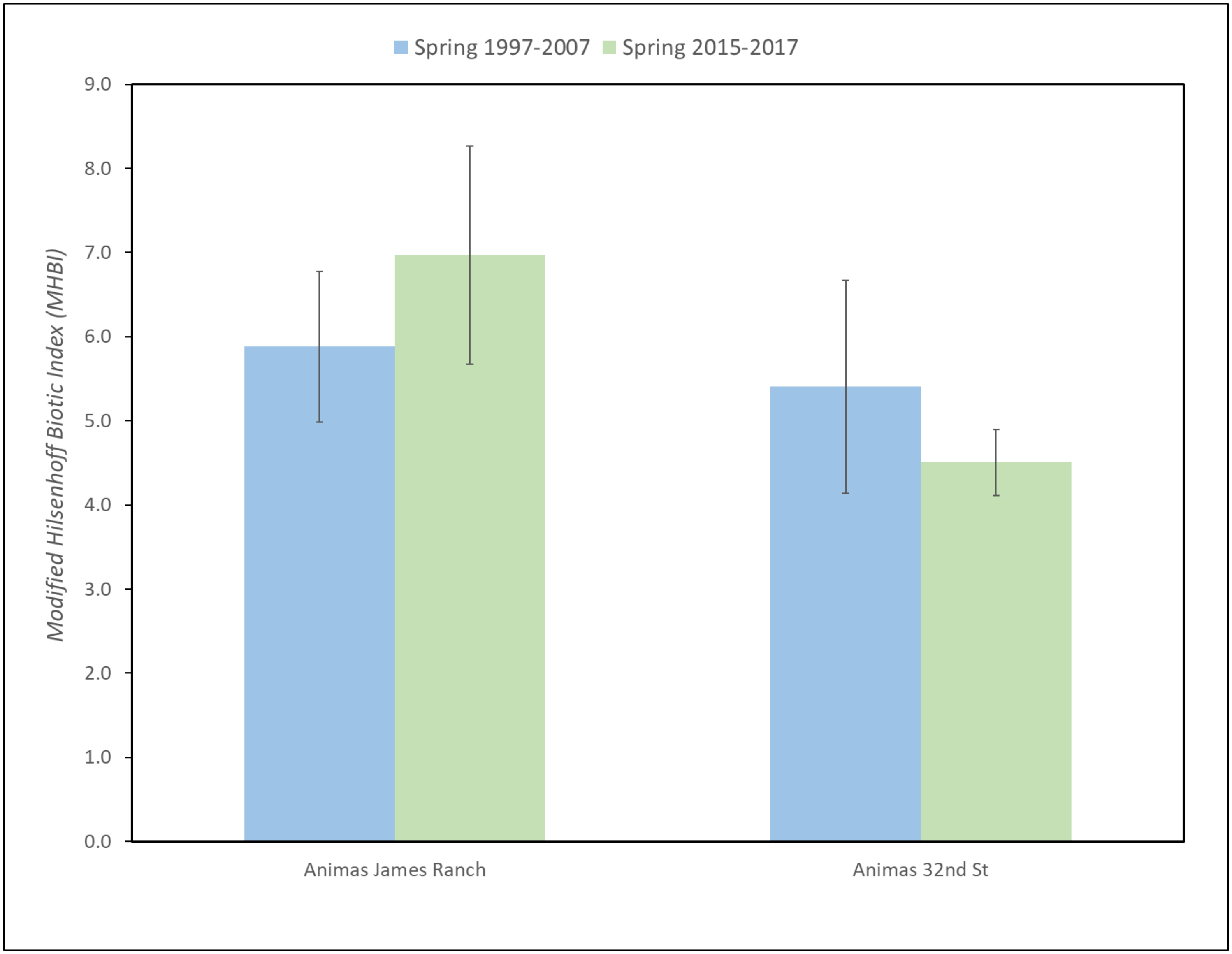


Figure 44: Modified Hilsenhoff Biotic Index (MHBI) – Spring 1997-2007 compared to Spring 2015-2017
Note: see table 4 for an explanation of BMI metrics.

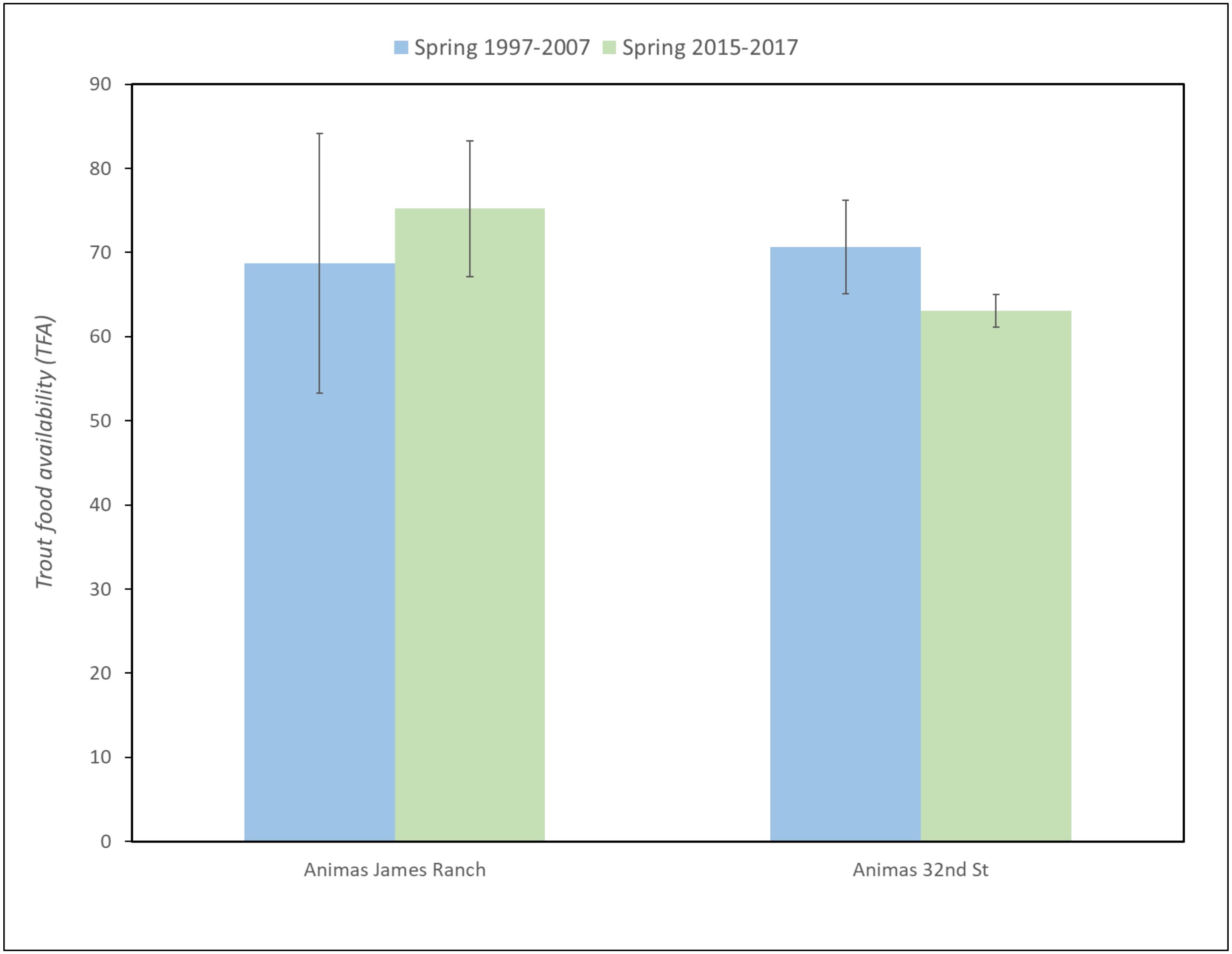


Figure 45: Trout Food Availability (TFA) – Spring 1997-2007 compared to Spring 2015-2017

Note: see table 4 for an explanation of BMI metrics.

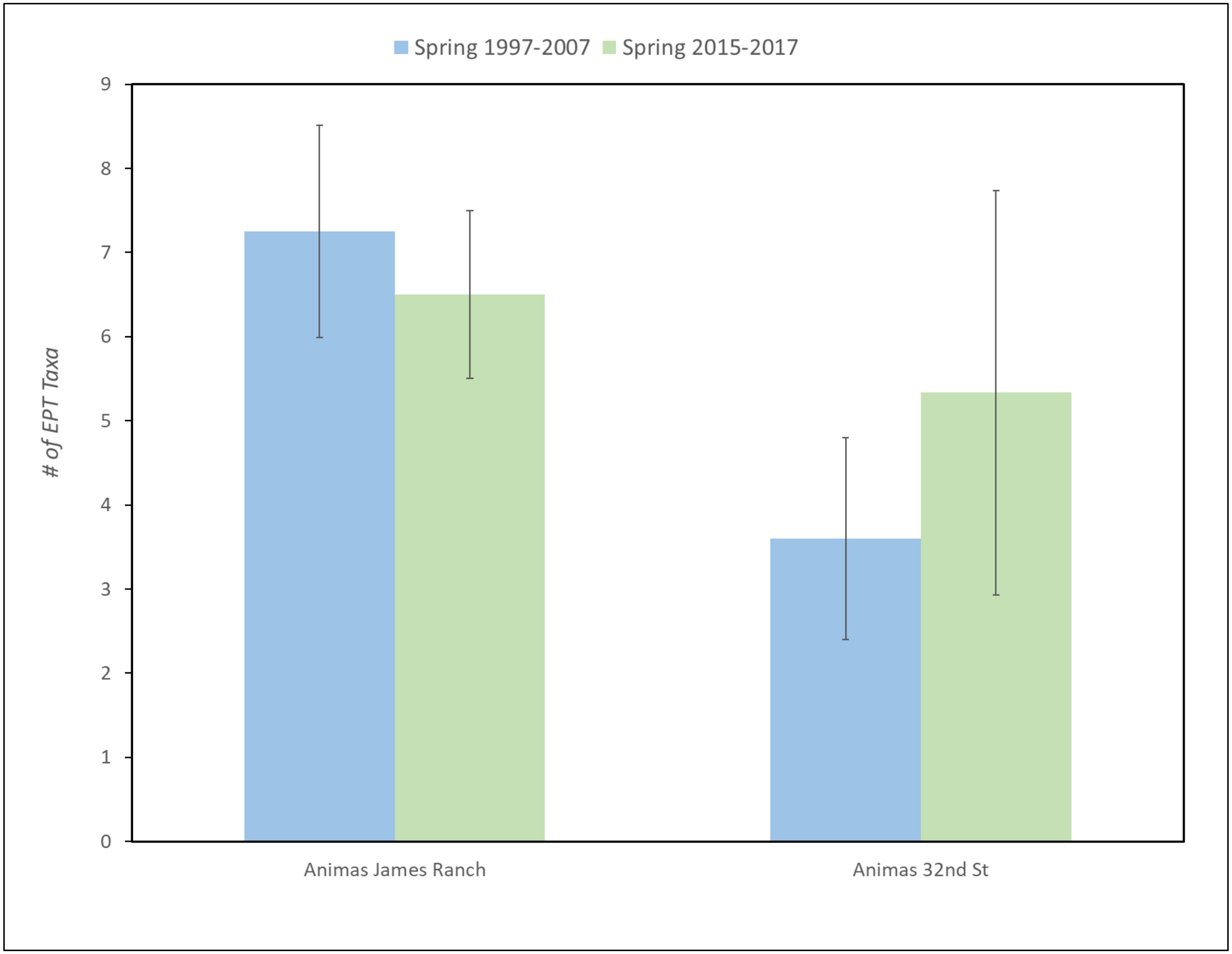


Figure 46: EPT taxa richness – Spring 1997-2007 compared to Spring 2015-2017
Note: see table 4 for an explanation of BMI metrics.

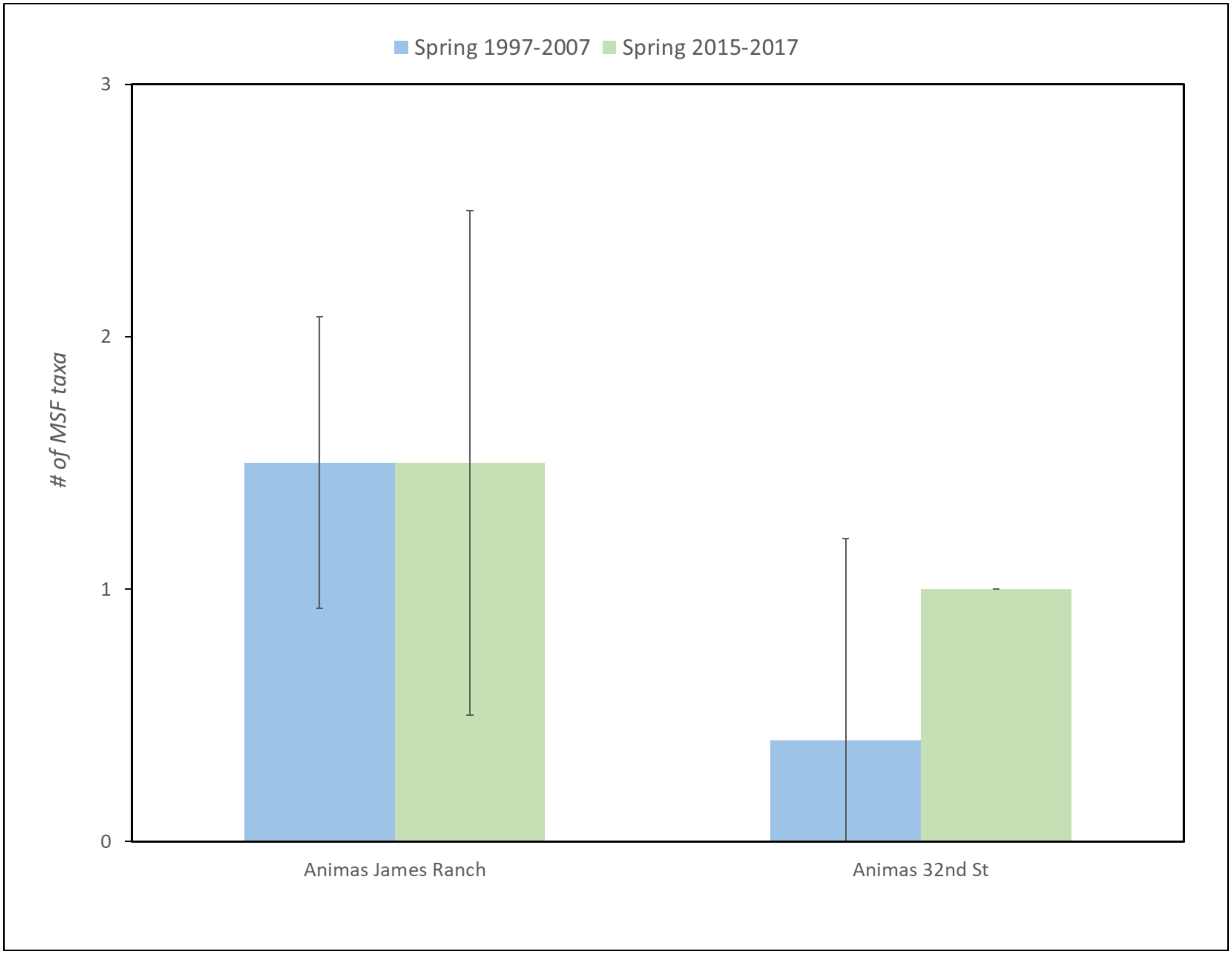


Figure 47: Metal-sensitive family (MSF) taxa richness – Spring 1997-2007 compared to Spring 2015-2017
Note: see table 4 for an explanation of BMI metrics.

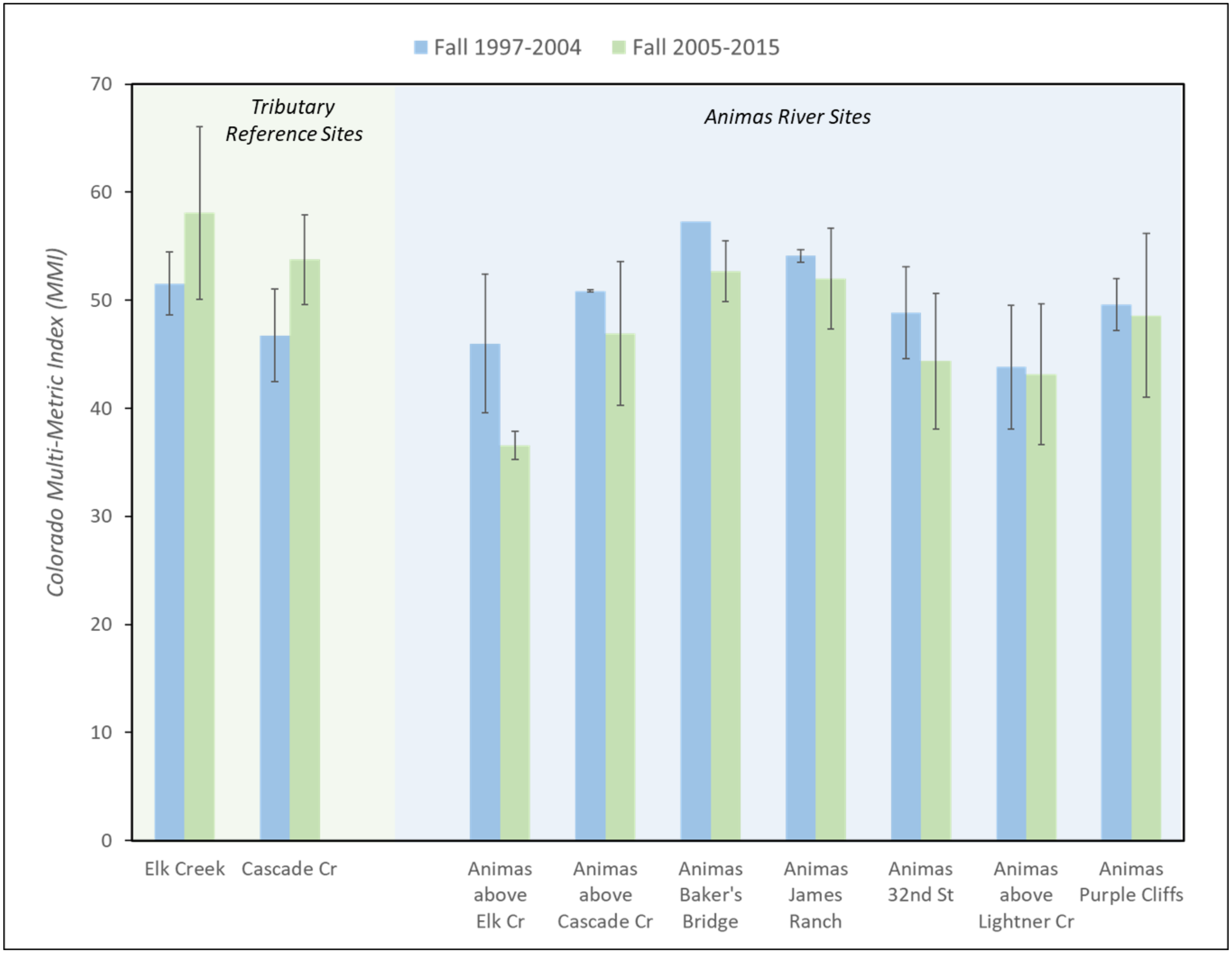


Figure 48: Colorado Multi-Metric Index (MMI) – Fall 1997-2004 compared to Fall 2005-2015

Note: See table 4 for an explanation of BMI metrics.

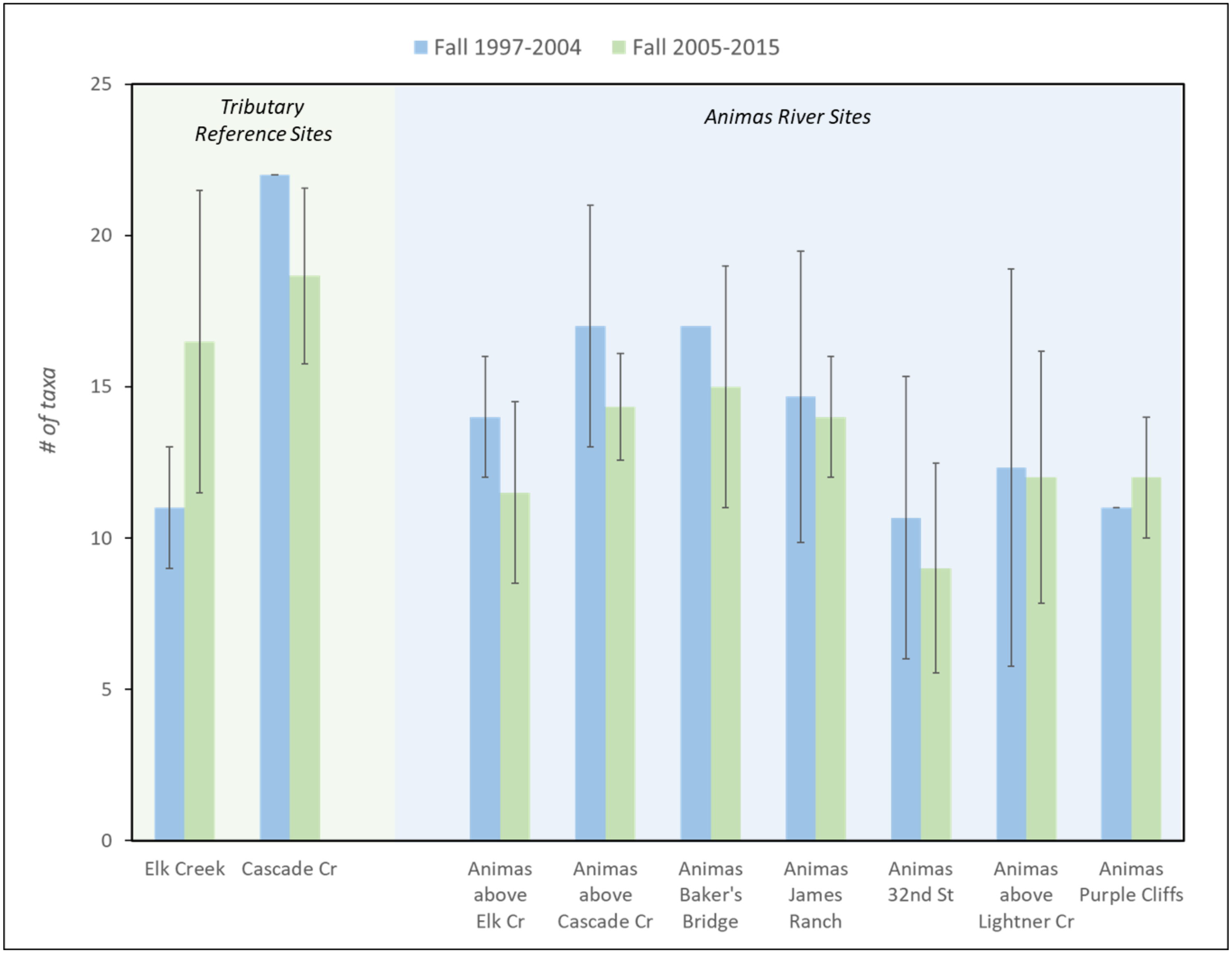


Figure 49: Taxa richness – Fall 1997-2004 compared to Fall 2005-2015

Note: see table 4 for an explanation of BMI metrics.

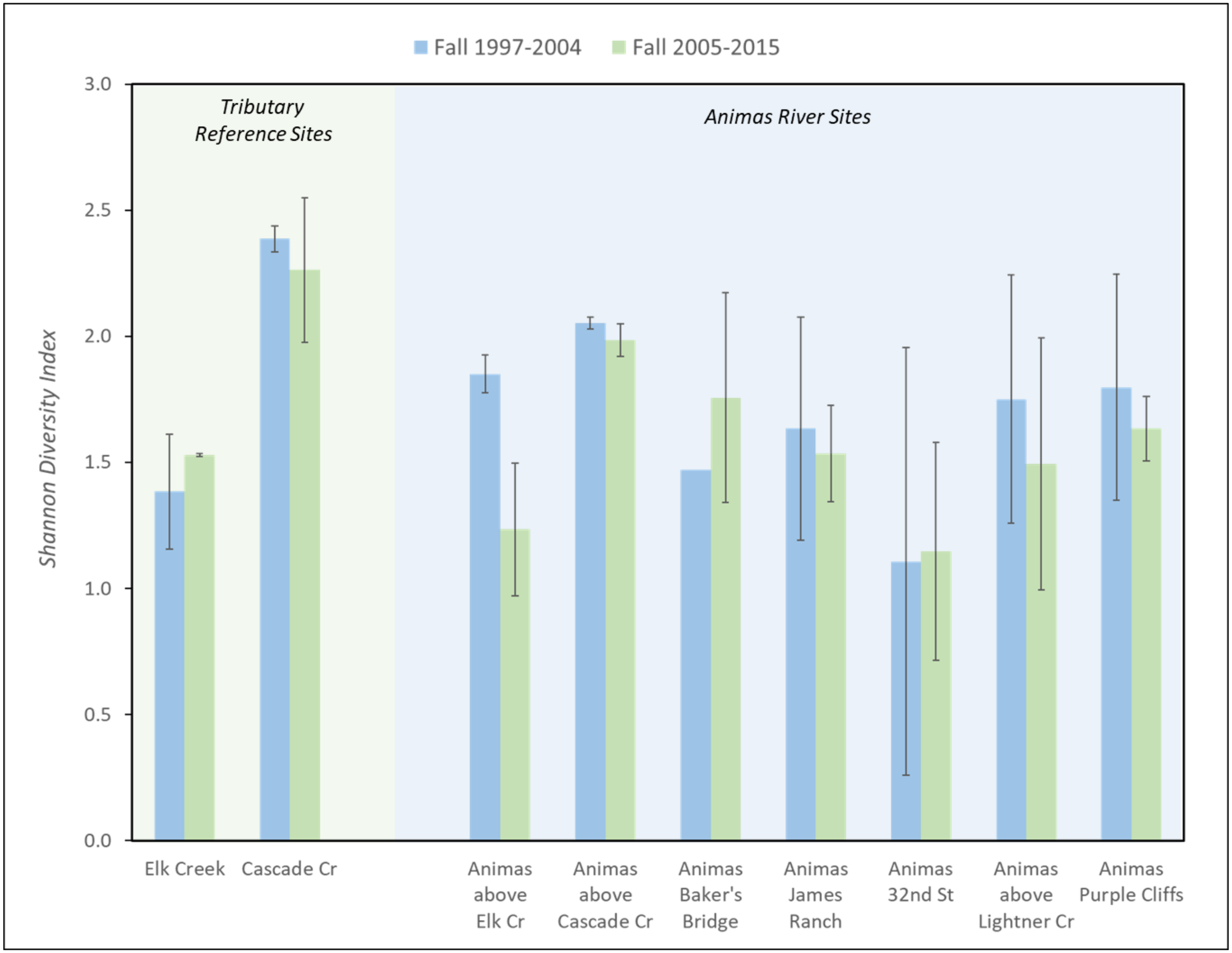


Figure 50: Shannon Diversity Index (SDI) – Fall 1997-2004 compared to Fall 2005-2015

Note: see table 4 for an explanation of BMI metrics.

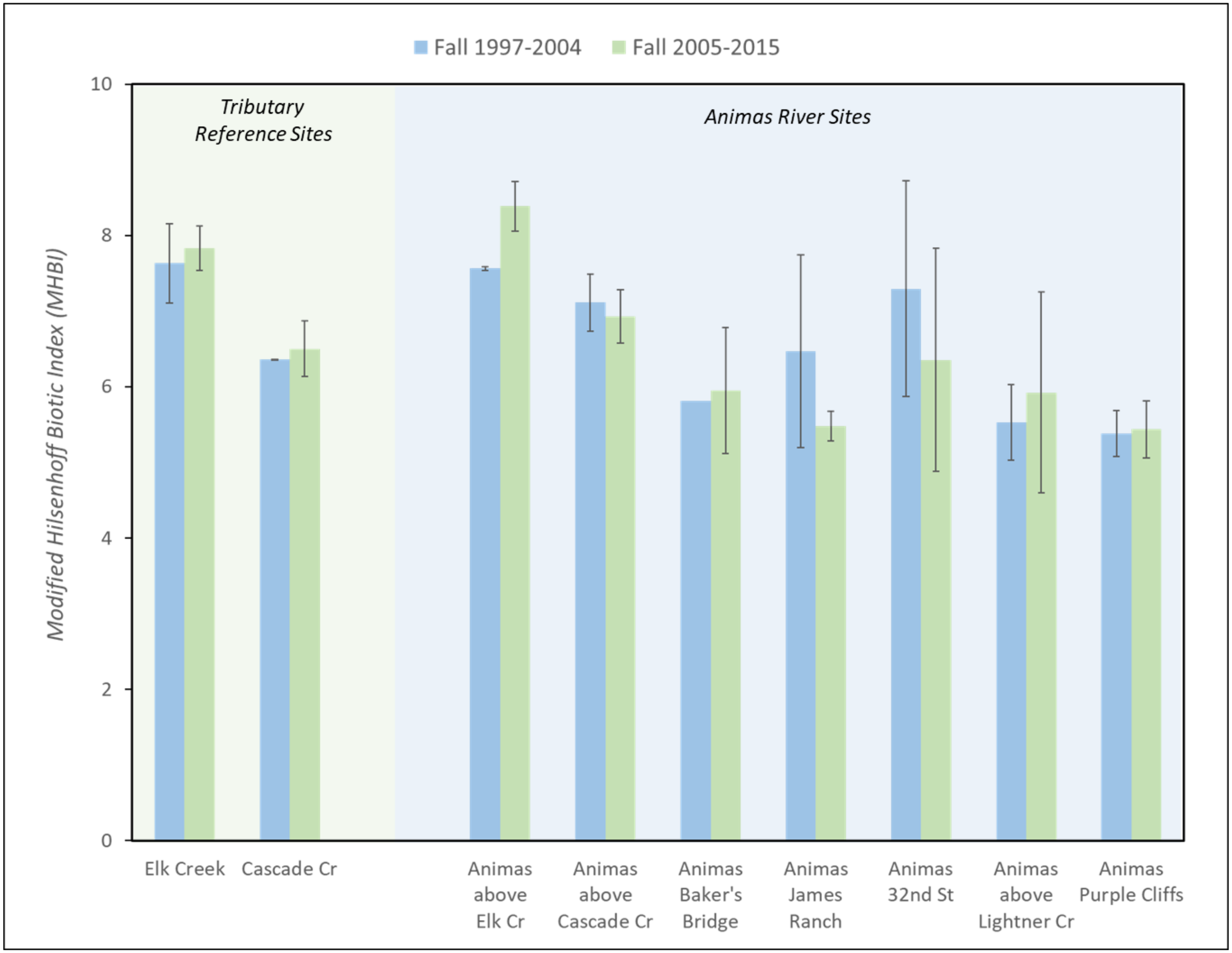


Figure 51: Modified Hilsenhoff Biotic Index (MHBI) – Fall 1997-2004 compared to Fall 2005-2015

Note: see table 4 for an explanation of BMI metrics.

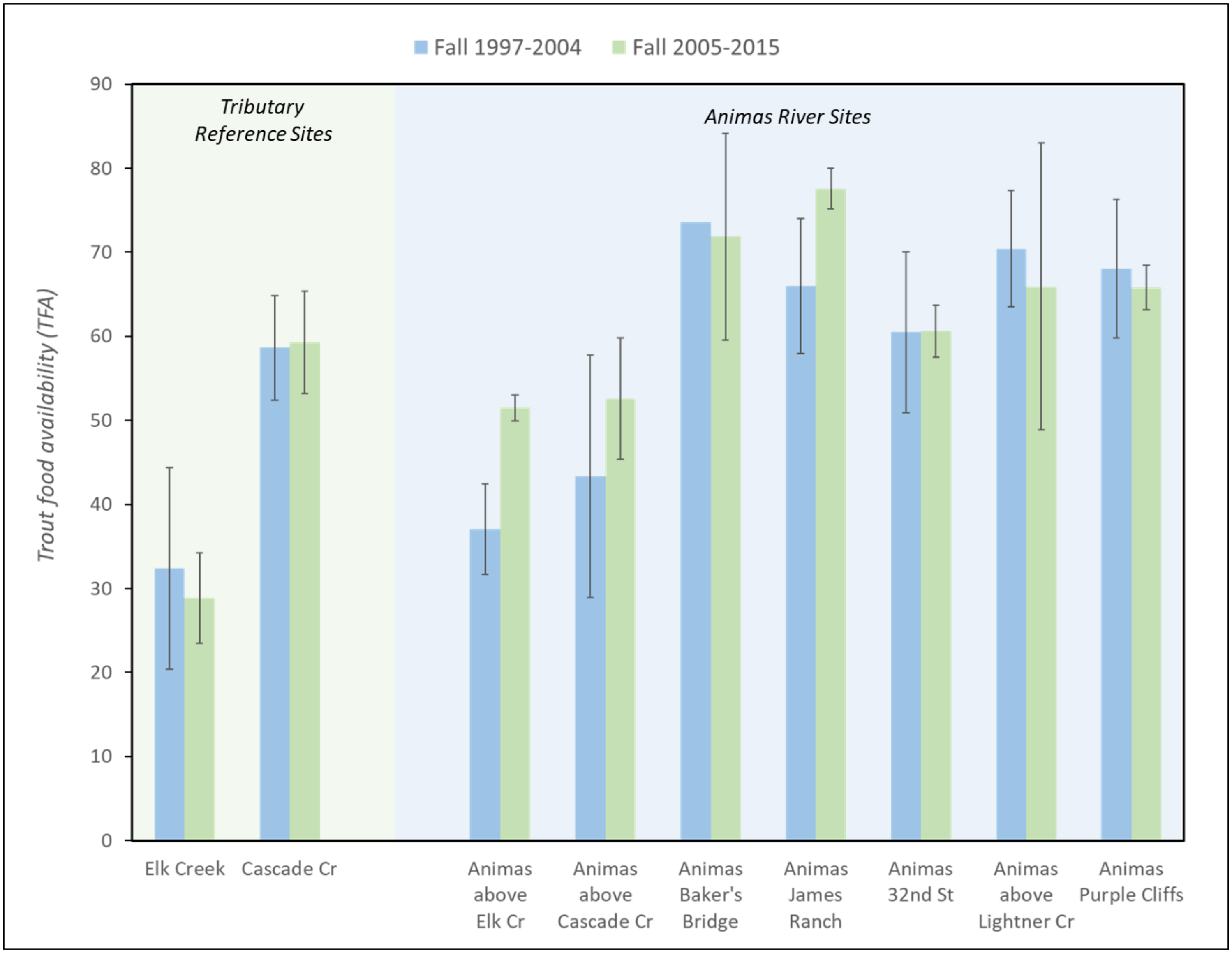


Figure 52: Trout Food Availability (TFA) – Fall 1997-2004 compared to Fall 2005-2015
Note: see table 4 for an explanation of BMI metrics.

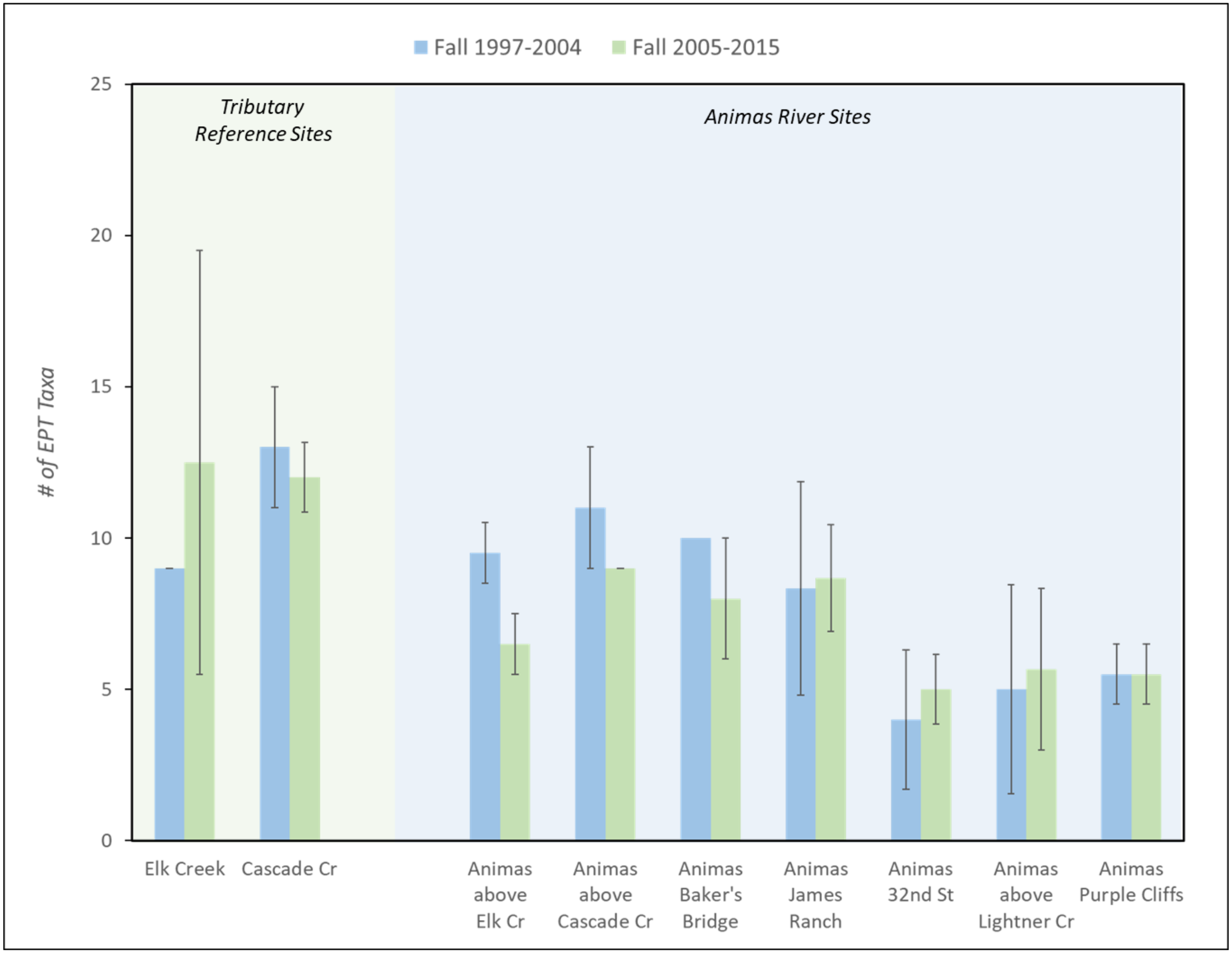


Figure 53: EPT taxa richness – Fall 1997-2004 compared to Fall 2005-2015

Note: see table 4 for an explanation of BMI metrics.

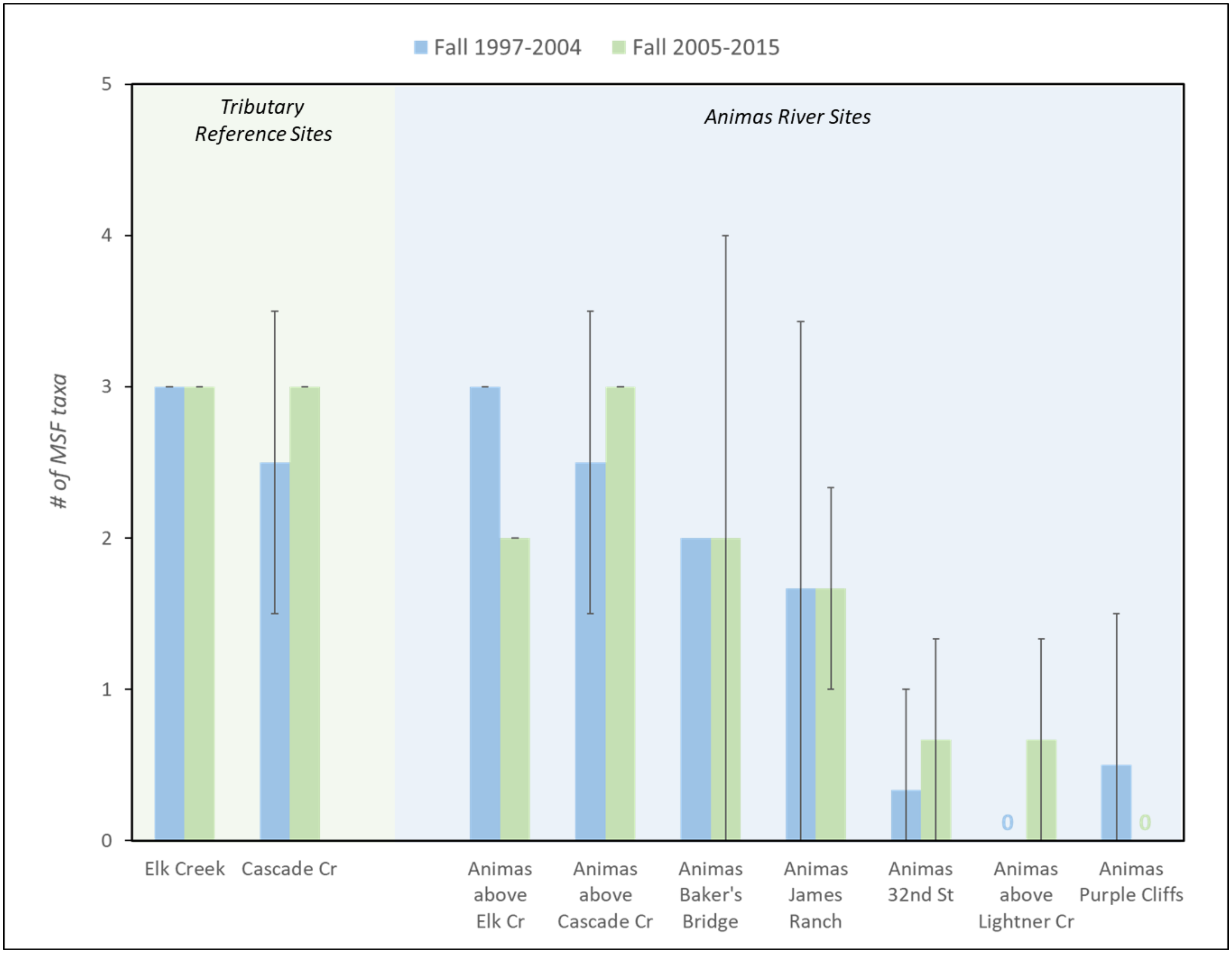


Figure 54: Metal-sensitive family (MSF) taxa richness – Fall 1997-2004 compared to Fall 2005-2015

Note: 0 indicates that a sample was collected, but no MSF taxa were present; see table 4 for an explanation of BMI metrics.

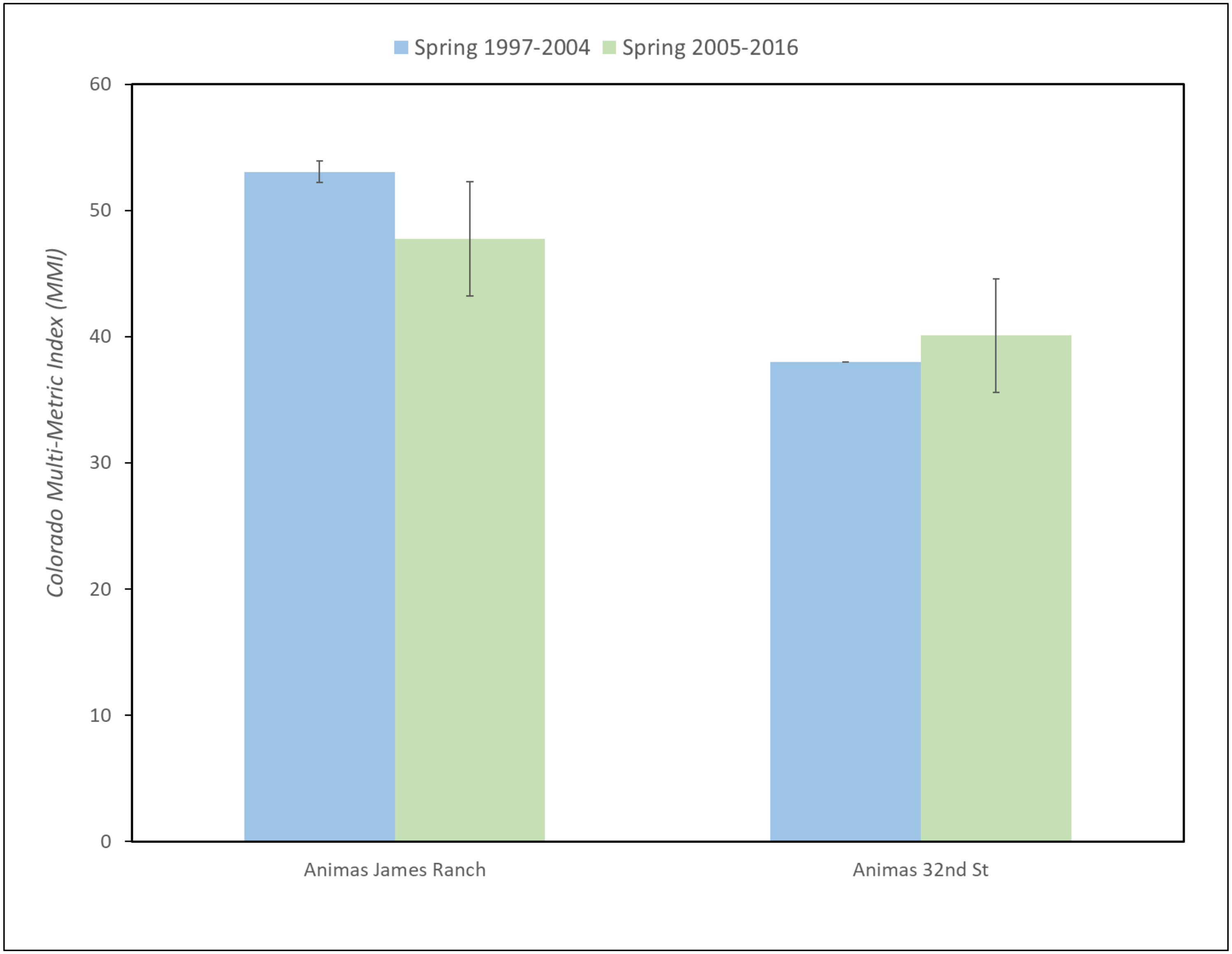


Figure 55: Colorado Multi-Metric Index (MMI) – Spring 1997-2004 compared to Spring 2005-2016
Note: See table 4 for an explanation of BMI metrics.

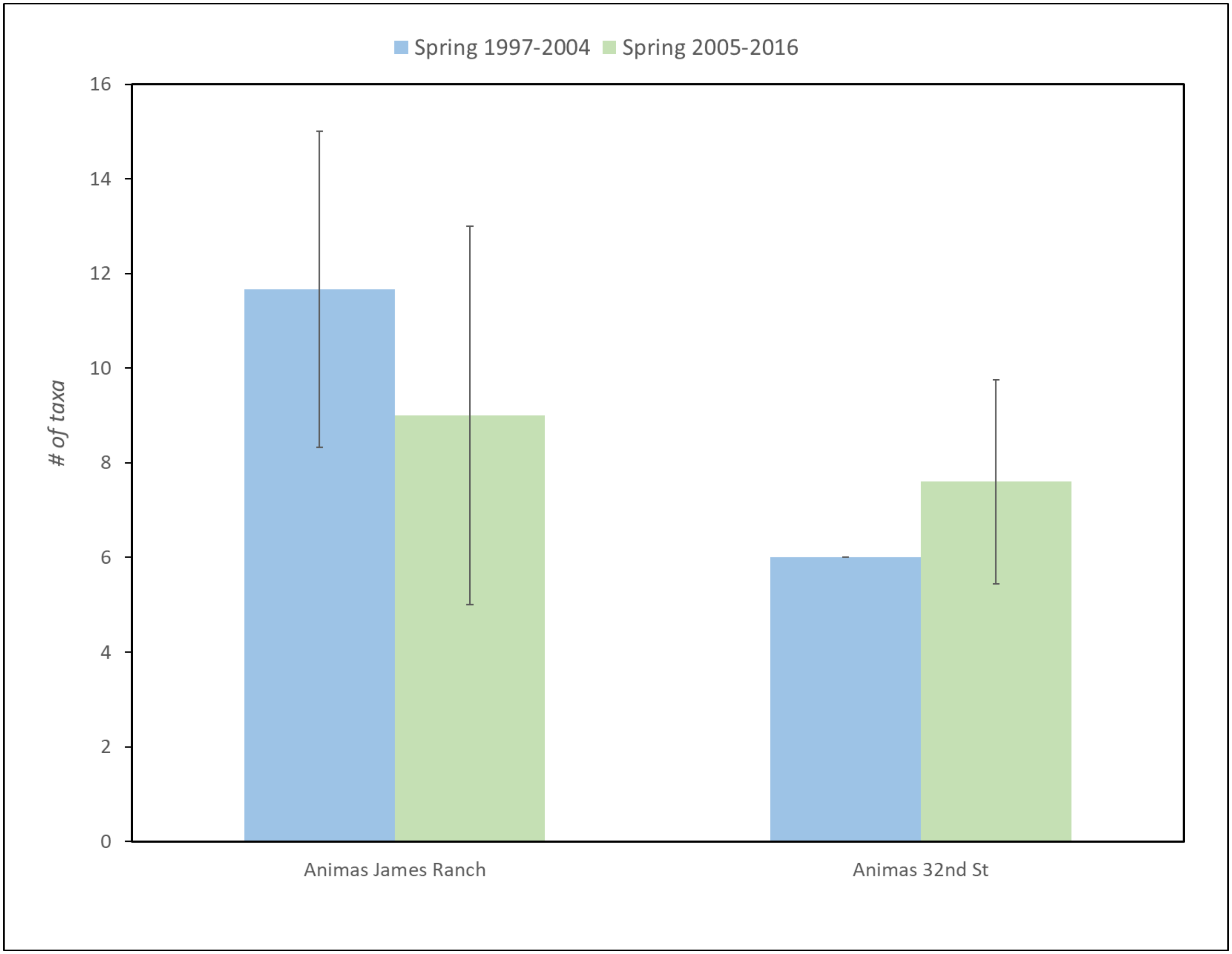


Figure 56: Taxa richness – Spring 1997-2004 compared to Spring 2005-2016
Note: see table 4 for an explanation of BMI metrics.

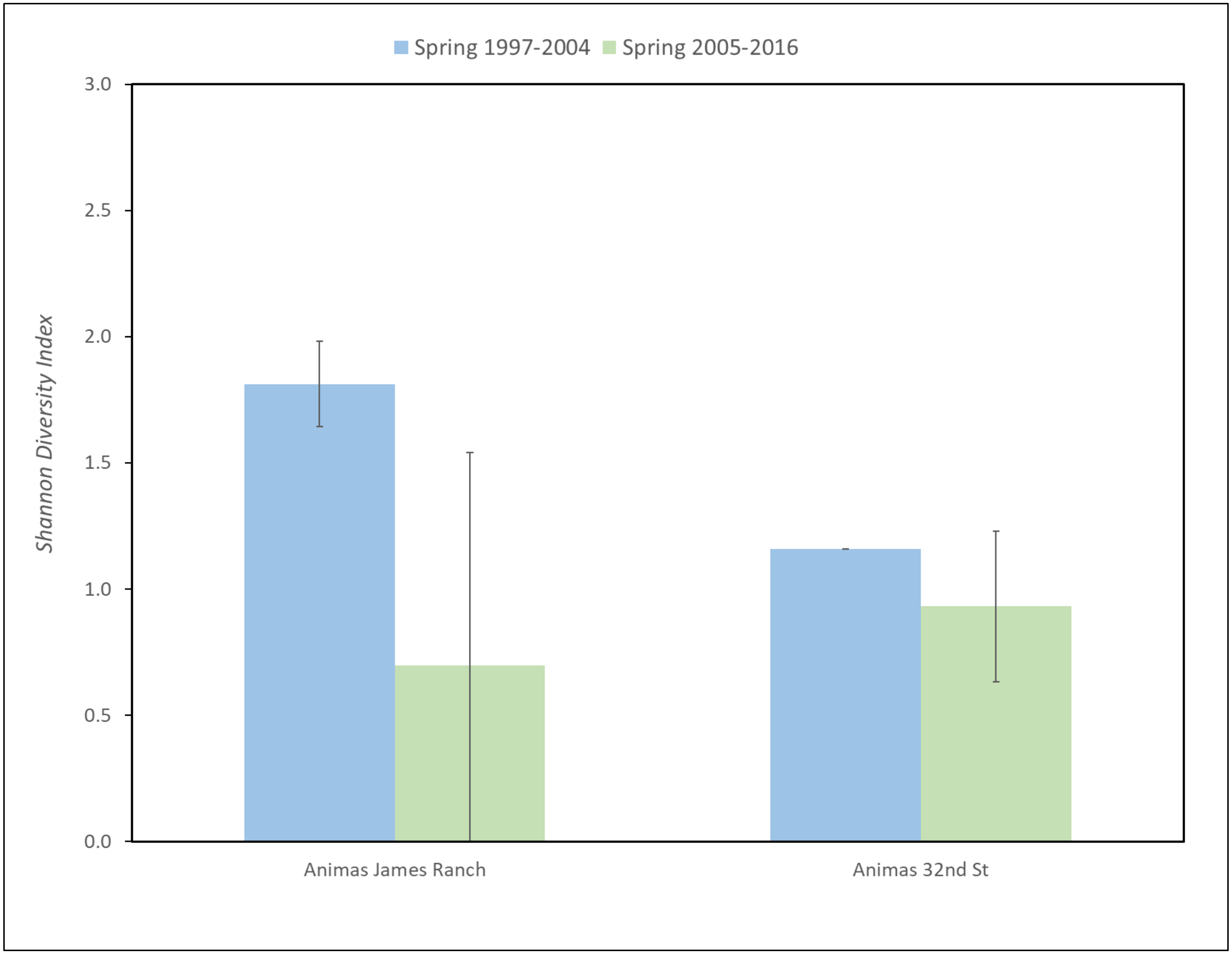


Figure 57: Shannon Diversity Index (SDI) – Spring 1997-2004 compared to Spring 2005-2016
Note: see table 4 for an explanation of BMI metrics.

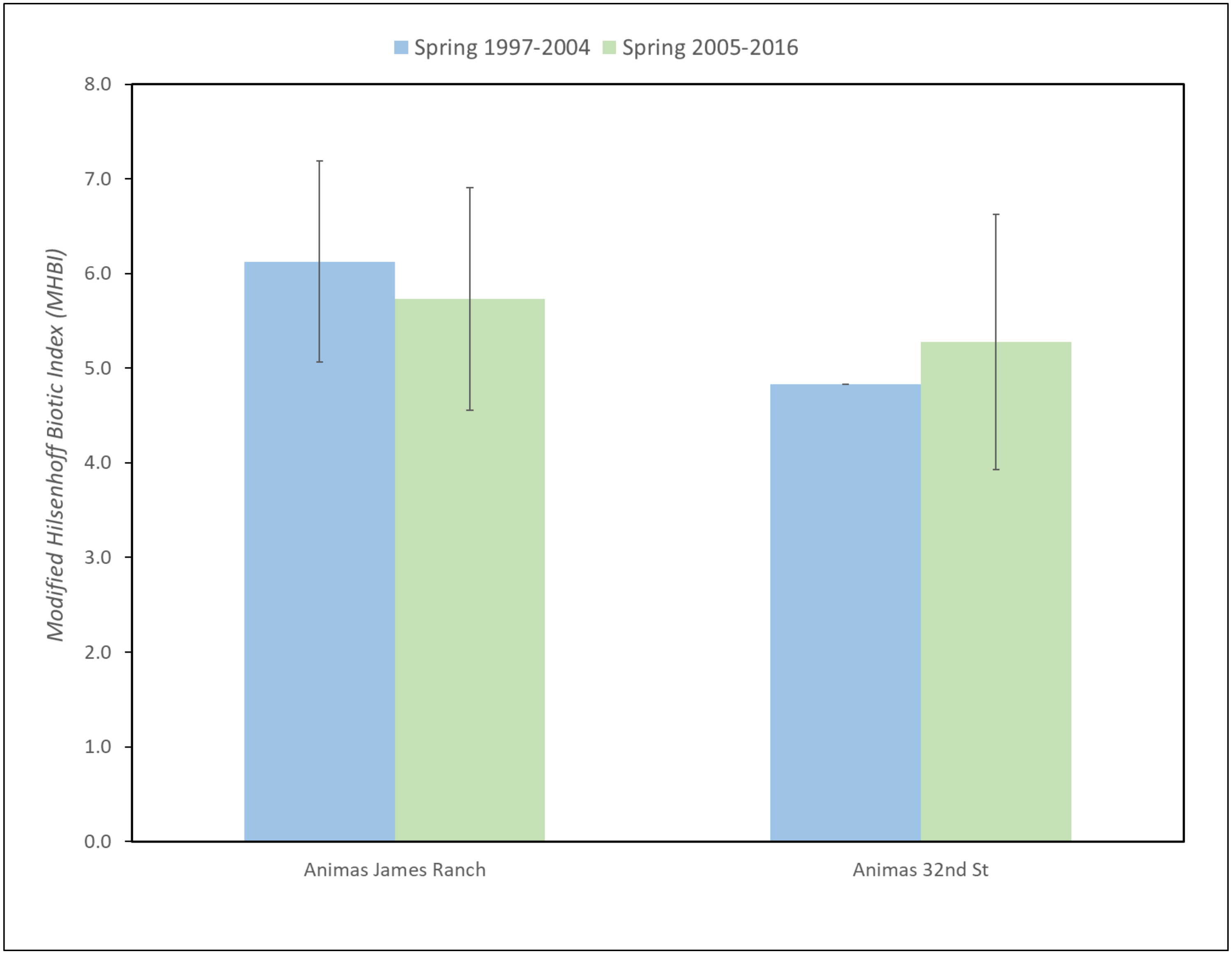


Figure 58: Modified Hilsenhoff Biotic Index (MHBI) – Spring 1997-2004 compared to Spring 2005-2016

Note: see table 4 for an explanation of BMI metrics.

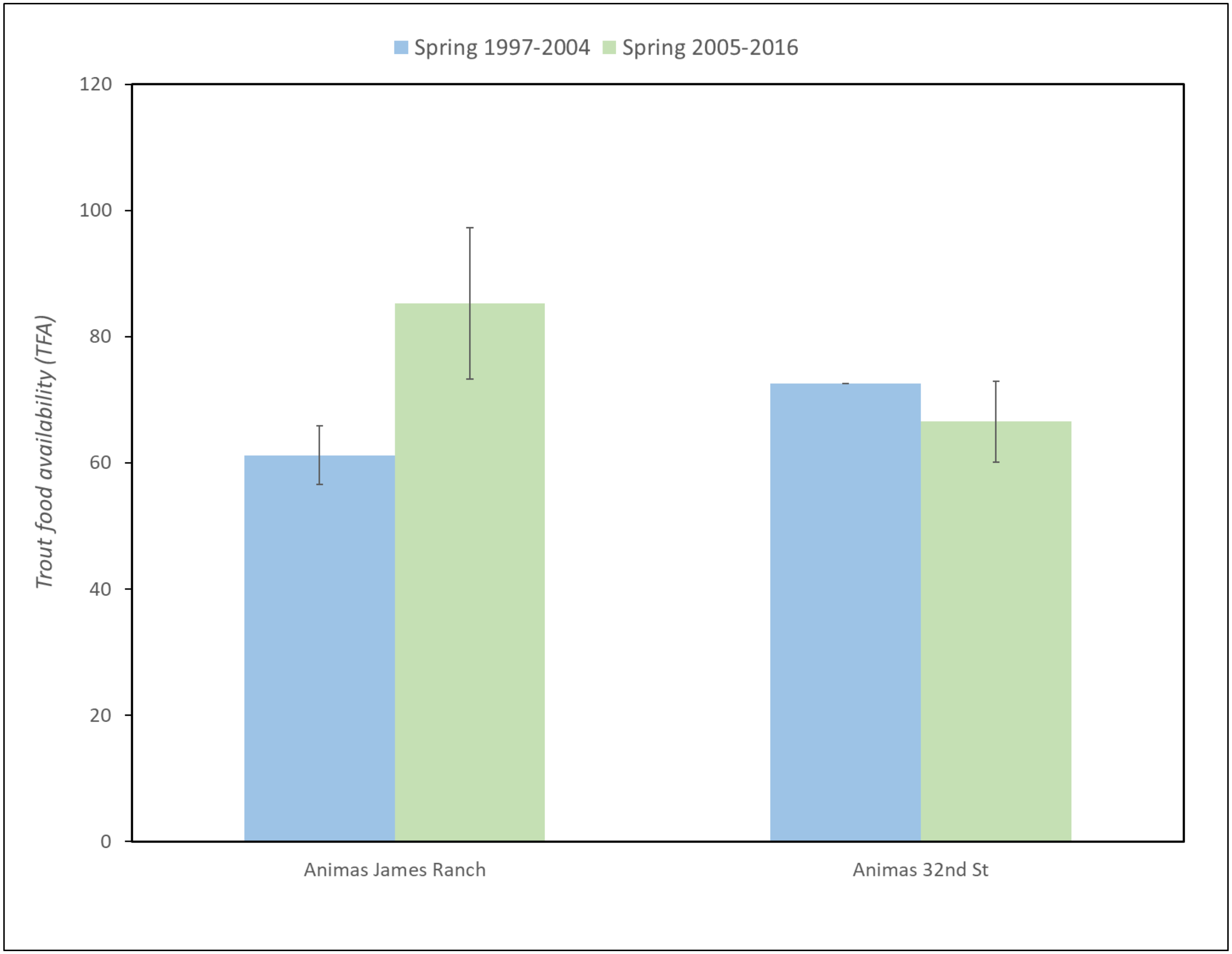


Figure 59: Trout Food Availability (TFA) – Spring 1997-2004 compared to Spring 2005-2016
Note: see table 4 for an explanation of BMI metrics.

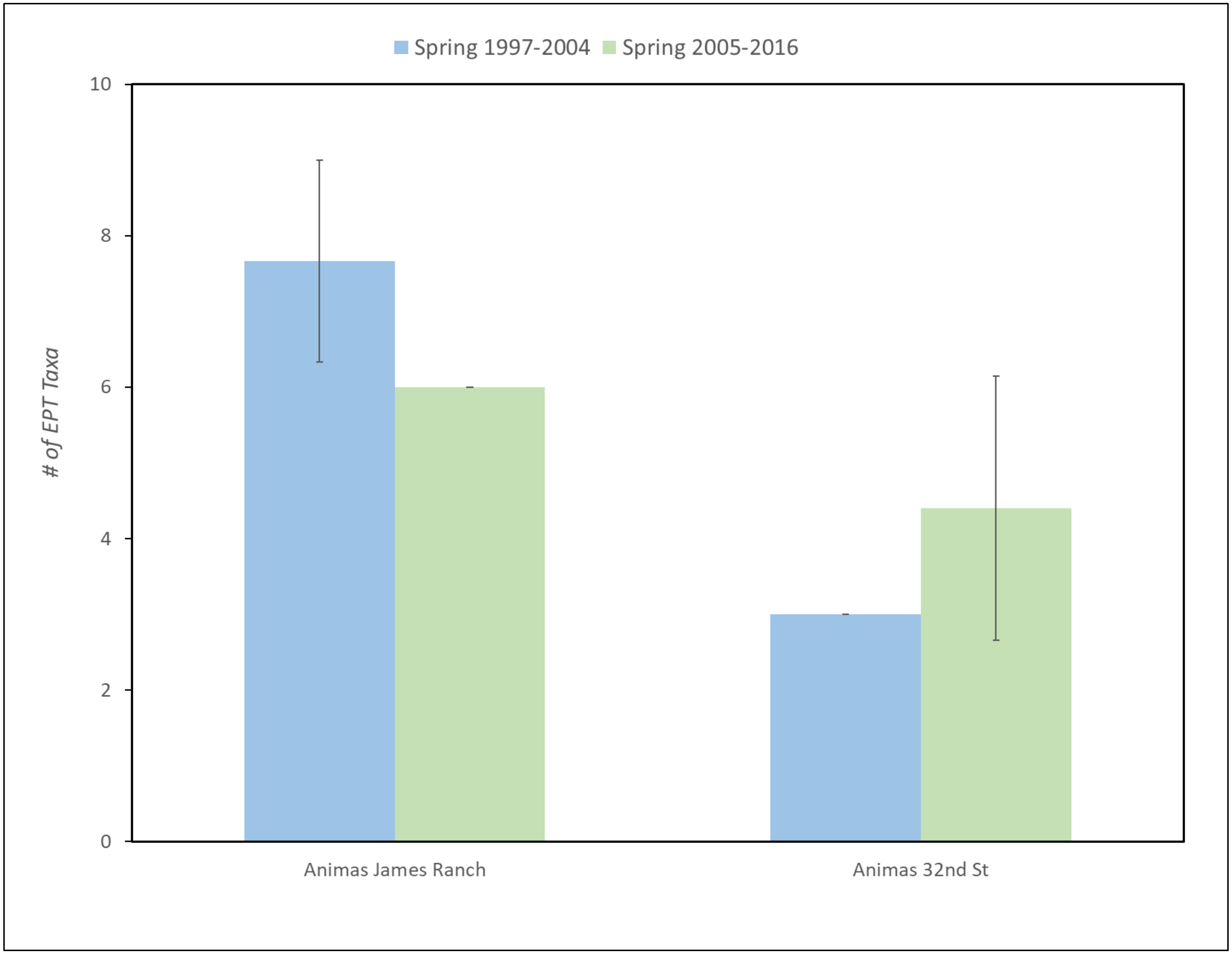


Figure 60: EPT taxa richness – Spring 1997-2004 compared to Spring 2005-2016
Note: see table 4 for an explanation of BMI metrics.

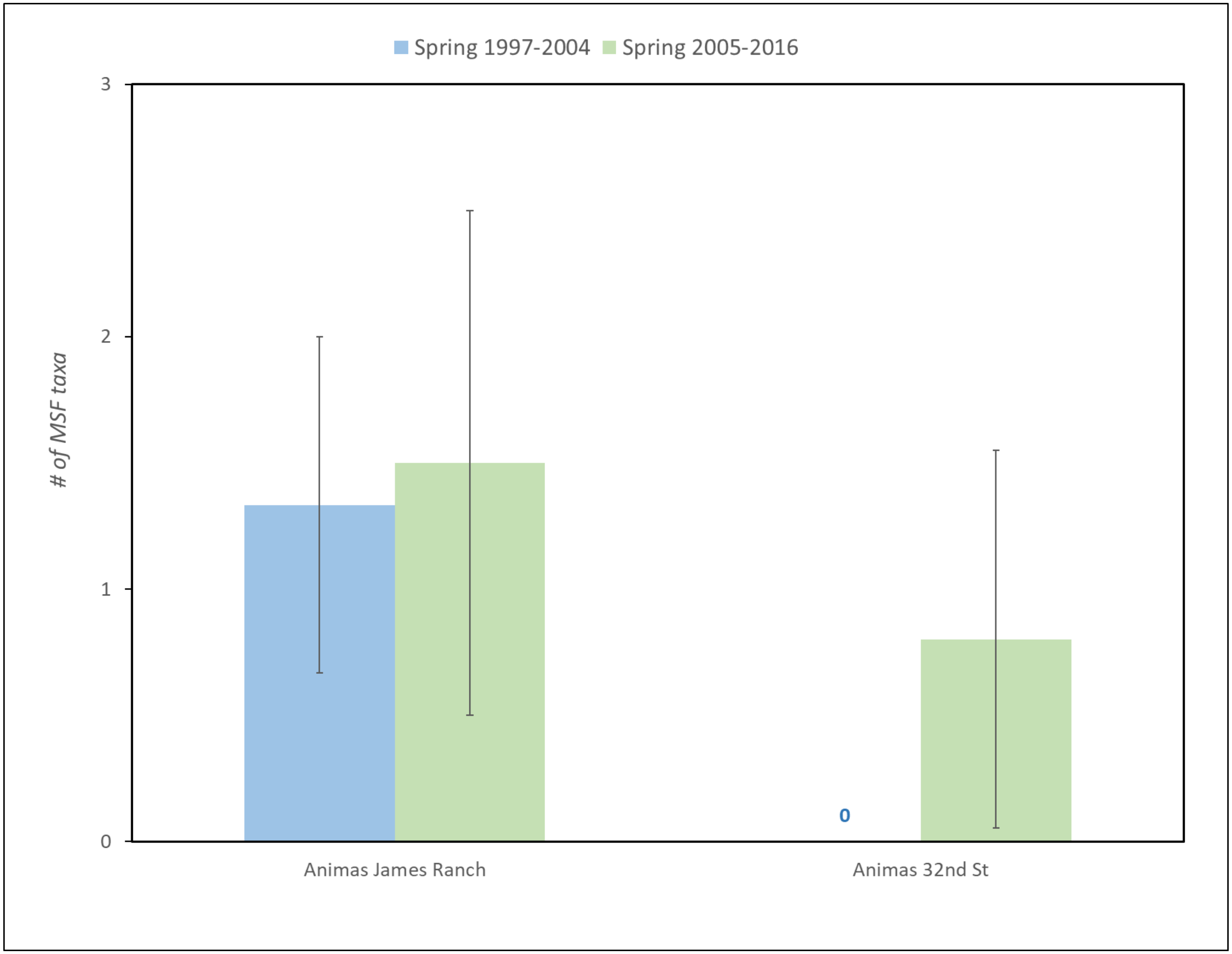


Figure 61: Metal-sensitive family (MSF) taxa richness – Spring 1997-2004 compared to Spring 2005-2016

Note: 0 indicates that a sample was collected, but no MSF taxa were present; see table 4 for an explanation of BMI metrics.

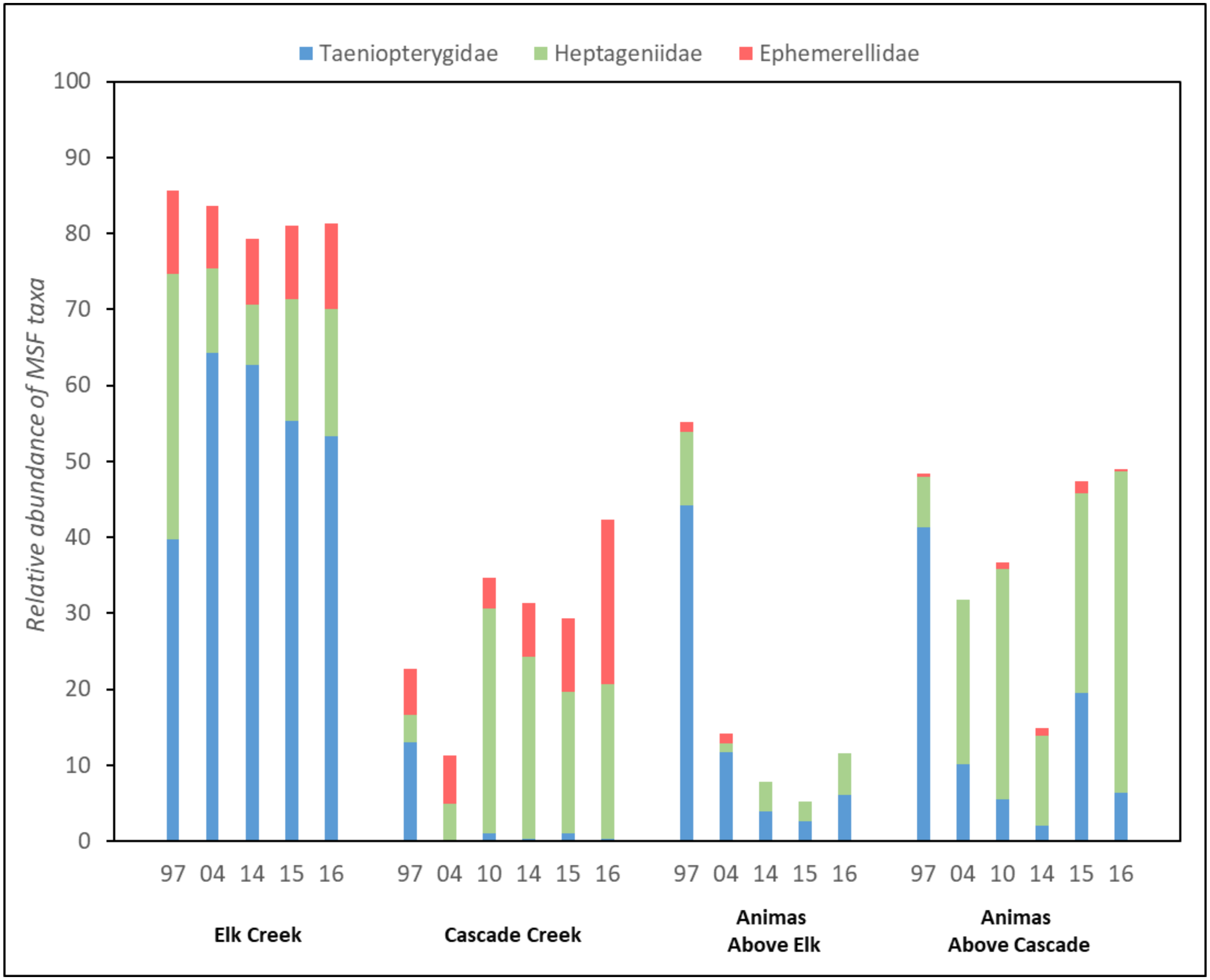


Figure 62: Relative abundance of metal-sensitive families (MSF) – Upper sites – Fall 1997-2016

Note: See table 4 for an explanation of BMI metrics.

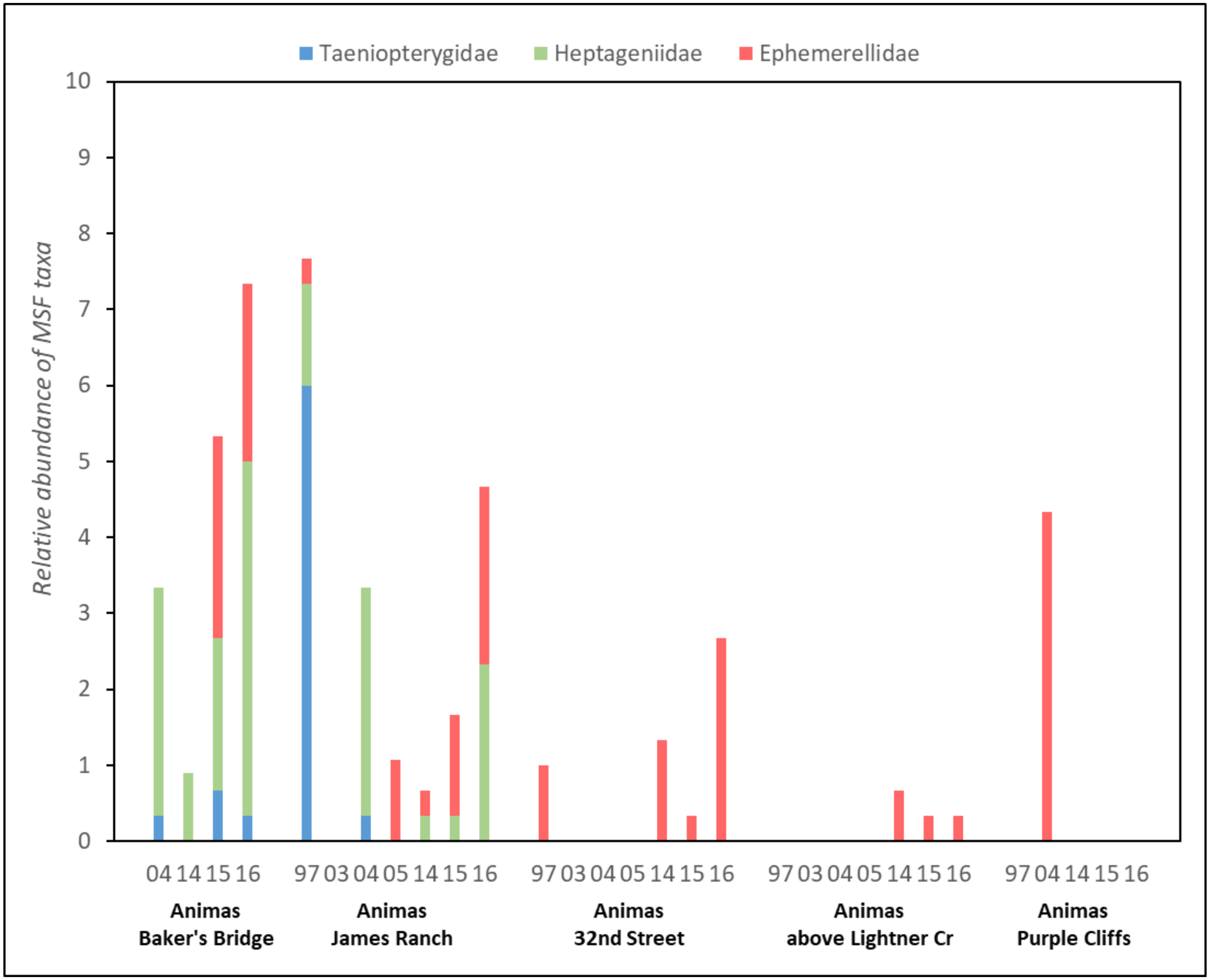


Figure 63: Relative abundance of metal-sensitive families (MSF) – Lower sites – Fall 1997-2016

Note: See table 4 for an explanation of BMI metrics.

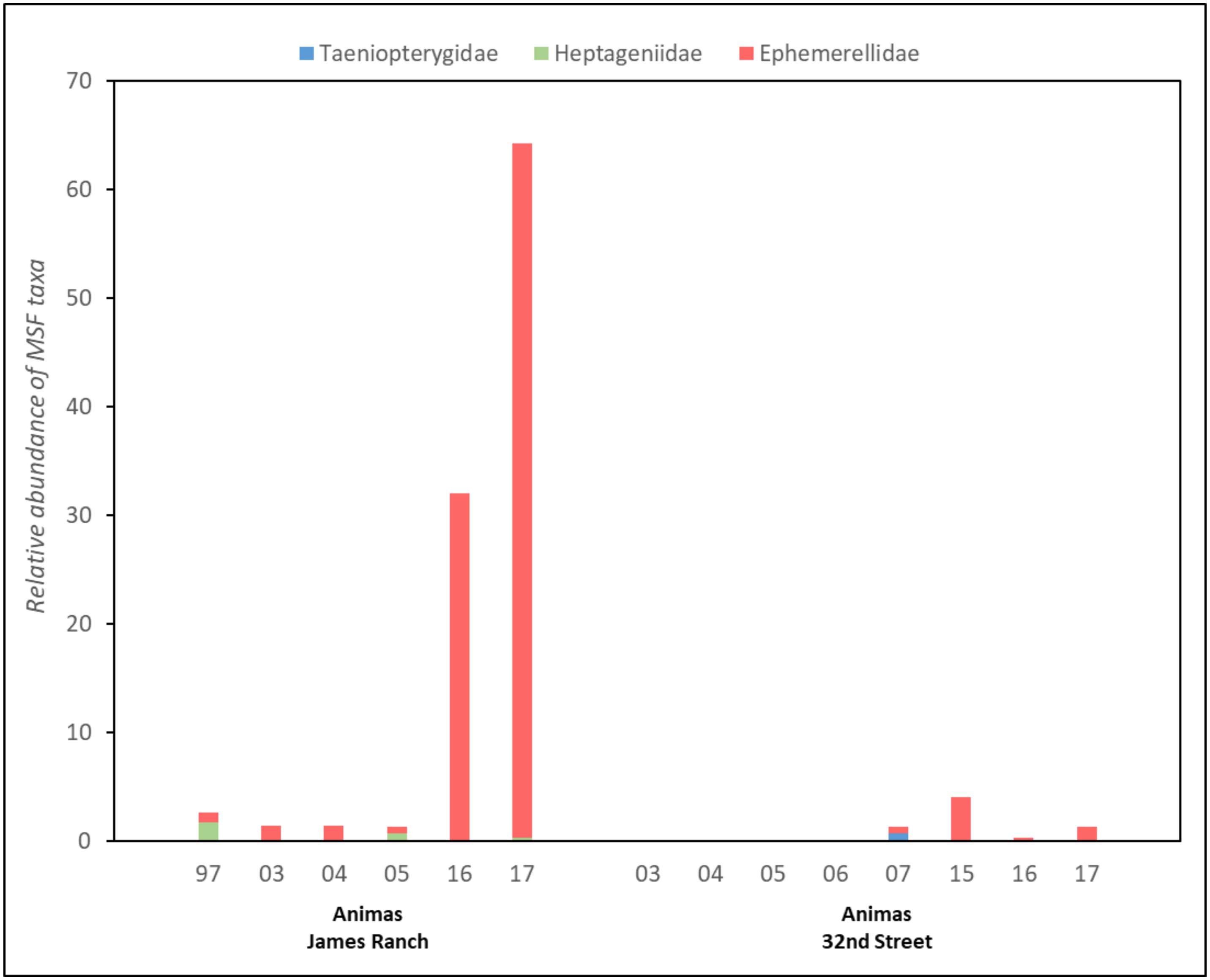


Figure 64: Relative abundance of metal-sensitive families (MSF) – Spring 1997-2017

Note: See table 4 for an explanation of BMI metrics.

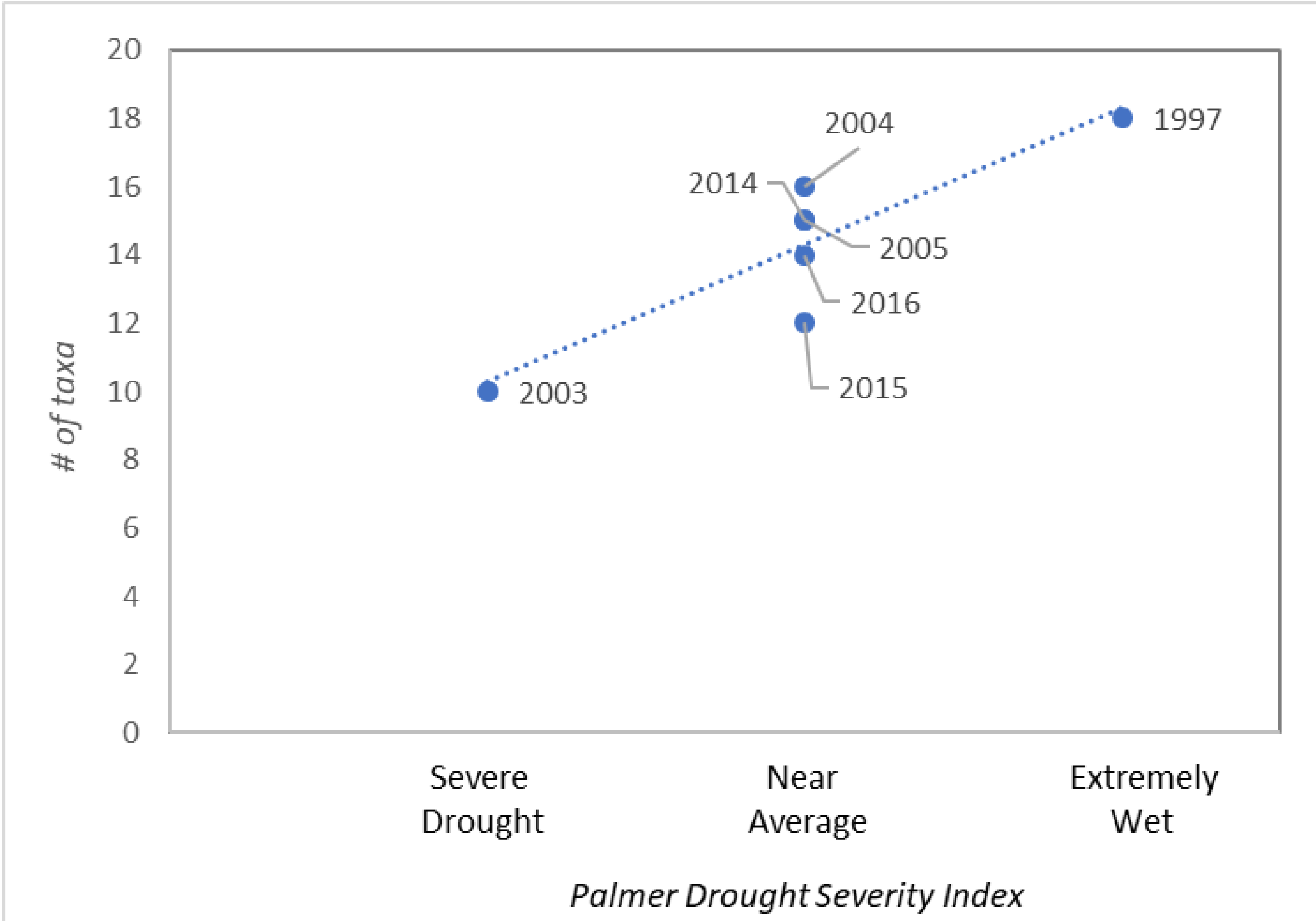


Figure 65: Relationship between taxa richness and the Palmer Drought Severity Index – AJR
Note: For full correlation results of BMI metrics and measures of drought, see tables 10-11.

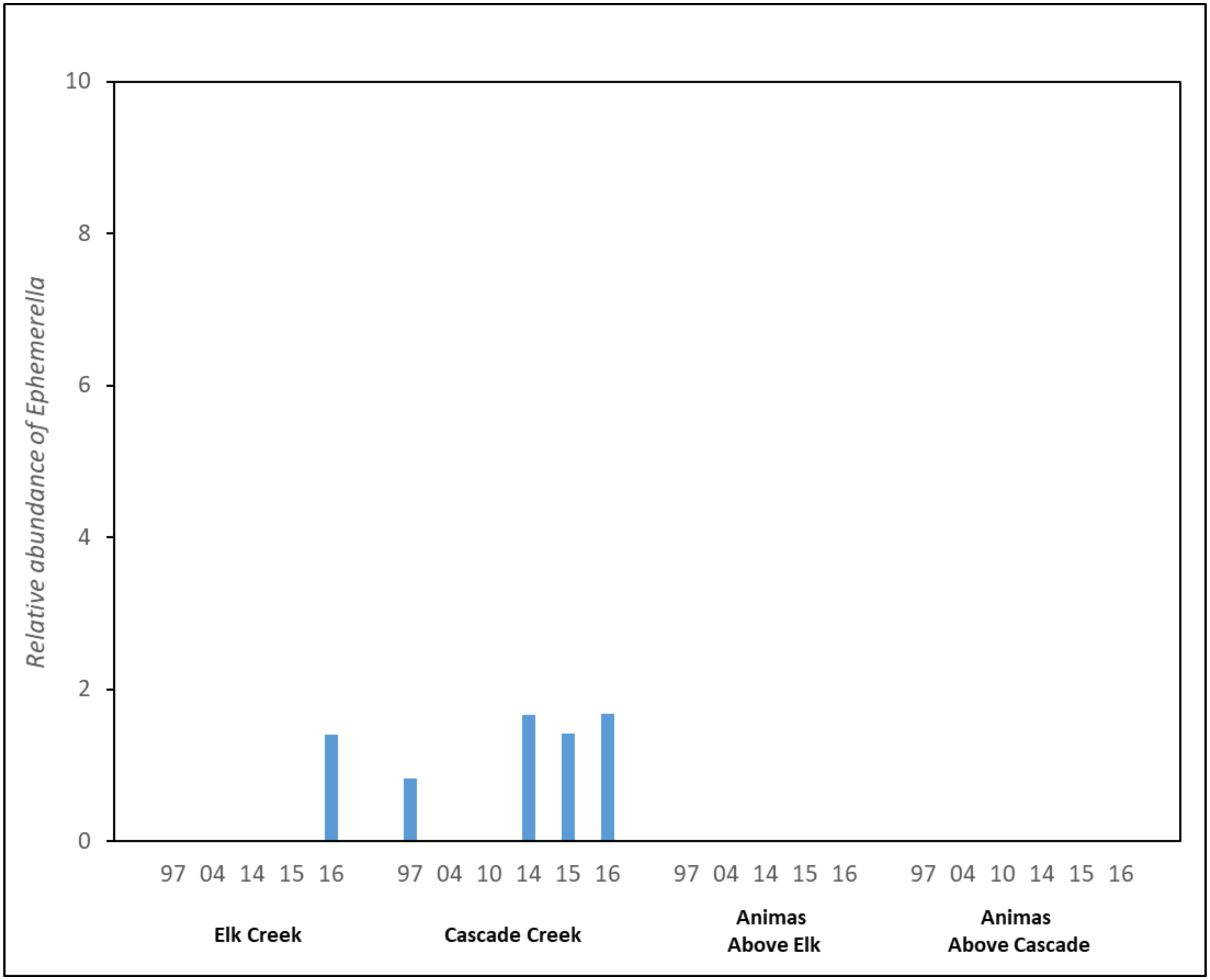


Figure 66: Relative abundance of *Ephemerella* (pale morning dun) – Upper sites – Fall 1997-2016
Note: See table 4 for an explanation of BMI metrics.

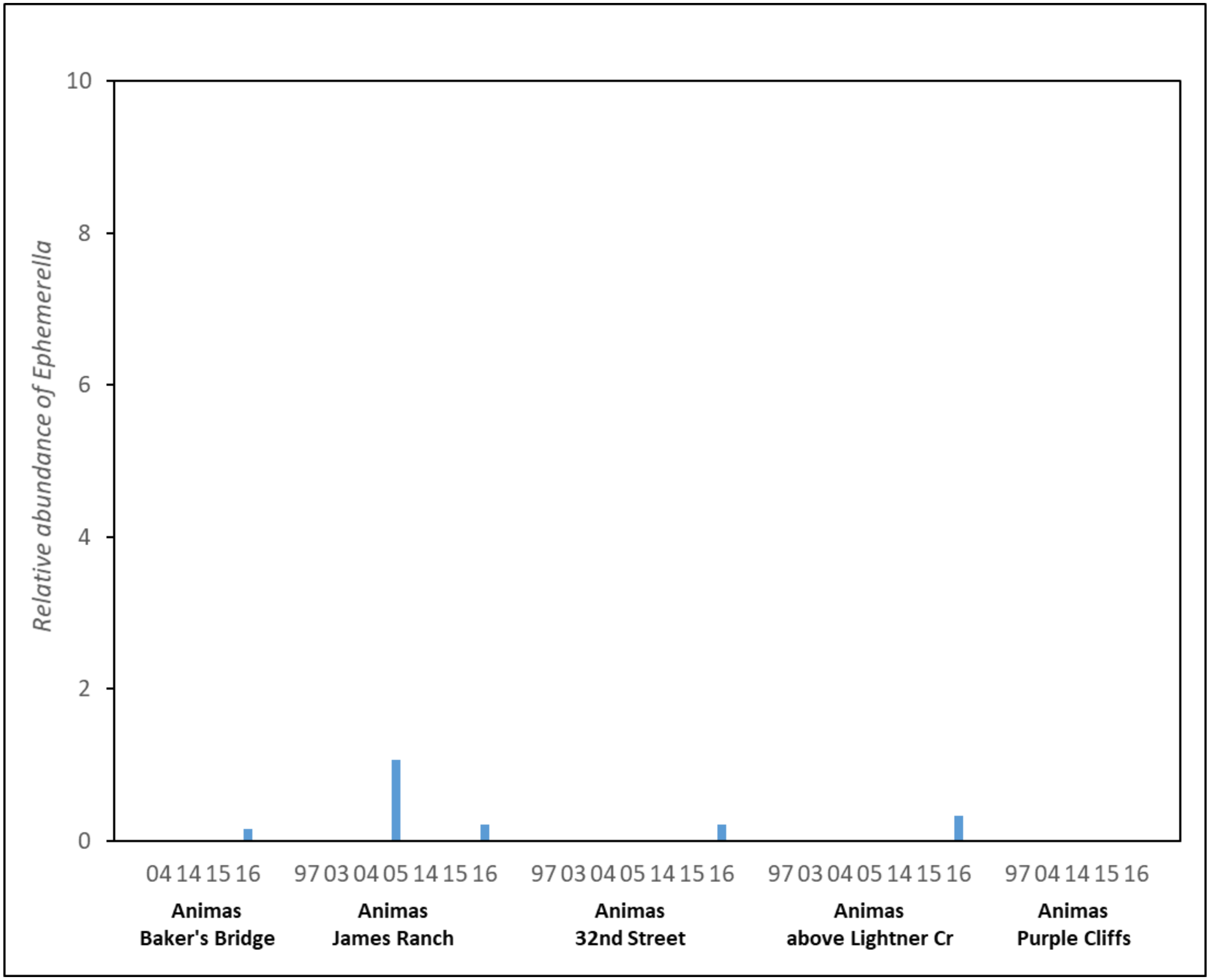


Figure 67: Relative abundance of *Ephemerella* (pale morning dun) – Lower sites – Fall 1997-2016

Note: See table 4 for an explanation of BMI metrics.

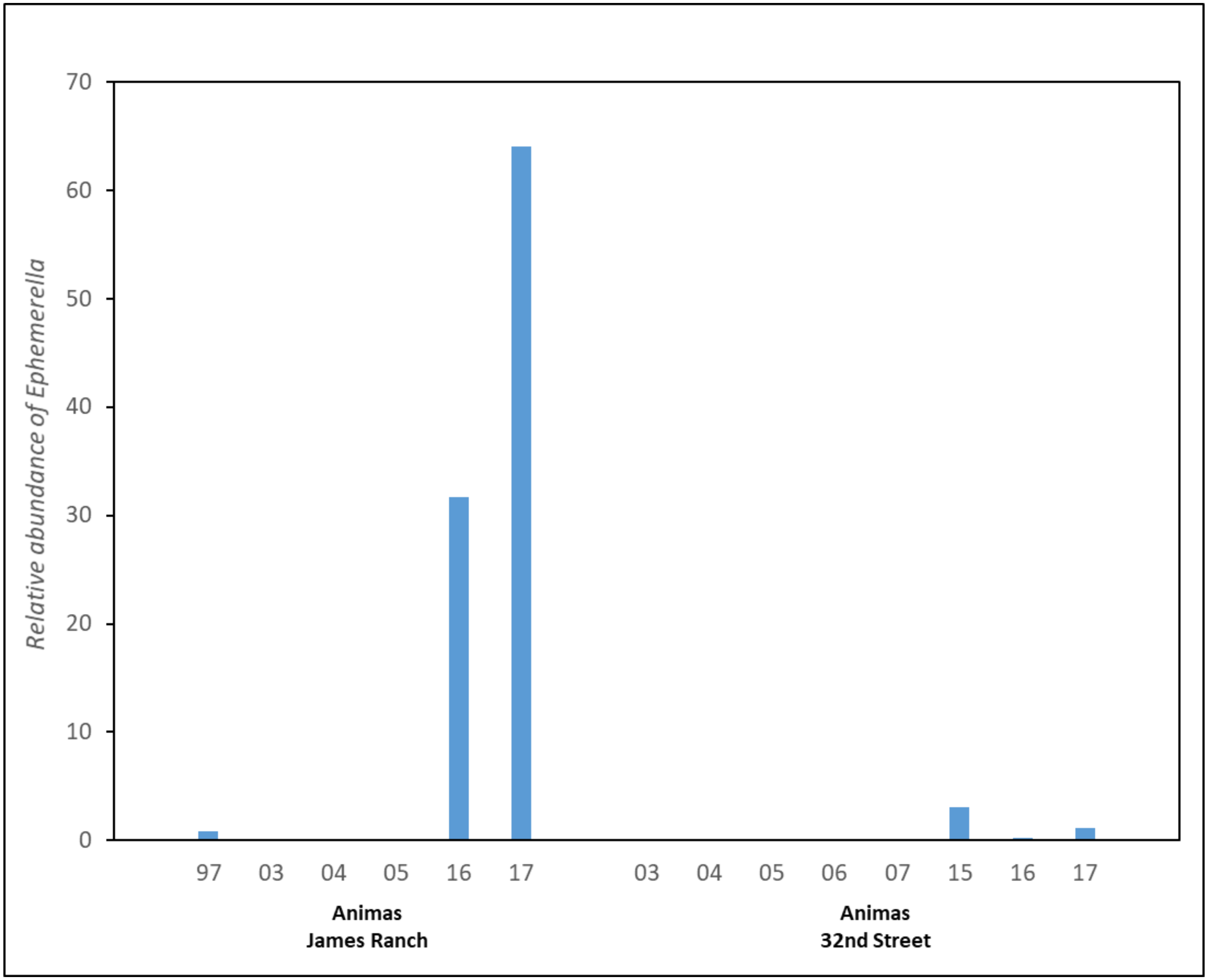
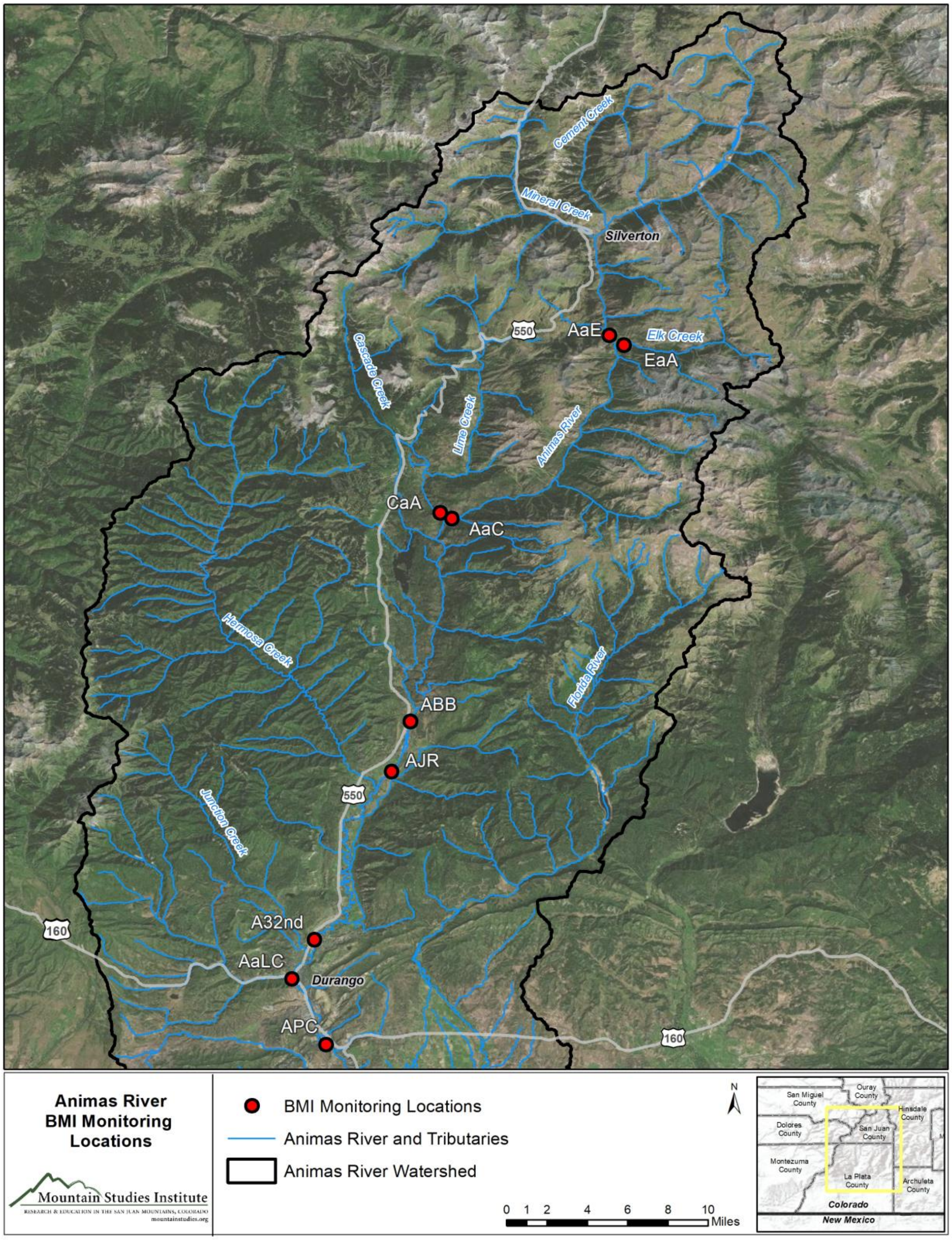


Figure 68: Relative abundance of *Ephemerella* (pale morning dun) – Spring 1997-2017
Note: See table 4 for an explanation of BMI metrics.

Appendix A – Maps



Map 1: BMI monitoring locations.

Appendix B – Field Photos

Image 1. Animas River, Above Elk Creek – 100m Reach – Photo taken at 0m, looking downstream.

Image 2. Animas River, Above Elk Creek – 100m Reach – Photo taken at 0m, looking upstream.

Image 3. Elk Creek, Above Animas River – 100m Reach – Photo taken at 0m, looking downstream.

Image 4. Elk Creek, Above Animas River – 100m Reach – Photo taken at 0m, looking upstream.

Image 5. Animas River, Above Cascade Creek – 100m Reach – Photo taken at 0m, looking downstream.

Image 6. Animas River, Above Cascade Creek – 100m Reach – Photo taken at 0m, looking upstream.

Image 7. Cascade Creek, Above Animas River – 100m Reach – Photo taken at 0m, looking downstream.

Image 8. Cascade Creek, Above Animas River – 100m Reach – Photo taken at 0m, looking upstream.

Image 9. Animas River, Baker's Bridge – 100m Reach – Photo taken at 0m, looking upstream.

Image 10. Animas River, Baker's Bridge – 100m Reach – Photo taken at 100m, looking downstream.

Image 11. Animas River, James Ranch – 100m Reach – Photo taken at 0m, looking upstream.

Image 12. Animas River, James Ranch – 100m Reach – Photo taken at 100m, looking downstream.

Image 13. Animas River, 32nd Street – 100m Reach – Photo taken at 0m, looking upstream.

Image 14. Animas River, 32nd Street – 100m Reach – Photo taken at 100m, looking downstream.

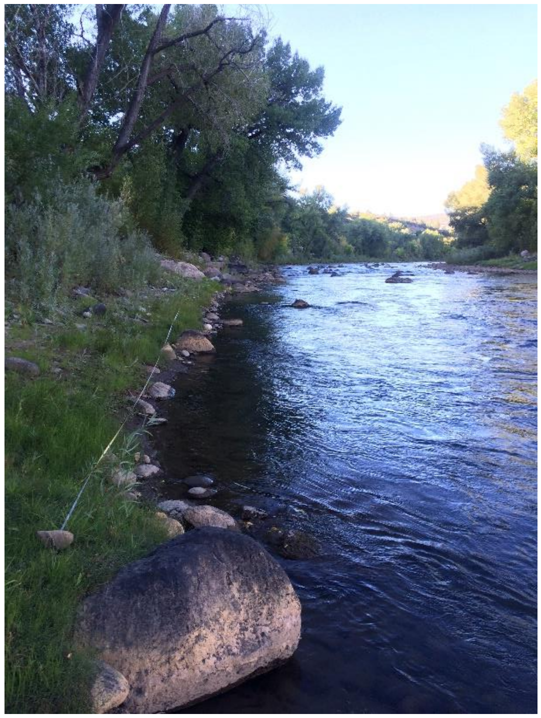


Image 15. Animas River, Above Lightner Creek – 100m Reach – Photo taken at 0m, looking upstream.

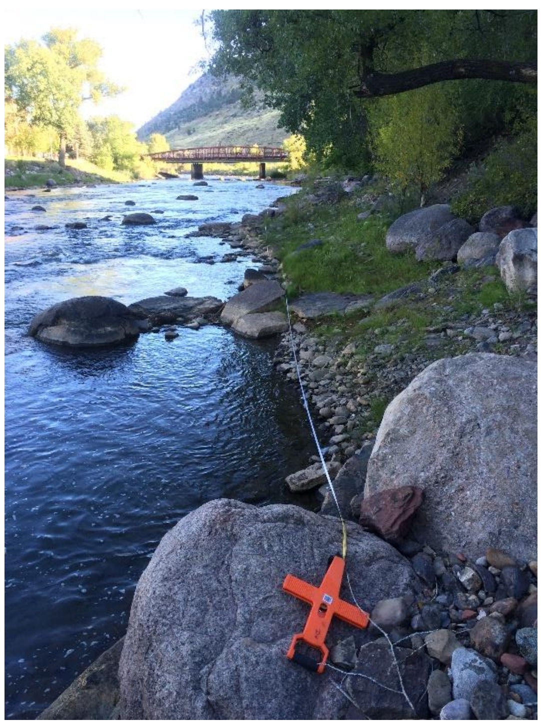


Image 16. Animas River, Above Lightner Creek – 100m Reach – Photo taken at 100m, looking downstream.

Image 17. Animas River, Purple Cliffs – 100m Reach – Photo taken at 0m, looking upstream.

Image 18. Animas River, Purple Cliffs – 100m Reach – Photo taken at 100m, looking downstream.

Appendix C – Physical Habitat Field Data Sheet

Site:								Date:							
Habitat:															
	1			2			3			4			5		
	S1	Mod	Em	S2	Mod	Em	S3	Mod	Em	S4	Mod	Em	S5	Mod	Em
10															
20															
30															
40															
50															
60															
70															
80															
90															
100															
Mod: A=Algae; M=Moss; V=Veg															
Rows are transects; Columns are Positions along transects															

Note: S = substrate; Mod = modifier; Em = embeddedness