ASSESSING MACROINVERTEBRATE COMMUNITIES IN THE CONASAUGA RIVER

by

CARLEISHA HANNS

(Under the Direction of Seth Wenger)

ABSTRACT

Recent observations have shown a decline in fish populations in the Conasauga River. Anecdotal evidence also suggests similar trends for macroinvertebrates, potentially indicating food limitations for fish in the area. This study examines macroinvertebrate communities in the Conasauga River to test the hypothesis that populations are lower in abundance compared to neighboring rivers through comparative analyses of macroinvertebrate biomass and abundance along the Conasauga River, Armuchee Creek, South Chickamauga Creek, and a global reference dataset. I found no statistically significant differences in biomass among these systems. There was a weak longitudinal gradient in macroinvertebrate abundance and biomass. Additionally, there were shifts in community composition from upstream to downstream. The findings highlight the influence of localized and historical factors, such as land use, over natural longitudinal gradients. This also emphasizes the need for conservation strategies that address multiple stressors to preserve freshwater ecosystems.

INDEX WORDS: Aquatic conservation, freshwater ecology, biodiversity

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by

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CHAPTER 1

INTRODUCTION

Freshwater ecosystems cover a small portion of the Earth's surface at only 1%. Though having a small surface area, they provide immense benefits. They contain a staggering 10% of all known species and 33% of vertebrate species (Collen et al. 2014). These ecosystems also provide essential ecosystem services including water filtration, carbon sequestration, and critical habitat for wildlife (Hanna et al., 2018). Freshwater systems also provide services that benefit the economy such as agricultural irrigation, fisheries, and hydropower (Vári et al., 2022). Given their small coverage area and immense ecological and economic importance, we must prioritize their conservation.

Unfortunately, freshwater ecosystems and heavily impacted by anthropogenic effects, which are only exacerbated by climate change (Lake et al., 2000). These combined impacts are reflected in freshwater species populations. For instance, the Freshwater Living Planet Index reported that populations of freshwater invertebrate species have plummeted by 83% globally from 1970 to 2018 (WWF, 2022). The World Wildlife Fund also reported that a third of freshwater vertebrate species are at risk of extinction (WWF, 2021). This rate of decline is a cause of concern because it exceeds that observed in terrestrial or marine ecosystems. While freshwater ecosystems are crucial to biodiversity, research on these species has lagged behind terrestrial ecosystem studies. This in return limits our understanding of their conservation status and population trends (Carrizo et al., 2017). Based on these statistics it is pertinent to better understand these systems and address these threats.

Among freshwater species, macroinvertebrates which include crustaceans, insects, and mollusks are often understudied compared to their vertebrate counterparts. Macroinvertebrates play key roles in the aquatic food web as primary consumers and prey items for many larger predators like fish. They also serve as biological indicators for assessing water quality and ecosystem health (López-López & Sedeño-Díaz, 2014). Though being a fundamental part of freshwater ecosystems and being more abundant than vertebrate species, macroinvertebrates face significant threats. For example, the International Union for Conservation of Nature (IUCN) reported that freshwater mussels are among the most at-risk groups for extinction, highlighting their global declines (Bogan, 2008 & IUCN, 2007). Notably, this trend of decline is pronounced in the Southeastern United States, which is a region recognized as a hotspot for mussel diversity (Haag & Williams, 2014). However, more recent studies have reported conflicting trends in freshwater macroinvertebrate population trends. A study published in 2023 in the United States observed a decrease in the total density of stream macroinvertebrates over the past 27 years, while simultaneously there was an increase in α diversity. Their results also indicated that divergences in community composition between human-impacted and forested streams have increased over time (Rumschlag et al., 2023). These results suggest that shifts in macroinvertebrate community structure are due to environmental and land use changes. A global analysis conducted by van Klink et al. (2020) found that aquatic insect communities appear to be increasing in both abundance and biomass, which contrasts with the declines observed in terrestrial insect populations. The complex and variable trends underscore the need for a deeper understanding of macroinvertebrate communities to effectively manage and conserve freshwater systems and challenge the prevailing narratives of widespread declines.

To gain better insight into macroinvertebrate roles and distribution in streams the River Continuum Concept (RCC) can be applied. The RCC is a theoretical framework developed to explain expected spatial patterns and structure in river ecosystems by explaining the change of biological, chemical, and physical factors from upstream to downstream (Vannote et al., 1980). According to the RCC headwater streams and typically dominated by macroinvertebrates categorized as shredders and collectors that process coarse particulate organic matter (CPOM), while mid-sized streams support a greater diversity of grazer and predators due to higher inputs of fine particulate organic matter (FPOM). Additionally, downstream sections are expected to be dominated by collectors and predators (Vannote et al., 1980). The RCC illustrates how both energy inputs and habitat complexity change along a river's course. A study conducted on the Little Tennessee River revealed that organic matter flowing through food webs increased significantly downstream (Rosi & Wallace, 2002). They also observed a shift in the primary food source consumed from leaf detritus upstream to amorphous detritus and animal material downstream aligning with the RCC's predictions (Rosi & Wallace, 2002). However, human activities, such as land use changes (e.g., agriculture, urbanization) can alter these natural patterns, further complicating the RCC predictions (Miserendino & Pizzolón, 2004). These insights emphasize the need to account for both natural gradients and human impacts when evaluating the health and biodiversity of freshwater ecosystems.

Historically, the southeastern United States was recognized as having some of the richest freshwater biodiversity including a wide variety of fish, mussels, and macroinvertebrates (Jelks et al., 2008). This region boasts the nation's highest number of endemic fish species (Lydeard and Mayden 1995), yet ongoing human-driven landscape changes continue to impact freshwater systems here (Benz and Collins, 1997). The Conasauga River located in the Valley and Ridge region, stands out for its ecological significance in this region. Previously it has served as a refuge for imperiled species, including the endangered Conasauga logperch (Percina jenkinsi) endemic to this river system (Walters, 1997). Recent studies have shown alarming declines in population numbers among several fish species within the Conasauga, which is potentially linked to pollution and habitat loss (Freeman et al., 2017).

Understanding the drivers behind these declines in fish populations is necessary for making effective conservation strategies and protecting the long-term health of the Conasauga River. Anecdotal evidence from ongoing annual monitoring suggests reduced densities of aquatic macroinvertebrates and fish in the Conasauga compared to the neighboring Etowah River hinting at potential food limitations driving fish population declines (Phillip Bumpers, personal communication, 2022). Research has also shown a longitudinal decrease in benthic macroinvertebrates in the Conasauga, likely tied to a macrophyte (Baker et al., 2012). Due to macroinvertebrates being key components of aquatic ecosystems and primary prey items for many fish species, their decline could signal broader ecological issues. As land use intensity increases downstream, we hypothesize a decrease in sensitive aquatic macroinvertebrate species resulting in an overall community shift.

My study aims to test the hypothesis that macroinvertebrate populations are lower in abundance in the Conasauga than in neighboring rivers, which could provide evidence that fish in the Conasauga River could be food limited. My research attempts to understand the distinct ecological dynamics within the Conasauga River and evaluate the implications for the broader ecosystem through comparative analyses of macroinvertebrate populations with those in Armuchee Creek, South Chickamauga Creek, and a global reference. Additionally, I will examine potential longitudinal gradients in macroinvertebrate abundance and biomass within the

Conasauga River itself utilizing datasets from 2009 and 2023. I hypothesize that the composition and biomass of macroinvertebrates will exhibit a longitudinal decline along the river, which is driven primarily by shifts from forested land cover in the headwaters to agricultural and urban land use downstream.

CHAPTER 2

METHODS

Study Area

The Conasauga River originates in the Cohutta Mountains of Georgia within the Chattahoochee National Forest and is approximately 93 miles long. The river then flows into southeastern Tennessee before returning to Georgia where it eventually meets the Coosawattee River to form the Oostanula River (Figure 1). The Conasauga is one of the few undammed rivers in the Coosa River basin, part of the Mobile River system. Regarding geology, the river’s course spans two physiographic regions, starting from the Blue Ridge and finishing in the Valley and Ridge. The varied geological landscapes contribute significantly to the river’s diverse ecological attributes. Descending from protected mountain areas, it flows into landscapes increasingly dominated by agricultural activity (Sharpe & Nichols, 2007).

The Armuchee and South Chickamauga Creek drainages are also in the Valley and Ridge physiographic region (Figure 1). Armuchee Creek is located in northwest Georgia in the Coosa River basin spanning approximately 50 miles. It has similar impacts from agriculture and development to the Conasauga. South Chickamauga Creek is in the Tennessee River basin and spans about 36 miles, flowing through a mix of land use from rural and urban areas.

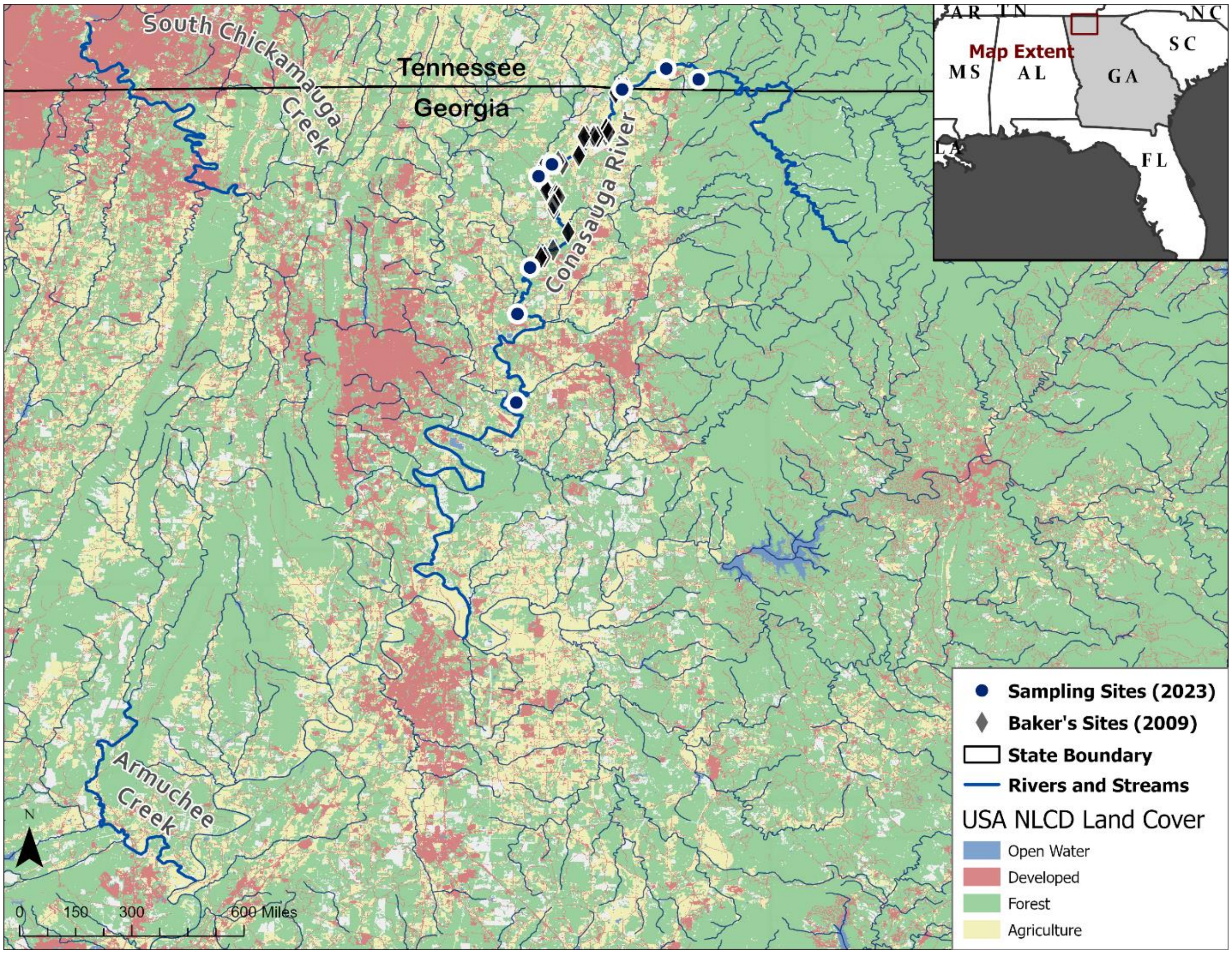

Figure 1 Map of 2023 sampling sites and NLCD landcover

Sample Collection and Processing

I conducted field surveys in the Conasauga River, Armuchee Creek, and South Chickamauga Creek to assess the diversity, abundance, and biomass of macroinvertebrates. I selected sampling sites in the Conasauga River along a longitudinal gradient of 37.4 miles that aligned with annually sampled fish survey sites (Nagy et al., 2024). The Armuchee Creek and South Chickamauga Creek were chosen as comparison sites due to their location within the same geophysical province as the Conasauga River and similar drainage size. We sampled for macroinvertebrates in late June through early August across 10 sites: 8 in the Conasauga, 1 in the Armuchee, and 1 in the South Chickamauga. At each site, we took point measurements for water temperature, dissolved oxygen, pH, conductivity, depth, velocity, and turbidity to assess their potential influence on the distribution and abundance of macroinvertebrates. At 6 of the 8 Conasauga River sites, I collected 3 replicates from cobble or gravel riffle habitats, and at 2 sites, I collected 4 replicates, totaling 26 samples. In the Armuchee and South Chickamauga I sampled 3 replicates in cobble/gravel riffle habitat. All samples were collected using a modified t-sampler with a 250-µm mesh catchnet (English, 1987). The substrate was agitated for a maximum of 90 seconds for each replicate. The collected material was rinsed into a pollination bag and preserved in the field with 70% ethanol.

In the lab, I sorted samples by elutriating the collected samples to separate the specimens from the larger substrate. I then divided the elutriated samples into two size classes by rinsing them over stacked 1-mm and 250-µm sieves and preserving the contents in 70% ethanol. Depending on the density of organisms, the <1mm samples were split into either half or quarter subsamples using a plankton splitter. The target was to obtain approximately 200 individuals per <1mm subsample. Using a dissecting microscope, I picked individuals from the remaining substrate. In the picking process all individuals, body parts, and caddisfly cases were picked. All picked specimens were identified to the lowest taxonomic level typically to genus, but with several exceptions. Members of the Chironomidae family were identified as Tanypodinae or non-Tanypodinae. Snails were identified only at the family level. Other exceptions included members of Ostracoda, Turbellaria, and Oligochaeta, as well as mites and limpets, none of which were identified further. I measured the length of individuals to the nearest millimeter to calculate ash-free dry biomass using a length-mass regression based on total body length (Benke et al., 1999). For taxa not included in Benke et al., the closest related listed taxon was used.

Previously Collected Data

I utilized a second macroinvertebrate dataset collected from the Conasauga by Baker (2012) to provide an additional test of whether there was a longitudinal gradient, and to explore differences through time. Baker collected 65 samples of macroinvertebrates from randomly selected shoals in the Conasauga River (Figure 2). The sampling spanned 16.7 river miles and occurred from 2 sampling dates in June and July of 2009 (Baker, 2012). Baker employed a similar macroinvertebrate field sampling and processing method to ours, but the key difference is they utilized a modified t-sampler with a 243 µm mesh catchnet whereas I used a 250 µm mesh catchnet. They identified taxa to the lowest taxonomic level possible, which was typically genus with the same exceptions. All macroinvertebrates were measured to the nearest 0.1mm (Baker, 2012). Baker (2012) calculated ash-free dry biomass using the same length-mass regression utilizing the same length-mass regressions (Benke et al., 1999).

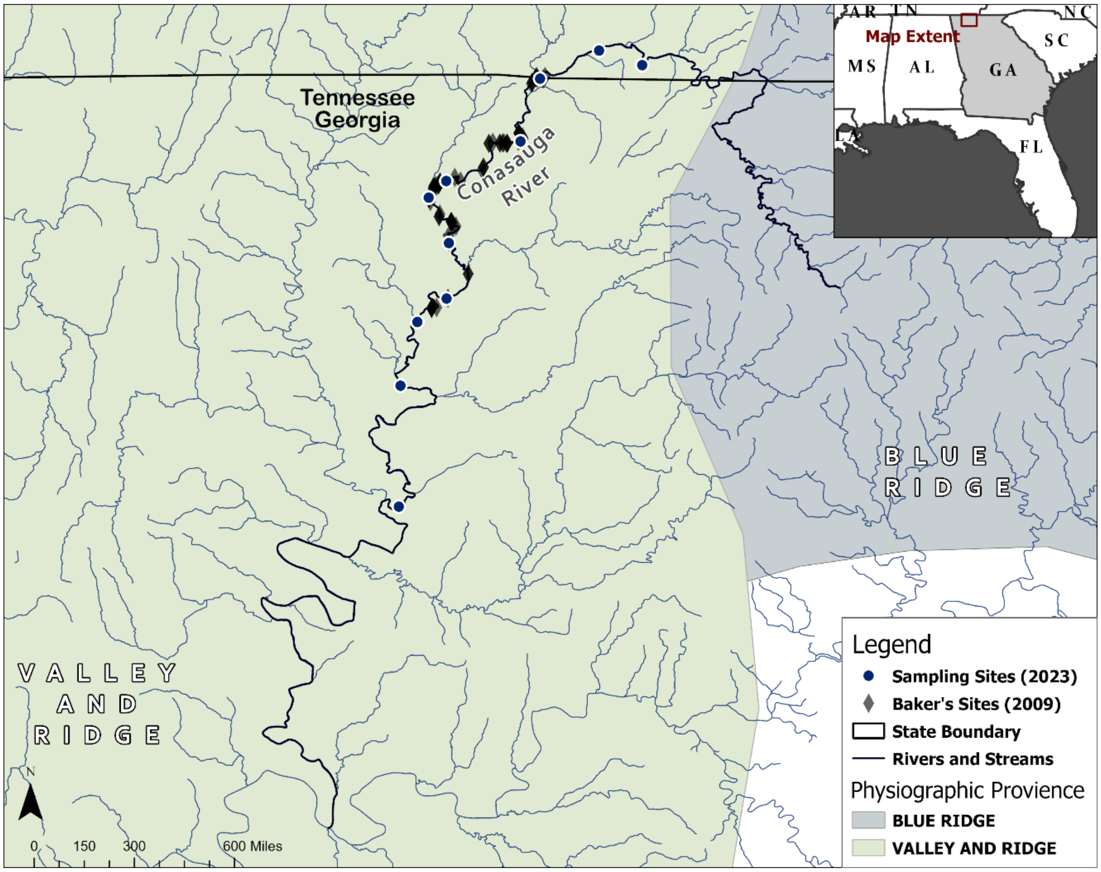

Figure 2 Map of 2009 and 2023 sampling sites (Baker ,2012)

Analysis

For the 2023 dataset, I standardized abundance and biomass by dividing the total values by the number of replicates per site to allow for comparisons among sites with different amounts of replicates. These standardized values were utilized in all the following analyses.

To standardize biomass measurements for comparative analysis, insect biomass was adjusted to a per-unit-area basis. Initially, the raw biomass values were recorded as collected with a t-sampler with an 11.5 cm diameter. The area of the t-sampler was calculated using the formula for the area of a circle, resulting in an area of approximately 103.9 square centimeters (t-area = $\pi \times (0.5 \times 11.5)$ 2). Next, the biomass values in milligrams per t-sample were converted to grams per square meter by dividing by 1000 (to convert milligrams to grams) and then scaling to a square meter using the calculated area:

$$\text{Insect Biomass (g/m2)} = \frac{\text{Biomass (mg)}}{1000} \times \frac{10000}{\text{t-area}}$$

Finally, the natural logarithm of insect biomass in milligrams per square meter was calculated to produce a log-transformed variable to normalize the data distribution and address potential skewness. I compared only the insect biomass of the Conasauga River to neighboring waterways and a global reference using boxplots. The global reference I used was Patrick et al. (2019), which included annual insect biomass estimates for 102 sites with stream orders between 2 and 5. I then conducted a one-way ANOVA to test the hypothesis that macroinvertebrate populations in the Conasauga River are lower in biomass compared to neighboring systems Armuchee Creek, and South Chickamauga Creek.

To test the null hypothesis that there was no shift in abundance and biomass along a longitudinal gradient I performed a linear regression. River mile was the predictor variable and total abundance, and biomass of macroinvertebrates were the response variables for the 2009 and 2023 datasets from the Conasauga River.

I examined the spatial patterns of dataset macroinvertebrate communities at the lowest taxonomic level for the 2009 and 2023 data using non-metric multidimensional scaling (NMDS), with a Bray-Curtis dissimilarity matrix (Oksanen, et al., 2024). This allowed for the visualization of community structure and the identification of potential environmental gradients influencing macroinvertebrate distribution between the 2 years. I conducted this analysis first with presence/absence data, and then with transformed biomass data (log (biomass +1)) to stabilize the variance. To test for longitudinal gradients in the distribution of these communities, I utilized the envfit function from the vegan package (Oksanen, et al., 2024.). This function allowed me to regress river miles against ordination scores to explore how macroinvertebrate communities vary along the longitudinal gradient of the river.

For the 2009 and 2023 macroinvertebrate datasets, I excluded all orders except Diptera, Ephemeroptera, Plecoptera, and Trichoptera. I then conducted a separate linear regression analysis for each order, using log-transformed biomass (log (biomass +1)) as the response variable and river mile as the predictor variable.

I then categorized the 2009 and 2023 Conasauga River dataset taxa into functional feeding groups (FFGs). The concept of FFGs classifies macroinvertebrate species according to their food intake method and the types of food they consume (Wallace & Webster, 1996). Focusing on FFGs rather than individual taxa provides a functional view of community composition, which can allow for a better understanding of ecological interactions (Cummins & Klug, 1979). I classified my data into 7 FFG categories filterer, gatherer, piercers-algae, predator, scraper, shredder, and unknown. I conducted a separate linear regression analysis for each FFG, using both abundance and log transformed biomass (log (biomass +1)) as response variables, and river mile as the predictor variable.

CHAPTER 3

RESULTS

The 2023 dataset included 26 samples from 8 sites spanning 37.4 river miles along the Conasauga River. The 2023 dataset included approximately 5100 individuals and a total taxa richness of 49 (Appendix A). The 2009 samples consisted of 65 samples along 16.7 river miles of the Conasauga. The 2009 dataset contained approximately 32,000 individuals and a total taxa richness of 56. Three sites were sampled in both the 2009 and 2023 datasets. Though the 2009 dataset had a higher mean abundance (8.93, se = 0.71) than the 2023 dataset (8.24, se=1.06), the 2023 dataset had a higher mean biomass (2.67, se = 0.78, compared with 0.24, se= 0.02 in 2009).

Comparison of Biomass to Global and Neighboring Streams

We found that the Conasauga River had lower mean biomass than the global dataset and the individual sites at Armuchee Creek and South Chickamauga Creek (Figure 3). However, there was high variability in biomass among sites in the Conasauga, and a few sites had higher biomass than the global mean and the neighboring reference sites. Figure 3 illustrates the biomass distribution of macroinvertebrate insects across four different datasets of Armuchee Creek, Conasauga River, South Chickamauga Creek, and a global reference (Patrick et al., 2019). The ANOVA results indicated no statistically significant difference in macroinvertebrate biomass among the Conasauga River, Armuchee Creek, and South Chickamauga Creek sites (p = 0.43) (Appendix B).

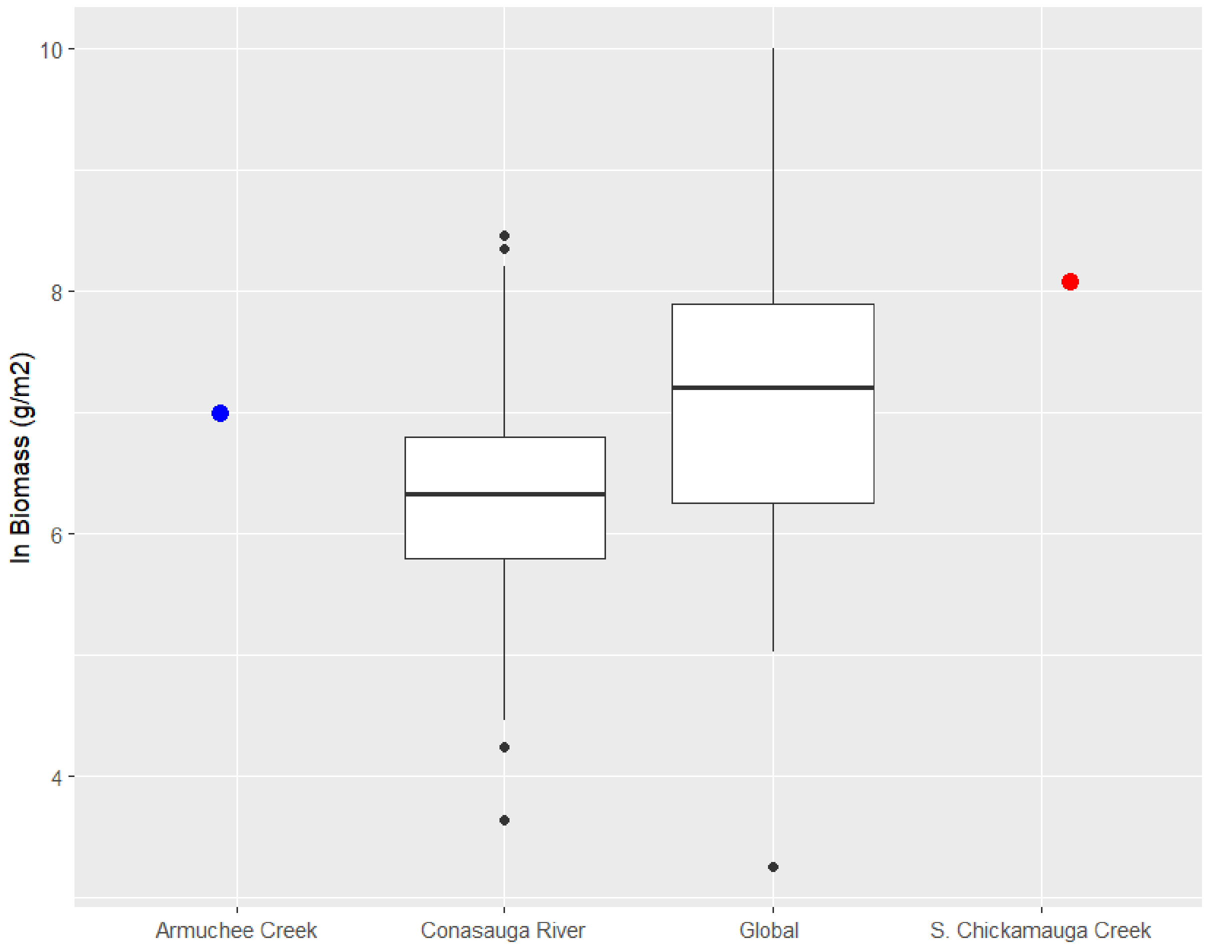

Figure 3 Boxplot of macroinvertebrate biomass across different sites: Armuchee Creek, Conasauga River (2009 & 2023), S. Chickamauga Creek, and Globa (Patrick et al., 2019)l. Armuchee Creek and S. Chickamauga Creek are represented by individual data points, highlighted in blue and red, respectively.

Longitudinal gradient of taxa

The linear regression analysis of the 2009 dataset analysis shows that river miles account for a very small proportion of the variance in the abundance ($r^2 = 0.01$, $p = 0.34$) and biomass ($r^2 = 0.01$, $p = 0.42$) of taxa per site (Figures 4 & 5). Parameter estimates indicated that slopes were not significantly different from zero (Table 1).

The linear regression analysis of the 2023 dataset indicated that river mile accounts for a modest portion of the variance in the abundance ($r^2 = 0.18$, $p = 0.29$) and biomass ($r^2 = 0.41$, $p = 0.09$) of taxa per site (Figures 4 & 5). However, biomass showed a negative trend that suggests as river miles decrease (moving downstream), the biomass decreases (Figure 5). Parameter estimates similarly indicated negative relationships with river mile, but slopes were not significantly different from zero (Table 1).

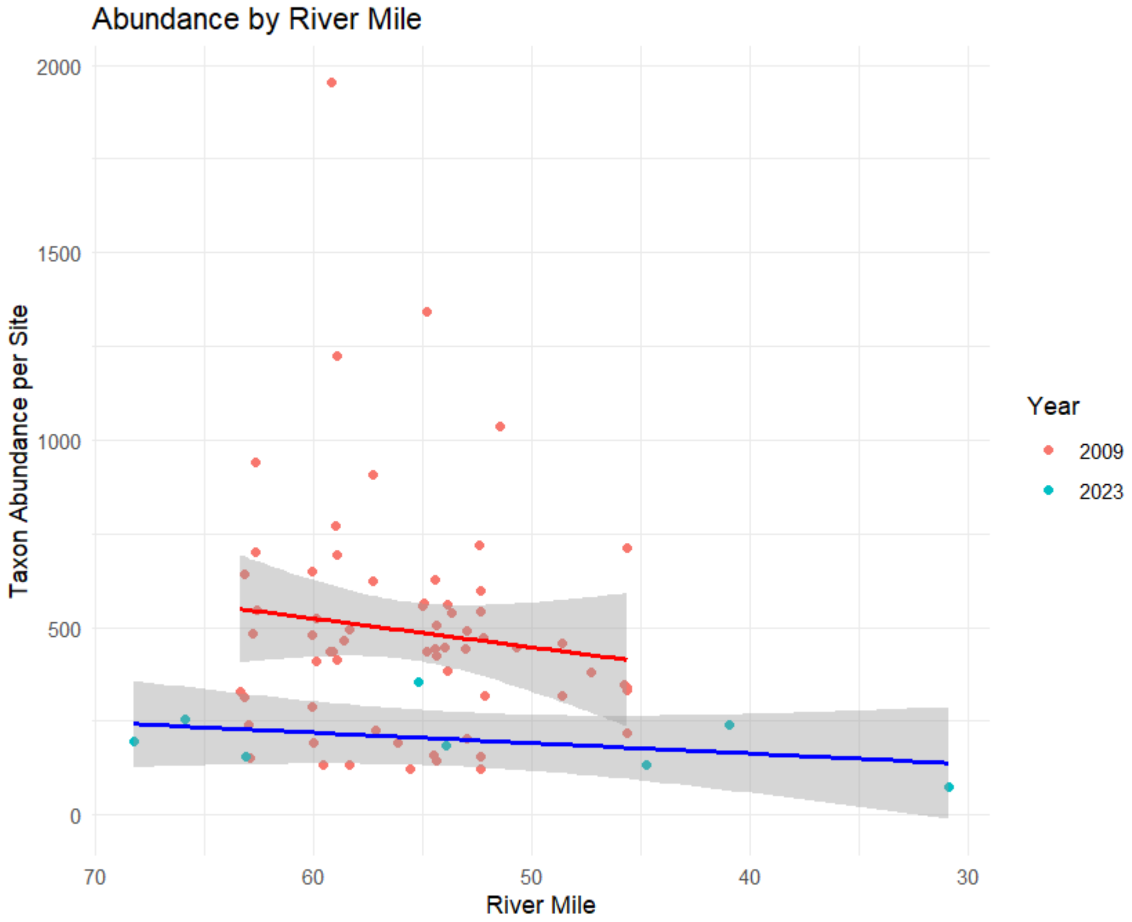

Figure 4 Linear regression of taxon abundance per site by river mile for the Conasauga River, comparing 2009 (red) and 2023 (blue) The x-axis is orientated from upstream to downstream. Shaded areas represent confidence intervals.

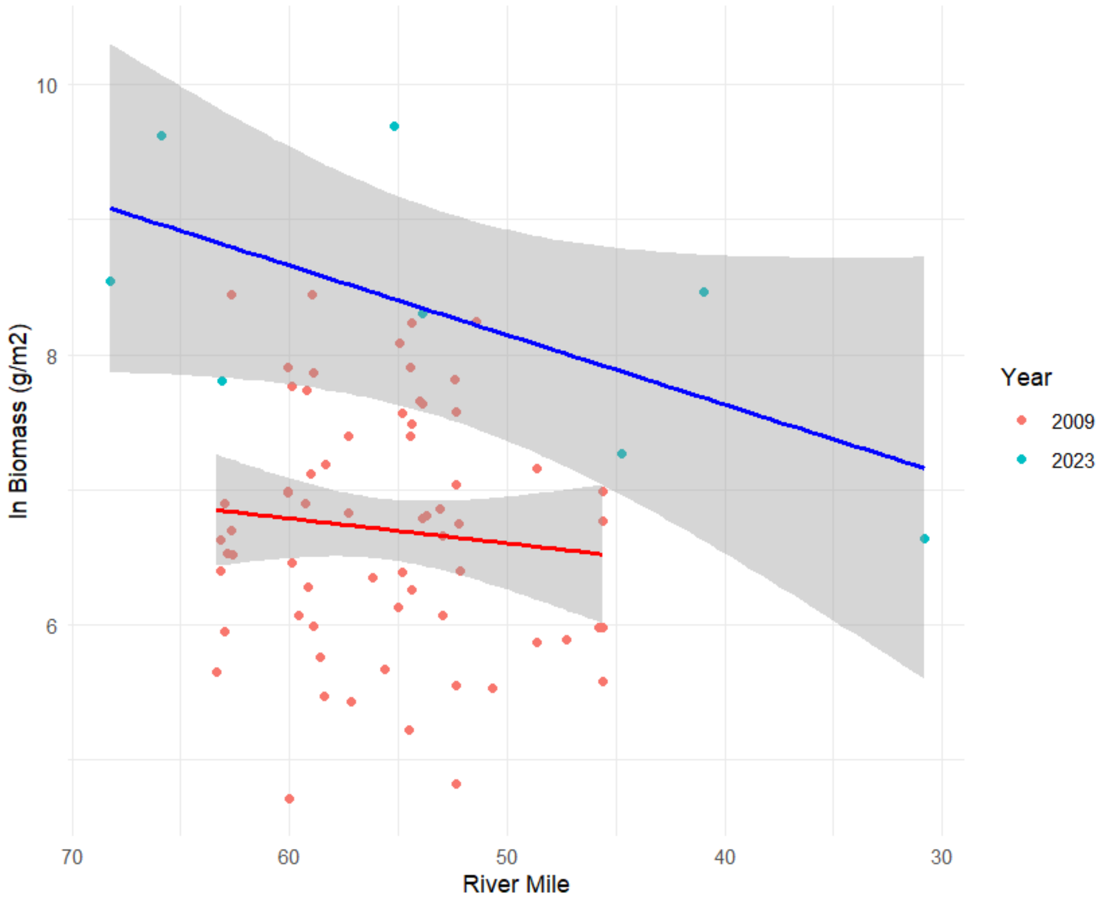

Figure 5 Linear regression of taxon biomass per site by river mile for the Conasauga River, comparing 2009 (red) and 2023 (blue). Shaded areas represent confidence intervals.

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Table 1. Summary of Linear Model Fit Statistics for Macroinvertebrate Abundance and Biomass in the Conasauga River Across 2009 and 2023

Year	Parameter	Residual Standard Error	Multiple R-squared	F-statistic	p-value	Slope (RM)
2009	Abundance	311.40	0.01	0.94	0.34	7.64
2009	Biomass	0.90	0.01	0.65	0.42	0.02
2023	Abundance	83.86	0.18	1.33	0.29	2.78
2023	Biomass	0.88	0.41	4.14	0.09	0.05

Comparative Community Composition of Macroinvertebrates

The comparison between the 2009 and 2023 taxon presence/absence and biomass datasets revealed distinct patterns of community composition between the years (Figures 6 & 7). Both NMDS plots had stress values below 0.2, indicating an effective representation of community composition in two-dimensional space (McCune and Grace 2002). The envfit line for presence/absence (r^2 =0.942, p = 0.001), suggest that river mile is strongly correlated with the 2 axes of the NMDS plot (Figure 6). The NMDS plot for biomass by taxon (Figure 7) reveals a more distinct separation between the 2009 and 2023 datasets compared to the presence/absence plot. The envfit line for biomass (r^2 =0.161, p = 0.003), implies that river mile has a moderate correlation with the 2 axes.

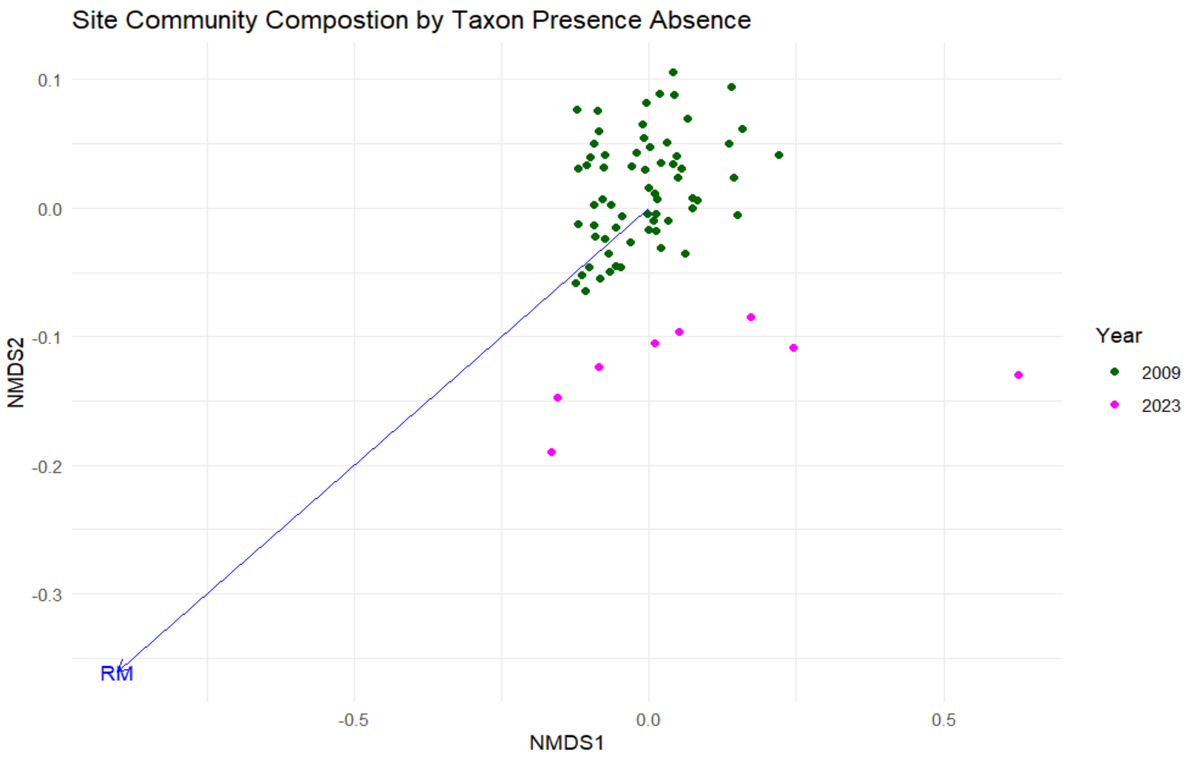

Figure 6 Non-metric multidimensional scaling (NMDS) plot illustrating site community composition based on taxon presence and absence for 2009 (green) and 2023 (pink). The blue arrow represents the correlation of river mile (RM) with the NMDS axes.

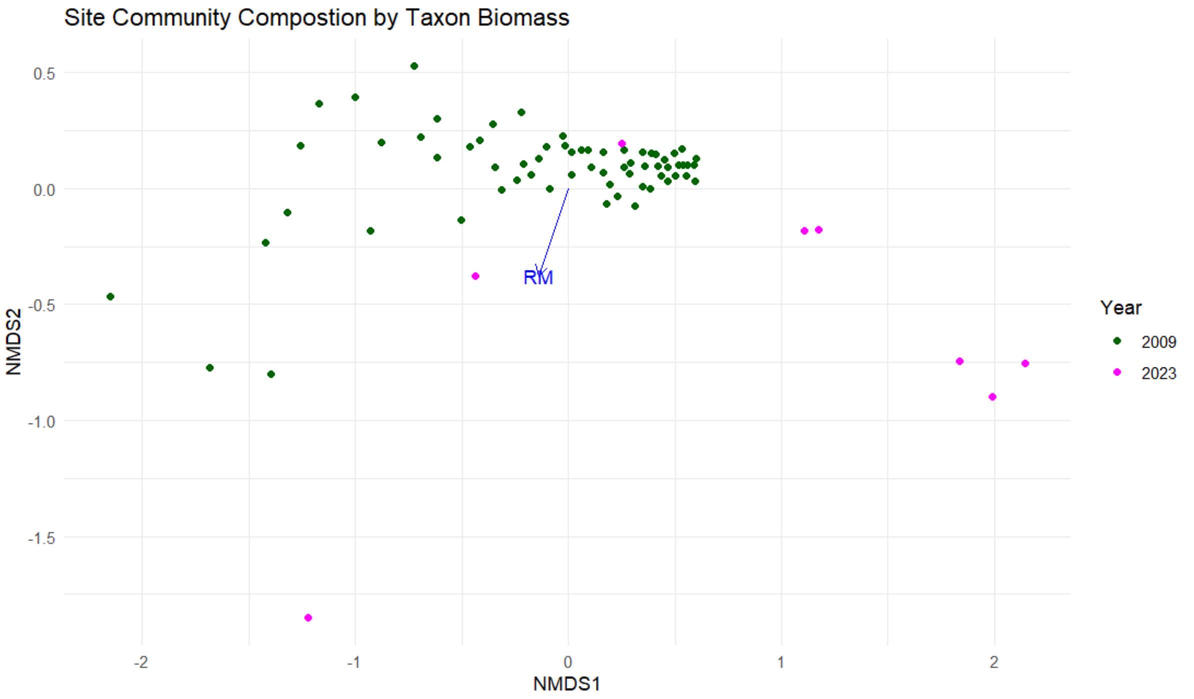

Figure 7 Non-metric multidimensional scaling (NMDS) plot illustrating site community composition based on taxon on natural log biomass (log (biomass+1) for 2009 (green) and 2023 (pink). The blue arrow represents the correlation of river mile (RM) with the NMDS axes.

Longitudinal Gradient of Orders

The linear regression analysis of the 2009 dataset indicated varying relationships between river mile and the biomass of each order. Plecoptera was the only order that exhibited a significant relationship with river mile ($r^2 = 0.125$, $p = 0.006$). This positive correlation suggests that as river miles decrease (moving downstream), the biomass of Plecoptera increases (Figure 8). The other orders Diptera, Ephemeroptera, and Trichoptera showed no significant relationships between biomass and river mile, which suggest no strong spatial patterns in their distribution along the river gradient (Appendix C).

The 2023 dataset showed no significant relationships between river mile and biomass for any of the analyzed orders. Diptera demonstrated a weak positive correlation ($r^2 = 0.106$, $p = 0.430$), indicating a slight increase in biomass with decreasing river miles (moving downstream), but this relationship was not statistically significant (Figure 9). Ephemeroptera and Trichoptera exhibited a moderate negative correlation, neither of which was significant. Plecoptera did not produce a meaningful regression outcome due to insufficient data (Appendix D).

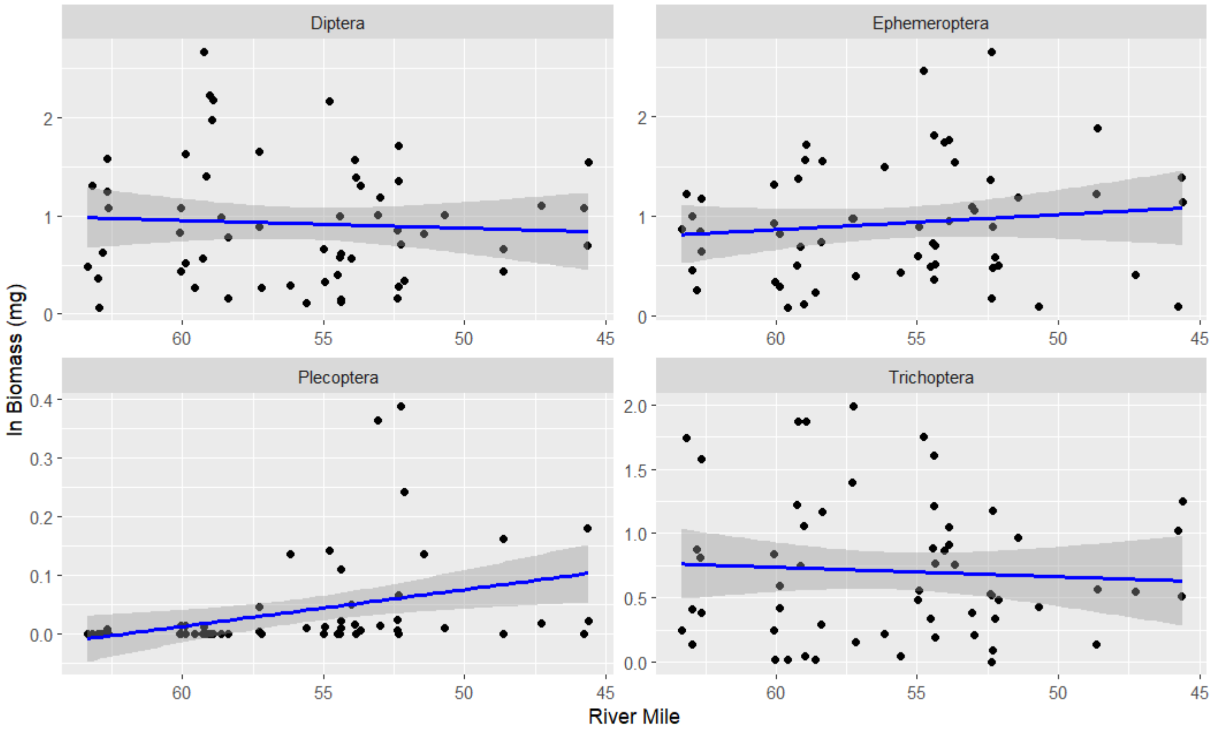

Figure 8 Linear regression plots for Orders showing biomass versus river mile for the 2009 Conasauga River dataset. Each plot depicts the relationship for a different Order. The regression line is shown in blue with shaded areas representing the confidence intervals. Plots outlined in green show significant trends.

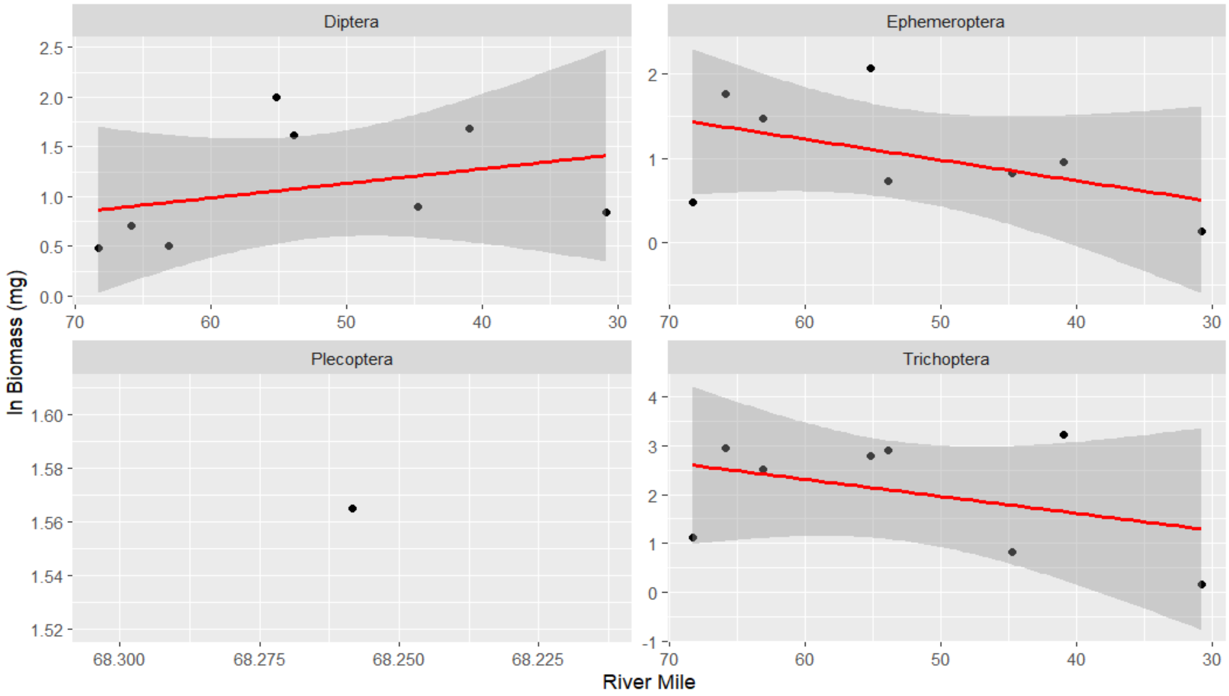

Figure 9 Linear regression plots for Orders showing biomass versus river mile for the 2023 Conasauga River dataset. Each plot depicts the relationship for a different Order. The regression line is shown in red with shaded areas representing the confidence intervals.

Longitudinal Gradient of Functional Feeding Groups

The linear regression analysis of the 2009 dataset indicated varying relationships between river mile and the abundance and biomass of FFGs (Figures 10 & 11). In terms of abundance, only piercers ($r^2 = 0.207$, $p = 0.0003$) and scrapers ($r^2 = 0.078$, $p = 0.034$) were significantly associated with river mile (Appendix E). The piercer group showed a positive correlation, indicating that abundance increases as river mile decreases suggesting that these organisms are more abundant in upstream conditions (Figure 10). Conversely, the scraper group exhibited a negative relationship with abundance decreasing as the river mile increases (Figure 11). For biomass, piercers were the only group that exhibited a significant relationship with river mile ($r^2 = 0.101$, $p = 0.015$) (Figure 11). The other FFGs showed no significant relationship between biomass and river mile (Appendix F).

The linear regression analysis of the 2023 dataset revealed that predators ($r^2 = 0.571$, $p = 0.03$) and scrapers abundance ($r^2 = 0.618$, $p = 0.021$) exhibited significant relationships with river mile (Appendix G). Both showed a negative correlation suggesting that as river miles decrease the abundance increases (Figure 12). For biomass, scrapers ($r^2 = 0.623$, $p = 0.020$) were the only group to show a significant correlation between biomass and river mile. Biomass decreased as river miles increased (Figure 13 & Appendix H).

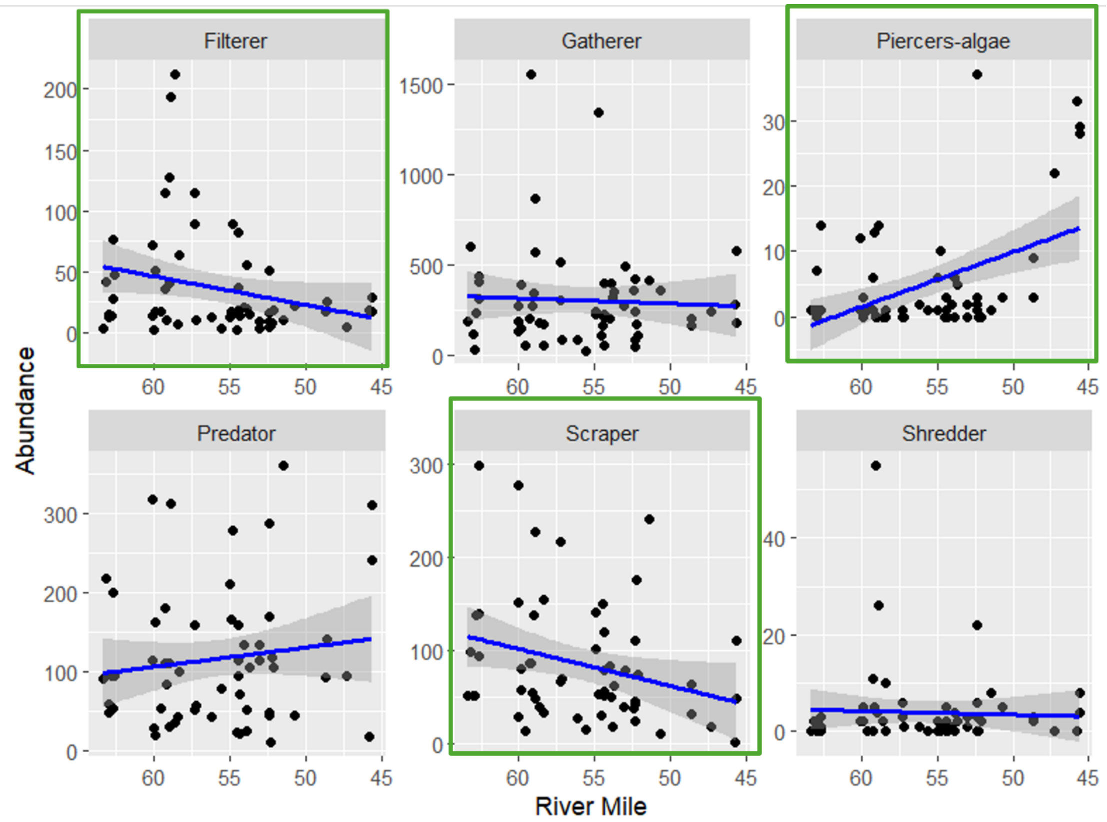

Figure 10 Linear regression plots for Functional Feeding Groups (FFGs) showing abundance versus river mile for the 2009 Conasauga River dataset. Each plot depicts the relationship for a different FFG. The regression line is shown in blue with shaded areas representing the confidence intervals. Plots outlined in green show significant trends.

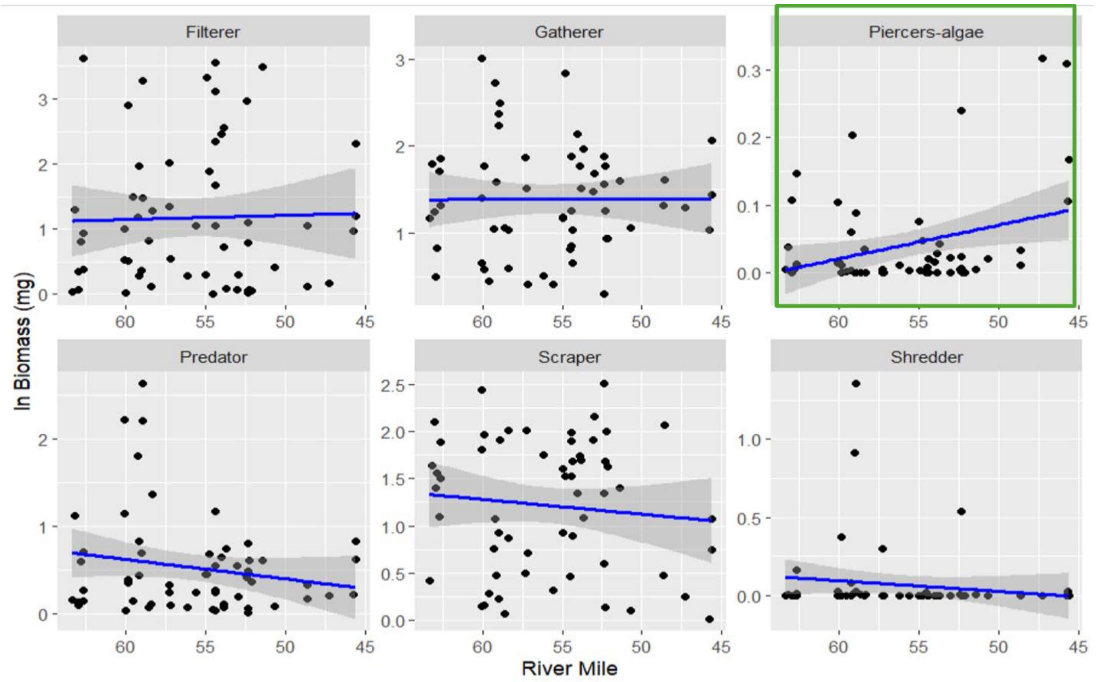

Figure 11 Linear regression plots for Functional Feeding Groups (FFGs) showing natural log biomass (log (biomass+1) versus river mile for the 2009 Conasauga River dataset. Each plot depicts the relationship for a different FFG. The regression line is shown in blue with shaded areas representing the confidence intervals. Plots outlined in green show significant trends.

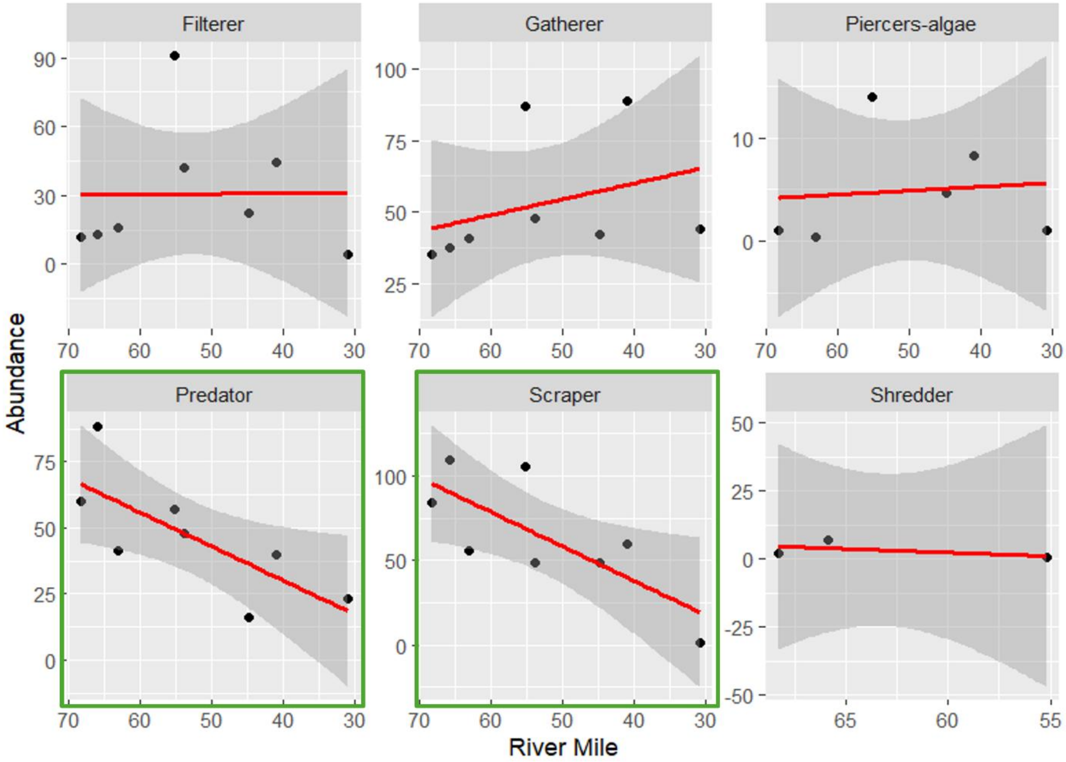

Figure 12 Linear regression plots for Functional Feeding Groups (FFGs) showing abundance versus river mile for the 2023 Conasauga River dataset. Each plot depicts the relationship for a different FFG. The regression line is shown in red with shaded areas representing the confidence intervals. Plots outlined in green show significant trends.

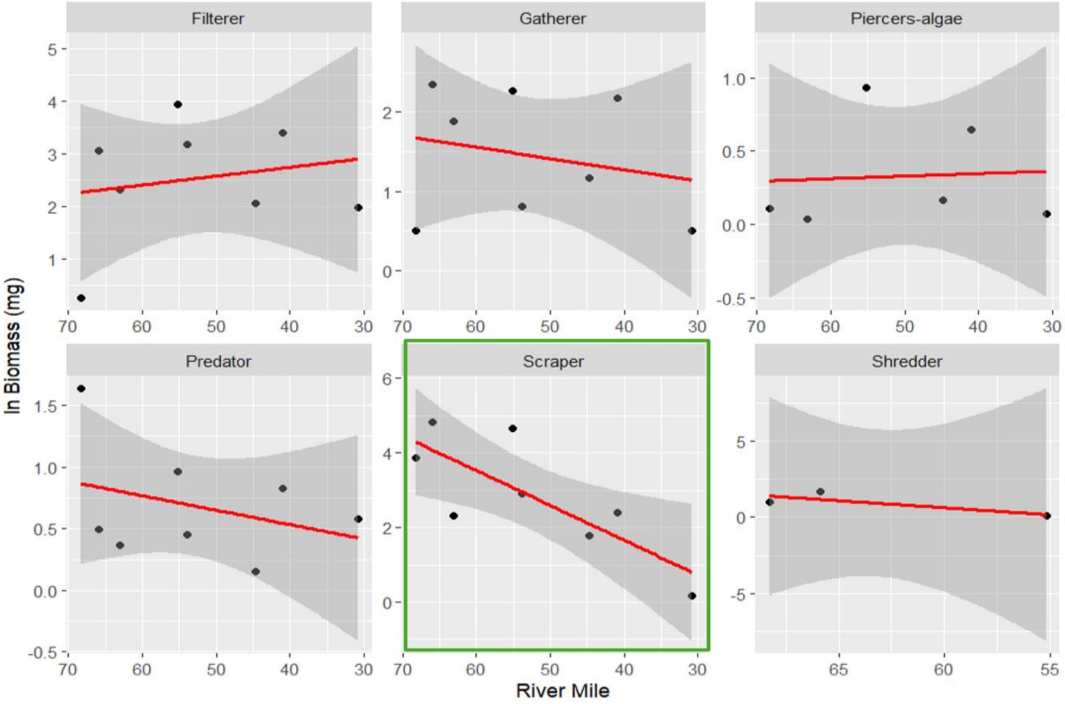

Figure 13 Linear regression plots for Functional Feeding Groups (FFGs) showing natural log biomass (log (biomass+1) versus river mile for the 2023 Conasauga River dataset. Each plot depicts the relationship for a different FFG. The regression line is shown in red with shaded areas representing the confidence intervals. Plots outlined in green show significant trends.

CHAPTER 4

DISCUSSION

We initially hypothesized that macroinvertebrate populations in Conasauga river would show lower biomass and abundance than those in neighboring communities and global reference sites. We also considered that a scarcity of macroinvertebrates could be a factor influencing observed declines in fish abundance. However, we did not find strong support for this hypothesis. Our findings suggest that the river's macroinvertebrate communities maintain biomass levels comparable to references. When looking at the 4 orders of Diptera, Ephemeroptera, Plecoptera, and Trichoptera we observed weak longitudinal gradient in macroinvertebrate abundance and biomass. In the 2009 dataset, Plecoptera was the only order that showed a significant positive relationship with river mile. In terms of functional feeding groups, the Conasauga River displayed a weak longitudinal gradient in macroinvertebrate abundance and biomass. The FFG scrapers particularly were driving the observed gradient. We observed a substantial influence of river mile on macroinvertebrate community composition when represented as presence/absence. However, we did not see significant influence of river mile on biomass of community composition. The study’s weak longitudinal gradient contrast with would typically be expected based on the RCC. The RCC typically suggests that headwaters are dominated by shredders and collectors, while mid-sized streams support a greater diversity of grazers and predators as habitat complexity and energy inputs change along the river course (Vannote et al., 1980). The results of this study align more closely with those of Grubaugh et al. (1996), who found that localized geomorphic and anthropogenic factors often play a more significant role in shaping macroinvertebrate communities than the RCC alone might predict (Grubaugh et al., 1996).

Although the limited relationship between river mile and macroinvertebrate biomass found in this study challenges the conventional understanding of longitudinal gradients in freshwater ecosystems, it’s important to consider that this study did not begin the Conasauga River’s headwaters. Our results may support the work of Harding et al. (1998), who demonstrated that land use legacies, especially historical agricultural practices, can have long-lasting impacts on stream biodiversity. This often leads to persistent alterations in community structure even after reforestation or other restoration efforts. Longitudinal gradients provide a useful starting point for understanding stream ecosystems. However, the distinct ecological and human influences in each river system can often lead to significant deviations from the RCC. As a result, managing and conserving freshwater ecosystems requires a more integrated approach. Delong and Brusven (1998) provide additional context for interpreting our results. They observed that agricultural impacts could significantly alter macroinvertebrate communities, which leads to reduced diversity and a shift towards pollution-tolerant species in certain sections of streams (Delong & Brusven, 1998). This suggests that agricultural practices may have disrupted the expected longitudinal patterns in the Conasauga River by altering habitat quality and food availability. These changes could potentially override the natural gradients predicted by the RCC. The presence of agricultural land use and its related stressors, such as sedimentation and nutrient loading, could help explain why the anticipated upstream-to-downstream changes in community structure and biomass were less pronounced in this study (Lasier et al., 2016).

The 2009 biomass dataset showed that Plecoptera was the only order exhibiting a clear positive correlation with river mile. This result is somewhat unexpected because Plecoptera are typically associated with cooler upstream environments (Hynes, 1970). In contrast, Diptera, Ephemeroptera, and Trichoptera showed no clear relationships with river mile. This suggests there is a lack of a strong spatial distribution patterns along the river gradient. The lack of spatial trends for these orders is consistent with research indicating that local environmental factors often exert a more significant influence on macroinvertebrate communities than longitudinal gradients alone (Karaouzas, Gritzalis, & Skoulikidis, 2007).

In the 2023 dataset there were no clear relationships observed between river mile and biomass for any of the orders analyzed. Diptera showed a weak positive correlation and Ephemeroptera and Trichoptera exhibited moderate negative correlations, but none were statistically significant. This highlights the potential influence of localized factors such as habitat quality or land use changes over time (Delong & Brusven, 1998). The absence of Plecoptera, a key indicator of water quality suggests potential ecological degradation. The lack of consistent patterns between the 2009 and 2023 data suggests that environmental conditions may have changed the macroinvertebrate community structure. This makes ongoing monitoring essential for understanding these dynamics and informing conservation strategies (Allan, 2004).

When comparing the 2009 and 2023 datasets, we found notable differences and similarities in how FFGs respond to variations in river mile. In 2009 the piercers-algae group showed positive relationships with river mile indicating a preference for upstream conditions in both abundance and biomass. Filters and scrapers displayed a negative relationship for abundance and river mile. By 2023 the significant groups had shifted with predators and scrapers showing significant relationships for abundance and just scrapers for biomass. This shift highlights the changing dynamics of these groups over time. The scraper group consistently showed a significant negative correlation with river mile in both years suggesting a stable preference for upstream environments.

These results indicate that efforts to preserve macroinvertebrate diversity may require more than traditional approaches like protecting headwaters or restoring downstream habitats in isolation. Considering the significant roles of localized and historical factors, conservation strategies in the Conasauga River should adopt a broader approach that addresses both past and present land use impacts (Nagy, 2024). Such a holistic strategy could better maintain the ecological health of the river and its macroinvertebrate communities.

The limited sampling scope and the substantial time gap between the 2009 and 2023 datasets might affect the interpretation of our results. These datasets offer valuable insights into macroinvertebrate community structure but should not be viewed as direct evidence of temporal changes. Differences in environmental conditions between the sampling periods could affect results. The NMDS analysis revealed that the macroinvertebrate communities differed between the two time points, which could suggest shifts driven by human activity or shifts in climate. Given that we only have two snapshots in time we cannot draw firm conclusions about the underlying causes of these differences.

Future research would benefit from longitudinal studies with more frequent sampling and a broader range of environmental variables to provide deeper insights into the complex interactions between macroinvertebrate communities, land use, and other anthropogenic factors. The weak longitudinal gradient observed in the study implies that localized factors such as land use changes and environmental conditions may play a more critical role in shaping community dynamics than previously thought. Conservation strategies should focus on addressing localized stressors and preserving the diverse ecological attributes that support the resilience of macroinvertebrate communities in the Conasauga River. Additionally, examining the potential role of aquatic macrophytes as suggested by previous research could help clarify their influence on benthic macroinvertebrates and inform more targeted conservation strategies (Baker, 2012).

Our study on the Conasauga River shows a potentially concerning decline in macroinvertebrate biomass downstream contrary to the predictions of the River Continuum Concept (RCC), which suggests that biomass should increase downstream due to more productive habitats. This downward trend together with low predator biomass and the absence of Plecoptera key indicators of water quality could suggest potential ecological degradation. Such changes could relate to the increasing land use intensity, pollution, and habitat loss downstream, possibly limiting food sources for fish and help explain the observed declines in fish populations. Conservation efforts should therefore focus on addressing these localized impacts to effectively preserve the ecological integrity of the Conasauga River and similar freshwater systems.

APPENDIX

Appendix A: The Taxon list for all the macroinvertebrates collected in 2023.

TaxonID	Order	Family	Genus
C.DIN	Coleoptera	Gyrinidae	Dineutus
C.ELM	Coleoptera	Elmidae (Larvae)	Unknown
C.MIC	Coleoptera	Elmidae (Larvae)	Microcylloepus
C.OPT	Coleoptera	Elmidae (Adult)	Optioservus
C.PSE	Coleoptera	Psephenidae	Psephenus
C.STE	Coleoptera	Elmidae (Adult)	Stenelmis
CORB	Veneroida	Corbiculidae	Corbicula
D.ANT	Diptera	Limoniidae	Antocha
D.CER	Diptera	Ceratopogonidae	Many genera
D.HEM	Diptera	Empididae	Hemerodromia
D.NON	Diptera	Chironomidae	Non-Tanypodinae
D.SIM	Diptera	Simuliidae	Simulium
D.TAN	Diptera	Chironomidae	Tanypodinae
E.ANT	Ephemeroptera	Potamanthidae	Anthopotamus
E.BAE	Ephemeroptera	Baetidae	Baetis
E.BUN	Ephemeroptera	Baetidae	Unknown
E.CAE	Ephemeroptera	Caenidae	Caenis
E.EPH	Ephemeroptera	Ephemerellidae	Ephemerella
E.EUN	Ephemeroptera	Ephemerellidae	Unknown
E.GOE	Ephemeroptera	Goeridae	Goerita

E.HUN	Ephemeroptera	Heptageniidae	Unknown
E.ISO	Ephemeroptera	Isonychiidae	Isonychia
E.STO	Ephemeroptera	Heptageniidae	Stenonema
E.TEL	Ephemeroptera	Ephemerellidae	Teloganopsis deficiens
E.TRI	Ephemeroptera	Leptohyphidae	Tricorythodes
G.PLA	Gastropoda	Planoribidae	
G.PLE	Gastropoda	Pleuroceridae	
LIMP	Patellogastropoda	Limpets	
M.COR	Megaloptera	Corydalidae	Corydalus
MITE	Acari	Hydrachnidae	
O.ARI	Odonata	Gomphidae	Arigomphus
OLIG	Oligochaeta		
OSTR	Ostracoda		
P.PEA	Plecoptera	Perlidae	Perlinella
T.BRA	Trichoptera	Brachycentridae	Brachycentrus
T.CHE	Trichoptera	Hydropsychidae	Cheumatopsyche
T.CHI	Trichoptera	Philopotamidae	Chimarra
T.HEL	Trichoptera	Helicopsychidae	Helicopsyche
T.HLA	Trichoptera	Hydroptilidae	Hydroptila
T.HUN	Trichoptera	Hydropsychidae	Unknown
T.HYD	Trichoptera	Hydropsychidae	Hydropsyche
T.LEP	Trichoptera	Lepidostomatidae	Lepidostoma

T.MIC	Trichoptera	Brachycentridae	Micrasema
T.NEC	Trichoptera	Leptoceridae	Nectopsyche
T.OEC	Trichoptera	Leptoceridae	Oecetis
T.PRO	Trichoptera	Glossosomatidae	Protoptila
T.SET	Trichoptera	Leptoceridae	Setodes
T.WOR	Trichoptera	Philopotamidae	Wormaldia
TURB	Turbellaria		

Appendix B: ANOVA table showing the source of variation, degrees of freedom (Df), sum of squares (Sum Sq), mean square (Mean Sq), F value, and p-value (Pr(>F)) for macroinvertebrate biomass across different rivers.

Source	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
River	2	2565	1282	0.847	0.43
Residuals	239	362044	1515		

Appendix C: Results of linear regression testing the relationship of Order natural log biomass and river mile 2009.

Order	**r.squared**	**p.value**	**Std Error**
Diptera	0.004	0.653	0.017
Ephemeroptera	0.015	0.365	0.017
Plecoptera	0.125	0.006	0.002
Trichoptera	0.004	0.635	0.015

Appendix D: Results of linear regression testing the relationship of Order natural log biomass and river mile 2023.

Order	r.squared	p.value	Std error
Diptera	0.1064068	0.430377	0.0172857
Ephemeroptera	0.2405799	0.217189	0.0177689
Plecoptera	0	NA	NA
Trichoptera	0.1528823	0.338166	0.0334705

Appendix E: Results of linear regression testing the relationship of FFG abundance and river mile 2009 dataset.

FFG	r.squared	p.value	Std Error
Filterer	0.062	0.059	1.214
Gatherer	0.003	0.708	7.790
Piercers-algae	0.207	0.000336	0.219
Predator	0.017	0.323	2.446
Scraper	0.078	0.034	1.822
Shredder	0.002	0.748	0.237

Appendix F: Results of linear regression testing the relationship of FFG natural log biomass and river mile 2009 dataset.

FFG	r.squared	p.value	Std Error
Filterer	0.001	0.835	0.031
Gatherer	0.00000834	0.983	7.790
Piercers-algae	0.101	0.015	0.002
Predator	0.034	0.166	0.016
Scraper	0.011	0.435	0.020
Shredder	0.020	0.295	0.006

Appendix G: Results of linear regression testing the relationship of FFG standardized abundance and river mile 2023.

FFG	r.squared	p.value	Std Error
Filterer	0.001	0.954	0.845
Gatherer	0.111	0.42	0.64
Piercers-algae	0.01	0.851	0.189
Predator	0.571	**0.03**	0.481
Scraper	0.618	**0.021**	0.653
Shredder	0.276	0.648	0.408

Appendix H: Results of linear regression testing the relationship of FFG natural log biomass and river mile 2023 biomass.

FFG	**r. squared**	**p. value**	**Std Error**
Filterer	0.038	0.645	0.035
Gatherer	0.055	0.577	0.024
Piercers	0.004	0.900	0.013
Predator	0.111	0.420	0.014
Scraper	0.623	0.020	0.030
Shredder	0.657	0.398	0.067

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