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RELATIONSHIPS BETWEEN WATERSHED FACTORS AND COMMUNITY SIZE SPECTRA OF THE FISH AND MACROINVERTEBRATE COMMUNITIES OF MID-APPALACHIAN STREAMS

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RELATIONSHIPS BETWEEN WATERSHED FACTORS AND COMMUNITY SIZE
SPECTRA OF THE FISH AND MACROINVERTEBRATE COMMUNITIES OF MID-
APPALACHIAN STREAMS

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Thesis submitted
to the Davis College of Agriculture, Natural Resources, and Design
at West Virginia University

in partial fulfillment of the requirements for the degree of

Master of Science in
Wildlife and Fisheries Resources

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2023

Keywords: Stream community structure, watershed factors, community size spectra, mid-order streams, Mid-Appalachian, ataxic, macroinvertebrates, fish, watershed assessment

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ABSTRACT

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Jarrett H. Landreth

Freshwater lotic communities are complex, dynamic, and quick to respond to changes to their environment. However, some of the specific mechanisms driving those changes are yet to be fully understood. Here, I will use the community size spectrum (CSS) approach to assess how the CSS slope, elevation, and food chain length of the fish and macroinvertebrate assemblages as well as the combined CSS of 15 mid-sized (2nd-4th order) streams in north-central West Virginia respond across gradients of watershed factors (drainage area, mean drainage slope, mean drainage elevation, drainage % forest, and stream specific conductance). Field sampling was carried out in summer of 2022, and laboratory analysis was completed in spring of 2023. \log_2 size bins vs. \log_2 densities were used to build CSS for each stream community. Results from the CSS were then used in an analytical procedure combining principal component analysis and univariate correlation which produced a set of significant correlations between certain watershed factors and CSS parameters that either followed established ecological knowledge or revealed caveats unique to the study design. Results provide evidence for including external watershed factors such as drainage elevation, slope, and land cover when assessing stream community structure as they could play a role in explaining variation between communities that cannot be accounted for by analyzing only biotic factors such as functional groups or diversity. Through this research, the community size spectrum approach to aquatic community assessment was shown as a valuable tool for stream ecologists due to its close link with changes to watersheds. It would be beneficial to add CSS to the repertoire of stream community assessment tools when seeking to better understand these diverse, dynamic, and vulnerable systems. Future research should focus on streams from a single major watershed to remove geographic location as a driver of variation so the importance of the other watershed factors on CSS parameters could be more effectively teased out, and then integrate these results into an analysis with well-studied biotic factors so that the level of explanatory power for each can be better understood. An ideal future goal for using CSS in stream community management would then be to develop a predictive model that can output estimated future CSS parameters (slope, elevation, and FCL) once biotic factors, geomorphology, and proposed land cover/water quality remediation effects are put into the model. This would give managers the capability to set targets for holistically improving community structure before breaking ground on any restoration/management projects along with thresholds for how much development can be done to a watershed before the stream community structure is significantly harmed.

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ABSTRACT

Freshwater lotic communities are complex, dynamic, and quick to respond to changes to their environment. However, some of the specific mechanisms driving those changes are yet to be fully understood. Here, I will use the community size spectrum (CSS) approach to assess how the CSS slope, elevation, and food chain length of the fish and macroinvertebrate assemblages as well as the combined CSS of 15 mid-sized (2nd-4th order) streams in north-central West Virginia respond across gradients of watershed factors (drainage area, mean drainage slope, mean drainage elevation, drainage % forest, and stream specific conductance). Field sampling was carried out in summer of 2022, and laboratory analysis was completed in spring of 2023. Log₂ size bins vs. log₂ densities were used to build CSS for each stream community. Results from the CSS were then used in an analytical procedure combining principal component analysis and univariate correlation which produced a set of significant correlations between certain watershed factors and CSS parameters that either followed established ecological knowledge or revealed caveats unique to the study design. Results provide evidence for including external watershed factors such as drainage elevation, slope, and land cover when assessing stream community structure as they could play a role in explaining variation between communities that cannot be accounted for by analyzing only biotic factors such as functional groups or diversity. Through this research, the community size spectrum approach to aquatic community assessment was shown as a valuable tool for stream ecologists due to its close link with changes to watersheds. It would be beneficial to add CSS to the repertoire of stream community assessment tools when seeking to better understand these diverse, dynamic, and vulnerable systems. Future research should focus on streams from a single major watershed to remove geographic location as a driver of variation so the importance of the other watershed factors on CSS parameters could be more effectively teased out, and then integrate these results into an analysis with well-studied biotic factors so that the level of explanatory power for each can be better understood. An ideal future goal for using CSS in stream community management would then be to develop a predictive model that can output estimated future CSS parameters (slope, elevation, and FCL) once biotic

factors, geomorphology, and proposed land cover/water quality remediation effects are put into the model. This would give managers the capability to set targets for holistically improving community structure before breaking ground on any restoration/management projects along with thresholds for how much development can be done to a watershed before the stream community structure is significantly harmed.

Keywords: Stream community structure, watershed factors, community size spectra, mid-order streams, Mid-Appalachian, ataxic, macroinvertebrates, fish, watershed assessment

INTRODUCTION

Freshwater lotic communities are known to be complex, dynamic, and quick to respond to changes to their environment, but some of the specific mechanisms driving those changes are yet to be fully understood (McGarvey and Kirk 2018, Shin et al. 2005). The structure of a stream community is a delicate balancing act, with a diverse and dense array of individuals competing for limited energy. Any changes to the surrounding watershed, whether natural or anthropogenic, can alter the available energy in a stream effectively forcing its community to restructure its food web to return to equilibrium (Vannote et al. 1980). Achieving an understanding of the major sources of change in watersheds, or watershed factors, that impact stream communities and how those impacts translate to community structure has been the topic of extensive research (Dimech et al. 2008, Blanchard et al. 2014, Marin et al. 2023).

Historically, efforts have focused on studying factors such as stream hydrology and sediment loading to develop theoretical frameworks predicting community structure, e.g. the river continuum (Vannote et al. 1980) and flood pulse (Junk 1999) concepts. Another path historically taken was developing diversity assessments, defining functional groups, and creating other taxon-based approaches that used the presence and/or abundance of taxa with widely accepted ecological roles to inform conclusions on community structure and impairment levels in stream ecosystems (Caetano et al. 2016, Roth et al. 1996, Rueda et al. 2015, Cooper et al. 2018). Ataxic approaches to assessing aquatic community structure were developed more recently in marine systems and weren't adapted to stream research until much later (Shin et al. 2005, Blanchard et al. 2014, Petchey et al. 2010). These approaches, commonly called size spectra, study the size structure of an entire stream community which is directly linked to the available energy in the system (White et al. 2007, Edwards et al. 2017). Links can then be drawn between changes in an aquatic system and specific changes to its community's size structure (Dimech et al. 2008, Blanchard et al. 2014, Marin et al. 2023). Using this community size spectrum (CSS) technique in stream systems to assess the mechanisms that govern stream community structure in a holistic manner will add to the understanding of what makes these dynamic ecosystems tick, giving watershed managers an effective tool for stream community conservation (McGarvey and Kirk 2018, Shin et al. 2005, Marin et al. 2023).

Watershed Factors

Freshwater lotic systems (streams and their watersheds) are defined by many factors that vary in time and space, and these can be influenced by anthropogenic activity. Several studies have focused on which watershed factors are the primary mechanisms driving community structure. Broad theoretical concepts such as the river continuum (Vannote et al. 1980) and flood pulse (Junk et al. 1999) highlighted stream hydrology and sediment loading along longitudinal and horizontal axes of watersheds for playing major roles. Some other examples include land cover (altered by agricultural and urban development), runoff input (altered by industrial, municipal, and agricultural pollution), and topography (driven by drainage area, slope, and elevation) (Pomeranz et al. 2019, Liao et al. 2018, Poff et al. 1997). Lotic systems are sensitive to such factors because they act as a funnel for biotic and abiotic influences from throughout their watersheds (Poff et al. 1997). Altering the land cover of a drainage by increasing the impervious surface area and/or the agricultural land area can lead to flashier flood events, increased pollution from runoff, and increased stream temperatures (Tong and Chen 2002, Du et al. 2012). These impacts could then alter community structure; flashy systems can scour out habitat and wash away vital nutrients which decreases the number of individuals that can live in that system, while high pollution and temperatures can force out sensitive taxa that are then replaced by tolerant taxa (Liao et al. 2018, Poff et al. 1997, Pomeranz et al. 2019, Merriam et al. 2011). Research has found that a decrease in pH caused by acid mine drainage and an increase in phosphorus concentrations caused by agricultural runoff are both associated with a decreased abundance of large fish and an increase in smaller, acidity-resilient fish due to large fish having slower reproductive rates, larger home ranges, and higher energy demands (Brose et al. 2017, Brown et al. 2004, Woodward et al. 2012). This results in an altered community structure lacking large predators and containing unnaturally heightened and unstable abundances of small taxa (Schorr and Backer 2006). Lastly, the topography (e.g. drainage area, slope, and elevation) of a watershed is directly tied to the structure of a stream community through driving the flow regime (Poff et al. 1997, King et al. 2012, Benejam et al. 2018). For example, streams with larger drainages tend to have relatively stable flows along with higher amounts and types of available energy sources that encourage diverse and dense communities, while smaller drainages can show the opposite trend (Vannote et al. 1980, Poff et al. 1997). Studying the relationships these and other watershed factors have with stream communities is crucial for the successful conservation

of stream communities, and several assessment approaches have been used by researchers to accomplish this goal.

Stream Assessment Approaches

Aquatic ecologists have a variety of tools and approaches at their disposal to assess stream community structure. These include the selection of indicator species (Caetano et al. 2016), the use of physical/chemical/biological indices of biological integrity (a.k.a. IBIs) (Roth et al. 1996), and species-specific condition (Rueda et al. 2015) among others. However, each of these methods give only partial pictures of the stream community being studied. The use of a single indicator species or even a multispecies IBI will inevitably leave out information about the complex interactions of an aquatic community, since only one or a fraction of the species that make up the community are being studied (Caetano et al. 2016, Roth et al. 1996). To account for these limitations, researchers often use several of these tools at once to achieve a more complete assessment (Cooper et al. 2018). It would therefore benefit aquatic ecologists to add a holistic food web-based assessment tool to the repertoire that simplifies the vast complexity of stream food webs and complements other approaches, and in recent years some began developing such a tool for stream assessment practices.

Community Size Spectra

Over the last 50 years, an indicator and management paradigm has been slowly developing called community size spectra (CSS), an ataxic approach that uses community body-size distributions to make inferences into community health and effectively considers the variable and vital roles of individuals at different life stages (Shin et al. 2005, Blanchard et al. 2014). CSS analysis was first developed in marine systems by Sheldon (1972) and the theory was further developed in the next 3 decades until it was summarized by Kerr and Dickie (2001). Since the publishing of their seminal book, *The Biomass Size Spectrum* (2001), CSS research moved from theory to application (Petchey et al. 2010). CSS are typically displayed as log x log linear regression plots of organism biomass, abundance, or density versus body size bins (White et al. 2007, Edwards et al. 2017, Blanchard et al. 2009). A non-binning CSS approach has arisen in recent years that some argue could standardize the use of CSS for better meta-analyses (White et al. 2007, Edwards et al. 2017), but for the purposes of this research a log₂ binning approach

was used. An example diagram of the type of CSS used for this research is shown in Figure 1. The slope of the relationship between abundance (or biomass) and body size is typically negative in aquatic communities due to a higher density of small individuals than large individuals (White et al. 2007, Petchey et al. 2010) and represents the ecological efficiency of the community (the rate that abundance of organisms decreases within increasing body size) (Daan et al. 2005, Sweeting et al. 2009, White et al. 2007, Murry and Farrell 2014, Mehner et al. 2016). A CSS slope can flatten when large size classes become dominated by large-bodied low-trophic species that compete for resources with individuals of small size classes, and conversely can steepen when large size classes are dominated by low densities of predatory species (White et al. 2007, Mehner et al. 2016). This is due to dominance of low-trophic taxa reducing the energy in the food web lost through predator-prey interactions, thereby increasing ecological efficiency above that of a typical aquatic community containing primarily large-bodied predators (White et al. 2007, Mehner et al. 2016). The y-intercept of the regression is typically centered, called the spectral elevation, and provides an indicator of food-web capacity (an index of the number of organisms that the food web can support) (White et al. 2007, Murry and Farrell 2014). The range of size bins shown in the regression measures the food chain length (FCL) and is an indicator of trophic complexity (Collyer et al. 2023). These three parameters make CSS useful for aquatic ecosystem management decision-making (Dimech et al. 2008, Blanchard et al. 2014).

Several studies have shown evidence of the usefulness of CSS in aquatic ecosystem management decision-making. Dimech et al. (2008) used CSS to determine that a marine fisheries protection zone was effective in increasing biomass of the demersal community, showing evidence that the management decision was a success. Blanchard et al. (2014) developed a predictive CSS model for a marine fish assemblage that effectively replicated past and current assemblage structure, as well as predicting future effects due to varying levels of fishing pressure. Marin et al. (2023) tested the effectiveness of CSS in assessing ecosystem health compared to taxon-based management approaches and determined that using CSS could make management programs more efficient. Novak et al. (in prep) are developing a CSS-based framework as a possible tool for early detection and setting management targets for invasive species control. Murry et al. (in review) suggest CSS-based management targets toward optimizing recreational fisheries. Among other studies not mentioned, these examples provide

evidence that the CSS approach has qualities that could complement taxon-based approaches when assessing a stream community (Shin et al. 2005, Marin et al. 2023).

The CSS approach has several qualities that complement taxon-based approaches such as the commonly used Index of Biotic Integrity (IBI) when carrying out aquatic community assessments (Marin et al. 2023, Shin et al. 2005). The grouping of sampled individuals into ataxic body-size bins (either by weight or length) is representative of typical aquatic predator-prey interactions (especially in temperate regions) where larger individuals generally consume individuals of a sufficiently low size class with little regard to taxa (Hury and Benke 2007, White et al. 2007). CSS are also very adaptable to different aquatic systems across ecoregions, with the same basic analytical framework able to be used on virtually any aquatic community (White et al. 2007). In addition, CSS can be developed for an aquatic community with little knowledge of the community composition prior to field sampling due to its ataxic nature. Lastly, CSS can be developed using preexisting survey data for a stream community if it includes length and/or weight data for the individuals captured (Shin et al. 2005, Kerr and Dickie 2001). These strengths allow community structure parameters to be identified for an aquatic community using less time and resources than taxon-based approaches (Shin et al. 2005, Marin et al. 2023). IBIs for example must use extensive research to become highly specialized to certain geographic regions/habitats in order to effectively function (Roth et al. 1996, Cooper et al. 2018), and CSS could utilize the early-stage IBI survey data to produce additional and distinct results on community structure to support and provide context to the eventual IBI findings (Shin et al. 2005). The strengths of each assessment method tend to complement each other, showing the potential for using a combination of the CSS approach with species-based methods to assess an aquatic community holistically. One major part of achieving that goal is describing the relationship between watershed factors and the CSS of aquatic communities (Mehner et al. 2016, Pomeranz et al. 2019, Clement et al. 2015).

CSS is used to assess changes in an aquatic community's size structure over time and how that size structure compares to other communities (McGarvey and Kirk 2018, Broadway et al. 2015, Murry and Farrell 2014). Changes in CSS within a system over time (temporal CSS) can be used to assess long-term changes in a community and relate them to processes such as climate change with increased temperatures associated with shallower CSS slopes (higher ecological efficiency) (Lindmark et al. 2019), or to assess seasonal changes in aquatic

community dynamics (McGarvey and Kirk 2018). CSS analysis can also be used to assess and compare spectra of multiple independent aquatic communities (spatial CSS) (Mehner et al. 2016, Clement et al. 2015). Both temporal and spatial CSS can answer questions about the effects of other anthropogenic alterations such as land cover transformation (Lenat and Crawford 1994, Martínez et al. 2016), pollution (Pomeranz et al. 2019), and nutrient inputs (Murry and Farrell 2014, Tsuda et al. 1992). For example, one study used temporal CSS to find that increasing non-native predator populations and decreasing phosphorus levels were associated with decreased CSS elevation (food web capacity) in a large river fish assemblage (Murry and Farrell 2014). A different study used CSS from 6 individual lakes to test the effects of natural disturbance regime, fish species richness, and lake size on fish CSS. They discovered that in half the lakes the assemblages were driven by disturbance regime and were largely stochastic, while the other half were large enough to weather disturbances with little effect on their fish assemblages (Clement et al. 2015). These temporal and spatial forms of CSS analysis have been very useful where applied, but relationships with many watershed characteristics are still poorly understood for mid-order streams.

The CSS approach and the management issues it was developed to address were largely focused on marine systems and the effects of commercial fishing (Petchey et al. 2010). This presented challenges when the approach was adapted to freshwater systems (Petchey et al. 2010) and many ecosystems (e.g. mid-order streams) were understudied. Freshwater and marine systems are fundamentally different, so researchers had to develop an understanding of CSS dynamics, drivers, and temporal/spatial variation in freshwater systems before addressing freshwater management issues (Poff et al. 1993). CSS has been used in many freshwater studies, but the majority are focused on lakes (Arranz et al. 2019, Clement et al. 2015, Lindmark et al. 2019, Tsuda et al. 1992) and large rivers (Murry and Farrell 2014, Broadway et al. 2015). Low-order lotic systems have received less attention and most focused on either benthic macroinvertebrate assemblages (Cattaneo 1993, Morin et al. 1995, Ramsay et al. 1997, Martínez et al. 2016, Pomeranz et al. 2019) or fish assemblages (Benejam et al. 2018). Few combined the fish and macroinvertebrate assemblages of a stream into one cohesive CSS (McGarvey and Kirk 2018, Poff et al. 1993, Huryn and Benke 2007). Observing the size structure of mid-order stream communities that include both fish and macroinvertebrate assemblages in relation to the factors defining their watersheds could shed light on stream drivers and dynamics, allowing for the

subsequent development of CSS-based tools for watershed management (McGarvey and Kirk 2018).

Objectives/Hypotheses

In order to shed light on the drivers and dynamics of mid-order stream community size-structure and lay the groundwork for developing CSS-based tools for watershed management, my major objective was to assess how CSS slope (ecological efficiency), elevation (food web capacity), and FCL (trophic complexity) of the fish and macroinvertebrate assemblages as well as the combined communities among 15 mid-order streams in north-central WV respond respectively across gradients of watershed factors. I hypothesized that at least some of the watershed factors listed in Table 1 (drainage area, mean drainage slope, drainage slope STDEV, mean drainage elevation, drainage % forest, and stream specific conductance (SPC)) would have strong and predictable relationships with CSS parameters due to ecological mechanisms that have been previously determined in literature (Table 1). If the responses of stream CSS to the watershed factor differences are predictable based on clear ecological mechanisms, these relationships can form the basis for a holistic, ataxic tool to evaluate stream community structure and indicate potential anthropogenic stressors.

METHODS

Study Site Description

Sampling was focused on 15 mid-sized streams located in northern and central West Virginia. I defined mid-sized streams for this study as wadable 2nd, 3rd, and 4th order. I chose the streams to represent a wide range of differences in watershed factors, from high anthropogenic impact (e.g. high deforestation, with significant historic and current acid mine drainage (AMD) inputs) to low impact (e.g. highly forested, with low AMD) (Figure 2). I selected two ~200m reaches (100-299m range) to sample from each study stream in order to collect sufficient abundances of individuals to build reliable size spectra through pooling the two site datasets. Sample reaches were bordered on the upstream and downstream ends by natural barriers such as shallow riffles and/or islands when possible. The reaches selected were generally close to a road to facilitate the transportation of sampling equipment to and from the site. When possible, the entire length of each reach was wadable for researchers wearing chest waders and backpack

electrofishers/pulling a PRAM barge electrofisher, and the widths of each reach did not exceed that which is adequately sampled by 2 backpack electrofishers and 1 PRAM barge electrofisher. Study stream sites and major characteristics are listed in Table 2.

Sampling Methods

I chose the sample reaches for each stream based on the above criteria prior to data collection. WV DNR biologists assisted with the reconnaissance and selection of the sample reaches. Sites were sampled for fish and macroinvertebrates from June 15, 2022, and finished August 4, 2022. If subsurface visibility was low enough to render the collecting methods inaccurate when we arrived at a site, we postponed sampling there until conditions improved. At each reach we recorded coordinates of the downstream end of the reach (Lat, Lon) using a GPS, length of the reach (m) using a rangefinder, and width (m) and depth (cm) of the reach at 3 evenly spaced points using a measuring tape. We also measured stream SPC (mS/cm) at 3 evenly spaced points along the reach using a Multiparameter Sonde. Variables collected multiple times for each stream were averaged to calculate the mean value of each variable for each stream. In addition to the field data collected, with ArcGIS Pro spatial analyst tools I used the “NLCD 2019 (CONUS)” online GIS database to record stream drainage % forest land cover (USGS 2023) and the “NHDPlus High Resolution” online GIS database to record stream drainage area (km²), mean drainage slope and STDEV, and mean drainage elevation (m) for each study stream. Each of these geomorphological watershed factors were calculated using all of the land area draining into the study stream from headwaters to mouth (USGS 2023) (Table 3).

Macroinvertebrate Sampling Protocol

I used a 0.09 m² Surber Sampler with 500 µm mesh to collect macroinvertebrates from 9 sites that are evenly divided between 1-3 riffles within the reach (depending on the number of riffles available in the reach), according to the NAMC Protocol for the Collection of Aquatic Macroinvertebrate Samples (NAMC 2015). I selected each of the 0.09 m² areas of the substrate for sampling based on the location having consistent water flow and less depth than the Surber Sampler’s height (to facilitate accurate sampling). At each selected sample site, the Surber Sampler’s mouth was placed facing directly upstream, with the area surrounded by the attached 0.09 m² net frame. All substrate 2-3cm deep within the delineated area was scrubbed clean (if

larger rocks) or thoroughly disturbed (if gravel or fine sediment), releasing macroinvertebrates to drift downstream into the Surber Sampler. The contents were emptied into a 70% alcohol-filled 100 mL bottle on the stream bank (NAMC 2015). The 9 bottles of macroinvertebrate samples from each study site were taken to the laboratory for further processing.

In the laboratory after field sampling was completed, I identified all preserved individuals to family (Benke et al. 1999). After individuals were identified I used an AmScope Trinocular Stereo Zoom Microscope with an AmScope MU1603 camera attached to take 16MP images of the samples under 1.7X magnification. These images gave accurate lengths (mm) of each specimen when the camera's associated software package was used (AmScope V4.11.04022022). Individuals were measured according to established methods (e.g., anterior of the head to the final abdominal segment, excluding cerci, for most taxa) (Benke et al. 1999, Pomeranz et al. 2019). Previous publications have established dry weight estimates based on measured lengths of the taxa collected, so I used these length/weight relationships to calculate the dry weight (g) of each macroinvertebrate sampled (Benke et al. 1999, Smock 1980). If length/weight equations couldn't be found in literature for a particular family, the next closest related family that did have an equation developed was used (Table 4).

Fish Sampling Protocol

Following 2011 WVDEP-WAB Wadable Stream Fish Collection protocols, researchers formed a line evenly spaced across the width of the stream at the downstream end of the reach. The number of netters, electrofishers, etc. were adapted to the size of each reach sampled to keep the sampling effort consistent. In reaches where two backpack electrofisher operators were adequate, four additional crew members were evenly distributed around them for netting support. In reaches that required assistance from the 2-3 WV DNR biologists and their PRAM barge electrofisher (ETS SDC-1 model), they were evenly spaced across the deepest part of the stream channel with the rest of the team split evenly on either side. In sites small enough to only require a single backpack electrofisher (APB-4-MR model), the electrofisher operator walked in the center of the channel with one netter on each side.

We then walked upstream at a pace that allowed the electrofisher operators to sweep their anode probes across the entire width of the stream about every 2 meters walked upstream. Netters captured as many stunned fish as possible and placed them in the closest water-filled 5-

gal bucket or the holding tank on the PRAM barge. We attempted to capture every fish observed, regardless of species or size. The probe sweeps were kept roughly ≤ 10 cm below the surface to draw the stunned fish upward for ease of capture. Once we reached the upstream end of the site, all captured fish were put into a large container filled with water on the stream bank and oxygenated using battery powered aerators. A single pass with proper electrofishing methods has been shown to accurately represent the fish assemblage, and its effectiveness is statistically similar to that of multi-pass methods (Reid et al. 2009).

Team members first processed the largest individuals, identifying each to species and measuring total length (mm) of every fish caught. Each fish was also weighed (g) on a field scale. All processed fish were then promptly released back into the sample reach. This process continued until only individuals less than 100mm in length were yet to be measured. The remaining fish were preserved in 10% formalin-filled 1-gal containers to be taken back to the laboratory where they were identified to species, measured for total length (mm), and weighed (g) post-sampling (WVDEP-WAB 2011). Fish wet weights were converted to estimated dry weight with an established conversion factor (1 g wet weight = 0.2 g dry weight, McGarvey and Kirk 2018, Waters 1977) (Table 5). Fish weight data were used along with the macroinvertebrate weight data to develop CSS for each stream site.

Data Analysis

I constructed community size spectra (CSS) for each assemblage/community from each sample stream, combining the data from the up- and downstream sites on each stream. To accomplish this, I first binned all individuals by assemblage (macroinvertebrates and fish) by dry weight into \log_2 size bins. I then centered those bins on the y-axis to make the resulting CSS y-intercept (elevation, a.k.a. food web capacity) comparable between stream communities, using the equation below for the mean \log_2 mass of each bin:

$$\text{Centered Mean of Bin } x = \text{Bin } x \text{ Mean} - \text{Average of All Bin Means}$$

I calculated the density (number of individuals per size bin / sampled stream area in m^2) of each size bin, and \log_2 transformed the densities. It is well established that the smallest size classes of individuals are typically under-sampled in aquatic sampling protocols for both

macroinvertebrates and fish, so I removed the smallest fish and macroinvertebrate size bins that had unnaturally low-density values (i.e. lower than the density of the next largest size bin) (McGarvey and Kirk 2018). The minimum cutoff for macroinvertebrate estimated dry weight was 6.26×10^{-5} g (removing the bottom six \log_2 size bins) and the minimum cutoff for fish estimated dry weight was 1.26×10^{-1} g (removing the bottom three \log_2 size bins). This was done to prevent the assemblage ecological efficiencies from being skewed due to sampling error (McGarvey and Kirk 2018). For the combined community, I first identified any overlapping size bins between the two assemblages that would need to be merged. Then, I took the weighted mean of each overlapping size bin's mean dry weights to calculate a new mean \log_2 dry weight for the affected bins. I next took the mean of the two densities of each overlapped size bin and made that the new density value for the affected bins, as opposed to summing the overlapping bins due to the overlapped bins being sampled twice through a combination of macroinvertebrate and fish collection protocols. This method of merging overlapping size bins was developed for the purposes of this research in order to include them in analysis to achieve as holistic a picture of the communities as possible; past studies with similar methods omitted overlapping macroinvertebrate and fish size bins entirely (McGarvey and Kirk 2018). Once overlapped size bins were merged, the combined community mean \log_2 weights of each size bin were centered and each bin's density was \log_2 transformed as with the individual assemblages. I next created linear regressions of the macroinvertebrate assemblage, fish assemblage, and combined community for each study stream using centered mean \log_2 mass of each size bin vs. \log_2 density of each size bin (a.k.a. community size spectrum), which gave me the three parameters of each assemblage/community that I needed for producing results: the CSS slopes (ecological efficiency), elevations (centered y-intercepts, a.k.a. food web capacity), and FCL's (size bin ranges, a.k.a. trophic complexity).

I used a combination of univariate correlation and principal component analysis (Murry and Farrell 2014, Murry et al. in review) to assess relationships between the three CSS parameters for each assemblage/community and the watershed factors listed in Table 1. To begin, I used Pearson correlation analyses to verify independence among the CSS response variables and for multicollinearity among watershed factors. I conducted principal component analyses (PCA) on the environmental and watershed factors in Table 1 (including an added numeric variable representing the major watershed each stream drained into based on relative size: Cheat

River = 1, Monongahela River = 2, and Ohio River = 3). This produced loadings for each watershed factor, shown in Table 7. I next ran the new variables built from the PCA's in Pearson correlation analyses against the three CSS parameters for each assemblage/community to identify environmental and watershed factors that were strongly influential (Murry and Farrell et al. 2014, Murry et al. in review). I used the results of the analyses to produce 10 correlation plots (Figures 2a-c, 3a-b, and 4a-c) which show the principal component(s) that were most strongly associated with each CSS parameter. Principal components were then decomposed to identify the environmental and watershed factors related to CSS parameters.

RESULTS

Across all 15 study streams, I identified, measured, and estimated dry weight for 39,425 macroinvertebrates which included 14 orders and 64 families (Table 4). Their estimated dry weights ranged from < 0.0001 g to 5.3 g. An average of 2628 macroinvertebrates (range 759-5686) were sampled per stream. Fish assemblage data collection produced 21,709 individuals that included 14 families, 36 genera, and 60 species as well as 8 different hybrids (Table 5). Their estimated dry weights ranged from 0.1 g to 1,289.1 g. An average of 1447 fish (range 248-3508) were sampled per stream.

CSS parameters (slope, elevation, and FCL) for each stream assemblage/community can be seen in Table 6 and Figure 3. CSS slope on average was lowest in the combined communities (mean = -1.04) and highest in fish assemblages (mean = -0.61). It was the most variable among fish assemblages (STDEV = 0.22) and least variable among combined communities (STDEV = 0.07). Size spectrum elevation on average was highest in the macroinvertebrate assemblages (mean = 4.78) and lowest in the fish assemblages (mean = -7.42). It was most variable among combined communities (STDEV = 0.82) and least variable among macroinvertebrate assemblages (STDEV = 0.71). FCL on average was highest in the combined communities (Mean = 18.9) and lowest in the fish assemblages (Mean = 9.9). It was most variable between macroinvertebrate assemblages (STDEV = 2.77) and least variable equally between both fish assemblages and combined communities (STDEV = 1.34 for both). Summarizing all assemblages/communities by study stream, slopes tended to be lowest in Whiteday Creek and highest in Dry Fork. Elevations tended to be highest in Paw Paw Creek and lowest in Three Fork Creek. FCLs tended to be highest in Wheeling Creek and lowest in Three Fork Creek. Univariate

correlations between fish assemblage and macroinvertebrate assemblages produced no relationship between their size spectrum slopes ($r = 0.210$, $P = 0.471$) or elevations ($r = 0.017$, $P = 0.954$) (Figure 4a-b).

The PCA-correlation approach produced several notable relationships between stream CSS parameters and watershed factors. Macroinvertebrate assemblage slope, although insignificant ($P = 0.073$) was negatively associated with Principal Component 2 (33.42% variation explained), which contrasted mean drainage elevation with major watershed, specific conductance, and mean drainage slope (Figure 5a, Table 7). This means that macroinvertebrate assemblage size spectrum slopes tend to flatten with increased mean drainage elevation and tend to steepen with a change in major watershed (Cheat → Monongahela → Ohio) and with increased stream specific conductance and mean drainage slope (Table 8). Fish assemblage slope was positively associated with Principal Component 3 (14.83% variation explained), which contrasted drainage % forest and major watershed with stream specific conductance, mean drainage slope STDEV, and drainage area (Figure 5b, Table 7). This means that fish assemblage size spectrum slopes tend to flatten with increased drainage % forest and a change in major watershed (Cheat → Monongahela → Ohio) and tend to steepen with increased stream specific conductance, mean drainage slope STDEV, and drainage area (Table 8). Fish assemblage FCL was positively associated with Principal Component 2 (33.42% variation explained), which contrasted mean drainage elevation with major watershed, specific conductance, and mean drainage slope (Figure 5c, Table 7). This means that fish assemblage size spectrum FCLs tend to decrease with increased mean drainage elevation and tend to decrease with a change in major watershed (Cheat → Monongahela → Ohio) and with increased stream specific conductance and mean drainage slope (Table 8). Combined community FCL was positively associated with Principal Component 2 (33.42% variation explained), which contrasted mean drainage elevation with major watershed, specific conductance, and mean drainage slope (Figure 5d, Table 7). This means that combined community size spectrum FCLs tend to decrease with increased mean drainage elevation and tend to decrease with a change in major watershed (Cheat → Monongahela → Ohio) and with increased stream specific conductance and mean drainage slope (Table 8).

DISCUSSION

The results of this study show a number of significant correlations between aspects of stream community structure and watershed factors, both geomorphological and anthropogenically-driven. The majority of these findings support the original hypotheses of this research and can therefore be explained by ecological mechanisms. This research shows the evidence for including external watershed factors such as drainage elevation, slope, and land cover when assessing stream community structure as they could play a role in explaining variation between communities that cannot be accounted for by analyzing only biotic factors such as functional groups or diversity. Trophic complexity (CSS FCL) and ecological efficiency (CSS slope) both displayed significant correlations with various watershed factors, but food web capacity (CSS elevation) failed to correlate with any watershed factors. This suggests that the capacity of stream food webs in the study area could be driven primarily by factors not included in this research, such as the aforementioned internal biotic variables or other specific water quality parameters such as stream temperature, pH, or dissolved O₂. Additionally, unmeasured habitat variables (substrate composition, cover, riparian habitat, etc.) may be also be important factors that were not captured in this study. All of the watershed factors were found to correlate with at least one of the CSS parameters, showing that aspects of geography, stream size, topography, land cover, and water quality could all play a role in the community structure of the study streams. These findings provide support for future research into these and other unassessed watershed factors such as the water quality and habitat variables previously mentioned.

Size Spectrum Slopes

Macroinvertebrate assemblage slope tended to flatten as mean drainage elevation increased while steepening as major watershed changed and mean drainage slope and stream specific conductance increased. Although these trends were present the correlations were not significant, suggesting that these relationships may be weak or involve other unmeasured factors that significantly influence this parameter. The higher the elevation of a drainage, the more likely it is to be near the upper bounds of its larger watershed, therefore receiving the majority of its energy from allochthonous sources (Vannote et al. 1980). This has been known to result in high densities of large-bodied taxa that feed low on the food web and compete with small sized individuals for resources (Vannote et al. 1980, Benejam et al. 2018). Additionally, high drainage

slopes can lead to high flow velocity and low buildup of sediment on the streambed, making those drainages less suitable for large-bodied invertebrates that bury themselves in the substrate (King et al. 2012). Large size classes have been known to be more vulnerable to poor water quality (represented by high SPC) due to slower reproductive rates, larger home ranges, and high energy demands (Brose et al. 2017, Brown et al. 2004, Woodward et al. 2012). The correlation between macroinvertebrate assemblage slope and major watershed could suggest that certain watershed characteristics tied to geographic location (possibly unmeasured) were influential to these results. Ultimately, these findings may suggest that macroinvertebrate assemblage slope is likely tied closer to geography, topography, and water quality than to land cover or stream size (King et al. 2012, Benejam et al. 2018).

In comparison to the macroinvertebrate results, fish assemblage slope tended to flatten with an increase in drainage area, mean drainage slope STDEV, and stream SPC while steepening with a change in major watershed and an increase in drainage % forest. As the size of a stream increases, communities tend to gain a higher abundance of large-bodied taxa due to the shifting of available energy sources (Vannote et al. 1980). For example, large streams with large drainages receive more energy from internal sources such as phytoplankton than from external sources such as leaf litter, which form the base of a food web dominated by large fishes that feed on plankton and their macroinvertebrate predators such as Catostomidae, a.k.a. suckers (Vannote et al. 1980). High drainage slope variability produces highly variable stream gradients, which has been found to result in notable differences in macroinvertebrate assemblages (King et al. 2012) and could produce similar results for fish assemblages. This result could occur because a variable stream gradient can form a diverse range of habitat types, which could possibly encourage higher densities of large macroinvertebrate taxa that are known habitat specialists (e.g. Odonata). Drainage slope variability has not been the object of as much previous research as the other watershed factors in this study, so the true ecological mechanisms driving the relationship seen here between fish assemblage slope and drainage slope variability is difficult to determine without focused future study. The contrasting influence of percent forest and SPC demonstrates the potential to predict that fish size spectrum slopes in streams should increase (flatten) with increasing development in their drainages and decrease (steepen) after remediation (Collyer et al. 2023). This relationship is thought to be due to development simplifying the stream community structure to be dominated by tolerant species that reduce energy lost along the trophic gradient

(Collyer et al. 2023). In contrast, streams that experience less disturbance from anthropogenic impacts (i.e., mining, urbanization, agriculture, etc.) that alters natural habitat (include forested land) should have more intact natural communities and diverse taxa that form complex predator-prey interactions (Collyer et al. 2023). This set of relationships notably includes stream size and land cover while lacking stream altitude, which sets it apart from the relationships found with macroinvertebrate assemblage slopes. This could support the lack of correlation found between the two assemblages' slopes, as they appear to each be influenced by different combinations of watershed factors. Like the macroinvertebrate results, fish assemblage slope correlating with major watershed could suggest that certain watershed conditions tied to geographic location had a substantial influence on the results. Note the opposite trends that macroinvertebrate and fish slopes show with stream SPC. This demonstrates the intricacy of assessing CSS slopes using portions of a stream community, as fish and macroinvertebrate assemblage slopes can react differently to the same change in water quality or another factor due to differences in life history and/or physiology. Because of this, it could be difficult to designate certain CSS slope values or trends as “healthy” or “impaired” for management purposes; a case-by-case approach when assessing stream community slopes might be ideal.

Combined community slopes were found to be consistently steeper than their respective assemblages. This is due to individual assemblages receiving external energy subsidies that are not accounted for in their size spectra thereby increasing their ecological efficiency (flattening their slopes) (Blanchard et al. 2009). Combined CSSs reduce the number of external subsidies unaccounted for, so their ecological efficiencies more accurately show the loss of energy through the food web (Blanchard et al. 2009). Ultimately, no significant correlations were found between the various watershed factors and combined community slopes, possibly indicating the need for further research incorporating other unmeasured variables that may better capture any variation in this parameter.

Size Spectrum FCLs

Fish assemblage and combined community FCLs both tended to increase with a change in major watershed and an increase in mean drainage slope and stream SPC while decreasing as mean drainage elevation increased. Higher sloped drainages tend to have more diverse and complex food webs due to higher flow variability and low buildup of sediment on the streambed

forming a wider range of habitats (King et al. 2012). Consequently, a wider range of habitats may support a more diverse community leading to more complex food webs (King et al. 2012). The higher the elevation of a drainage, the more likely it is to be near the upper bounds of its larger watershed, therefore receiving less energy input (Vannote et al. 1980), which restricts the size of its food web, decreasing FCL (Benejam et al. 2018). A commonly-known extreme example of this relationship can be observed in high elevation Appalachian streams dominated by *Salvelinus fontinalis* (brook trout) with little else in terms of fish diversity barring the occasional *Cottus* sp. (sculpin). To our knowledge, the observed correlation between FCL and stream SPC is not supported by published literature on CSS, which has shown the opposite result likely due to anthropogenic impacts truncating and simplifying the food web to be tolerant generalist-dominated (Pomeranz et al. 2019). It should be noted that existing literature studying this relationship focuses primarily on macroinvertebrates (e.g. Pomeranz et al. 2019) and studies focused on fish FCL versus SPC is limited, so future publications with such a focus could shed more light on this relationship. Since the four streams with the lowest SPCs were also all four of the streams from the Cheat River major watershed and the four highest SPC values were from the Monongahela River major watershed, this unexpected correlation could be a spurious biproduct of the stronger relationship that FCL had with major watershed and might not show evidence of possible causality. For instance, streams in the Cheat River watershed were mainly higher elevation streams with low SPC. Natural fish species richness is often lower in these higher elevations streams which could explain, in part, the lower food web complexity (Benejam et al. 2018). In contrast, the streams in the Monongahela River watershed were mainly low elevation streams, often with higher SPC and relatively close to a large navigable river (Monongahela River). These low elevation streams near a large river may support higher species richness and thus more complex food webs (Benejam et al. 2018). This suggests that FCL in stream fish assemblages and combined communities naturally differ between drainages due to ties to their major watersheds and where they are located within those watersheds, not water quality, land cover, or stream size (Benejam et al. 2018, King et al. 2012, Pomeranz et al. 2019).

CONCLUDING REMARKS

There is a gap in our understanding of how specific watershed factors are impacting stream community structure, due to the high complexity and diversity of stream ecosystems. Current methods used to identify these relationships are often taxon-based and fail to consider the energetic component of stream community structure that the CSS approach incorporates. Because of this, the primary goal of this research was to use the CSS approach to assess the mechanisms that govern stream community structure in a holistic manner to add to the understanding of how these dynamic ecosystems function and give watershed managers an additional tool for stream community conservation that complements existing approaches.

Findings from this study describe several relationships between watershed factors and stream community structure using CSS that have explainable ecological mechanisms and could help future researchers determine what drives differences/changes in CSS parameters and how to predict the magnitude of such effects. Macroinvertebrate and fish assemblage slopes were found to be correlated with different sets of factors, showing that macroinvertebrates could be more sensitive to differences in drainage elevation than fish while fish could be more sensitive to differences in drainage area and land cover than macroinvertebrates. This could be useful to researchers in the future when determining which factors to collect data on when assessing a certain assemblage. Every watershed factor studied was associated with either size spectrum slope, FCL, or both, and this included both geomorphological (major watershed, drainage area, mean drainage slope, drainage slope variability, and mean drainage elevation) and anthropogenically-driven (drainage % forest and stream specific conductance) factors. While typically stable in stream drainages, geomorphology can in certain cases be altered significantly by anthropogenic activity, e.g. through large-scale mountaintop mining practices and interstate highway construction projects that still occur in West Virginia today. Watershed managers could use this research as support for protecting the natural geomorphology of watersheds from such practices. To further tease out the level of importance the hypothesized watershed factors have on influencing CSS parameters, future research into these relationships could focus on streams from a single major watershed to remove geographic location as a driver of variation. Adding long-term data collection for other water chemistry factors (pH, dissolved O₂, turbidity, etc.) and habitat factors (substrate composition, cover, riparian habitat, etc.) to analyze as well will strengthen that research. To make this and similar studies more useful for watershed managers,

next steps could be to integrate these watershed factors into an analysis with well-studied biotic factors so that the level of explanatory power for each can be better understood. A different analytical technique like the regression approach might be more effective for assessing associations with such a large number of factors than the PCA-correlation approach used in this research would be. This is because the chances of spurious results when accounting for patterns of variance in the PCA-correlation approach increase as the number of environmental covariates increases. If this integration of factor relationships is successful, an ideal goal for using CSS in stream community management would be developing a predictive model that can output estimated future CSS parameters (slope, elevation, and FCL) once biotic factors, geomorphology, and proposed land cover/water quality remediation effects are put into the model. This would give managers the capability to set targets for holistically improving community structure before breaking ground on any restoration/management projects along with thresholds for how much development can be done to a watershed before the stream community structure is significantly harmed, and is a goal sought by others as well (Murry et al., in review). The findings from this research could add to our understanding of these mechanisms and put stream ecologists one step closer to developing a targeted and predictive stream community model that can be added to the toolbox with existing taxon-based methods such as the IBI (Shin et al. 2005, Marin et al. 2023). The community size spectrum approach to aquatic community assessment has been shown as a valuable tool for stream ecologists due to its close link with differences between watersheds and would be beneficial to develop further with the goal of adding CSS to the repertoire when seeking to better understand our diverse, dynamic and fragile mid-order stream communities.

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TABLES

Table 1. Hypothesized associations of CSS slope (ecological efficiency), elevation (food web capacity), and FCL (trophic complexity) with individual watershed factors, including citations.

Watershed Factor	CSS Slope	CSS Elevation	CSS FCL	Literature Source
Drainage Area	Flatten	+	+	Vannote et al. 1980
Mean Drainage Slope	Steepen	+	+	King et al. 2012
Mean Drainage Slope STDEV	None	None	+	King et al. 2012
Mean Drainage Elevation	Flatten	–	–	Benejam et al. 2018
Drainage % Forested	Steepen	+	+	Collyer et al. 2023
Stream Specific Conductance	None	–	–	Pomeranz et al. 2019

Table 2. 30 Study sites for the 15 study streams, with basic geographic information. Study site coordinates are the downstream ends of each sample reach. Counties and major watersheds where the sites were located are also listed. Sourced from WVDEP website.

Site Name	Site Coordinates	County	Watershed
Beaver Creek DS	N 39° 37' 36.4", W 79° 35' 58.2"	Preston	Cheat
Beaver Creek US	N 39° 36.679', W 79° 31.031'	Preston	Cheat
Big Sandy Creek DS	N 39° 39.480', W 79° 38.339'	Preston	Cheat
Big Sandy Creek US	N 39° 43.142', W 79° 39.561'	Preston	Cheat
Buffalo Creek DS	N 39° 29.919', W 80° 11.066'	Marion	Monongahela
Buffalo Creek US	N 39° 30.690', W 80° 14.885'	Marion	Monongahela
Dry Fork DS	N 39° 00.791', W 79° 31.738'	Tucker	Cheat
Dry Fork US	N 38° 59.554', W 79° 31.826'	Tucker	Cheat
Dunkard Creek DS	N 39° 43.054', W 80° 07.126'	Monongalia	Monongahela
Dunkard Creek US	N 39° 42.932', W 80° 09.928'	Monongalia	Monongahela
Elk Creek DS	N 39° 15.634', W 80° 19.086'	Harrison	Monongahela
Elk Creek US	N 39° 13.560', W 80° 18.371'	Harrison	Monongahela
Fish Creek DS	N 39° 46' 00.7", W 80° 42' 33.4"	Marshall	Ohio
Fish Creek US	N 39° 46.040', W 80° 38.705'	Marshall	Ohio
Fishing Creek DS	N 39° 35.447', W 80° 48.750'	Wetzel	Ohio
Fishing Creek US	N 39° 33.794', W 80° 42.322'	Wetzel	Ohio
Horseshoe Run DS	N 39° 09.242', W 79° 39.677'	Tucker	Cheat
Horseshoe Run US	N 39° 10.844', W 79° 36.140'	Tucker	Cheat
Paw Paw Creek DS	N 39° 33.204', W 80° 10.050'	Marion	Monongahela
Paw Paw Creek US	N 39° 33.646', W 80° 11.218'	Marion	Monongahela
Simpson Creek DS	N 39° 18.528', W 80° 16.734'	Harrison	Monongahela
Simpson Creek US	N 39° 17.353', W 80° 15.935'	Harrison	Monongahela
Tenmile Creek DS	N 39° 22.408', W 80° 20.863'	Harrison	Monongahela
Tenmile Creek US	N 39° 20.703', W 80° 24.038'	Harrison	Monongahela
Three Fork Creek DS	N 39° 20.181', W 79° 59.473'	Taylor	Monongahela
Three Fork Creek US	N 39° 22.844', W 79° 54.825'	Taylor	Monongahela
Wheeling Creek DS	N 39° 58.542', W 80° 37.846'	Marshall	Ohio
Wheeling Creek US	N 39° 57.528', W 80° 32.953'	Marshall	Ohio
Whiteday Creek DS	N 39° 31.849', W 80° 02.652'	Monongalia	Monongahela
Whiteday Creek US	N 39° 29.256', W 80° 00.415'	Monongalia	Monongahela

Table 3. Watershed factors measured for each study stream. The upper table lists geomorphological factors and the lower table lists anthropogenically driven factors. Factor means and STDEV are listed below each column. The numerical designation for each major watershed used in analysis is shown in parentheses.

Stream	Major Watershed	Drainage Area (km ²)	Mean Drainage Slope	Mean Drainage Slope STDEV	Mean Drainage Elevation (m)
Beaver	Cheat (1)	33.9659	6.5075	4.5238	649.0608
Big Sandy	Cheat (1)	538.3227	8.6937	6.0144	598.1086
Buffalo	Monongahela (2)	324.7127	17.1725	6.8015	373.4073
Dry Fork	Cheat (1)	1269.0630	15.0751	8.6455	987.5103
Dunkard	Monongahela (2)	602.8421	16.0046	6.4361	370.3938
Elk	Monongahela (2)	312.6247	13.7639	7.4684	384.9662
Fish	Ohio (3)	648.7640	18.9161	7.0029	372.8829
Fishing	Ohio (3)	565.4472	21.2104	7.1003	341.7056
Horseshoe	Cheat (1)	143.0542	19.2824	8.6355	732.7213
Paw Paw	Monongahela (2)	108.4840	16.0717	6.4981	370.9559
Simpson	Monongahela (2)	188.6707	13.6243	7.5665	377.0313
Tenmile	Monongahela (2)	323.6045	16.9763	6.8991	370.7112
Three Fork	Monongahela (2)	261.9187	12.8882	7.1546	508.3217
Wheeling	Ohio (3)	770.9441	14.6995	6.6664	364.1501
Whiteday	Monongahela (2)	85.2591	13.6004	6.2018	444.9793
Mean	<i>1.9</i>	<i>411.8452</i>	<i>14.9658</i>	<i>6.9077</i>	<i>483.1271</i>
STDEV	<i>0.7</i>	<i>328.1126</i>	<i>3.8251</i>	<i>1.0107</i>	<i>184.5289</i>

Stream	Drainage % Forest	Stream Specific Conductance (mS/cm)
Beaver	63.58	0.0736
Big Sandy	75.28	0.1284
Buffalo	83.59	0.3658
Dry Fork	89.52	0.0996
Dunkard	80.97	0.6058
Elk	68.08	1.1155
Fish	86.47	0.2497
Fishing	91.75	0.2026
Horseshoe	88.14	0.0658
Paw Paw	81.01	0.4473
Simpson	64.51	0.9688
Tenmile	83.29	0.7757
Three Fork	79.16	0.2885
Wheeling	70.94	0.5407
Whiteday	79.81	0.1459
Mean	<i>79.07</i>	<i>0.4049</i>
STDEV	<i>8.89</i>	<i>0.3341</i>

Table 4. Complete list of macroinvertebrate families sampled from 15 study streams, including: total abundances of each family, literature-based taxon used to calculate dry weight for each family, *a*-value from literature for reference taxon's length/weight equation, *b*-value from literature for reference taxon's length/weight equation, and literature source of the family's length/weight equation. N = 39425.

Family	Abundance	Source Taxa for Length/Weight Equation	<i>a</i>	<i>b</i>	Source
Aeshnidae	4	Aeshnidae	0.0082	2.813	Benke et al 1999
Ameletidae	20	Ameletidae	0.0077	2.588	Benke et al 1999
Asellidae	1	Asellidae	0.0054	2.948	Benke et al 1999
Athericidae	236	Athericidae	0.004	2.586	Benke et al 1999
Baetidae	3095	Baetidae	0.0053	2.875	Benke et al 1999
Blephariceridae	26	Blephariceridae	0.0067	3.292	Benke et al 1999
Brachycentridae	90	Brachycentridae	0.0083	2.818	Benke et al 1999
Caenidae	1447	Caenidae	0.0054	2.772	Benke et al 1999
Cambaridae	39	Decapoda	0.0147	3.626	Benke et al 1999
Ceratopogonidae	5	Ceratopogonidae	0.0025	2.469	Benke et al 1999
Chironomidae	2164	Chironomidae	0.0018	2.617	Benke et al 1999
Chloroperlidae	32	Chloroperlidae	0.0065	2.724	Benke et al 1999
Chrysomelidae (larvae)	1	Chrysomelidae (larvae)	0.039	3.111	Benke et al 1999
Coenagrionidae	35	Coenagrionidae	0.0051	2.785	Benke et al 1999
Corbiculidae	202	Corbiculidae	0.0142	2.835	Benke et al 1999
Corydalidae	256	Corydalidae	0.0037	2.873	Benke et al 1999
Crambidae	11	Pyralidae	0.0033	2.918	Benke et al 1999
Elmidae (adult)	1505	Elmidae (adult)	0.0618	2.5	Smock 1980
Elmidae (larvae)	10003	Elmidae (larvae)	0.0074	2.879	Benke et al 1999
Empididae	98	Empididae	0.0054	2.546	Benke et al 1999
Ephemerellidae	106	Ephemerellidae	0.0103	2.676	Benke et al 1999
Ephemeridae	10	Ephemeridae	0.0034	2.764	Benke et al 1999
Gammaridae	55	Gammaridae	0.0085	2.8705	Benke et al 1999
Gerridae	6	Gerridae	0.015	2.596	Benke et al 1999
Glossosomatidae	14	Glossosomatidae	0.0082	2.958	Benke et al 1999
Goeridae	3	Brachycentridae	0.0083	2.818	Benke et al 1999
Gomphidae	53	Gomphidae	0.0088	2.787	Benke et al 1999
Helicopsychidae	141	Helicopsychidae	0.0125	3.096	Benke et al 1999
Heptageniidae	3053	Heptageniidae	0.0108	2.754	Benke et al 1999
Hydropsychidae	9953	Hydropsychidae	0.0046	2.926	Benke et al 1999
Hydroptilidae	688	Glossosomatidae	0.0082	2.958	Benke et al 1999
Isonychiidae	690	Isonychiidae	0.0031	3.043	Benke et al 1999
Lepidostomatidae	4	Lepidostomatidae	0.0079	2.649	Benke et al 1999
Leptoceridae	306	Leptoceridae	0.0034	3.212	Benke et al 1999
Leptohiphidae	869	Ephemerellidae	0.0103	2.676	Benke et al 1999
Leptophlebiidae	60	Leptophlebiidae	0.0047	2.686	Benke et al 1999
Leuctridae	389	Leuctridae	0.0028	2.719	Benke et al 1999

Limnephilidae	4	Limnephilidae	0.004	2.933	Benke et al 1999
Limoniidae	351	Tipulidae	0.0029	2.681	Benke et al 1999
Macromiidae	1	Corduliidae	0.0096	2.787	Benke et al 1999
Metretopodidae	5	Ameletidae	0.0077	2.588	Benke et al 1999
Nemouridae	1	Nemouridae	0.0056	2.762	Benke et al 1999
Neophemeridae	17	Caenidae	0.0054	2.772	Benke et al 1999
Odontoceridae	52	Odontoceridae	0.0077	2.988	Benke et al 1999
Oligoneuriidae	2	Isonychiidae	0.0031	3.043	Benke et al 1999
Pediciidae	18	Tipulidae	0.0029	2.681	Benke et al 1999
Peltoperlidae	7	Peltoperlidae	0.017	2.737	Benke et al 1999
Perlidae	491	Perlidae	0.0099	2.879	Benke et al 1999
Perlodidae	6	Perlodidae	0.0196	2.742	Benke et al 1999
Philopotamidae	1021	Philopotamidae	0.005	2.511	Benke et al 1999
Polycentropodidae	102	Polycentropodidae	0.0047	2.705	Benke et al 1999
Polymitarciidae	2	Polymitarciidae	0.002	3.05	Benke et al 1999
Psephenidae (larvae)	1007	Psephenidae (larvae)	0.0123	2.906	Benke et al 1999
Psychomyiidae	54	Psychomyiidae	0.0039	2.873	Benke et al 1999
Pteronarcyidae	13	Pteronarcyidae	0.0324	2.573	Benke et al 1999
Rhyacophilidae	4	Rhyacophilidae	0.0099	2.48	Benke et al 1999
Saldidae	5	Gerridae	0.015	2.596	Benke et al 1999
Sericostomatidae	1	Sericostomatidae	0.0074	2.741	Benke et al 1999
Sialidae	75	Sialidae	0.0037	2.753	Benke et al 1999
Simuliidae	307	Simuliidae	0.002	3.011	Benke et al 1999
Siphonuridae	2	Siphonuridae	0.0027	3.446	Benke et al 1999
Thremmatidae	1	Brachycentridae	0.0083	2.818	Benke et al 1999
Tipulidae	25	Tipulidae	0.0029	2.681	Benke et al 1999
Veliidae	43	Veliidae	0.0126	2.719	Benke et al 1999
Viviparidae	138	Pleuroceridae (*AFDM)	0.0077	3.001	Benke et al 1999

Table 5. Complete list of fish species/hybrids sampled from 15 study streams, including: scientific names, common names, total abundances, sampled length ranges (mm), and number of streams present of each species/hybrid. N = 21709.

Scientific Name	Common Name	Abundance	Length Range (mm)	# of Streams Present
<i>Etheostoma zonale</i>	banded darter	671	38-77	11
<i>Pomoxis nigromaculatus</i>	black crappie	7	56-197	1
<i>Moxostoma duquesnei</i>	black redhorse	40	125-434	6
<i>Rhinichthys atratulus</i>	blacknose dace	87	42-70	5
<i>Percina maculata</i>	blackside darter	103	40-79	8
<i>Lepomis macrochirus</i>	bluegill	232	35-200	11
<i>Lepomis macrochirus x ?</i>	bluegill hybrid	2	86-102	1
<i>Pimephales notatus</i>	bluntnose minnow	2651	37-97	12
<i>Noturus miurus</i>	brindled madtom	15	51-77	3
<i>Labidesthes sicculus</i>	brook silverside	3	52-73	2
<i>Salvelinus fontinalis</i>	brook trout	21	60-330	2
<i>Salmo trutta</i>	brown trout	21	254-350	3
<i>Camptostoma anomalum</i>	central stoneroller	2101	33-140	13
<i>Ictalurus punctatus</i>	channel catfish	24	274-625	4
<i>Cyprinus carpio</i>	common carp	13	38-780	1
<i>Semotilus atromaculatus</i>	creek chub	282	36-166	13
<i>Etheostoma flabellare</i>	fantail darter	739	33-76	12
<i>Pylodictis olivaris</i>	flathead catfish	6	255-659	2
<i>Aplodinotus grunniens</i>	freshwater drum	34	59-686	6
<i>Dorosoma cepedianum</i>	gizzard shad	13	45-383	2
<i>Oncorhynchus mykiss</i>	golden rainbow trout	3	202-320	3
<i>Moxostoma erythrurum</i>	golden redhorse	297	121-453	10
<i>Lepomis cyanellus</i>	green sunfish	381	38-191	15
<i>Lepomis cyanellus x ?</i>	green sunfish hybrid	19	43-143	4
<i>Etheostoma blennioides</i>	greenside darter	2101	33-96	14
<i>Etheostoma nigrum</i>	johnny darter	227	41-63	12
<i>Micropterus salmoides</i>	largemouth bass	83	37-350	11
<i>Lampetra aepyptera</i>	least brook lamprey	10	70-170	2
<i>Percina caprodes</i>	logperch	171	47-169	9
<i>Lepomis megalotis</i>	longear sunfish	109	43-190	8
<i>Rhinichthys cataractae</i>	longnose dace	152	46-125	5
<i>Rhinichthys cataractae x Nocomis micropogon</i>	longnose dace x river chub	37	49-116	1
<i>Lepisosteus osseus</i>	longnose gar	16	111-702	3
<i>Notropis volucellus</i>	mimic shiner	723	37-68	7
<i>Gambusia affinis</i>	mosquitofish	3	42-45	1
<i>Cottus bairdii</i>	mottled sculpin	899	36-95	5
<i>Moxostoma sp.</i>	redhorse sp.	401	37-100	9
<i>Hypentelium nigricans</i>	northern hogsucker	634	40-387	15

<i>Ichthyomyzon bdellium</i>	ohio lamprey	1	211-211	1
<i>Lepomis gibbosus</i>	pumpkinseed	2	74-150	2
<i>Etheostoma caeruleum</i>	rainbow darter	370	40-70	10
<i>Oncorhynchus mykiss</i>	rainbow trout	7	307-403	3
<i>Lepomis auritus</i>	redbreast sunfish	30	45-188	1
<i>Nocomis micropogon</i>	river chub	428	39-253	8
<i>Nocomis micropogon x ?</i>	river chub hybrid	19	53-79	1
<i>Nocomis micropogon x Notropis stramineus</i>	river chub x sand shiner	20	47-78	1
<i>Ambloplites rupestris</i>	rock bass	580	33-248	14
<i>Notropis rubellus</i>	rosyface shiner	1436	40-99	12
<i>Notropis rubellus x Luxilus chrysocephalus</i>	rosyface shiner x striped shiner	20	52-101	1
<i>Clinostomus funduloides</i>	rosyside dace	8	45-78	1
<i>Notropis stramineus</i>	sand shiner	2404	39-74	11
<i>Sander canadensis</i>	sauger	2	302-353	2
<i>Percina oxyrhynchus</i>	sharpnose darter	1	82-82	1
<i>Moxostoma anisurum</i>	silver redhorse	44	124-526	6
<i>Notropis photogenis</i>	silver shiner	274	44-124	10
<i>Notropis buccatus</i>	silverjaw minnow	68	40-85	6
<i>Micropterus dolomieu</i>	smallmouth bass	871	33-465	14
<i>Ictiobus bubalus</i>	smallmouth buffalo	2	44-508	2
<i>Moxostoma breviceps</i>	smallmouth redhorse	48	134-607	4
<i>Cyprinella spiloptera</i>	spotfin shiner	377	44-115	9
<i>Micropterus punctulatus</i>	spotted bass	2	41-90	2
<i>Noturus flavus</i>	stonecat	32	38-187	6
<i>Luxilus chrysocephalus</i>	striped shiner	762	43-160	11
<i>Luxilus chrysocephalus x ?</i>	striped shiner hybrid	1	103-103	1
<i>Salvelinus fontinalis x Salmo trutta</i>	tiger trout	2	250-340	1
<i>Etheostoma variatum</i>	variegated darter	397	35-93	7
<i>Morone chrysops</i>	white bass	1	66-66	1
<i>Pomoxis annularis</i>	white crappie	14	57-381	1
<i>Catostomus commersonii</i>	white sucker	108	43-457	9
<i>Ameiurus natalis</i>	yellow bullhead	47	34-267	9

Table 6. CSS slope (ecological efficiency), elevation (food web capacity), and FCL (trophic complexity) of both assemblages and the combined community for each study stream. Means and standard deviations for each column are also included.

Stream	<i>Macroinvertebrate</i>			<i>Fish</i>			<i>Combined</i>		
	Slope	Elevation	FCL	Slope	Elevation	FCL	Slope	Elevation	FCL
Beaver	-0.6295	3.5001	16	-0.5767	-7.3675	8	-0.9706	-1.4803	17
Big Sandy	-0.7254	4.4140	15	-0.7579	-7.0127	10	-0.9994	-1.2907	19
Buffalo	-1.0026	5.7607	9	-0.7031	-7.3418	10	-1.0505	-1.9737	19
Dry Fork	-0.6345	5.2472	13	-0.1570	-8.5554	9	-1.1015	0.1103	18
Dunkard	-0.8312	4.9723	15	-0.7370	-6.4991	10	-1.0293	-0.4998	19
Elk	-0.8684	4.3357	13	-0.3477	-7.2365	11	-0.9576	-1.4005	20
Fish	-0.8273	4.2974	13	-0.5241	-7.8343	11	-0.9990	-1.9041	20
Fishing	-0.7353	3.4501	14	-0.8412	-7.6570	11	-0.9947	-1.8164	20
Horseshoe	-0.8867	5.4961	9	-0.7444	-7.0914	10	-1.0325	-1.6068	19
Paw Paw	-0.7530	5.3553	16	-0.5286	-5.8082	10	-0.9588	0.1009	19
Simpson	-0.8740	4.9888	12	-0.7730	-6.9774	10	-1.0605	-0.8145	19
Tennile	-0.9433	5.5405	12	-0.6460	-8.6673	10	-1.1835	-1.8328	19
Three Fork	-1.0915	4.9554	6	-0.6313	-8.8392	8	-1.1102	-3.0094	17
Wheeling	-0.8720	5.0634	13	-0.4576	-7.4014	13	-0.9567	-1.6777	22
Whiteday	-0.8210	4.3121	12	-1.0551	-7.0059	8	-1.0552	-1.3476	17
Mean	-0.8330	4.7793	12.5	-0.6320	-7.4197	9.9	-1.0307	-1.3629	18.9
Std Dev	0.1264	0.7063	2.77	0.2154	0.8114	1.34	0.0652	0.8168	1.34

Table 7. Watershed factor eigenvectors (loadings) for the top three principal components (PC#). The percent variation explained by each PC# is shown in parentheses. PCs 4-7 were not significantly associated with any CSS parameter and therefore were not included. *PC1 was not significantly associated with any CSS parameter and is shown only for context.

Watershed Factor	PC1* (37.23)	PC2 (33.42)	PC3 (14.83)
Major Watershed	0.1462	0.5641	-0.2129
Drainage Area	0.3766	-0.1242	0.2411
Mean Drainage Slope	0.5121	0.3098	-0.0965
Mean Drainage Slope STDEV	0.4699	-0.0233	0.5425
Mean Drainage Elevation	0.1391	-0.6167	0.1852
Drainage % Forest	0.5572	-0.0552	-0.3368
Specific Conductance	-0.1548	0.4319	0.6672

Table 8. Significant (and nearly significant) correlations between macroinvertebrate assemblage, fish assemblage, and combined community size spectrum slope (ecological efficiency), elevation (food web capacity), food chain length (trophic complexity) and individual watershed factors found from statistical analyses. P-values are included with statistically insignificant correlations.

Watershed Factor	<i>Macroinvertebrate Assemblage</i>			<i>Fish Assemblage</i>			<i>Combined Community</i>		
	Slope	Elevation	FCL	Slope	Elevation	FCL	Slope	Elevation	FCL
Major Watershed	Steepen p=0.07			Steepen		+			+
Drainage Area				Flatten					
Mean Drainage Slope	Steepen p=0.07					+			+
Mean Drainage Slope STDEV				Flatten					
Mean Drainage Elevation	Flatten p=0.07					-			-
Drainage % Forested				Steepen					
Stream Specific Conductance	Steepen p=0.07			Flatten		+			+

FIGURES

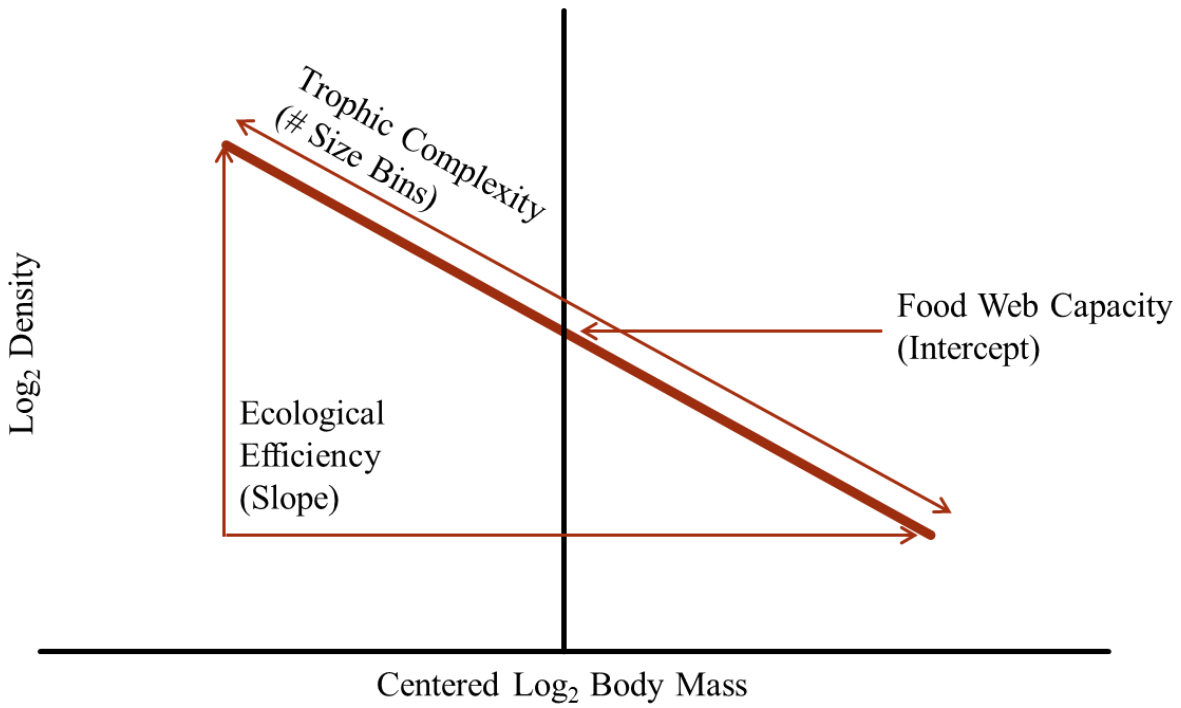


Figure 1. Example diagram of a community size spectrum and the three food web parameters that it produces.

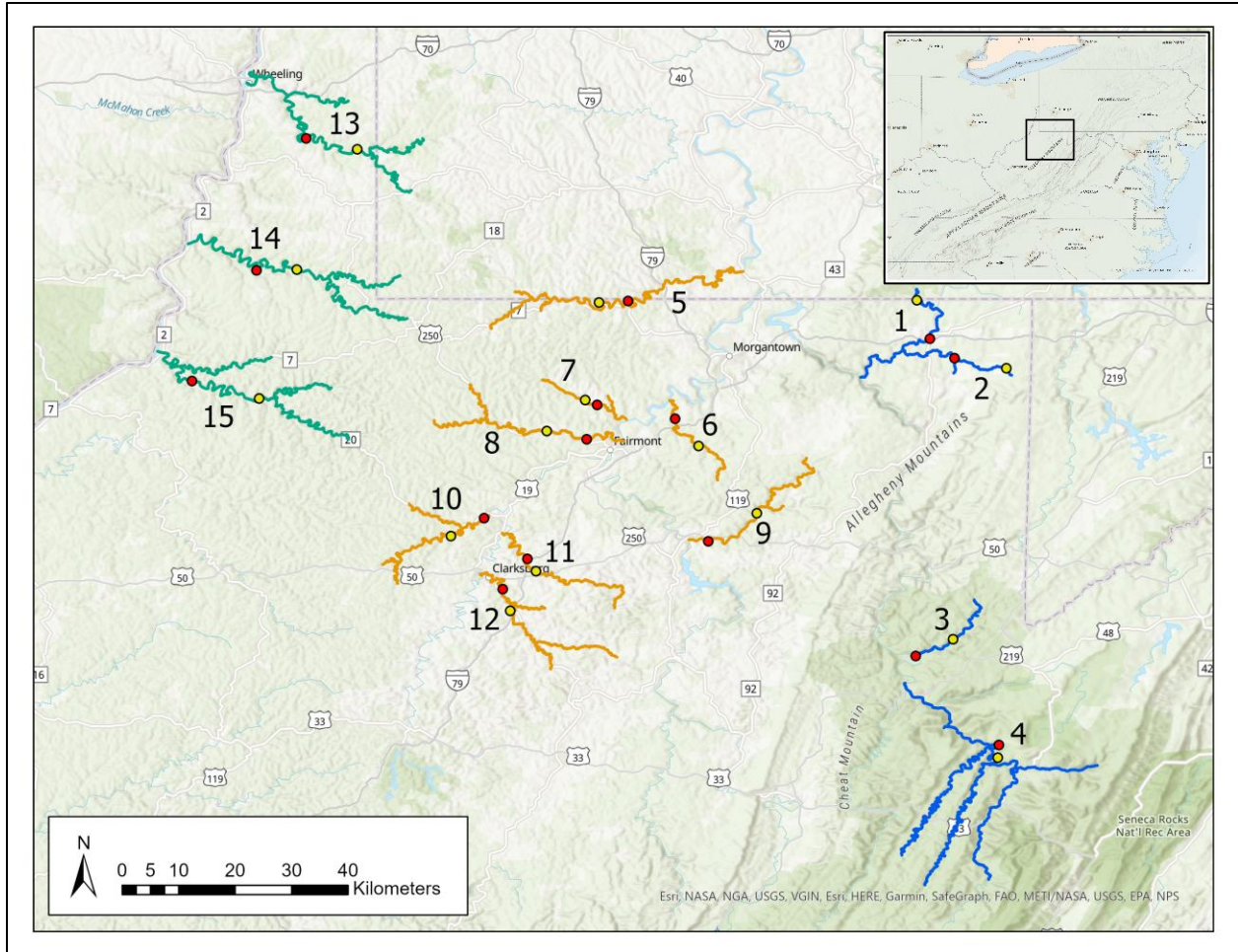


Figure 2. Map of 15 study streams (including major tributaries) and 30 sample sites in northcentral West Virginia. Blue-colored streams are in the Cheat R. watershed, orange-colored streams are in the Monongahela R. watershed, and green-colored streams are in the Ohio R. watershed. Red markers are the downstream sample sites for each stream, and yellow markers are the upstream sample sites for each stream. Streams are labeled as such: Big Sandy Crk. (1), Beaver Crk. (2), Horseshoe Rn. (3), Dry Fork (4), Dunkard Crk. (5), Whiteday Crk. (6), Paw Paw Crk. (7), Buffalo Crk. (8), Three Fork Crk. (9), Tenmile Crk. (10), Simpson Crk. (11), Elk Crk. (12), Wheeling Crk. (13), Fish Crk. (14), Fishing Crk. (15). Developed with ArcGIS Pro software.

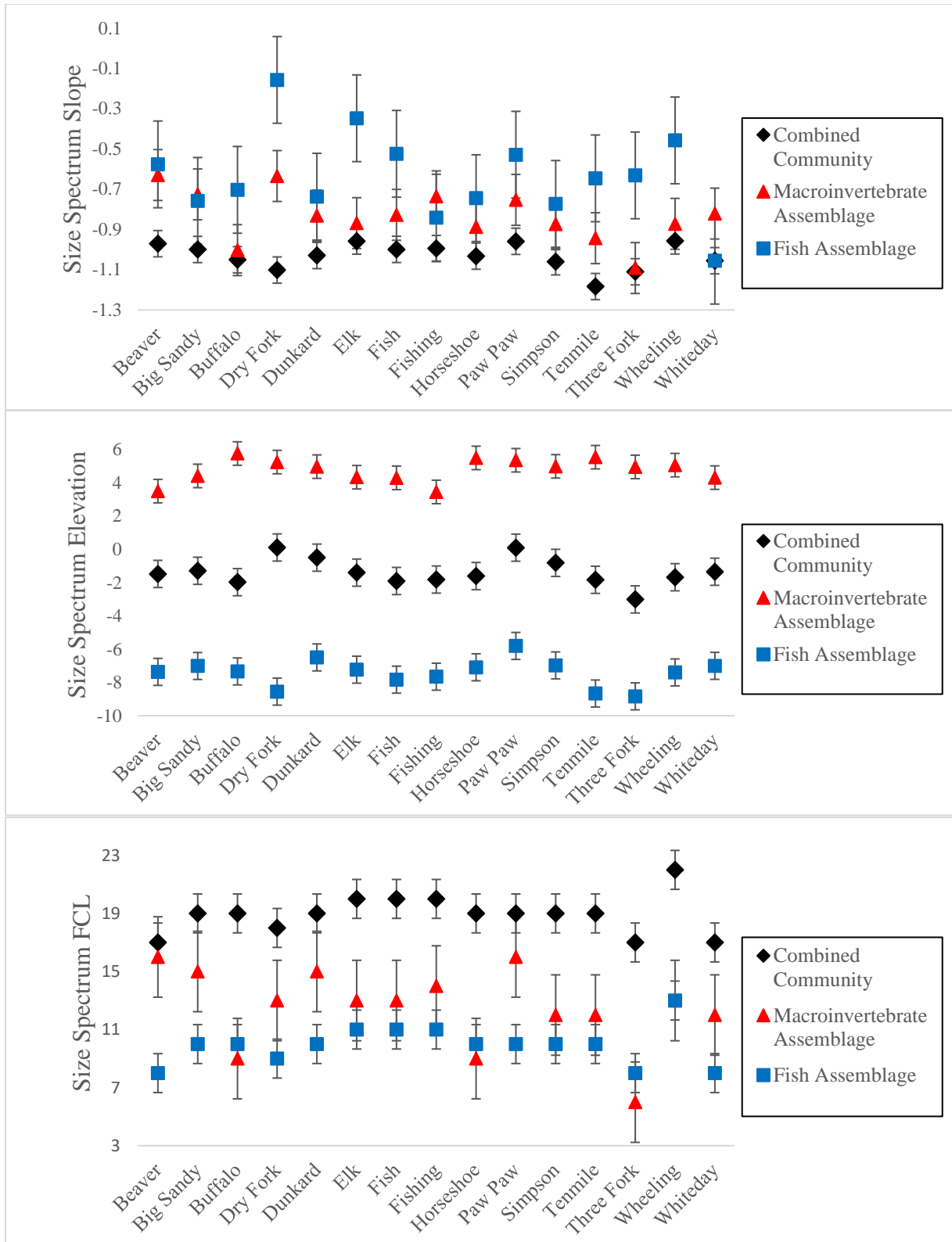


Figure 3. Size spectrum parameters for the macroinvertebrate assemblages, fish assemblages, and combined communities of the 15 study streams, with standard deviation bars.

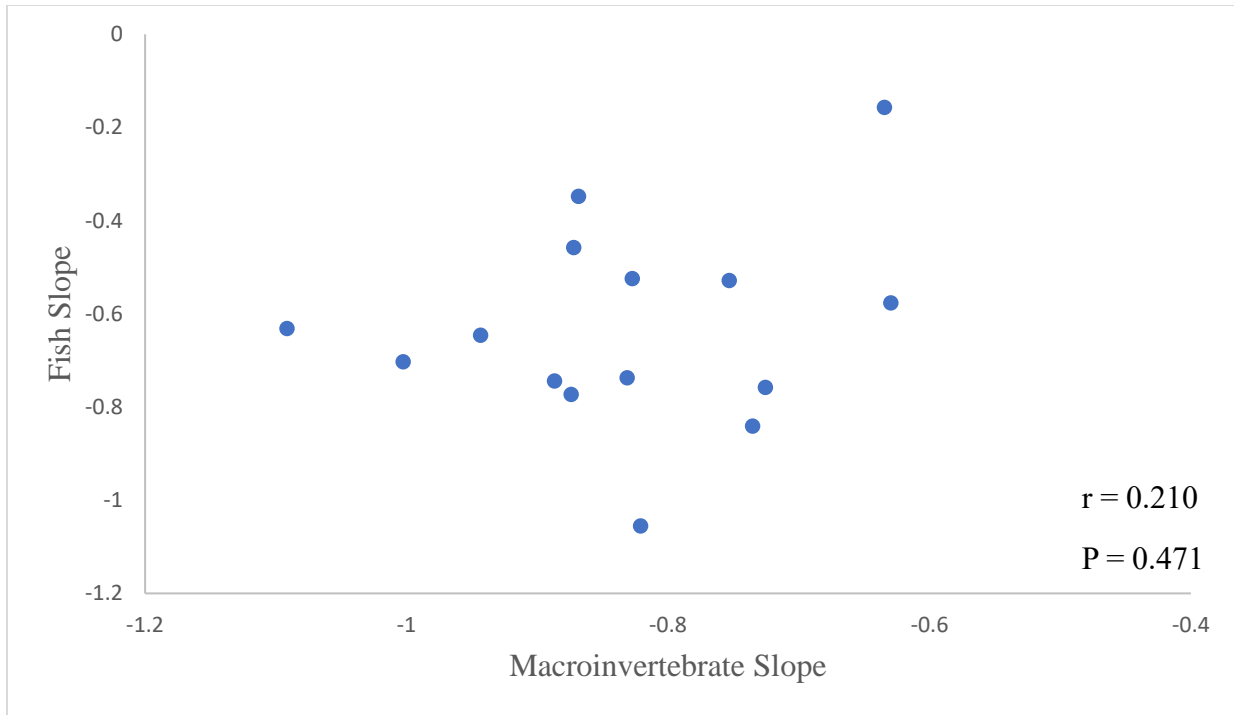


Figure 4a. Univariate correlation between the size spectrum slopes of the macroinvertebrate and fish assemblages.

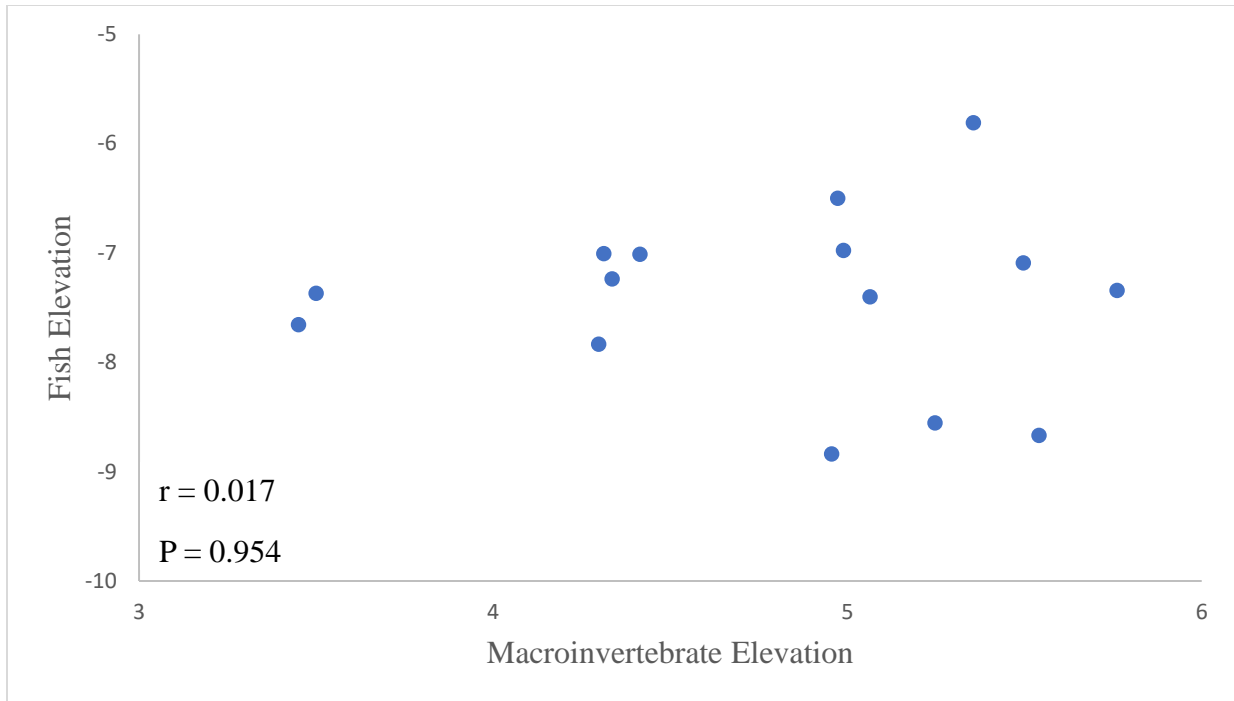


Figure 4b. Univariate correlation between the size spectrum elevations of the macroinvertebrate and fish assemblages.

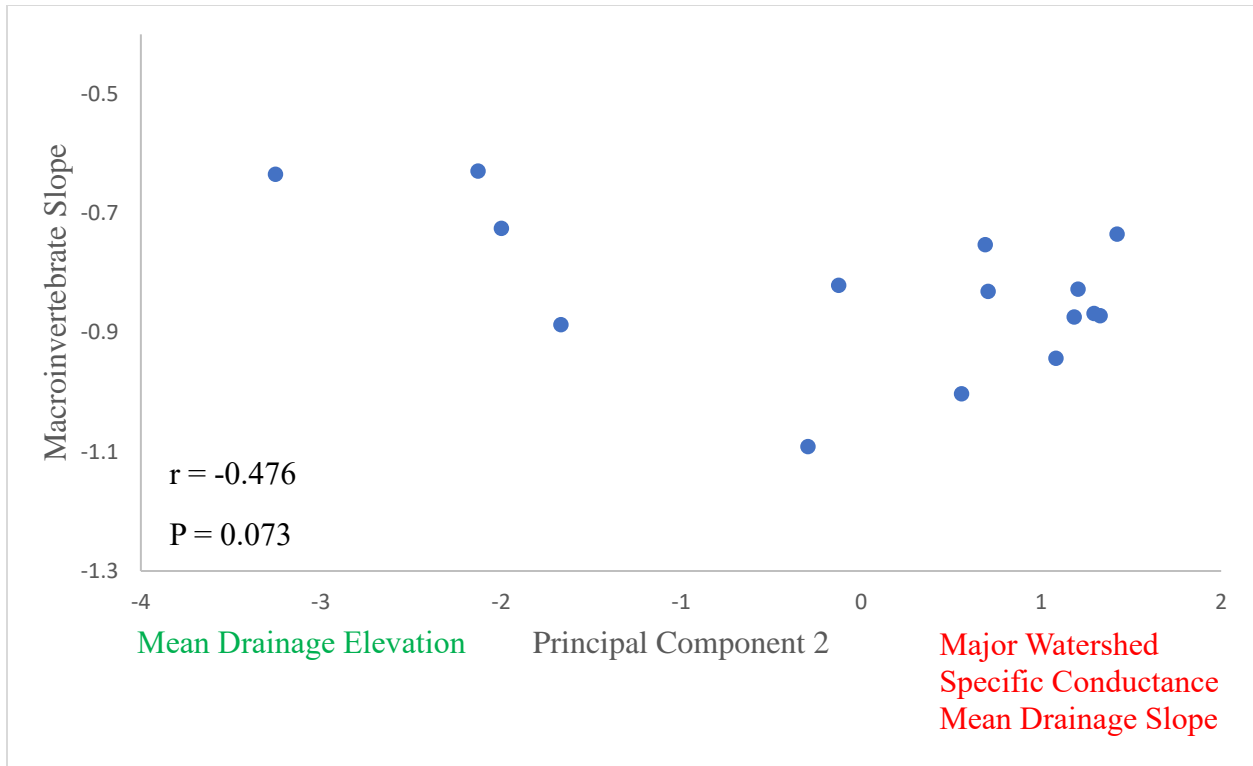


Figure 5a. Principal component 2 (33.42% variation explained) plotted against macroinvertebrate assemblage slope with r-value and p-value shown. PC2 contrasted mean drainage elevation against major watershed, specific conductance, and mean drainage slope. Red watershed factors are negatively associated while green watershed factors are positively associated with macroinvertebrate assemblage slope.

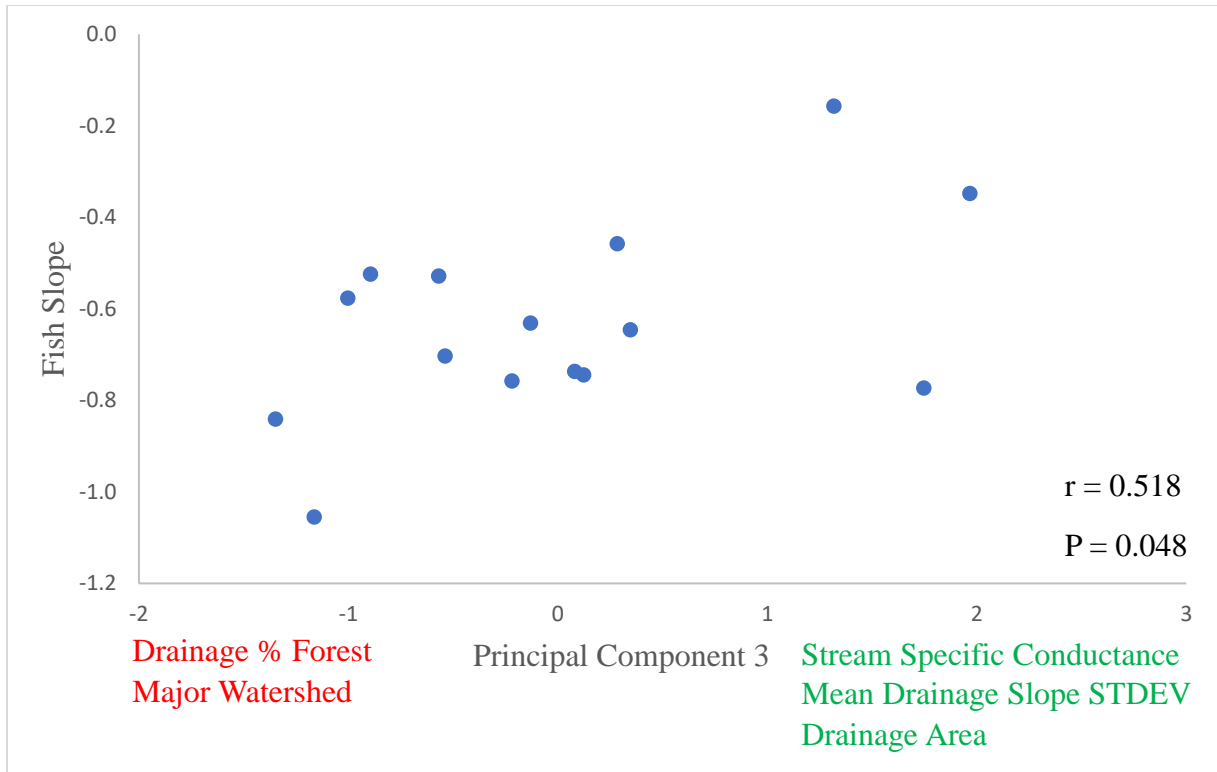


Figure 5b. Principal component 3 (14.83% variation explained) plotted against fish assemblage slope with r-value and p-value shown. PC2 contrasted drainage % forest and major watershed against stream specific conductance, mean drainage slope STDEV, and drainage area. Red watershed factors are negatively associated while green watershed factors are positively associated with fish assemblage slope.

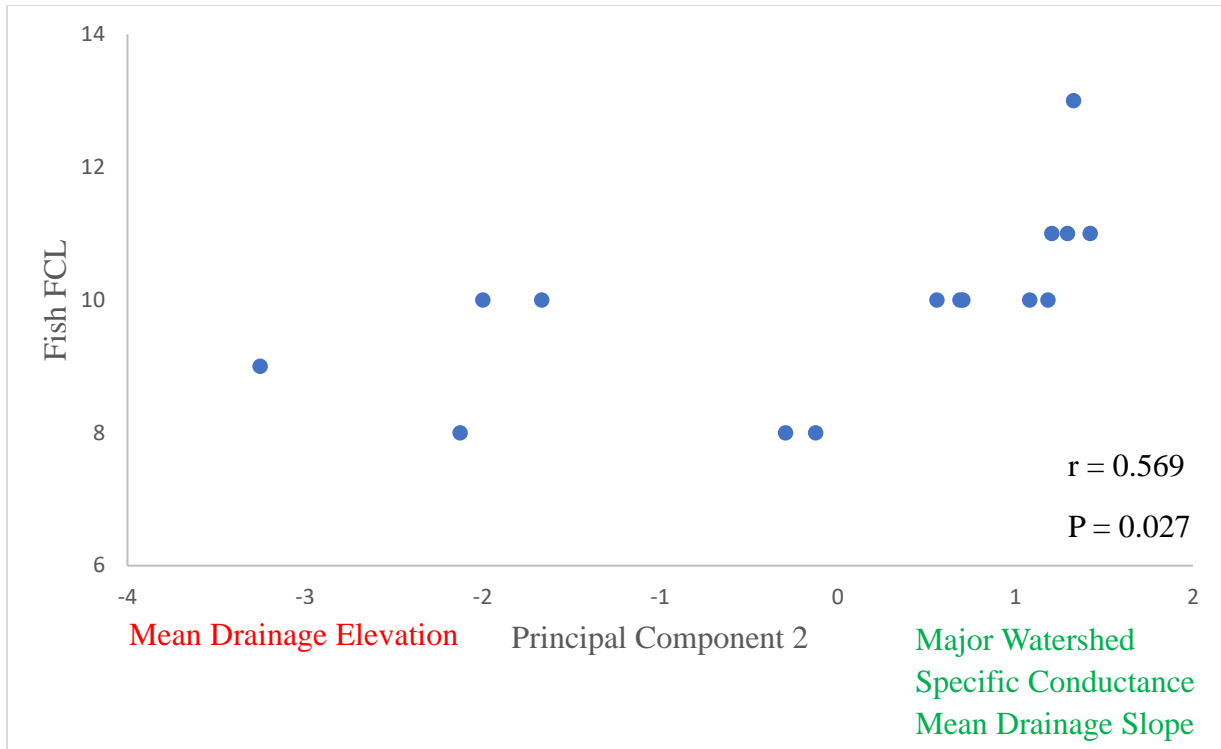


Figure 5c. Principal component 2 (33.42% variation explained) plotted against fish assemblage FCL with r-value and p-value shown. PC2 contrasted mean drainage elevation against major watershed, specific conductance, and mean drainage slope. Red watershed factors are negatively associated while green watershed factors are positively associated with fish assemblage FCL.

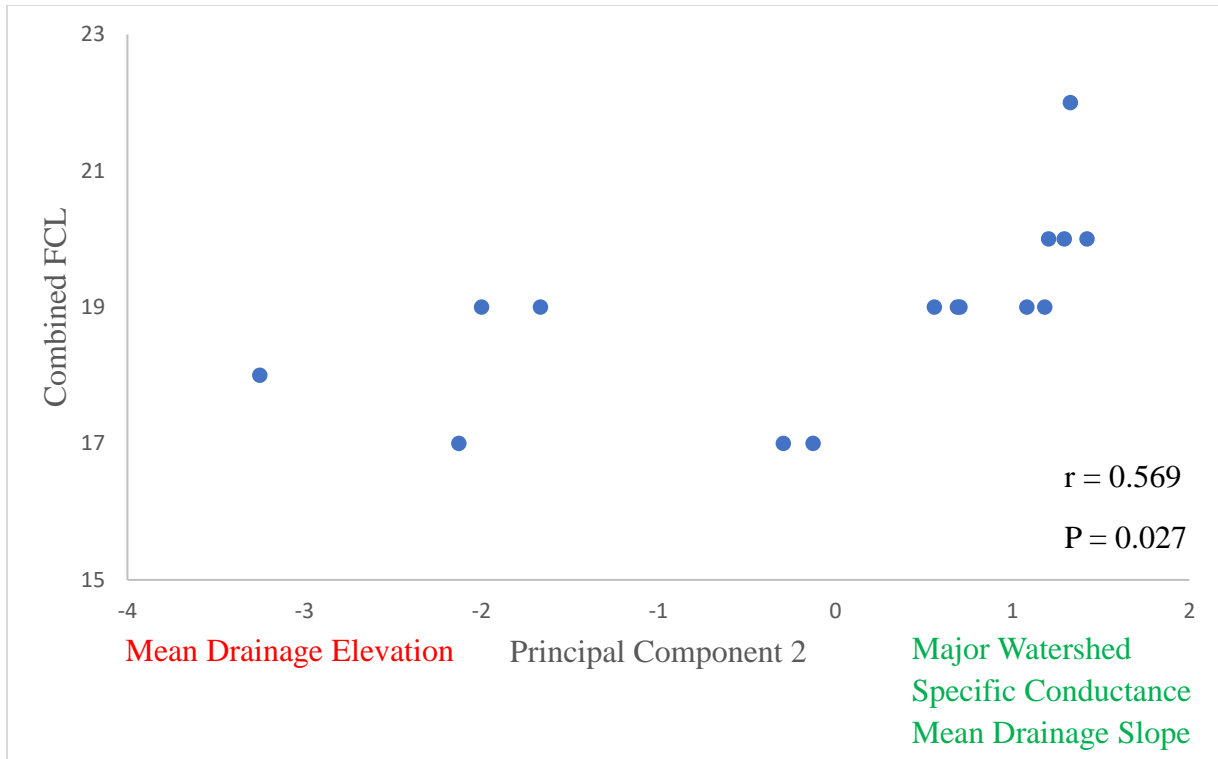


Figure 5d. Principal component 2 (33.42% variation explained) plotted against combined community FCL with r-value and p-value shown. PC2 contrasted mean drainage elevation against major watershed, specific conductance, and mean drainage slope. Red watershed factors are negatively associated while green watershed factors are positively associated with combined community FCL.