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Identification of taxonomic and functional ichthyofaunal zones within the James River Basin, Virginia

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IDENTIFICATION OF TAXONOMIC AND FUNCTIONAL ICHTHYOFAUNAL ZONES WITHIN THE JAMES RIVER BASIN, VIRGINIA

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Master of Science, Virginia Commonwealth University 2018

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Table of Contents

Abstract

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Environmental gradients structure ichthyofaunal communities longitudinally along river networks via the selective filtering of species' traits. In many instances, these environmental influences have created distinct zones of co-occurring fish species. Zonation studies have most often been conducted with taxonomic data (species x site matrices), but the increasing availability of functional trait data creates an opportunity to build more rigorous understanding of species' co-occurrence patterns. Notably, zonation studies that use taxonomic data may not reveal the same patterns as studies based on trait data. In this study, we tested for distinct ichthyofaunal zonation in James River Basin, VA using a combination of historical (1950-1987) and contemporary fish occurrence records (1986-2016) that were aggregated within 12-digit hydrologic units (HU). Zonation tests were performed separately for taxonomic data and functional trait data, using a combination of non-metric multidimensional scaling and k-means cluster analysis. We detected three distinct taxonomic zones and three functional trait zones within the James River Basin. In addition, through identifying that taxonomic dissimilarity between HUs was strongly correlated with functional dissimilarity, these zonation patterns were determined to not be significantly different.

Introduction

Background Information

In the 21st century, natural resources and biodiversity continue to be threatened by human activities, particularly within freshwater systems (Jelks et al., 2008; Sala et al., 2010; Kanno et al., 2012). Projected extinction rates are approximately five times greater for freshwater organisms compared to terrestrial fauna (Ricciardi and Rasmussen, 1999). The most serious contributors to these extinction rates include habitat fragmentation, habitat loss, hydrologic alteration, climate change, over-exploitation, pollution, and invasive species (Dudgeon et al., 2006). Traditionally, wildlife managers and environmental regulations focused on restoring the populations of imperiled species through single species conservation plans (Hutto et al., 1987; Arpin and Cosson, 2018). However, a species' long-term persistence depends on the sustained integrity of its habitat and community which are often irreversibly degraded or destroyed before species-level conservation actions are initiated. Because of this degradation and destruction, the success of these single species conservation programs is notably limited (Orians, 1993; Welner, 1995; Angermeier and Winston, 1999).

In contrast, a habitat-level conservation approach focused on protecting entire freshwater communities is (1) a more effective and practical use of limited resources (i.e., time constraints, financial funding, and scientific knowledge) and (2) more likely to prompt conservation actions before species populations become threatened or endangered (Franklin, 1993; Welner, 1995; Beattie, 1996; Koontz and Bodine, 2008). However, before developing management plans and securing legal protections, natural resource managers and policy makers need to delineate the habitats within their management area (Tracy and Brussard, 1994; Brussard et al., 1998). To be most effective, the habitat classification scheme utilized must provide a framework for identifying the non-random associations between communities and their physical habitats (Orians, 1993; Brussard et al., 1998). The identification of longitudinal zonation in river systems satisfies this requirement (Lasne et al., 2007).

Ichthyofaunal zonation occurs in running waters that encompass physical, chemical, and biological gradients that change from headwaters to mouth (Hawkes, 1975). The variation in these gradients along a river produces a series of longitudinally-arranged habitats, which each have a set of environmental constraints (e.g., landscape filters) that selectively filters for a different subset of functional traits (Poff, 1997). As a result, each riverine habitat is theoretically characterized by a functionally and taxonomically distinct fish assemblage (Heino et al., 2013; Kang et al., 2013). Traditionally, taxonomic identities have been used in zonation studies. For instance, Huet (1959) described Western European rivers as being composed of ‘trout’, ‘grayling’, ‘barbel’, and ‘bream’ zones largely caused by differences in stream gradient, water temperature, and current velocity. Similarly, fish zones have been identified within the Luongo River in Zaire, Africa (Balon and Stewart; 1983) and the Napo River in Ecuador, South America (Ibarra and Stewart, 1989). In the United States, ichthyofaunal zonation has been observed in the Southeast (Burton and Odum, 1945; McGarvey and Ward, 2008), Southwest (McGarvey, 2011), and Northwest (Hughes and Gammon, 1987; Rahel and Hubert, 1991; McGarvey and Hughes, 2008; Mee et al., 2018).

The classic zonation concept suggests that ichthyofaunal zones exist as discrete entities with sharp borders (i.e., regions of abrupt faunal turnover) between successive zones (Hawkes, 1975). In reality, fish assemblage composition gradually changes longitudinally in response to the continuous nature of the changing river gradients (e.g., Vannote et al., 1980). Ichthyofaunal zones are recognizable within this continuum because a notably greater change in habitat constraints occurs in short sections along the river (e.g., at the boundary between physiographic provinces) leading to regions along the continuum of marked increase in faunal turnover (i.e., transition zones; Hawkes, 1975; Lasne, 2007). Moreover, although fish are primarily arranged into zones of co-occurring species by their trait-environment relationships, taxonomic analyses may reveal different spatial patterns than functional trait analyses. Historical processes (e.g., speciation and dispersal) also structure the taxonomic composition of fish assemblages (Hocutt and Wiley, 1986; Tedesco et al., 2005) and interact with the isolated nature of river drainages to develop them into well-delimited biogeographic provinces (Matthews, 1998; Kang et al., 2013; Tedesco et al., 2005). As a result, taxonomic patterns may chiefly reflect drainage boundaries rather than habitat differences. In contrast, similar environmental conditions consistently lead to similar species' traits within assemblages despite large differences in taxonomy and phylogenetic history (Lamouroux et al., 2002; Ibanez et al., 2009; Olden and Kennard 2010; Oliveira et al., 2012; Kang et al., 2013). In a study that spanned several river drainages, Hoeinghaus et al. (2007) found that taxonomic analyses reveal patterns based on species' distributions while functional trait analyses separate fish assemblages based on habitat constraints. In practice, restricting analyzes to a single drainage basin (i.e., a single regional species pool) should result in a zonation study yielding similar spatial patterns when taxonomic versus functional trait data are used. However, there is still a lack of information concerning whether this is accurate.

Objectives

In this study, the existence of ichthyofaunal zonation within the James River Basin, Virginia was examined based on taxonomic identities and functional traits. The James River is a temperate coastal river in the Atlantic slope region of the southern United States that originates at the confluence of the Cowpasture River and Jackson River in Botetourt County, Virginia

(Smock et al., 2005). This confluence occurs within the Ridge and Valley physiographic provinces of Virginia at 328 m above sea level. From there, the James River flows southeast for 540 km until it reaches the Chesapeake Bay at 37° latitude. In total, the James River Basin drains 26,164 km^2 of central Virginia (Smock et al., 2005). The four physiographic provinces that the James River and its tributaries flow through – the Ridge and Valley, the Blue Ridge, the Piedmont, and the Coastal Plain – differ substantially in topography, water chemistry, channel morphology, hydrologic regime, and disturbance pattern (Jenkins and Burkhead, 1994; Cook et al., 2004). This strong longitudinal variation in major river gradients makes the James River Basin ideal for studying ichthyofaunal zonation.

A combination of historical (1950-1987) and contemporary (1986-2016) fish occurrence records aggregated within the 12-digit hydrologic units (HUs) of the James River Basin were used. The first three sub-questions were (1) whether taxonomic and functional zones exist within the James River Basin, (2) whether these taxonomic and functional zones roughly overlap with the physiographic provinces of Virginia and (3) whether taxonomically-dissimilar fish assemblages are also functionally-dissimilar. Previous community-level studies in Virginia have shown that the taxonomic composition (Angermeier and Winston, 1999; Cook et al., 2004) and functional composition (Angermeier and Winston, 1999) of fish assemblages are significantly different between physiographic provinces. Moreover, a significant, positive correlation was found to exist between taxonomic and functional composition (Angermeier and Winston, 1999). However, the finest spatial resolution of the study extents analyzed by these studies were 8-digit HUs (i.e., subdrainages), which are on average 11-45 times larger than 12-digit HUs (U.S. Geological Survey & U.S. Department of Agriculture-Natural Resources Conservation Service, 2011). Therefore, the fourth sub-question was whether the trends stated above also occur at smaller spatial scales than previously identified.

Methods

Study Area

The complete stream and river network of the James River Basin was represented by the 1:100,000-scale National Hydrography Dataset Plus ver. 2 (NHDPlus ver. 2) digital stream network (McKay et al., 2015; Figure 1). Each stream segment within the NHDPlus ver. 2 is uniquely identified by a common identifier (ComID); these ComIDs provide a universal indexing system for appending attribute data to individual segments. The Watershed Boundary Dataset (WBD) partitioned the basin into 298 12-digit hydrologic units (HUs) at the 1:24,000-scale (U.S. Geological Survey, 2015). The surface area of each 12-digit HU ranged from 40-162 km^2 (U.S. Geological Survey & U.S. Department of Agriculture-Natural Resources Conservation Service, 2011).

Fish Presence Data

Fish presence records were obtained from two independent sources. First, we queried all James River Basin records from the Interactive Stream Assessment Resource (INSTAR) database. INSTAR is primarily a compilation of rapid bioassessment type surveys (i.e., single-pass backpack electrofishing) conducted throughout the wadable streams of Virginia; however, it also contains a few surveys that were focused on species of interest (e.g., game fish) rather than the entire fish assemblage. INSTAR is an ongoing collaboration between the VCU Center for Environmental Studies and several agencies, both governmental and non-profit (http://instar.vcu.edu). Many HUs within the James River Basin were sampled multiple times by these collaborators. As of March 2018, INSTAR included 30 years (1986-2016) of fish survey data.

The second data source, IchthyMaps, is a compilation of fish occurrence records for the contiguous United States (Frimpong et al., 2015). Each fish occurrence is paired with the specific stream segment (i.e., ComID) in NHDPlus ver. 2 that it was collected from, with the potential for multiple records to occur on the same stream segment (i.e., multiple species collected at the same site). IchthyMaps records for the James River Basin were compiled from *Freshwater Fishes of Virginia* (Jenkins and Burkhead, 1994*), Atlas of North American Freshwater Fishes* (Lee et al., 1980), and the Multistate Aquatic Resources Information System. Among these three sources, field collection dates ranged from 1950-1987.

Spatial Data Aggregation

Several pre-processing steps were necessary before the fish presence data could be used in analyses of fish assemblage structure. First, minor data errors in IchthyMaps (e.g., stream segments that straddled two HUs) were corrected. INSTAR fish occurrence records were then superimposed onto the NHDPlus ver. 2 stream segments based on the geographic coordinates of their sampling sites. Next, the IchthyMaps and INSTAR records were catalogued within their respective 12-digit HUs, followed by queries that removed the resultant duplicate fish occurrence records (i.e., ensuring that a species would only be listed once within a given HU). All species names were standardized in accordance with the Integrated Taxonomic Information System (www.itis.gov).

To determine which HUs were adequately sampled and therefore suitable for inclusion in our analyses, species accumulation curves were built for the INSTAR and IchthyMaps fish data in R (R Core Team, 2017). However, the measure of sampling effort used to build the species accumulation curves differed between the two data sources because of variable sampling methods. For IchthyMaps, sampling effort was the number of sampled stream segments (i.e., ComIDs). In contrast, the sampling effort for INSTAR was the number of sampling events. Both species accumulation curves were graphed as the sampling effort within a given HU plotted against the cumulative species richness within that HU.

The second derivative of a logarithmic regression model fit to each curve was used to identify the specific sampling effort beyond which a marked decline in the return on effort (i.e., the species richness detected within a HU) was observed and further effort would not yield appreciable gains. Specifically, the lowest negative value calculated by the second derivative indicated the species effort at which the logarithmic model had the highest decrease in the rate of species detection. This specific sampling effort was used throughout the study area as a threshold to identify HUs that did not have an adequate number of samples collected within them to represent local fish assemblage structure. If neither INSTAR nor IchthyMaps had sufficient sampling effort to describe a given HU, then that HU was removed from the study. Fish occurrence records within HUs that met or exceeded the threshold sampling effort criterion were then combined and converted to a single binary presence-absence matrix of all species known to occur within each HU.

Taxonomic Zones

The master fish presence-absence matrix was converted to a Sorensen’s dissimilarity matrix of all pairwise site comparisons (Sorensen, 1948) using the *vegdist* function in the R package *vegan* (Oksanen et al., 2017). Next, *k*-means clustering of the HUs based on their pairwise dissimilarities was used to test for taxonomic ichthyofaunal zonation. The HUs were partitioned into 2-group to 7-group clustering solutions using the *cascadeKM* function in *vegan*. *K*-means clustering attempts to find the partitioning of individual objects within a dataset (HUs in this study) that minimizes the total error sum of squares (i.e., the sum of squared Euclidean distances between the objects and their cluster centroids; Legendre and Legendre, 2012).

The six clustering solutions were visualized using ArcMap 10.5 to examine the spatial distribution of the clustered HUs. Clustering solutions that exhibited moderate to strong spatial cohesion (i.e., groups of HUs that were assigned to the same clusters and clearly aggregated in space) were retained for further analysis. Non-metric multidimensional scaling (NMDS) of the Sorensen's dissimilarity matrix was also used to examine the distribution of the HUs within a three-dimensional ordination. NMDS was conducted with 1,000 random starts using the *metaMDS* function in *vegan*. An ordination plot was drawn for each of the six clustering solutions with colors indicating cluster membership for each HU and 95% confidence ellipses drawn around clusters. Ordination plots were built with the R package *ggplot2*, using the *stat_ellipse* function to generate confidence ellipses (Wickham, 2009). If distinct clusters of HUs were evident in the NMDS ordination plot for a given clustering solution and a clear pattern of spatial clustering was evident when the HUs were mapped in ArcMap, we concluded that that clustering solution provided evidence of taxonomic ichthyofaunal zonation in the James River Basin.

Functional Trait Zones

A matrix of fish species' functional traits was obtained from Woods and McGarvey (in press) for species included in the master presence-absence matrix. This trait matrix included trophic, habitat, behavioral, and life history characters that were compiled from multiple fish atlases, journal articles, and online governmental/academic databases (see original sources in

Woods and McGarvey, in press). A complete list of the functional traits used in this study is provided in Table A1.

Species' traits were then appended to the fish presence-absence matrix to create a species' trait x HU matrix. Each HU in the dataset was then reduced to a single row of summarized species' traits using the R package *dplyr* (Wickham et al., 2017). Numeric species' traits were summarized by their median values and the widths of their overall ranges. Categorical traits were summarized by their modal values and the numbers of discrete trait levels. A Gower's dissimilarity matrix (Gower, 1971) was then calculated for all pairwise HU contrasts using the *daisy* function in the R package *cluster* (Maechler et al., 2017). Gower's dissimilarity coefficient was used rather than Sorensen's dissimilarity because it accommodated the mixed data types and missing values of the summarized species' trait dataset (Olden and Kennard, 2010). Next, the zonation of ichthyofaunal species' traits within the James River Basin was identified using the same procedure that identified taxonomic ichthyofaunal zonation.

Indicator Species Analysis

The characteristic species of each ichthyofaunal zone (i.e., indicator species) were identified using the *multipatt* function in the R package *indicspecies* (De Caceres and Legendre, 2009). Species replacement patterns were examined by comparing the characteristic species between faunal zones in the same zonation pattern (e.g., the taxonomic zones). The characteristic species of overlapping taxonomic and functional zones were also examined. If the taxonomic and functional zones truly had the same zonal structure (i.e., spatial layout within the James River Basin), then we expected the characteristic species of the overlapping zones to be identical.

A species was designated as characteristic for the faunal zone, or the combination of zones, in which its indicator value was highest. Each indicator value was calculated as the product of a species' specificity and fidelity for a given faunal zone. Specificity was the probability that a HU belonged to the faunal zone if that species was found. In contrast, fidelity was the probability of finding the species within a HU of the faunal zone. Only characteristic species with a specificity and fidelity greater than or equal to 0.70 and 0.30 respectively were retained. A significance test was independently calculated by permutations for each reported species-zone combination, requiring that a Bonferroni correction be applied (De Caceres et al., 2010).

Significance Testing

A Mantel test (Mantel, 1967) was used to determine whether the spatial clustering patterns were significantly different when taxonomic and functional trait data were used in clustering and NMDS analyses. This test used Pearson product-moment correlation coefficients to quantify and assess congruence between the Sorensen's dissimilarity matrix (i.e., taxonomic dissimilarity between 12-digit HUs) and the corresponding Gower's distance matrix (i.e., functional dissimilarity between HUs). The correlation coefficient was determined using the *mantel* function in *vegan*. This function determined the statistical significance of the correlation coefficient using permutations of the rows and columns of the first dissimilarity matrix. In addition, the correlation was plotted using *ggplot2* to examine how functional dissimilarity varied with taxonomic dissimilarity between HUs.

Results

Fish Presence Data and Spatial Aggregation

The sampling effort necessary for the fish occurrence records to adequately represent the taxonomic composition of a HU was determined to be 2 sampled stream segments (i.e., ComIDs) in IchthyMaps or 2 sampling events in INSTAR. However, because IchthyMaps is a compilation of atlas records rather than direct survey results, the minimum sampling effort required for IchthyMaps was increased to 3 sampled stream segments. Minimum sampling effort was met for 274 (91.9%) of the 12-digit HUs. The combined fish presence dataset detailed the known occurrences of 145 fish species from 27 Families and 17 Orders within the James River Basin.

Taxonomic Zones

Three taxonomic ichthyofaunal zones were identified within the James River Basin; a montane zone, a piedmont zone, and a tidal zone. These taxonomic zones were distinguished in HU maps of the James River Basin using the 3-group clustering solution produced by *k*-means (Figure B1). A NMDS ordination plot of the HUs also reflected this zonation pattern (Figure B2). The transition from montane zone to piedmont zone was marked by a notable overlap in the confidence ellipses of the two zones. In contrast, only minor overlap was present at the transition from piedmont zone to tidal zone. A second taxonomic zonation pattern was identified using the two-group clustering solution (Figure B1). The montane and piedmont zones were combined into a non-tidal (i.e., freshwater) zone, and the tidal zone expanded into the lower fourth of the Piedmont physiographic province. In the NMDS plot, the transition from non-tidal zone to tidal zone was marked by notable overlap (Figure B3). The juxtaposition of the zones in both ordination plots reflected their geographic distribution in the HU maps (Figure B1).

Functional Trait Zones

Three functional ichthyofaunal zones were identified in the HU maps using the 4-group clustering solution produced by *k*-means (Figure B4). Because the functional zones were similar in shape and location to the three taxonomic zones, the functional zones were designated with the same nomenclature. The fourth cluster in the 4-group cluster solution was composed of HUs distributed across the James River Basin with no coherent structure; thus, it was determined to be an artifact of the methodology. A NMDS ordination plot of the HUs reflected these zonation patterns (Figure B5). The artifacts were dispersed around the left-side of the ordination plot rather than tightly clustered, providing further evidence that they did not constitute a meaningful grouping of the HUs. The juxtaposition of the three functional zones within the ordination reflected the layout of the taxonomic clusters.

A second functional zonation pattern was identified using the three-group clustering solution (Figure B4). The montane zone remained within its own cluster, while the piedmont and tidal zones were combined to make up the second cluster. Again, the *k*-means algorithm partitioned the artifacts into their own cluster. This result contradicted expectations based on the NMDS of the 4-group clustering solution, which showed the piedmont functional zone as having the tightest cluster. Logic suggested that the piedmont zone should have been within its own cluster rather than the montane zone. Instead, the NMDS plot of the 3-group clustering solution (Figure B6) indicated that the HUs within the montane zone were more distinct in terms of functional traits than the HUs within the piedmont or tidal zones.

Physiographic Provinces

The tidal taxonomic zone coincided with the Coastal Plain physiographic province, with the exception that the lower third of the Appomattox River was included (Figure B1). In contrast, the Piedmont physiographic province was roughly overlapped by the piedmont zone. Moreover, a section of the piedmont zone was also present within the lower part of the Ridge and Valley physiographic province. The rest of the Ridge and Valley province was overlapped by the montane zone. The Blue Ridge physiographic province, the physiographic province between the Ridge and Valley and Piedmont provinces, was evenly divided between the piedmont and montane taxonomic zones. In general, the transition between taxonomic ichthyofaunal zones roughly corresponded with a transition from one physiographic province to the next.

As was the case for the taxonomic tidal zone, the functional tidal zone overlapped the Coastal Plain province, plus a portion of the lower Appomattox River (Figure B4). Similarly, the piedmont functional zone had the same rough boundaries as the piedmont taxonomic zone. In addition, as was the case for the taxonomic zonation pattern, the Blue Ridge province was evenly divided between the piedmont and montane zones. However, a large portion of the HUs adjacent to and south of the Appomattox River were assigned to the montane zone. Moreover, the montane functional zone lacked the notable section of piedmont zone that characterized the lower half of the montane taxonomic zone.

Indicator Species Analysis

A Bonferroni correction lowered the alpha value for the significance tests to 0.0003. The characteristic species of the corresponding taxonomic and functional ichthyofaunal zones were very similar, albeit not identical. Three species were shared between the montane taxonomic zone and montane functional zone; *Cottus caeruleomentum, Salvelinus fontinalis*, and *Oncorhynchus mykiss* (Table B2). Another species, *Exoglossum maxillingua*, was also a strong indicator of whether a HU belonged to the montane taxonomic zone. In contrast, many species were characteristic of the tidal taxonomic zone and tidal functional zone, 12 and 14 respectively.

More than half of these species were shared between the tidal zones. Interestingly, the piedmont zones were characterized by fewer species than the montane zones. Both piedmont zones were characterized by *Percina roanoka*, while the piedmont taxonomic zone was also characterized by *Etheostoma vitreum* and *Lepomis cyanellus*. Yet, the montane-piedmont zones and piedmont-tidal zones were characterized by many strong indicator species (Table B2), suggesting that many fish species within the James River Basin had flexible tolerances to the differences in habitat constraints between adjacent zones.

Significance Testing

A Mantel test indicated that the taxonomic dissimilarity between HUs was strongly correlated ($R = 0.83$; $N = 75\ 076$; $P < 0.001$) with functional dissimilarity (Figure B7). Therefore, the differences between spatial clustering patterns were not statistically significant when taxonomic versus functional trait data were used. According to the correlation plot, an increase in taxonomic dissimilarity (i.e., species turnover) corresponded with a disproportionately small increase in functional dissimilarity. This suggested that replacement by a functionally similar species characterized most species turnover. Moreover, although some HUs had complete taxonomic dissimilarity (i.e., no shared species; value of 1), relatively few HUs had functional dissimilarity exceeding 0.50, which suggested that some functional traits were roughly constant within the James River Basin.

Discussion

Fish Patterns in the James River Basin

Using *k*-means cluster analysis and NMDS on fish assemblage data obtained from INSTAR and IchthyMaps, three taxonomic and functional ichthyofaunal zones were detected within the James River Basin, Virginia. Similar zonation patterns (i.e., 3-4 successive ichthyofaunal zones) have been identified in other rivers across the United States (e.g., Hughes and Gammon, 1987; McGarvey and Hughes, 2008; McGarvey and Ward, 2008; McGarvey, 2011). Moreover, through identifying that taxonomic dissimilarity between HUs was strongly correlated with functional dissimilarity ($R = 0.83$; $N = 75{,}074$; $P = 0.001$), these zonation patterns were determined to not be significantly different. In extension, this study suggests that community analyses of taxonomic versus functional trait data may show similar or identical spatial patterns when restricted to a single river drainage.

This conclusion disagreed with Hoeinghaus et al. (2007) that patterns revealed by community analyses of fish assemblages depend on whether taxonomic versus functional data are used. However, the overall zonation patterns revealed by this study agreed with their conclusion that taxonomic analyses identify patterns driven by species distributions while functional analyses detect patterns directed by habitat characteristics (i.e., a habitat template). This contradiction was a result of restricting analyses to a single drainage while Hoeinghaus et al. (2007) examined fish assemblages distributed across several drainage basins. Because the regional species pool was constant within the James River Basin, species distributions were predominantly driven by the same habitat constraints that structured the functional traits. Thus, at the drainage-level, the spatial patterns displayed by species composition and functional traits should be expected to be largely similar. In contrast, because their analyses spanned drainages, patterns in species distributions detected by Hoeinghaus et al. (2007) were also structured by drainage boundaries and differences in the regional species pool between drainages, while habitat type continued to structure functional traits regardless of these additional influences.

The zonation concept states that distinct fish assemblages of ecologically similar species exist in discrete zones with clear, definable boundaries caused by high turnover between adjacent zones (Hawkes, 1975). To the contrary, by arrange the 12-digit HUs of the faunal zones along a continuous gradient, the NMDS plots (Figures B2 and B5) showed that taxonomic and functional composition vary continuously (i.e., along a continuum) within the James River Basin rather than in discrete entities. Other authors have acknowledged this reality (Vannote, 1980; Zalewski and Naiman, 1985; Matthews, 1998; Lasne et al., 2007). Rather than share a common response to environmental stressors and changing habitat conditions, which would lead to the development of discrete fish zones through large-scale species turnover, the individual species that comprise fish assemblages respond in varying degrees to alterations to their habitat (Pont et al., 2005). This variability in species response was also suggested by the indicator species analysis. Numerous species were determined to be most characteristic of one of the three ichthyofaunal zones (Table B1), but none of these species had a specificity equal to 1 which would have signified that a given species occurred only within its zone (De Ceceres and Legendre, 2009). Similarly, as shown by their respective fidelity values, none of these characteristic species were present within all the sites (i.e., 12-digit HUs) of their respective zone. The notable variability in the indicator values of even the most characteristic species of our three zones hint that the boundaries between ichthyofaunal zones are not as distinct as suggested by classic zonation studies.

Interestingly, the overall slope of the correlation between taxonomic and functional dissimilarity between 12-digit HUs suggested that species composition is notably more variable along this continuum than functional composition. An increase in the taxonomic dissimilarity between HUs corresponded with a disproportionately small increase in functional dissimilarity and sometimes no change at all (Figure B7). Although the stressors of a given habitat may select for a given subset of functional traits, and thus produce a functional faunal zone, local-scale habitat factors and historical processes (e.g., dispersal and extinction) determine which species from the regional species pool are within the local fish assemblage. In other words, replacement by a functionally similar species likely characterizes most species turnover between fish assemblages (Angermeier and Winston, 1999). In contrast, an increase in functional dissimilarity, which likely reflects differences in habitat constraints, significantly corresponded to a high degree of species turnover (Figure B7). To summarize, although fish assemblages of similar taxonomic composition are inevitably similar functionally, and taxonomically-dissimilar assemblages may still have similar functional composition (Angermeier and Winston, 1999), functionally-dissimilar fish assemblages explicitly have dissimilar taxonomic composition. Angermeier and Winston (1999) found that these relationships also exist at the state-wide and subdrainage level (i.e., within 8-digit HUs) in Virginia.

These interpretations help explain the zonation results. By clustering the HUs into faunal zones that corresponded to the physiographic provinces (Figures B1 and B4), the *k*-means clustering algorithm indicated that the difference in species and functional traits between HUs was notably higher amongst provinces than within provinces. Previous studies have found these ichthyofaunal differences between physiographies to be significant (Angermeier and Winston 1999; Cook et al., 2004). At the boundaries between these physiographies, which signified considerable changes in topography, channel morphology, hydrologic regime, water chemistry, and disturbance patterns (Swanson et al., 1988; Jenkins and Burkhead, 1994), an increase occurred in the turnover rate of species and functional traits. In turn, this higher turnover rate caused the areas of greater contrast within the continuum that made HUs more similar within physiographies than between physiographies. Gradual change along the length of a river with marked changes over shorter distances at intervals is a classic pattern in zonation studies (Hawkes, 1975). Although the NMDS plots could not signify how sharp these transition areas were, they did support that higher similarity existed between HUs of the same faunal zone by grouping them together into different sections along the continuum.

The strong association between fish assemblage composition and physiographies implied by the three ichthyofaunal zones may be partially deceptive. Pflieger (1971) and Hawkes et al. (1986) indicated that the major ichthyofaunal regions within Missouri and Kansas coincided with physiographic province boundaries respectively, but physiographies failed to explain the distribution of distinct ichthyofaunal regions within Oregon (Hughes et al., 1987). Instead, the broad faunal differences between fish assemblages in Oregon were well predicted by ecoregions (Hughes et al., 1987; Whittier et al., 1988). Similarly, ecoregions were concordant with fish assemblages in Arkansas (Rohm et al., 1987), Ohio (Larsen et al., 1986), Nebraska (Bazata, 1991), Wisconsin (Lyons, 1989) and Washington (Plotnikoff, 1992). Physiographies by themselves are poor surrogates for the variables (e.g. water temperature, chemistry, clarity, or streambed substrate) that determine the current distributions and abundances of fish species (Hughes, 1978). In contrast, ecoregions integrate the spatial differences in multiple landscape characteristics such as geology, soils, vegetation, climate, land-use, wildlife, hydrology, and physiography to more holistically identify regions of homogenous ecosystem (Omernik, 1995;

Woods et al., 1996). Five level III ecoregions run parallel through the James River Basin (Omernik and Griffith, 2014) and roughly correspond to the physiographic provinces. Therefore, the three ichthyofaunal zones also share general boundaries with ecoregions suggesting that more than physiographic differences are likely structuring the fish assemblages of the James River Basin.

We failed to detect subsetting of the regional species pool at a scale smaller than previously identified in Virginia. Instead, our results supported the finding by Cook et al. (2004) that regional species pools are most nested at the drainage-physiography level. Finer-scale zonation or nestedness of the regional species pool was not detected because these regional classifications combined localities differing substantially in the local-scale factors controlling species' distributions, such as incorporating multiple stream orders (e.g., Vannote et al., 1980; Zalewski and Naiman, 1985). By aggregating the local species pools of these fish assemblages to the 8- or 12-digit HUs, the structuring effects of the local-scale biotic and abiotic factors on assemblage composition became largely undetectable (Jackson et al., 2001). As such, regional-scale abiotic factors, which influence these fish assemblages regardless of locality, were the major drivers of ichthyofaunal patterns at these spatial scales (Huston, 1999; Jackson et al., 2001). Angermeier and Winston (1998) supported that regional factors outweigh the influence of local-scale factors on fish assemblage structure in Virginia. Moreover, zonation or nestedness was not detected at smaller spatial scales because the regional-scale factors did not meaningfully differ between these hydrologic units and the larger drainage-physiographies in which they were nested. Whereas drainage-physiography combinations describe regions of distinctive fish assemblages and relatively homogenous environmental conditions (Angermeier and Winston, 1999), and thus outline meaningful biotic zones, hydrologic units are not designed to depict differences in either of these features (Omernik, 1995; Cook et al., 2004).

Mechanisms Causing the Fish Assemblage Patterns

Through analyzing the known habitat requirements and distributions of the indicator species, major underlying drivers of ichthyofaunal zonation within the James River Basin were identified. Moreover, like other zonation studies conducted in mountainous regions (e.g., Huet, 1959; Rahel and Hubert, 1991; Lasne et al., 2007), we detected a montane headwater zone dominated by coldwater trout assemblages and a downstream warmwater zone dominated by cyprinid-catostomid assemblages. The descriptions of habitats, habitat preferences, and species distributions provided in this section were obtained from Jenkins and Burkhead (1994) unless stated otherwise.

The fish species characteristic of the montane zone corresponded to the high-gradient headwater assemblages. As expected, the trout species *Salvelinus fontinalis* and *Oncorhynchus mykiss* were present within these assemblages. These coldwater stenotherms are restricted from persisting within the warmer, moderate-gradient valley streams between mountain ranges due to physiological constraints imposed by water temperature and dissolved-oxygen concentration (Quist et al., 2004). In contrast, although *Cottus caeruleomentum* co-occurs with these salmonids in moderately high gradient coolwater streams, it also persists within warmwater faunas downstream. In fact, many of the montane-piedmont species also deviate from the downstream warmwater zone classification by inhabiting both cool and warm waters rather than strictly warmwater habitats. A few of these species, such as the dace *Rhinichthys atratulus* and the chub *Nocomis micropogon*, even inhabit the lower range limits of the aforementioned trout species. The piedmont zone, piedmont-tidal zone, and tidal piedmont zone species conform to the classification by generally inhabiting only warm waters. As a result, regions of the James River Basin can be roughly divided into sections that support either cold, cool-warm, or warmwater assemblages.

The distribution of these fish assemblages is also structured by two correlates of stream gradient, water velocity (Rahel and Hubert, 1991; Gorman and Karr, 1987; Pont et al., 2005) and streambed substrate (Hawkes et al., 1986; Lyons, 1989; Gorman and Karr, 1987; Petry and Schultz, 2006). As stream gradient slowly declines from the headwaters in the montane zone to the Chesapeake Bay, water velocity generally changes from swift to moderately flowing to slow or stagnant. Moreover, this gradient corresponds to rocky streambeds gradually giving way to increased dominance by sand substrate in the Piedmont followed by sand, mud, detritus, and vegetation in the Coastal Plain. Montane species generally only occupy rocky substrates in high to moderate-high gradient (i.e., fast-flowing) streams, whereas montane-piedmont species extend into the Piedmont in varying degrees based on their tolerance to sand or preference for gravel-sand substrate. The availability of rocky substrate is a requirement for many of these species. Several physically manipulate the rocky substrate of their habitat by excavating gravel redds or building nesting mounds. Furthermore, several other montane-piedmont species are nest associates of mound builders. Also, as a chiefly anthropomorphic influence on the distribution of fish species, the increased intensity of streambed siltation at lower stream gradients is a limiting factor for some species like *Ambloplites rupestris, Campostoma anomalum*, and *Cottus caeruleomentum*.

Interestingly, despite being identified as one of the few piedmont species, the darter *Percina roanoka* has a notable distribution within the montane zone. However, the other two piedmont species are fitting of their classification. *Lepomis cyanellus* tolerates all the substrates prevalent within the Piedmont. This would suggest that it may also successfully colonize montane streams, but like most centrarchids its laterally compressed body form make fast-flowing habitats energetically prohibitive (Angermeier, 1987). In contrast, the darter *Etheostoma vitreum* is highly specialized in both body morphology and spawning behavior to persist within sand bars and flats. The shovel-like snout of *Etheostoma vitreum* acts as a wedge for both foraging and burrowing its slender body into the sand. Long pectoral fins facilitate covering its body with sand and possibly act as hydrofoils that anchor it to the streambed while resting. Moreover, its semi-transparent, pallid flesh with dark flecking provides camouflage against sand substrates. Eggs are always attached above the streambed on solid structures and facing strong currents to preclude or reduce smothering by shifting sands.

As the tidal zone encompasses the estuarine portion of the James River, a transitional ecosystem at the interface between marine and freshwater environments, the fish assemblages of this area include a combination of freshwater, marine, and estuarine species. Aquatic macrophytes are abundant in the fine-grained organic substrates of the low-gradient rivers that characterize streams of the Coastal Plain but are largely absent from the sandy and rocky streams of the montane and piedmont zone (Garman and Nielsen, 1992). *Ancantharcthus pomotis*, which is a strictly freshwater species, prefers nests on these soft substrates (i.e., mud, detritus, and silt) among the vegetation in sluggish waters. *Ennecanthus gloriosus*, *Centrarchus macropterus*, and *Umbra pygmaea* also share these habitat preferences; however, they also tolerate marginally brackish waters. *Gambusia holbrooki* is even more tolerant of salinity and may inhabit waters stagnant enough to become near-anoxic. The anadromous river herrings *Alosa aestivalis* and *Alosa pseudoharengus* both spawn upon entering tidal-fresh or brackish waters, and larvae and juveniles remain in their natal streams during development. The anadromous *Morone saxatilis* and largely-estuarine *Morone americana* spawn in similar habitat conditions, though they tend to use the moderate to strong currents in the rocky or rock-sand areas around the Fall Zone to prevent their eggs and larvae from settling and becoming smothered in the soft sediments.

A discussion of the piedmont-tidal species would be repetitive as these habitats and their major gradients have already been adequately described. The trends presented here strongly suggest that water temperature, substrate composition, and stream gradient function together to longitudinally structure fish assemblages. As previously discussed, our NMDS results (Figures B2 and B5) indicated that fish assemblages change along a continuum rather than in discrete events. Not only is this reality supported by the variable specificity and fidelity of the indicator species for their respective zones, and by the variable tolerances of indicator species for major environmental gradients, but also by the existence of the montane-piedmont and piedmont-tidal zones. The NMDS plot for taxonomic dissimilarity indicated this sharing of species between adjacent zones by plotting the faunal zones along the continuum in the same order as their geographic distribution. Similarly, the NMDS plot for functional dissimilarity (Figure B5) also ordered the faunal zones this way.

The location of the piedmont zone within the ordination plots is also interesting because it only has two characteristic species, *Etheostoma vitreum* and *Lepomis cyanellus*, which contrasts greatly to the numerous species that it shares with either the montane or tidal zone. It seems that the piedmont zone is chiefly a transition zone between montane and tidal fish assemblages. In general, less montane-piedmont species and more tidal-piedmont species are present within the piedmont HUs from west to east. This is logical given that upper piedmont HUs are montane-like (i.e., rocky or rock sand with moderate current) in habitat while lower piedmont HUs are more tidal-like (slow waters with soft substrates and vegetation). This mixing of fish assemblages from the two zones also explains why montane and tidal HUs were more functionally-similar to the piedmont HUs that to each other.

Interesting Patterns within the Zonation Schemes

The section of piedmont HUs displaced within the taxonomic montane zone (Figure B1) were populated by several piedmont-tidal species and the piedmont species *Percina roanoka*. An examination of the distributions of these piedmont-tidal species within the fish presence data revealed that these species were well-dispersed across the piedmont and tidal zone with disjunct populations within this disjunct piedmont section. Moreover, *Percina roanoka* is introduced to the James River Basin and was first recorded within this area, specifically Craig Creek, during the 1950s (Jenkins and Burkhead, 1994). Furthermore, this cluster of HUs had usually high species richness compared to the surrounding montane HUs. The combination of montane-piedmont and piedmont-tidal species, which describes the overall composition of the piedmont zone, and the high species richness likely functioned together to make these HUs more similar to the piedmont than to the montane zone.

It was not unexpected that the 2-group clustering solution for the taxonomic zonation (Figures B1 and B3) divided the James River Basin into a freshwater zone and tidal zone. As previously stated, the species of the estuarine zone combine both freshwater, estuarine, and marine fish species, which caused the tidal zone to have more characteristic species than the montane zone. In other words, the taxonomic difference between the montane and piedmont zones were not as great as the difference between the piedmont and tidal zones. However, the interpretation of the 3-group clustering solution for the functional zonation (Figures B4 and B6) was more complex. Although it seemed to be identifying the montane region as functional distinct from the piedmont and tidal zones, which would be logical considering that steep elevation gradients are known to cause greater variation in key physiographic gradients (Cook et al., 2004), it was actually displaying the distribution of *Anguilla rostrata* within the fish presence data. The combined piedmont-tidal zone was the distribution of *Anguilla rostrata*, while the montane zone was indicative of its absence. This included the sizable section of montane HUs dispersed along the lower half of the Piedmont.

Repeating the zonation analyses after removing *Anguilla rostrata* resulted in the 4-group and 3-group clustering solutions clustering the HUs into a similar configuration to the 2-group clustering solution for taxonomic zonation (i.e., freshwater versus tidal; Figure B1), except that the tidal zone began closer to the Fall Line. In other words, the division of the freshwater portion of the James River Basin into a functional montane zone and functional piedmont zone by the *k*-means algorithm relied on the presence of *Anguilla rostrata.* In contrast, removing it from the presence records yielded no discernable change in the taxonomic zonation patterns. Furthermore, and very importantly, its removal neither effected the significance nor the slope of the correlation between taxonomic and functional dissimilarity between 12-digit HUs.

Anguilla rostrata is important to the functional zonation scheme because it had a wide distribution and several functional attributes that deviated from the norm. It had the second highest max fecundity and highest average fecundity of all fishes in the analyses. It also had a high maturation age, maximum longevity, and maximum total length, but more importantly it contributed a swim mode (i.e., anguilliform) and migratory behavior (i.e., catadromous) that no other fish species had. Several estuarine or marine fish species also had similar characteristics, with the exception of the catadromous migratory behavior and anguilliform movement. However, their distribution ranges were restricted to the tidal zone, whereas *Anguilla rostrata* was widely dispersed across the tidal and piedmont zones due to its generalist habitat preferences. The several extreme functional attributes of *Anguilla rostrata* acted on the range metrics (e.g., number of different spawning substrates) within the summarized species' trait x HU matrix to make the piedmont HUs more similar to the tidal HUs. Simultaneously, its functional attributes that were within the norm for species inhabiting the piedmont zone allowed the piedmont HUs to maintain some similarity with the montane HUs. As a result, the functional attributes of *Anguilla rostrata* caused the piedmont HUs in which it was present to be a functional intermediate between montane and tidal HUs.

Similarly, the artifacts group within the functional zonation patterns were caused by repeated occurrence of rare values. For instance, the most common swim mode for most HUs was cruiser but a select few HUs scattered throughout the James River Basin had creeper or maneuver denoted instead. Another example would be that the most prevalent trophic behavior in most HUs was invertivore but for a few scattered HUs it was either herbivore or omnivore. According to these functional attributes, these outlier HUs w ere more similar to each other than to the HUs around them. As these rare values tended to occur within the same subset of HUs, these small partial similarities acted additively to cause the *k*-means algorithm to cluster them together. Overall, the strong influence of *Anguilla rostrata* on the functional zonation patterns and the existence of the artifacts group indicates that the methodology used to analyze the functional trait data is easily influenced and may strongly benefit from adding additional metrics to the species' trait x HU matrix to capture more of the variability in the functional composition of the fish assemblages.

Conclusions

Because the three ichthyofaunal zones identified by this study roughly corresponded to the physiographic provinces and ecoregions within Virginia, and therefore delineated welldefined regions of relatively homogenous habitat and ichthyofaunal composition, they can be used by natural resource managers and restoration planners to identify the areas in which the same conservation and management practices are necessary and where different practices are needed (Hawkes et al., 1986; Lyons, 1989). In fact, these faunal zones satisfy the requirements to be considered at least preliminary ecoregions, which are explicitly designed for use in management decisions and as conservation units (Abell et al., 2000). Moreover, all of the analyses in this study were performed based solely the manipulation of standardized point samples, simple shapefiles, and species' trait data retrieved from large databases and fish atlases. These zonation patterns revealed by the combined use of ordination and classification techniques are only an example of the numerous patterns that may be discerned from the data contained within these simple point samples. Furthermore, and importantly, these analyses were notably more cost-effective and less time consuming than collecting extensive samples of local habitat variables.

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Zalewski, M. & Naiman, R.J. (1985). The regulation of river fish communities by a continuum of abiotic-biotic factors. In J.S. Alabaster (Ed.), *Habitat management and freshwater fisheries* (pp. 3-9). Buttersworths, London.

Appendix A

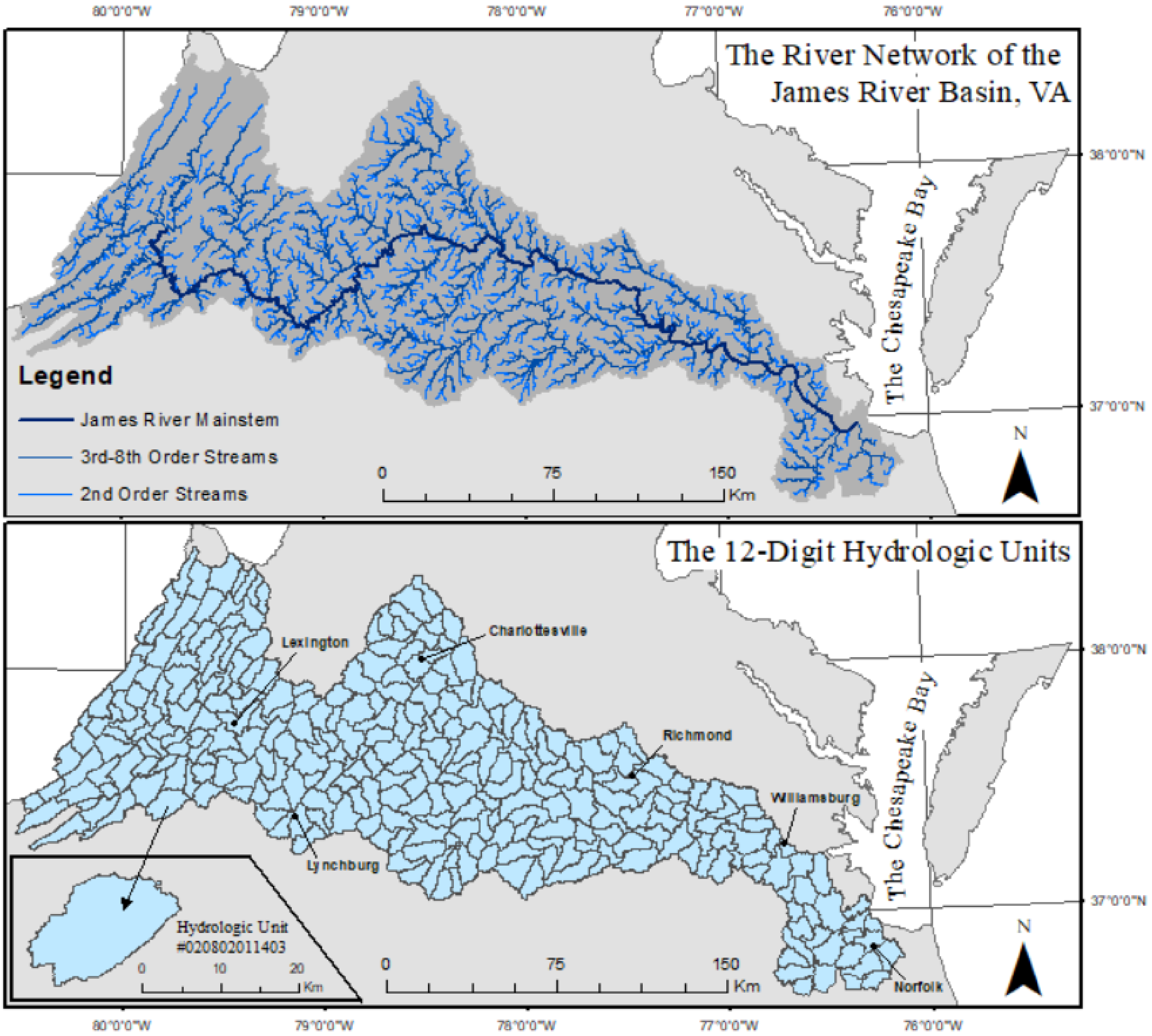

Figure A1. The James River Basin, Virginia. The top panel shows the river network of the James River Basin, as represented by the National Hydrography Dataset Plus Version 2 (NHDPlus ver. 2). First order streams were included in the study but excluded from the river network map to improve clarity. The bottom panel shows the 292 12-digit hydrologic units (Watershed Boundary Dataset) that lie within the James River Basin. A magnified 12-digit hydrologic unit with an inset scale bar is provided for reference. Locations of several major cities are included as landmarks to aid in interpretation.

Table A1. A list of the functional traits used in the study.

Functional Trait	Trait Description
Maturation Age (Female)	The youngest recorded age at maturation for females

Total Length at Maturation (Female)	The shortest recorded length at maturity for females, in centimeters
Maximum Fecundity	The highest recorded absolute fecundity
Average Fecundity	The average absolute fecundity
Maximum Egg Diameter	The largest cited mature egg diameter, in millimeters
Egg Adhesiveness	Whether eggs are adhesive or non-adhesive
Egg Guarding Behavior	Whether adults guard the eggs/nest during spawning
Pelagic/Demersal Egg Behavior	Whether the mature egg sinks or floats within the water column
Time to Hatch	The shortest cited egg incubation time, in hours
Egg Laying Behavior	Egg laying behavior (ex. brood hider, nest associate, speleophil)
Spawning Substrate	The preferred spawning substrate
Adult Substrate	The preferred adult (non-spawning) substrate
Velocity Preference for Adults	The water velocity preferred by adults
Velocity Preference for Spawning	The water velocity preferred during spawning
Spawning Month(s)	The month(s) during which spawning occurs
Average Spawning Temp	The average water temperature during spawning
Spawning Frequency	Whether spawning occurs once per year or multiple times
Maximum Longevity	The longest cited lifespan (male or female), in years
Average Longevity	The average lifespan (male or female), in years
Maximum Total Length	Longest cited total length (male or female), in centimeters
Average Total Length	The average total length (male or female), in centimeters
Parental Care	The degree of parental care provided to eggs and/or young, same definition as provided by Winemiller and Rose (1992)
Migratory Behavior	Migratory behavior
Depth Distribution	The location within the water column preferred by adults
Adult Habitat	The habitat type preferred by adults (ex. pool, riffle, river channel, backwater)
Trophic Level	The adult trophic level
Stream Size	The stream size preferred by adults
Swim Mode	The morphology/method of locomotion

Appendix B

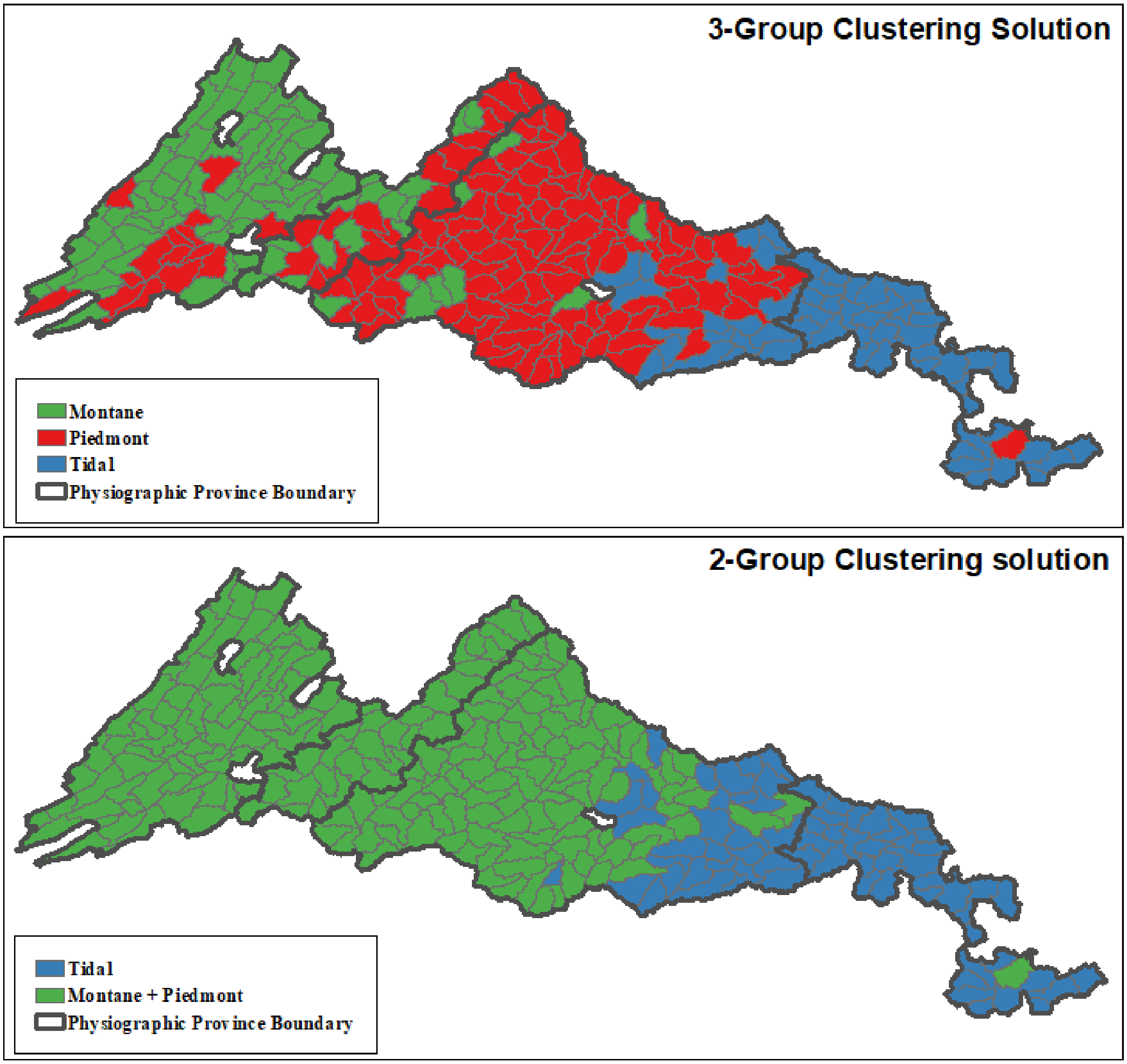

Figure B1. The top panel shows the three taxonomic ichthyofaunal zones identified by the 3-group clustering solution for the 12-digit hydrologic units produced by *k*-means clustering. The bottom panel shows the two taxonomic zones identified by the 2-group clustering solution. Boundaries of the physiographic provinces within the James River Basin are delineated by thick black lines. From left to right, these provinces are the Ridge and Valley, the Blue Ridge, the Piedmont, and the Coastal Plain.

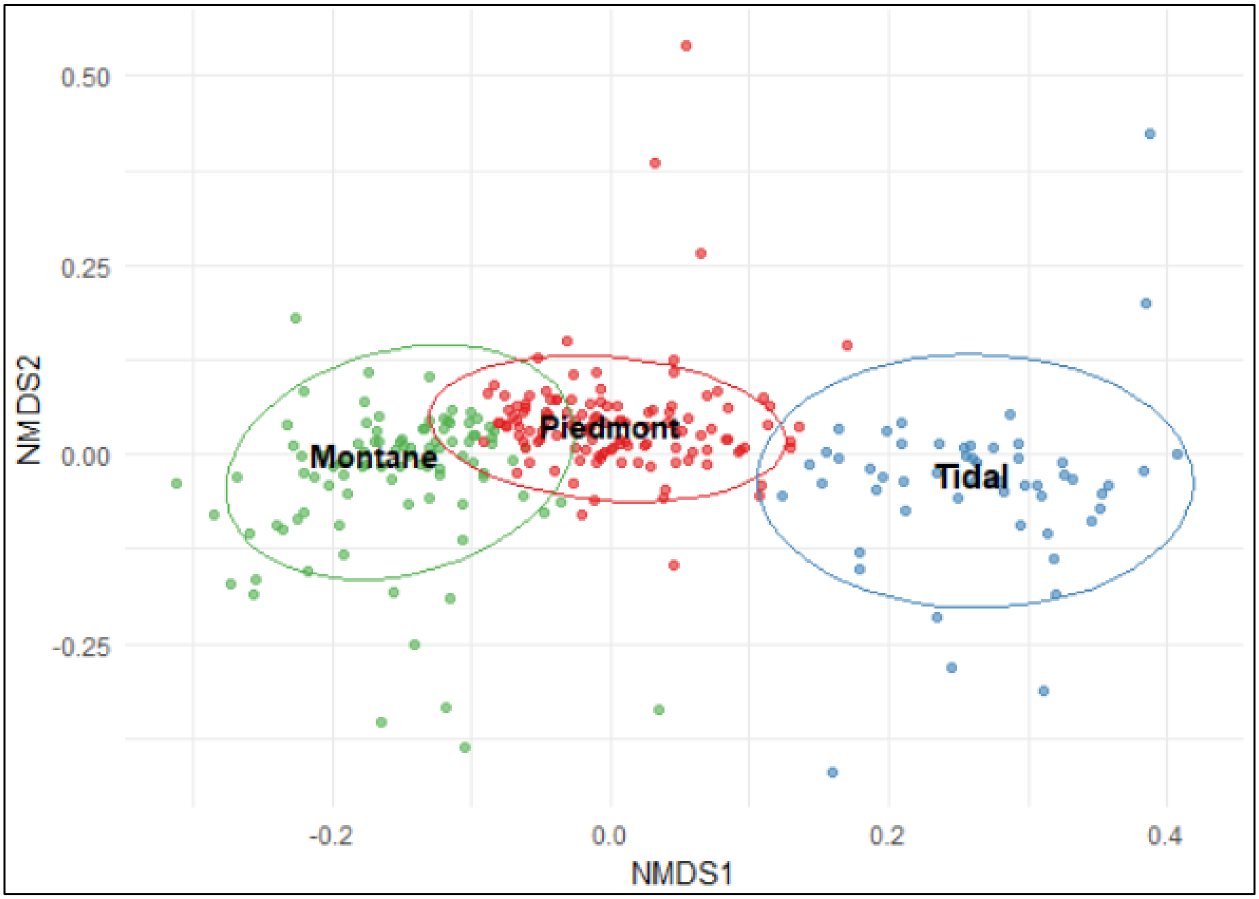

Figure B2. A non-metric multidimensional scaling of the Sorensen's dissimilarity matrix calculated from the taxonomic composition of the 12-digit hydrologic units (HUs). The HUs are colored according to their group assignments in the 3-group cluster solution produced by *k*-means clustering. In addition, a 95% confidence ellipse is drawn around each cluster of HUs.

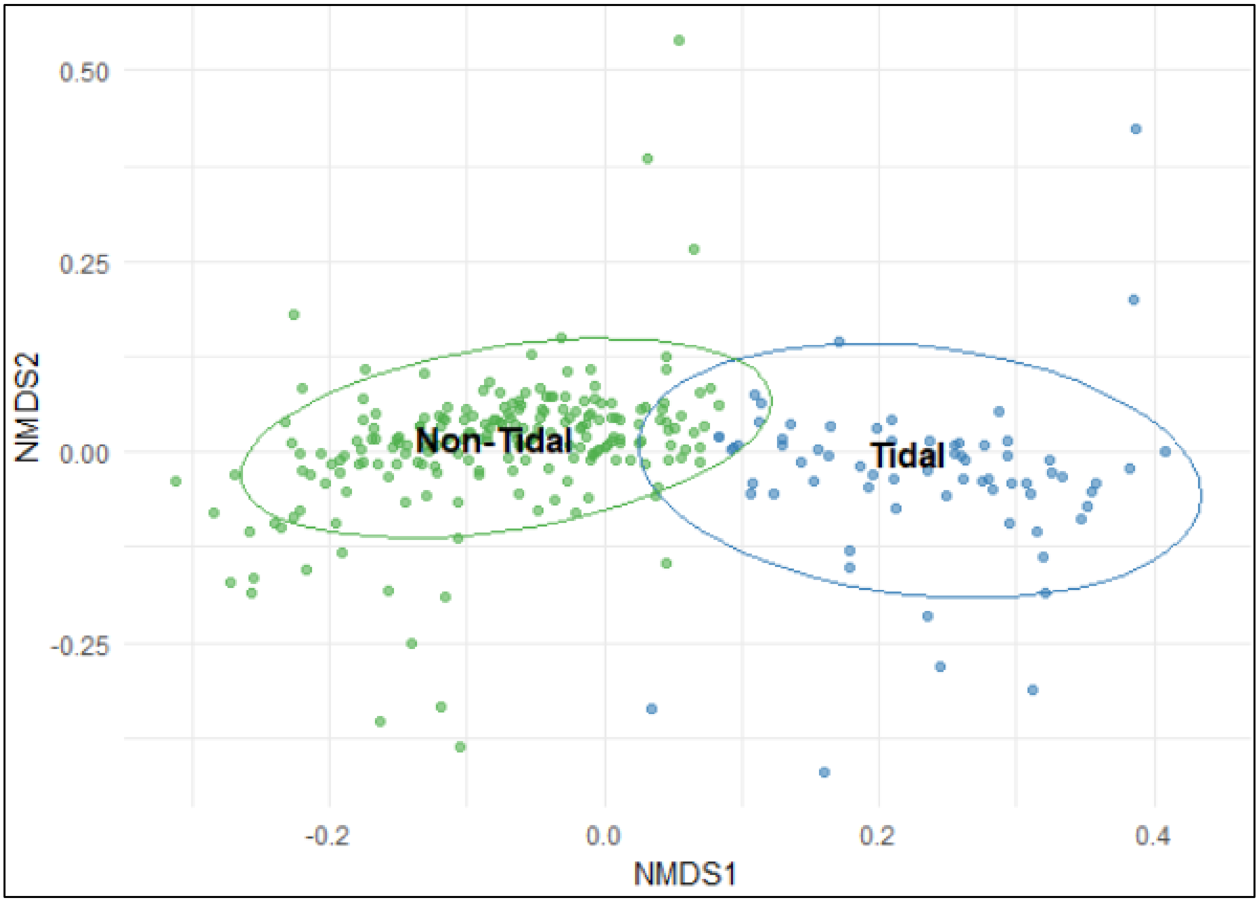

Figure B3. A non-metric multidimensional scaling of the Sorensen's dissimilarity matrix calculated from the taxonomic composition of the 12-digit hydrologic units (HUs). The HUs are colored according to their group assignments in the 2-group cluster solution produced by *k*-means clustering. In addition, a 95% confidence ellipse is drawn around each cluster of HUs.

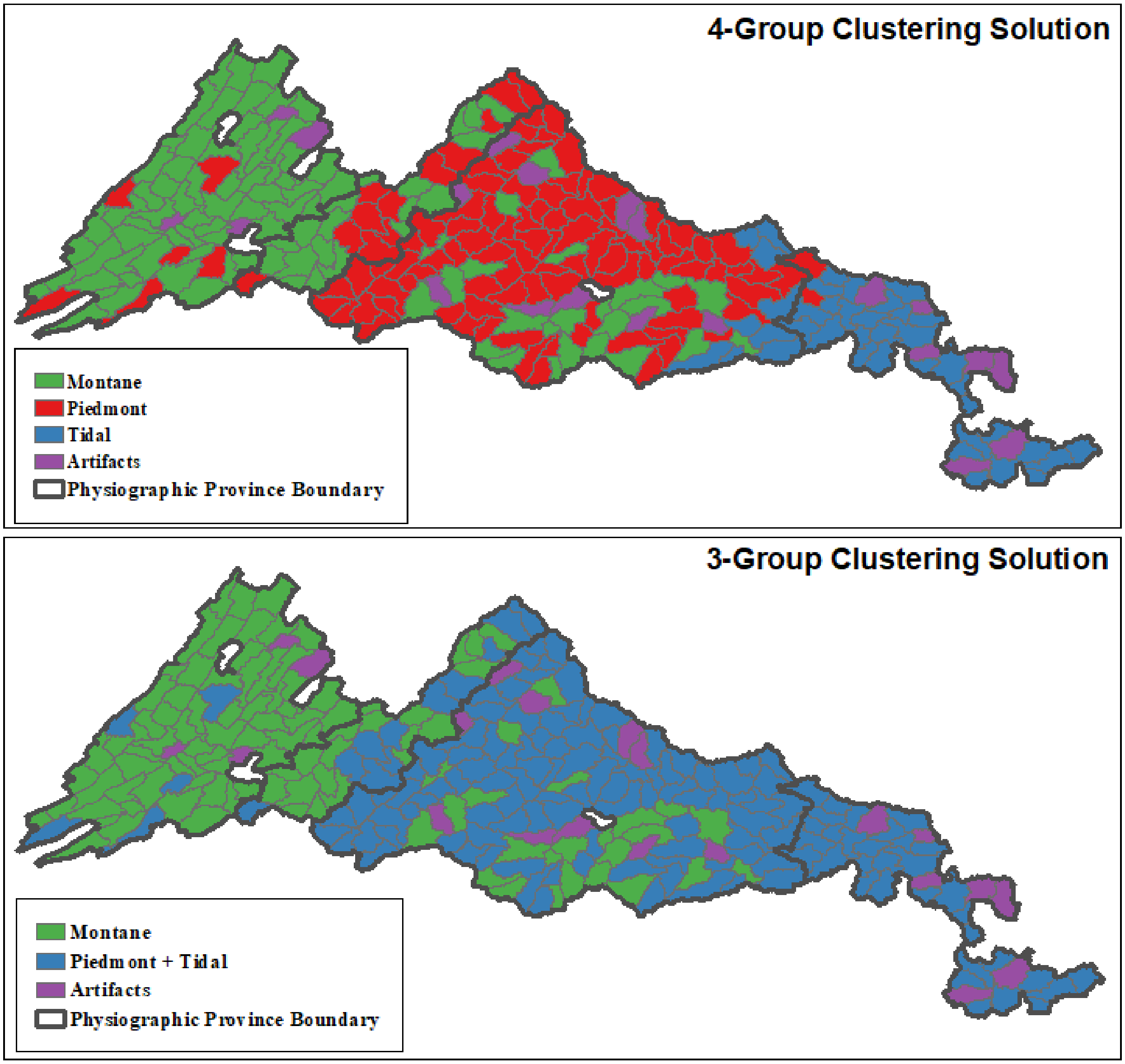

Figure B4. The top panel shows the three functional trait ichthyofaunal zones identified by the 4-group clustering solution for the 12-digit hydrologic units produced by *k*-means clustering. The Artifacts group was not an actual fish zone within the study, but a group of outliers produced by the methodology, which was partial indicated by the random scattering of the associated HUs. The bottom panel shows the two functional trait zones identified by the 3-group clustering solution. Boundaries of the physiographic provinces within the James River Basin are delineated by thick black lines. From left to right, these provinces are the Ridge and Valley, the Blue Ridge, the Piedmont, and the Coastal Plain.

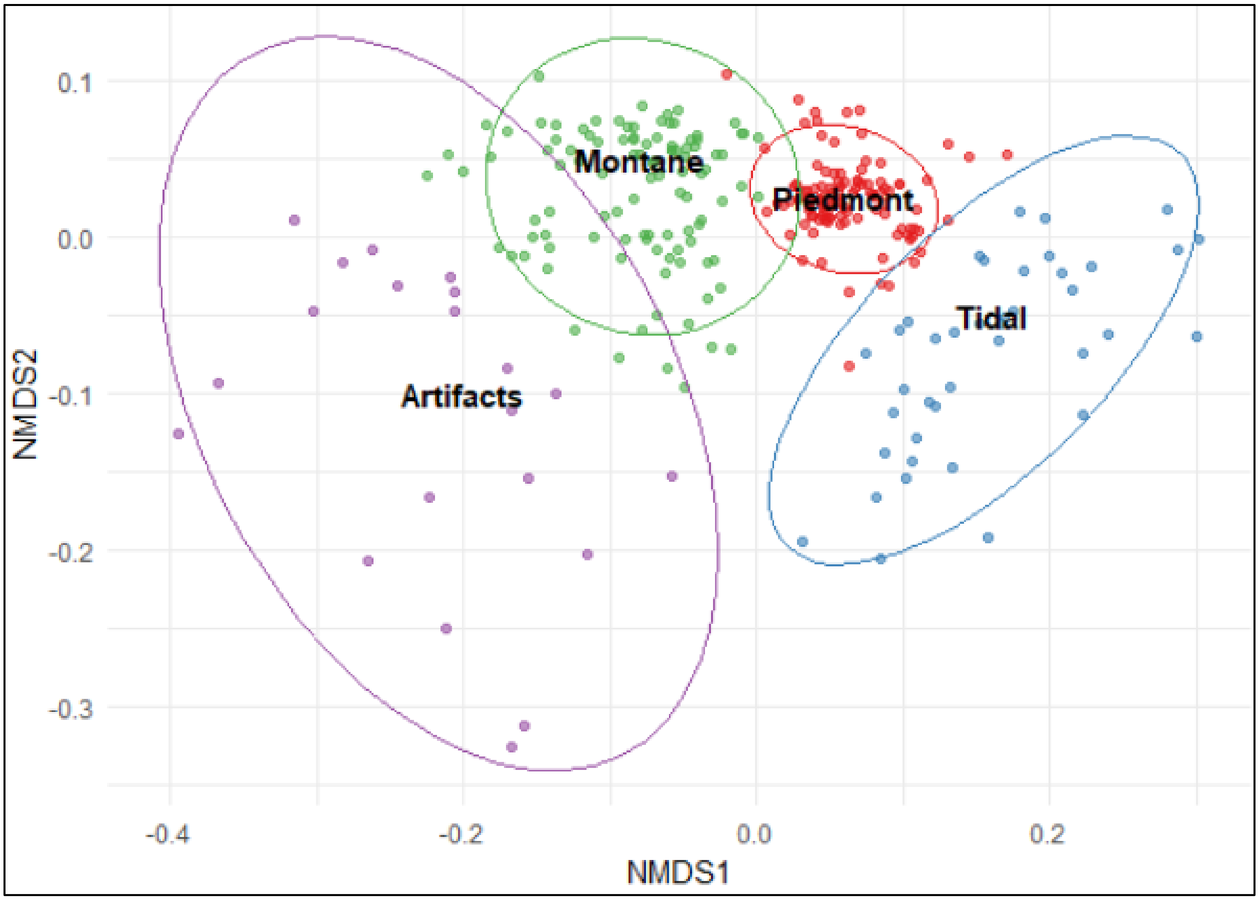

Figure B5. A non-metric multidimensional scaling of the Gower's distance matrix calculated from the summarized functional traits of the 12-digit hydrologic units (HUs). The HUs are colored according to their group assignments in the 4-group cluster solution produced by *k*-means clustering. In addition, a 95% confidence ellipse is drawn around each cluster of HUs. The Artifacts group was not an actual fish zone within the study, but a group of outliers produced by the methodology, which was partially indicated by the dispersion of the associated HUs within the ordination plot.

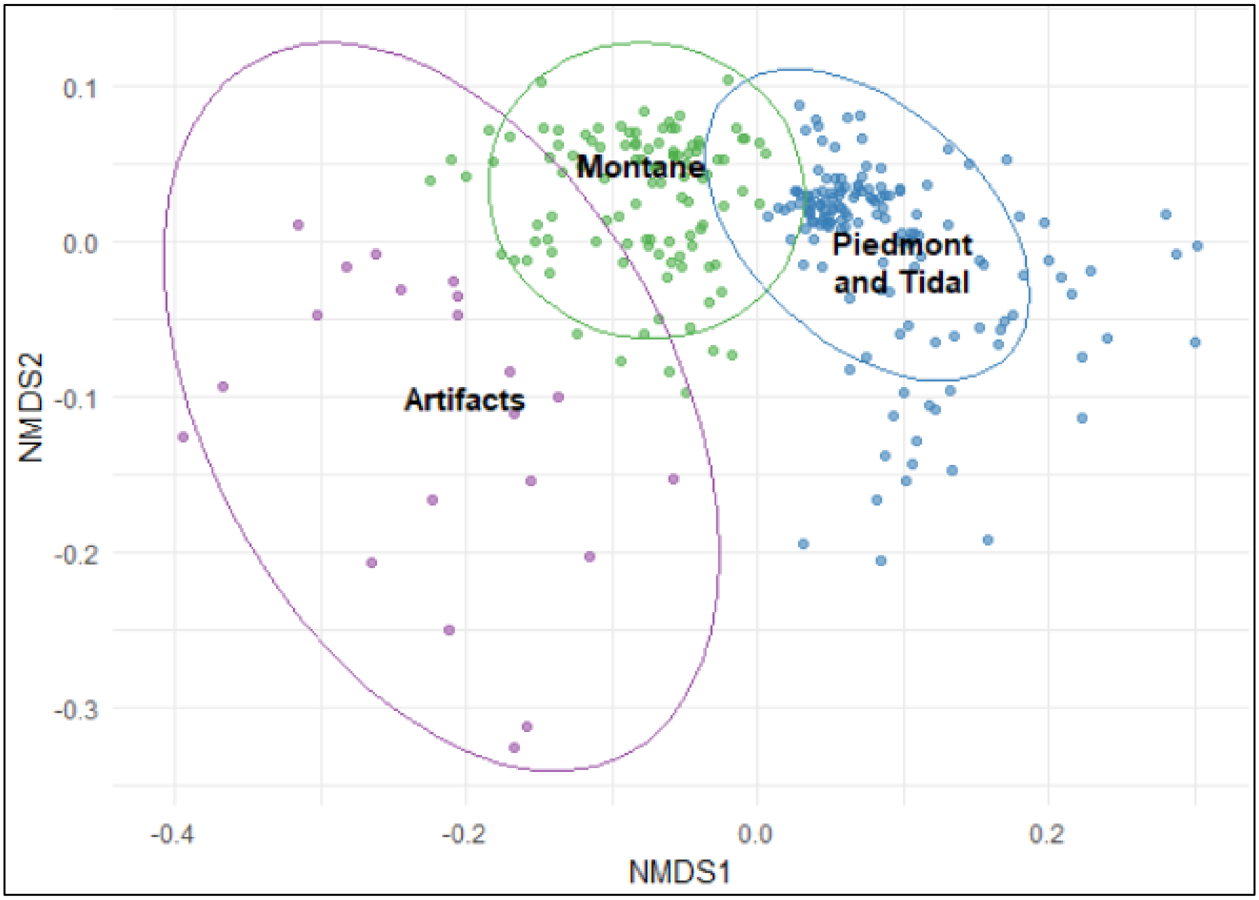

Figure B6. A non-metric multidimensional scaling of the Gower's distance matrix calculated from the summarized functional traits of the 12-digit hydrologic units (HUs). The HUs are colored according to their group assignments in the 3-group cluster solution produced by *k*-means clustering. In addition, a 95% confidence ellipse is drawn around each cluster of HUs. The Artifacts group was not an actual fish zone within the study, but a group of outliers produced by the methodology, which was partially indicated by the dispersion of the associated HUs within the ordination plot.

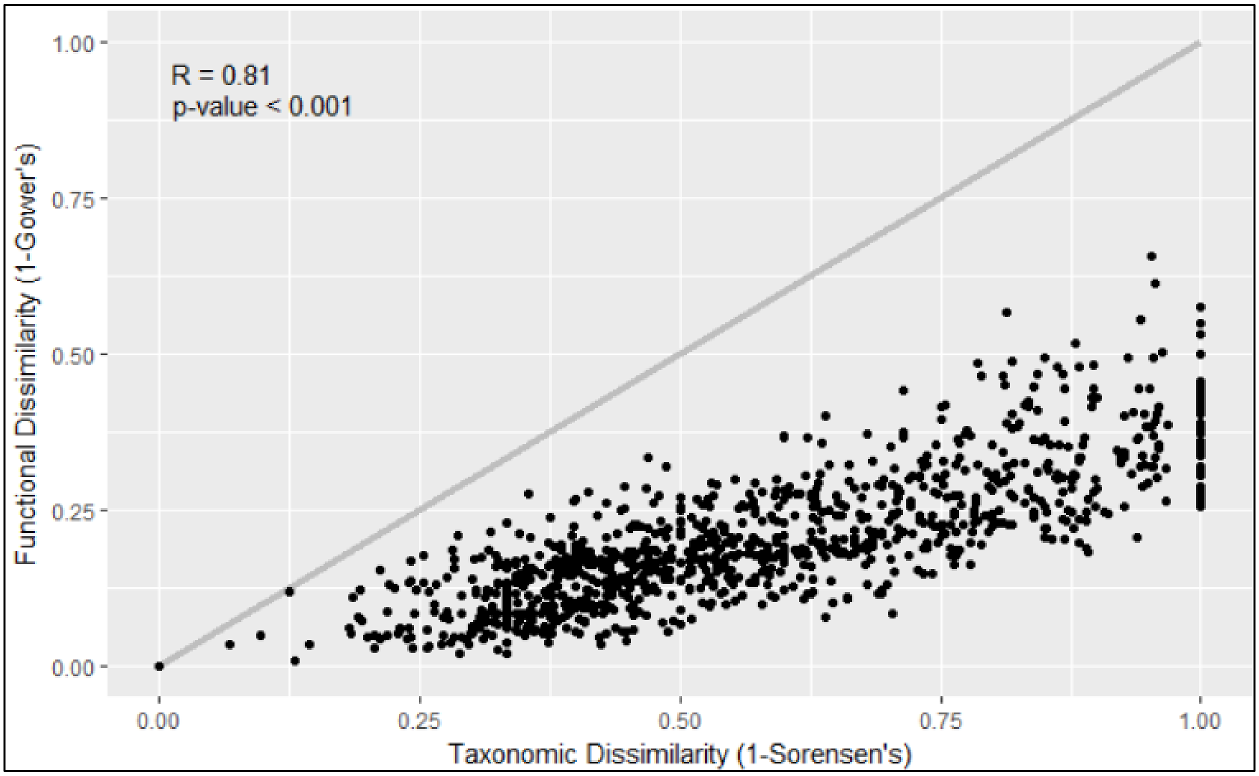

Figure B7. A plot of the correlation between the Sorensen's dissimilarity matrix (i.e., taxonomic dissimilarity) and the Gower's distance matrix (i.e., functional dissimilarity). A Mantel test using the Pearson product-moment correlation coefficient identified the correlation as significant (*R*=0.81, *p*-value <0.001). Each of the 1,000 plotted points represents a hydrologic unit x hydrologic unit comparison and was selected randomly from 75,076 possible points. A 1:1 straight reference line was included in the plot.

Table B1. The results of the indicator species analysis in which the characteristic species for each ichthyofaunal zone were identified. Characteristic species were also identified for combinations of zones (e.g., montane-piedmont zone in the taxonomic zonation pattern). Grey shading indicates characteristic species that were shared between corresponding taxonomic and functional zones (e.g., the montane taxonomic zone and the montane functional zone). Indicator species were only listed if their statistical significance satisfied the Bonferroni correction.

Taxonomy					**Functional Traits**				
Montane Zone	# of Characteristic Species: 4				Montane Zone	# of Characteristic Species: 3			
	A	B	IndVal	*p-value*		A	B	IndVal	*p-value*
Cottus bairdii	0.84	0.78	0.81	0.0001	Cottus caeruleomentum	0.78	0.66	0.72	0.0001
Oncorhynchus mykiss	0.86	0.38	0.57	0.0001	Oncorhynchus mykiss	0.81	0.34	0.53	0.0001
Salvelinus fontinalis	0.89	0.55	0.70	0.0001	Salvelinus fontinalis	0.80	0.44	0.59	0.0001
Exoglossum maxillingua	0.70	0.51	0.60	0.0001					
Piedmont Zone	# of Characteristic Species: 3				Piedmont Zone	# of Characteristic Species: 1			
	A	B	IndVal	*p-value*		A	B	IndVal	*p-value*
Percina roanoka	0.84	0.37	0.56	0.0001	Percina roanoka	0.80	0.41	0.57	0.0001
Etheostoma vitreum	0.80	0.45	0.60	0.0001					
Lepomis cyanellus	0.72	0.54	0.62	0.0001					
Tidal Zone	# of Characteristic Species: 12				Tidal Zone	# of Characteristic Species: 14			
	A	B	IndVal	*p-value*		A	B	IndVal	*p-value*
Acantharchus pomotis	0.92	0.35	0.57	0.0001	Acantharchus pomotis	0.88	0.40	0.60	0.0001
Ameiurus catus	0.78	0.31	0.50	0.0001	Ameiurus catus	0.82	0.40	0.57	0.0001
Centrarchus macropterus	0.77	0.63	0.70	0.0001	Centrarchus macropterus	0.73	0.71	0.72	0.0001
Dorosoma cepedianum	0.77	0.33	0.51	0.0001	Dorosoma cepedianum	0.77	0.40	0.56	0.0001
Enneacanthus gloriosus	0.80	0.74	0.77	0.0001	Enneacanthus gloriosus	0.74	0.79	0.76	0.0001
Etheostoma olmstedi	0.77	0.74	0.76	0.0001	Etheostoma olmstedi	0.73	0.81	0.77	0.0001
Morone americana	0.95	0.39	0.61	0.0001	Morone americana	0.94	0.43	0.64	0.0001
Morone saxatilis	0.94	0.37	0.59	0.0001	Morone saxatilis	0.94	0.45	0.65	0.0001
Perca flavescens	0.87	0.48	0.65	0.0001	Perca flavescens	0.87	0.62	0.73	0.0001
Umbra pygmaea	0.78	0.72	0.75	0.0001	Umbra pygmaea	0.71	0.74	0.73	0.0001
Etheostoma fusiforme	0.75	0.33	0.50	0.0001	Alosa aestivalis	0.92	0.31	0.53	0.0001
Gambusia holbrooki	0.74	0.78	0.76	0.0001	Alosa pseudoharengus	0.93	0.33	0.56	0.0001
					Fundulus diaphanus	0.94	0.33	0.56	0.0001
					Lampetra aepyptera	0.95	0.33	0.56	0.0001

Table B1 Continued. The results of the indicator species analysis in which the characteristic species for each ichthyofaunal zone were identified. Characteristic species were also identified for combinations of zones (e.g., montane-piedmont zone in the taxonomic zonation pattern). Grey shading indicates characteristic species that were shared between corresponding taxonomic and functional zones (e.g., the montane taxonomic zone and the montane functional zone). Indicator species were only listed if their statistical significance satisfied the Bonferroni correction.

Taxonomy					**Functional Traits**				
Montane-Piedmont Zone	# of Characteristic Species: 19				Montane-Piedmont Zone	# of Characteristic Species: 23			
	A	B	IndVal	*p-value*		A	B	IndVal	*p-value*
Ambloplites rupestris	0.98	0.43	0.65	0.0001	Ambloplites rupestris	0.98	0.46	0.67	0.0001
Campostoma anomalum	0.99	0.62	0.78	0.0001	Campostoma anomalum	0.98	0.63	0.79	0.0001
Catostomus commersonii	0.97	0.80	0.88	0.0001	Catostomus commersonii	0.97	0.83	0.90	0.0001
Clinostomus funduloides	0.88	0.91	0.90	0.0001	Clinostomus funduloides	0.91	0.92	0.91	0.0001
Etheostoma flabellare	0.94	0.83	0.88	0.0001	Etheostoma flabellare	0.95	0.87	0.91	0.0001
Etheostoma nigrum	0.88	0.74	0.81	0.0001	Etheostoma nigrum	0.90	0.77	0.83	0.0001
Hypentelium nigricans	0.98	0.61	0.78	0.0001	Hypentelium nigricans	0.98	0.64	0.79	0.0001
Luxilus cornutus	0.96	0.83	0.89	0.0001	Luxilus cornutus	0.95	0.86	0.90	0.0001
Lythrurus ardens	0.95	0.60	0.76	0.0001	Lythrurus ardens	0.96	0.64	0.79	0.0001
Micropterus dolomieu	0.96	0.60	0.76	0.0001	Micropterus dolomieu	0.95	0.64	0.78	0.0001
Nocomis micropogon	0.98	0.35	0.59	0.0001	Nocomis micropogon	0.97	0.37	0.60	0.0001
Notropis rubellus	1.00	0.43	0.65	0.0001	Notropis rubellus	1.00	0.44	0.67	0.0001
Percina notogramma	0.90	0.55	0.70	0.0001	Percina notogramma	0.92	0.59	0.74	0.0001
Chrosomus oreas	0.94	0.76	0.85	0.0001	Phoxinus oreas	0.97	0.79	0.88	0.0001
Phoxinus tennesseensis	1.00	0.45	0.67	0.0001	Phoxinus tennesseensis	1.00	0.46	0.68	0.0001
Rhinichthys atratulus	0.87	0.89	0.88	0.0001	Rhinichthys atratulus	0.88	0.90	0.89	0.0001
Rhinichthys cataractae	1.00	0.56	0.75	0.0001	Rhinichthys cataractae	1.00	0.59	0.77	0.0001
Semotilus corporalis	0.88	0.79	0.84	0.0001	Semotilus corporalis	0.91	0.85	0.88	0.0001
Thoburnia rhothoeca	0.99	0.86	0.92	0.0001	Thoburnia rhothoeca	0.99	0.86	0.92	0.0001
					Etheostoma longimanum	1.00	0.47	0.69	0.0001
					Exoglossum maxillingua	1.00	0.36	0.60	0.0001
					Nocomis leptocephalus	0.88	0.97	0.93	0.0001
					Pimephales notatus	1.00	0.30	0.55	0.0001
Piedmont-Tidal Zone	# of Characteristic Species: 10				Piedmont-Tidal Zone	# of Characteristic Species: 6			
	A	B	IndVal	*p-value*		A	B	IndVal	*p-value*
Ameiurus natalis	0.93	0.53	0.70	0.0001	Ameiurus natalis	0.84	0.56	0.69	0.0001
Anguilla rostrata	0.93	0.72	0.82	0.0001	Anguilla rostrata	0.99	0.95	0.97	0.0001
Aphredoderus sayanus	0.98	0.62	0.78	0.0001	Aphredoderus sayanus	0.87	0.61	0.73	0.0001
Erimyzon oblongus	0.89	0.64	0.76	0.0001	Erimyzon oblongus	0.82	0.65	0.73	0.0001
Lepomis macrochirus	0.89	0.90	0.89	0.0001	Lepomis macrochirus	0.82	0.93	0.87	0.0001
Micropterus salmoides	0.94	0.66	0.79	0.0001	Micropterus salmoides	0.87	0.73	0.80	0.0001
Cyprinella analostana	0.82	0.71	0.76	0.0001					
Esox niger	0.86	0.57	0.70	0.0001					
Lepomis gibbosus	0.91	0.71	0.81	0.0001					
Notropis amoenus	0.86	0.37	0.57	0.0001					